GROWTH AND DEVELOPMENT OF THE ROOT AND SHOOT APICAL MERISTEMS

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STATEMENT

This is to certify that none of the work presented here with myself as sole author has been previously submitted, in whole or in part, for any other degree at Edinburgh or elsewhere. The work represented by these papers was carried out by me, but often with technical help for which I have been grateful.

The papers with M.B. Miller (22, 23, 25) and N.H. Battey (38, 44) represent work which was successfully submitted by these students for the degree of Ph.D. at Edinburgh. The paper with F.E.W. Griffiths (43) represents work successfully submitted by this student for the degree of M. Phil. at Edinburgh, Dr M.D. Bennett being the external supervisor. The paper with N.H. Battey (42) was a joint work. These postgraduate students worked under my supervision.

The work on the root (1, 2, 3, 4) was carried out while I was a Post-doctoral Fellow, under the general direction of Prof. R. Brown, F.R.S. In work done while I was Visiting Assistant Professor at Cornell University, U.S.A., in the laboratory of Prof. F.C. Steward, F.R.S., Dr Barber helped in the early development of the acrylamide gel electrophoresis method (5).

The work in Parts 2 and 3 was all initiated by me. E.S. Robertson provided invaluable technical assistance in preparing material for electron microscopy (14). S. Grose was a Research Assistant working under my direction (36). Dr Cunningham (20, 21) and Dr Francis (26, 27, 31) worked as Post-doctoral Fellows under my direction. Later papers with Dr Francis (39, 40) were joint works.
The joint work with Dr Goodwin (34) relied entirely on his technical skill. The joint work with Dr Jacqmard (35, 37) was originated by me; some of it was carried out principally by me (35) and some principally by Dr Jacqmard (37), with antibodies prepared with Prof. Salmon's help (37), while I was visiting the laboratory of Prof. G. Bernier (35).
ABSTRACT

The growth and development of the root and shoot apical meristems have been studied to gain an understanding of the mechanisms of cell maturation in the root and of leaf and flower initiation in the shoot.

A method for isolating nuclei from small sections of root tips was devised and used to follow changes in nuclear composition and growth during cell maturation. The first use of acrylamide gel electrophoresis to demonstrate directly changes in the protein complement during cell maturation is described.

The measurement of cell division rates and planes within the shoot apex has shown that an adequate growth rate and the occurrence of periclinal divisions are prerequisites for primordium initiation rather than the immediate cause, which appears to be changes in the properties of the cell walls to allow localised bulging of the apical surface.

The cytological and histological changes observed at the shoot apex during floral evocation seem to be symptoms of flowering rather than its cause, as shown by their apparent non-essentiality in experiments in which they could be dissociated from flowering. The reduction in size of primordia on initiation at the transition to flowering, which was shown in Silene, seems a necessary and characteristic feature of flowering and is closely linked to changes in size of the shoot apical dome which are also characteristic of flowering. A comparison of flowering and its
reversion has shown that flower initiation consists of two sets of events. The first is a change in primordial arrangement which results from changes in partitioning of the apex each plastochron and may be accompanied by changes in apical growth rate. The second consists of the changes in competence and determination which lead to differentiation of the floral organs.
GROWTH AND DEVELOPMENT OF THE ROOT AND SHOOT APICAL MERISTEMS

The apical meristems are the regions of the plant autonomous for the generation of primary pattern and structure, as shown by their ability to continue to generate the same patterns and structures when they are excised from the rest of the plant and maintained in culture.

The cells produced by the meristem cease division, enlarge and vacuolate, and undergo maturation as the meristem grows away from them. Cell maturation is superimposed on, and simultaneous with, cell differentiation. The root meristem, because it does not produce organs near the tip, is the choice for studying cell maturation and how it is controlled. In order to understand how the leaves and flowers are formed it is necessary to understand how the shoot apical meristem functions.

This thesis represents that part of my work which has been concerned with the structure, functioning and development of the root and shoot apical meristems. The work is presented in 3 parts:

Part 1: Growth of the root apical meristem: cell maturation (Papers 1-5)

Part 2: Growth of the vegetative shoot apex, and the mechanism of leaf initiation (Papers 6-21)

Part 3: The transition to flowering at the shoot apex, and the mechanism of flower formation (Papers 22-46)
Part 1: Growth of the root apical meristem: cell maturation

In a growing root the linear spatial sequence of cells from the tip to the more mature, basal regions represents a temporal sequence of cell development. Serial sections behind the root meristem therefore provide samples of successive stages in cell maturation. Changes in enzyme activities along the root had been interpreted as indicating that the enzyme complement of the average cell changed as it matured. If such changes were regulated by changes in the transcription of DNA, then this would imply a changing environment within the nucleus and would be consistent with a changing influence of the nucleus on the rest of the cell and of the cytoplasm on the nucleus. In order to find out how the environment within the nucleus might be changing during cell maturation, one approach was to attempt to isolate nuclei from different parts of the root - from cells at different stages of maturation - and to compare their structure and enzymic activity.

A method was therefore developed for the isolation of pure preparations of intact nuclei from three sections of the root: the meristem, the region of rapid cell enlargement, and the region of cessation of cell enlargement and maturation (1).

The structure of the nuclei changed during cell maturation. The mean DNA and protein contents of the nuclei increased by about 30% from the meristem to the enlarged cells (4). This was mainly because the cells in the meristem were a mixture of 2C and 4C cells in different phases of the mitotic cycle, whereas in the enlarged cells the nuclei were mainly 4C or higher, because of some polyploidy which is associated with differentiation in the root (3). The RNA
content of the nuclei halved as the cells enlarged although the RNA content of the cells themselves increased three-fold (1, 4). This implied that RNA synthesis, which takes place in the nucleus, continued in the enlarging cells but less RNA was retained in the nucleus. RNA was apparently being released to the cytoplasm as fast as it was made, so that the enlargement of the nucleolus which occurs during interphase of the cell cycle (4) was not maintained in the enlarging cells. The way in which the flux of ribosomal and pre-ribosomal particles through the nucleus, and hence the size and rate of growth of the nucleolus, is controlled is a problem that still requires investigation.

A striking feature of the growth of the nucleus in the root cells was that much of its enlargement, both within the meristem and in the enlarging cells, was a result of increasing hydration which was not matched by a similar hydration of the cytoplasm (1, 3). This is presumably related to the physical properties of the proteins specific to the nucleus as it enlarges.

When the growth of the cells was followed in more detail it became apparent that there was much less variation between cells within a file than there was between cells in different files (3). This is perhaps to be expected since cells within a file are daughters or recent descendents of a common ancestor. However it does mean that the general relationships between cell and nuclear sizes and between cell size and DNA content, which have sometimes been invoked as being important in cell differentiation, are average values which obscure the differences between cells in adjacent files even within the same tissue. The root appears to be composed of diverse cells conforming to some common pattern or template and is
not easily interpretable as a structure resulting from rigid controls at the level of the individual cells (3).

The loss of RNA as the nuclei matured prompted the hypothesis that the activity of ribonucleases in the nucleus might increase as the nucleus grew. There was speculation that histones might show ribonuclease activity. The isolated nuclear preparations could therefore be used to test this hypothesis. However, no ribonuclease activity was detected in the nuclei (2). Ribonuclease activity was clearly shown to be confined to the cytoplasm, particularly the cytosol, and two ribonucleases with different pH optima could be measured.

Previous evidence had pointed to the probability that the protein complement of the root cells changed as they matured. When the new technique of acrylamide gel electrophoresis became available its much greater resolving power for separating proteins was used for the first time with higher plant material to examine the proteins of pea seedlings (5). This provided the first direct demonstration that the protein complement did indeed change during cell growth and maturation and that different parts of the seedling had different protein complements.
Part 2: Growth of the vegetative shoot apex, and the mechanism of leaf initiation

To understand what happens when a leaf forms it is necessary to know the physiological and cellular basis for the growth centres that give rise to the leaf primordia. Anatomical and histological studies did not provide sufficient quantitative data to describe apical growth. It was known that there was a greater frequency of mitotic figures on the flanks of the shoot apical dome than at its summit and it was therefore presumed that leaves arose because of a higher growth rate at the sites of leaf initiation.

To obtain quantitative data it was therefore necessary to measure growth rates directly throughout the shoot apical meristem. The pea (Pisum sativum) was used because of the convenient distichous arrangement of its leaves and primordia. The time taken for the apex to progress through morphologically definable stages of a single plastochron was measured experimentally so that the changes with time in the numbers of cells in all parts of the apex could be determined (6). Cell accumulation was faster in the primordium in the first 30h of the plastochron and faster in the apical dome in the last 16h. This was consistent with the young primordium arising as a new growth centre in the first part of the plastochron and then the apical dome being the main growing region in the second part of the plastochron, just prior to the initiation of the next leaf primordium.

To check these conclusions by an independent method, the rates of cell division throughout the apex were measured from the rate of metaphase accumulation in the presence of colchicine (7). The
somewhat surprising findings were that the rates of cell division throughout the apex and throughout the plastochron were much more uniform than the cell number data had suggested. Cell divisions were more frequent than expected in the axial part of the apex, although it did not grow much in terms of cell number. Also, in the apical dome and primordium, divisions were insufficiently frequent to account for the observed growth. Throughout the plastochron there was insufficient difference in rates of division to account for the phasic changes in cell numbers in different parts of the apex.

The necessary conclusion was that the apparent changes in growth rate in the different parts of the apex during the plastochron were the result of changes in the directions, rather than the rates, of growth. The prediction was therefore made that a change in the direction of growth to form a new primordium should be preceded by a change in orientation of division, from anticlinal to periclinal, and that this should be measurable as an increase in the frequency of periclinal mitoses in the $I_1$ region of the apical dome. This is what in fact was found (8). Sixteen hours before primordium initiation periclinal divisions appeared in the $I_1$ region where previously there had been very few or none. One third of the divisions were periclinal and the other two thirds were anticlinal, the latter equally distributed between those in the plane of the sections and those normal to it. These relative frequencies did not change during the 16h before primordium initiation. If periclinal divisions were the result of some stimulus it might have been expected that they would either show a peak or would continue increasing in frequency. The implication of a constant distribution between the three planes of space was that there was indeed no preferred plane but that orientation of division
was random in the \( I_1 \) region during the second part of the plastochron. The occurrence of periclinal divisions and the outgrowth of the primordium could therefore be interpreted as the lifting of a restraint on the apical dome which prevented periclinal divisions rather than the imposition of a new direction of growth (19).

A quantitative stereological study of the ultrastructure of the pea shoot apex showed that there were no changes in organelle composition of the cells which could be related to the plastochron and to the timing of leaf initiation. The only change observed during the plastochron was a decrease in starch granules in the \( I_1 \) region and an increase everywhere else in the apex at the precise time during the plastochron that periclinal divisions first appeared, i.e. 16h before leaf initiation (14). This raises the possibility that the changes in the amount of starch and the occurrence of periclinal divisions are both permitted by a change in concentration of some substance within the apex at this time, and that the restraint on primordial formation is a chemical one.

Despite the initiation of the leaf primordium in pea being more a result of changes in the direction of growth than in its rate, a localised increase of growth rate in the epidermis at the site of leaf initiation would be expected, because the surface must expand at this point. This is indeed found at the moment of leaf initiation when the surface starts to bulge (10, 19). This epidermal growth rate is greater on the abaxial than the adaxial surface, so that the young leaf tends to grow upwards (15). The faster growth of the epidermis at leaf initiation could be interpreted either as the epidermis having a controlling influence on leaf initiation or the
epidermis simply responding to events beneath it (10). P.B. Green and colleagues showed that changes in the orientation of cellulose wall microfibrils in the epidermis in *Graptopetalum* precede the initiation of leaves. They showed that the orientation of cellulose microfibrils in the epidermis, and the subsequent planes of cell division, changed through 90° at the sides of the putative primordium but remained unchanged at the potential upper and lower surfaces. If the same were necessary in the pea then it would be expected that divisions in the epidermis at the sides of the incipient primordium would be predominantly longitudinal (18) rather than being predominantly transverse as they were in the I₁ region (8). (Note: the orientation of the mitotic spindles as recorded in the papers is normal to the orientation of the plane of cell division). Measurements of the orientations of mitotic spindles on the sides of the pea apex showed that there was no evidence for a reorientation of epidermal division planes in this region (18). The pea primordium seemed to be formed by an outgrowth of the apex which preserved the predominantly longitudinal polarity of epidermal growth which was evident in the I₁ region. The polarity of the epidermis then seemed to become imposed on the underlying cells (18) which, in the I₁ region, had shown a random distribution of division planes and hence no polarity (8).

These data on the changes in the plane of cell division and polarity of growth had depended on the scoring of mitotic figures (8, 18), which are relatively infrequent in shoot apices. Methods involving transmission and scanning electron microscopy as well as light microscopy were therefore devised to obtain data from all cells which had divided less than one cell cycle ago. It was shown that these most recent divisions could be consistently recognised by
the thinness and position of the cell walls so that all recent periclinal divisions could be recorded and mapped (20). For the pea it was confirmed that the periclinal divisions corresponded in distribution with the surface extent of the future primordium and they appeared simultaneously within the whole of the I₁ region. They did not spread from a locus as might perhaps have been expected if they had been stimulated by some substance produced at a specific position such as the centre of the putative primordium.

However, Silene proved to be unexpectedly different. Periclinal divisions were present equally throughout the plastochron, in all parts of the apex, including both I₁ and I₂ regions of the apical dome and were also present at the sides of the apex where only the lateral bases of the primordia met (20). It was clear that in Silene the periclinal divisions did not prescribe either the time of primordium formation or the position and extent of the primordia. The frequency of divisions in the vegetative Silene apex, as shown by the distribution of colchicine-metaphases was greatest in the young primordia (23). These contrasting findings for Pisum and Silene suggest that in Pisum primordium initiation is accompanied by a change in the orientation of growth, against a background of an adequately rapid growth rate, but that in Silene there is an increase in growth rate at the primordial sites where periclinal divisions already occur (21).

Epidermal divisions in the region of primordium division in Pisum were predominantly transverse (21), as expected from the longitudinal polarity of growth (8, 18). However in Silene divisions were mostly longitudinal, indicating a predominantly
lateral growth polarity at the leaf site (21). Thus in Pisum, in which the leaf primordium is strongly dorsiventral, the longitudinal polarity and predominantly transverse divisions in the epidermis of the apical dome are maintained during primordium initiation. In Silene, in which the paired primordia extend round the apex to meet and form a collar, the division plane changes to longitudinal over the whole of the primordium surface. In Graptopetalum, in which the young primordium is essentially centric, the orientation of the wall microfibrils and the division planes change predominantly on the sides of the primordium site. The interpretation put forward (21) is that the division planes in the epidermis, and the changes in these planes, are more concerned with the shape of the young primordium than with its initiation per se. The further implication is that the occurrence of periclinal divisions, the direction of growth, and the rates of growth are all only permissive conditions for the outgrowth of the primordium. The overriding causal factor is presumably the physiological changes in the properties of the cell walls which alter the strain pattern and allow the walls to bulge out where primordia are formed. Ultimately attention will have to be directed to the nature of the morphogens in the shoot apex which affect wall plasticity, and for which auxins are plausible candidates (17).

Any explanation of the initiation of primordia must take into account the fact that they are initiated only on the flanks of the apical dome in the vegetative apex. The frequency of periclinal divisions tends to be higher on the flanks of the apex than at the summit (15). There is a gradient in the rates of growth and cell division from least at the summit to greatest on the flanks of the apical dome (13) and this in turn depends on the control of the rate
of cell division and the progression of cells through the cell cycle. The first measurements of all the phases of the cell cycle in cells throughout the shoot apex were made in the pea (13). They showed that mitosis (M) was the same length throughout the apex but all phases of interphase (G₁, S, G₂) were longer at the summit of the apex where the cell cycle was longer. The lengthening of S implied that the overall rate of DNA synthesis was inversely proportional to cycle length, and that entry into S and M were delayed in the longer cell cycles. This contrasted with what is known for the root in which the cell cycle is regulated mainly by the entry into S (i.e. by the length of G₁) whereas the length of time spent in DNA synthesis (S) tends to remain constant.

The gradient in growth rates in the apex is matched by a similar, but not exactly superimposed, gradient of cytological characteristics of the apical cells (7, 11). The cytochemical zonation of least intense staining in the central zone and more intense staining on the flanks of the apex is apparently mainly due to greater concentrations of proteins and RNA on the flanks (11, 19). In the pea, because the content per cell of RNA and protein is similar in all cells of the apical meristem, the cytochemical zonation is therefore a function of cell size, which is slightly greater in the central zone than on the flanks (6, 9). In other plants the same cytochemical zonation pattern does not depend on cell size and so the cytochemical gradient, which appears to be universal (19), is not coincident with the same cellular patterns (11). As in the root, the organisation of the shoot seems to be superimposed on the cell pattern rather than derived from it.

For the pea apex it can be calculated, from the growth rates
(7, 13) and the cellular contents of RNA and protein (9), that the rates of accumulation of RNA and protein are lower in the central zone than on the flanks of the apex. When radioactive precursors of RNA and protein were supplied to apices, their rates of incorporation were similar in all parts of the apex (12). This is interpreted as showing that the radioactive precursors were entering endogenous precursor pools that were smaller in the central zone, resulting in higher specific activity of precursors entering into the RNA and protein and producing a relatively high rate of incorporation but an erroneously high indication of the rates of synthesis. Smaller precursor pools in the apex in the more slowly growing cells would be in contrast to the root meristem in which the incorporation of labelled precursors tends to be proportional to the growth rate of the cells, implying that the sizes of the precursor pools are equal in all cells irrespective of growth rate. This seems yet another indication of differences between root and shoot meristems in the overall control of RNA and protein synthesis, which is also indicated by the differing rates of accumulation of nucleolar RNA and protein during the cell cycle in roots and shoots (16).

The cytochemical gradient in the shoot apex (11, 19) might be expected to be reflected in a gradient of subcellular structure. Quantitative examination of the ultrastructure of the pea shoot apex showed that, just as there were no differences in RNA and protein per cell (9), there were no significant differences in organellar composition per cell between the central zone and the flanks of the apex (14). The main basis for the cytochemical zonation in pea is therefore confirmed as being the slight differences in cell size resulting in differences in the concentrations of organelles and
almost certainly of soluble proteins and ribosomes, which were not measured in this study. The differences and changes in organellar composition per cell were related to the differentiation of the cells into different cell types. The replication of plastids was shown to be controlled independently of replication of mitochondria and each was linked to the control of cell division in different ways in different developmental pathways. The increase in the number of plastids per cell which occurs during leaf development could already be detected in the youngest leaf primordium (14).

In summary, this study of the growth of the shoot apex and the mechanism of leaf initiation has shown that

(a) the form of the apex is probably the result of the maintenance and release of restraints on growth rather than the imposition of new growth directions

(b) the occurrence of periclinal divisions and a maximal growth rate on the flanks of the apex are each apparently a prerequisite for primordium formation rather than being the immediate initiating events, which are probably concerned with changes in cell wall plasticity

(c) the occurrence of periclinal divisions may possibly depend on the concentration of some substance which also affects starch metabolism

(d) the changes in planes of cell division in the epidermis at leaf initiation seem to be more implicated in determining the form of the emergent primordium than in its actual initiation
(e) the rate of cell division in the shoot apex appears to be governed by multiple control points in the cell cycle viz: the entry into S, the rate of DNA synthesis, and the entry into M; unlike the root where the principal control point in normal growth is the entry into S

(f) cellular changes in the shoot apex at the organelle level are not related to leaf initiation but to cell differentiation.
Part 3: The transition to flowering at the shoot apex, and the mechanism of flower formation

The importance of changes in the orientation of growth rather than the rates of growth in the initiation of leaf primordia in the pea posed the question of whether, in the transition to flowering, key events at the apex leading to floral morphogenesis might be obscured by other events concerned with changing growth rates.

In order to try and isolate the crucial events of evocation—the processes in the shoot apex which lead to flowering—Silene coeli-rosa was chosen because the shoot apex transformed directly into a flower. A minimum change in growth rate might be therefore be expected. Also a direct comparison can be made between the formation of leaf primordia in the vegetative apex and floral organ primordia in the floral apex. This comparison cannot be made in plants with a terminal inflorescence such as a raceme or spike, or with axillary flowers. In such plants the appropriate comparison would be between the flower buds and the axillary buds of the vegetative shoot. However, vegetative axillary buds are slow to form after initiation and then after some growth are subject to inhibition by apical dominance, whereas the flower buds grow out from initiation, sometimes precociously, because of the loss of apical dominance on flowering.

S. coeli-rosa has the further advantages that it is an obligate long-day plant under the conditions used (41) so that the start of the flowering processes can be pinpointed, and it has opposite, decussate leaves which facilitate orientation and identification of primordia in histological preparations. In
addition the direction of the helix of the unequal vegetative axillary buds bears a constant relationship to the sequence and arrangement of the floral organs. Thus each of the leaves and floral organs can be individually identified routinely from sections and this has facilitated analysis of the growth of the flower in a way which has not been possible with any other plant (28, 29, 32).

Measurement of the rates of growth in the *Silene* shoot apex during evocation showed that the growth rate increased during the plastochron between the initiation of the last pair of leaves and the initiation of the sepals (23) and that the cell cycle shortened from 20 to 10h (22). It seemed that this increase in growth rate could be the cause of the enlargement of the apical dome relative to the sizes of the primordia at initiation, which would bring about the change in primordial arrangement associated with flowering. However, primordia also grew faster and so some other explanation had to be sought for the change in primordial arrangement and increase in apical dome size at the transition to flowering (24). It became evident that an important feature of the transition to flowering is the way in which the growth of the apex is partitioned between apical dome and primordia, and that it is this, and not growth rate, that determines how rapidly the apical dome enlarges.

From a series of scanning electron micrographs of the developing flower (28), measurements of the plastochron ratios of the floral organs allowed estimates to be made of the sizes of the primordia at initiation. These showed that there was a step-down in primordial size at sepal initiation, and a further reduction on initiation of the petals and stamens (29). In the only other flowers
for which data are available, Impatiens and Ranunculus, there is also a reduction in primordial size at flower initiation (42). Because the change in phyllotaxis to a higher order in the flower is a common or universal phenomenon, a reduction in primordial size, which this implies, is thought to be a characteristic and probably essential feature of flower formation. This again focuses attention on the mechanism which determines the formation, on the apical surface, of the bulge which becomes a primordium and the factors which control its size.

Although the sequence of initiation of the floral organs seems to be governed by the same rules as leaf initiation, and is therefore presumably governed by the same factors, it was found necessary to postulate a second set of factors governing the positions of the floral organ primordia (29). These latter factors may perhaps be physical structures - the cellulose reinforcement fields shown by P.B. Green to be features of the apical surface - on which chemical morphogens might act to allow primordia to form (21). However the occurrence of aberrant flowers showing meristic variation (30) shows that the specification of position is not always precise, especially for the stamens, which may not always reflect the positions of the sepals and petals. The sporadic occurrence of proliferous "flowers" (33) in which the reduction in primordial size was only partial and the organs did not differentiate into normal floral organs, or showed a whole range of anomalous forms, also suggests that the changes which normally occur in the apex on evocation and control primordial size are essentially quantitative although, because of the rapidity with which they normally occur, they seem to be qualitative.
Of the cellular changes during evocation the characteristic increase in RNA seemed to be more linked to changes in growth rate and gibberellin content than to flowering per se, as shown by the experimental attempts to dissociate the RNA increase and flowering (25). Neither the changes in the growth rate themselves nor the increases in RNA, which seemed to be linked to growth rate, were apparently essential for floral initiation since this could occur in plants at 13° before growth rate changed (24). Neither the initiation of primordia in the flower nor the form of the flower were determined primarily by the growth rate. Again the constant feature was the change in phyllotaxis on flower formation, and the more rapid primordium initiation that accompanied it, brought about by a reduction in primordial size at initiation (32).

Reduction in primordial size at flower initiation is also accompanied by a reduction in stem frustum size (42). The extent of the primordium on the apical surface therefore seems to be linked to the potential size of the incipient nodes and internodes. In the flower itself the lack of internodes is because internode initials apparently cease to be formed at the shoot apex (46). The further implication is that, in the apex, there are distinct cell layers from which nodes and internodes are initiated. The structure of the apical meristem may therefore be much more organised and layered than appears from histological examination.

A feature of evocation in photoperiodically induced plants is the occurrence of successive mitotic peaks which indicate some synchronisation of cell division in the shoot apex. Most of the events of evocation in Sinapis alba can be interpreted as being part of a synchronous cell cycle initiated by the inductive long day
The first long day of induction in *Silene* resulted in an increase in cell number in the apex, indicating an increased rate of cell division (23). Synchronisation was therefore looked for at this time in *Silene*. It was not found although the cell cycle shortened temporarily from 20 to 13h and the S phase also shortened, implying an increase in the rate of DNA synthesis (26, 27). This reduction in the cell cycle, which can be detected as soon as 30-60 min after the beginning of the changed photoperiod, seems to be essential for flowering in *Silene* (40).

Synchronisation was in fact found in *Silene*, but just before sepal initiation when the growth rate increased (31). When the plants were induced by 7 long days, then placed in darkness for 48h before being transferred to short days, synchronisation was suppressed although flower initiation went ahead normally, but was delayed by 48h because the growth of the apex was temporarily inhibited (36). Synchronisation is therefore not essential for flowering in *Silene*. If synchronisation is nevertheless a characteristic part of evocation, it should not be expected just before the formation of later-formed flowers and long after evocation and the transition to the flowering state have occurred.

When third order flowering buds of *Silene* were examined, using a novel method of analysis which does not rely on synchrony between plants in a population or sample, evidence for synchronisation was obtained for these buds too (45). This implies that in *Silene* synchronisation of cell division during evocation and flowering is not the direct result of a stimulus reaching the apex at a particular time but is more likely to be the result of changes, at the initiation of each flower, in the competence of the cells to respond to substances already present at the apex.
This was essentially the same conclusion as was reached from a study of the growth changes in the shoot apices of wheat lines with different vernalization requirements (43). Despite the apex being the site of both the perception and response in vernalization, no differences were found in the growth of the apices in the different lines which could be ascribed directly to vernalization. The only change vernalization brought about was in the ability, or competence, of the apex to react to subsequent long days.

The possibility that there is a key event in evocation involving only one or a few proteins was tested by examining the changes in the protein complement of the Sinapis apical meristem by autoradiographic (35) and immunological (37) techniques but the changes found either involved many proteins (35) or were very late in the flowering process (37).

When Sinapis, Silene and other plants are compared it seems that although there are a number of events, including increased growth rate, increased RNA and protein, and synchronisation of the cell cycle, which are characteristic of evocation, some events seem to be essential in some plants but not in others (39). If there is a single key event it has not yet been found. Events so far unsuspected no doubt also occur. When shoot apices of Silene were tested for the permeability of their cells to molecules of known sizes, it was shown that during synchronisation of cell division, just before sepal initiation, the cell-to-cell molecular exclusion limit decreased from about 650 to about 550 daltons (314). Whether this is a significant event of evocation is not known.

Another way of investigating evocation is to examine the
reversal of the process, to try and see which events might be reversed when flowering is reversed. Reversion of flowering usually occurs only sporadically, but in Impatiens an experimental system was devised to investigate flower reversion quantitatively (38). On flowering in Impatiens, the characteristic increase in growth rate of the shoot apex occurred and the sizes of the primordia at initiation were reduced. However, these changes were not reversed during reversion, showing that the mode of development was not directly correlated with the way the apex was growing (38). When reverted apices were made to re-flower they did so more readily than after the original induction and without any change in growth rate or primordial size (44). The apices in which these growth changes were retained from the pre-reverted state had apparently also retained a greater competence to form flowers, at least partly because the increased growth rate and smaller primordial size may be prerequisites for flowering, though not in themselves sufficient. This is reminiscent of the conditions for leaf initiation, for which a sufficient growth rate on the flanks of the apex and periclinal divisions are apparently prerequisites but not in themselves sufficient (21).

In the initiation of flowers at the shoot apex there seem, therefore, to be two principal sets of events. The first is the reduction in primordium and frustum size at initiation and the accompanying change in primordial arrangement to that characteristic of the flower. The second comprises those cellular changes which cause the apex to become competent to form potential floral organs and other changes which cause determination and development of the organs themselves as floral parts.
The problem of evocation of flowering therefore seems to be not so much a problem of trying to find a key event or set of events at the cytological or histological levels; these may be symptoms of flowering rather than causes. The problem seems to resolve itself into the need to understand the biochemical changes which cause the formation of primordia and control their extent and also the need to understand more generally the nature of the processes of competence and determination at the cellular and molecular levels. These are key problems of plant development which must now be tackled by new approaches and new techniques.
REFERENCE LIST OF PUBLICATIONS


APPENDIX

Published papers (1 - 46)
Changes in the Nucleus during Cellular Development in the Pea Seedling

R. F. LYNDON

Department of Botany, University of Edinburgh

WITH ONE PLATE

Received 18 October 1962

SUMMARY

Nuclei were isolated from three regions of the root and from the epicotyls of growing pea seedlings. As the dry mass of the nuclei was the same before and after isolation, and as the DNA content of the nuclei accounted for the DNA of the cells, the isolated nuclei are assumed to be a random sample of the nuclei in the intact tissues. The DNA, RNA, and protein contents per cell were much greater in the epicotyl than in the root, but the RNA and protein contents of the nuclei were similar in both epicotyl and root. Although the amounts of DNA, RNA, and protein per cell increased with increasing distance from the root tip, increase in the protein content of the nucleus occurred mainly between the meristematic and expanding regions, and at this same point nuclear RNA decreased. Nuclear volume increased with increasing distance from the root tip, whereas nuclear dry mass remained virtually unchanged. The nucleus therefore becomes increasingly hydrated as the cells mature. The data suggest a changing interaction between the nucleus and the cytoplasm during cellular development.

INTRODUCTION

As cells develop from the meristematic to the fully expanded state their metabolic pattern changes, and it has been suggested that this is an expression of a corresponding change in their protein complement (Robinson and Brown, 1952; Brown and Robinson, 1955). Any changes in the specific characteristics of the protein complement would presumably be under the ultimate control of the nucleus. If, as seems very probable, such changes do in fact occur, then a knowledge of the state of the nucleus at different stages of cell growth becomes important to the understanding of the process of cellular development.

The data presented here are based on observations with isolated nuclei. They represent the results of an investigation which was designed to test the validity of the procedure used for the isolation of the nuclei and, at the same time, to provide preliminary data on the extent to which gross changes may occur in the state and composition of the nucleus during cell growth. This investigation is intended as the first phase of a more extensive examination of the role of the nucleus in cellular development. At a later stage it is hoped to investigate, in detail, metabolic features of the development of the nucleus.

The validity of the isolation procedure has been examined by comparing the
composition of nuclei isolated from different parts of pea-seedling roots and from epicotyls. In some respects a comparison of the composition of isolated nuclei and the cells from which they were derived may be informative. Determinations on intact cells have therefore been included in the experimental design. In order to examine the possibility that material may have been lost from the nuclei owing to leaching into the sucrose media in which the nuclei were suspended, a comparison has been made of the dry mass of nuclei immediately after release from the cells and after the subsequent isolation procedure.

The studies on development have been based on nuclei isolated from three regions of the root: from the extreme apex, from the extending zone, and from the region of early maturity. In each case determinations have been made of the volume, dry mass, and the DNA, RNA, and protein content of the nuclei.

MATERIALS AND METHODS

Roots and epicotyls were obtained from seedlings of ‘Meteor’ peas (Pisum sativum). Seeds were germinated in vermiculite in the dark at 25°C, for two days when roots were required, and for five days when epicotyls were required. Segments were taken from roots which were 3.0–3.5 cm. in length. The segments were excised serially with a sharp razor-blade, the first cut being made 2 mm., the second 4 mm., and the third 9 mm. from the apex. Thus three segments were obtained which originally occupied the zones in the parent root 0–2, 2–4, and 4–9 mm. from the apex.

Isolation of nuclei. The method used was a modification of that developed by Johnston, Nasatir, and Stern (1957) in which nuclei are isolated in concentrated sucrose media containing calcium chloride. The modification was required by the use of a different experimental material and by the desirability of shortening the time necessary to complete the procedure. The earlier workers isolated nuclei from dry embryos, and the method they used for freeing the nuclei from the cells (homogenization of the tissue in an ‘Omnimixer’) could not be used with tissue composed of active, fully vacuolated cells. Some workers have released nuclei from vacuolated cells by grinding the tissue in a Potter-Elvehjem homogenizer provided with a loosely fitting pestle (Chayen and Denby, 1960; Accola, 1960). With this arrangement some of the nuclei that are released escape being squashed, but this method may be time-consuming. An apparatus consisting of contra-rotating rollers has also been used for large amounts of material (Rho and Chipchase, 1962).

A much simpler device was used in the present investigation. It was found that the tissue could be crushed sufficiently with a pestle enveloped in a wire gauze. With this device crushing is effected by the strands of the wire mesh. As the strands are some distance apart, some of the nuclei released from the cells remain within the meshes of the gauze and are not squashed. The tissue sample, consisting of epicotyls or 200–300 root segments, was crushed in 73 per cent. (w./v.) sucrose (about 2 ml./g. of tissue) in a Petri-dish. Then the whole fluid suspension was filtered through glass wool, together with 50 per cent. sucrose with which the pestle and the Petri-dish were rinsed. The filtrate, in which the sucrose concentration had been reduced to about 60 per cent. by the washing fluid, was centrifuged at 1,000 g for 7.5 min. The supernatant was then decanted away and the precipitate—consisting mainly of nuclei, starch grains, and cell-wall debris—was resuspended in about 0.05 ml. of 73 per cent.
sucrose. This suspension was layered over 96 per cent. sucrose and centrifuged at 50,000 g for 10 min. This treatment gave a sediment consisting of cell-wall debris and starch grains, and a suspension containing the nuclei. After being decanted from the sediment the suspension was diluted to bring the sucrose concentration to about 60 per cent. It was found that this dilution step was critical. If an appropriate volume of 17 per cent. sucrose was used to dilute the suspension (Johnston et al., 1957) then, on mixing, the nuclei tended to disintegrate. The dilution was therefore achieved by layering over the suspension an equal volume of 73 per cent. sucrose, and over this again 1.5 volumes of 27 per cent. sucrose. Subsequently the different layers were carefully mixed with a stirring-rod to ensure minimal disturbance to the nuclei. The suspension resulting from the mixing was centrifuged at 1,000 g for 7.5 min. This gave a wispy sediment of nuclei which was finally resuspended in 73 per cent. sucrose.

In all the sucrose solutions used as suspension media calcium chloride was also present at a concentration of 0.01 M. All operations were carried out at 0°C. All centrifugations (in both the isolation procedure and the analytical procedure described below) were done with the swing-out head of the centrifuge; this made easier the recovery of the small amounts of material involved.

The whole procedure occupied less than 1.5 hrs. and yielded preparations in which there was negligible cytoplasmic contamination, in which there were usually no unbroken cells, and in which many of the nuclei preserved the appearance of those in intact cells, although some were undoubtedly distorted or attenuated. A photograph of a typical preparation is shown in Plate I.

**Analytical techniques.** All analyses of DNA, RNA, and protein were done on a pellet obtained by precipitation of the nuclei. To the suspension of nuclei in sucrose solution was added an equal volume of ice-cold 10 N. perchloric acid. The resulting precipitate was sedimented by centrifugation and was washed successively, for only a few minutes in each, with ice-cold 0.5 N. perchloric acid, 80 per cent. ethanol, absolute ethanol, hot ethanol-ether (1:1), and finally ether. In some series of determinations the nuclei were precipitated with 70 per cent. ethanol and the acid washes were omitted. Similar values for the nucleic acid content of the nuclei were obtained after precipitation with either ethanol or acid.

For the determination of DNA the ether-dry pellet was first heated with 0.5 ml. of 0.5 N. perchloric acid for 20 min. at 70°C. After cooling, 1.0 ml. of diphenylamine reagent was added and the colour was developed according to the method of Burton (1956). The absorption of the solution was measured at 595 μm and 650 μm, the amount of DNA being proportional to the difference in extinction at the two wavelengths (Dische, 1955). The absorption measurements were made with a Unicam spectrophotometer using cuvettes with a 4-cm. light path. The method depends on a comparison with standard DNA preparations and these were included in each set of determinations.

Procedures involving the separation of RNA from DNA by precipitation, or the measurement of the UV absorption of an acid extract of the ether-dry pellet, proved to be unreliable for the determination of the small amounts of RNA in the nuclear preparations. RNA was therefore estimated as the difference between total nucleic acid and DNA, total nucleic acid being determined as phosphorus by the method of Allen (1940). The ether-dry nuclear pellet was boiled for 1 hr. with 0.3 ml. of 72 per cent. perchloric acid. After cooling, 0.05 ml. of 8.3 per cent. ammonium molybdate and 0.10 ml. of freshly prepared amidol-hisulphite solution were added. The total volume
Changes in the Nucleus during

was made up to 1.5 ml. with water. The solution was well mixed and after 15 min. the extinction at 725 m\(\mu\) was measured in a Unicam spectrophotometer using cuvettes with a 4-cm. light path. Phosphate standards were included in each set of determinations and total nucleic acid was estimated as \(P \times 10\).

For the determination of protein the total nitrogen content of the ether-dry pellet was measured by the Kjeldahl method. The pellet was digested for 3 hrs. with 0.1 ml. of concentrated sulphuric acid containing selenium as catalyst. After cooling and dilution duplicate aliquots were taken and the ammonia evolved on addition of alkali was determined by the microdiffusion method of Conway (1947). The values for N thus obtained were multiplied by the conventional factor of 6.25 to give the combined dry weight of the protein and of the nucleic acid in the sample. Protein was estimated as the difference between this value and that for total nucleic acid. Some determinations of total nitrogen were done by the micromethod of Sunderland, Heyes, and Brown (1957).

The DNA, RNA, and protein contents of intact tissues were also measured. The tissues, after being ground in 10 N. perchloric acid, were extracted with 0.5 N. perchloric acid, alcohol, and ether. The resulting residue was treated in exactly the same way as the ether-dry nuclear pellet.

The analytical determinations were in all cases accompanied by corresponding determinations of the number of nuclei in the original suspension or of the number of cells in the original tissue. This provision made it possible to express results on a unit nucleus or unit cell basis. For the determinations of nuclear numbers an aliquot was taken from the suspension from which a similar sample had already been taken for the preparation of the ether-dry pellet. The aliquot was pipetted into a test-tube and an equal volume of iodine solution was added. From this iodine suspension drops were transferred to a haemocytometer slide on which the number of nuclei per unit volume was determined. In this procedure the pipettes and test-tubes were siliconized to prevent the nuclei adhering to the glass. When intact tissues were analysed a comparable sample of tissue was prepared on which the number of cells was determined by the method of Brown and Rickless (1949) as modified by Brown and Broadbent (1951).

Determinations of dry mass of nuclei. Determinations of dry mass were made on isolated nuclei using the interference microscope and they were made on nuclei (a) immediately after release from the tissue and (b) after they had been subjected to the isolation procedure. As mentioned previously the two determinations were made to provide an assessment of the loss of components through leaching into the various suspension media during the isolation procedure. For this purpose the most appropriate first determination would have been one on the nucleus within the cell, but to determine the dry mass before release from the cell it is necessary to separate the intact cells from each other, and it was found impossible to do this without damage to the cytoplasm and the enclosed nucleus. Accordingly the compromise was adopted of making the determination immediately after release of the nucleus from the cell, when the release was made into alcohol. Tissue was crushed in a drop of absolute alcohol on a slide. A sediment containing nuclei adhered to the slide and this was washed with 70 per cent. ethanol, absolute ethanol, and ether. After drying, the preparation was mounted in water under a coverslip. Nuclei free from other material or displaced, sheared images were selected at random and their dry mass measured with a Baker interference microscope equipped with a shearing lens system. Illumination was provided by a Kohler lamp with a green filter having peak transmission...
at 546 m. The method of maximum darkness of background and object was used. In a system in which the object is immersed in water

\[ \text{Dry mass} = \frac{\text{Path difference} \times \text{area}}{0.18}. \]

The area of the nucleus was taken to be \( \pi \left( \frac{\text{length}}{2} \times \frac{\text{breadth}}{2} \right) \), and the length and breadth of the nucleus were measured directly against a calibrated ocular micrometer scale.

The isolation procedure yielded free nuclei. These were resuspended in 73 per cent. sucrose (containing 0.01 M. calcium chloride) having a measured refractive index \( (\mu_m) \) of 1.4340. The dry mass of nuclei in such preparations was determined by transferring drops of the suspension to a slide and making observations on nuclei selected at random. In a system in which the medium has a higher refractive index than water and in which the object is impregnated with the medium

\[ \text{Dry mass} = \frac{\text{Path difference}_{\text{medium}} \times \text{area}}{0.18} + (\mu_m - \mu_w) \left( \frac{\text{area}}{0.18} \right) t_e, \]

where \( t_e = \frac{\text{Path difference}_{\text{medium}}}{\mu_m - \mu_w} \)

and where \( \mu_m \) is the refractive index of the medium,
\( \mu_w \) that of water, and
\( \mu_n \) that of the nucleus (see Davies, 1958).

In addition to the determination of path difference and area it was also necessary (in this connexion) to determine the refractive index of the dry matter of the nucleus \( (\mu_n) \). For this purpose ether-dry preparations of tissue crushed in absolute alcohol on a slide, as described above, were used. The sediment adhering to the slide was immersed in various mixtures of paraffin oil and \( \alpha \)-bromonaphthalene and the mixture was found in which the nuclei in the preparation matched the background when observed with the interference microscope. The refractive index of the nucleus was then taken to be identical with that of the matching mixture. This procedure gave a value of 1.5422, which agrees with that usually used (1.54) for the refractive index of the dry matter of nuclei and cytoplasm (Davis, 1958; Stenram, 1961).

Since the nucleolus usually appears denser than the rest of the nucleus it was thought desirable to determine its dry mass separately and to make an appropriate correction to the value for the nucleus as a whole. It was found, however, that the volume of the nucleolus was small relative to that of the nucleus and that it was sufficient to apply a small standard correction to the overall value.

**Determinations of volume of nuclei.** These determinations were made on nuclei in intact cells in sections of tissue mounted in water and on isolated nuclei in 73 per cent. sucrose solution containing 0.01 M. calcium chloride. With both, the length and breadth of the nucleus was measured with a calibrated ocular micrometer and the volume was calculated as

\[ \frac{4}{3} \pi \left( \frac{\text{length} + \text{breadth}}{4} \right)^3. \]
EXPERIMENTAL RESULTS

The DNA, RNA, and protein contents of isolated nuclei, and of whole cells from the three root segments and the epicotyl, are given in Table I. The data show that the protein and RNA contents per cell increase considerably with increasing distance from the root apex. A similar result has been recorded by Brown and Broadbent (1951) and Heyes (1960). The data also show that the protein and RNA contents of the average epicotyl cell are considerably greater than those of the average root cell. The increase in the average content of DNA per cell is similar to that already recorded by Holmes, Mee, Hornsey, and Gray (1955), Heyes (1960), and Sunderland and McLeish (1961). In the epicotyl the average value for DNA per cell is considerably higher than it is in the root. It may be noted that the DNA values determined with intact tissues and with isolated nuclei agree remarkably closely. The DNA of the cells (certainly in the root) is therefore wholly accounted for by the DNA of the isolated nuclei.

The values for isolated nuclei show that protein tends to increase with increasing distance from the root apex whereas RNA tends to decrease, and, moreover, that the major change in both these components is between the apical segment of the root (0–2 mm. from the apex) and the region of rapid cell extension (2–4 mm. from the apex). The values for protein and RNA of the epicotyl nuclei are of the same order as the values for the nuclei of the root although the protein and RNA content per cell is much greater in the epicotyl than in the root.

The results of determinations of the dry mass of nuclei from the root and epicotyl are given in Table II. These data show that there is no significant increase in the dry mass with increasing distance from the root apex. Also, the dry masses of epicotyl nuclei and root nuclei are of the same order. There are no significant differences between the values for nuclei released from cells squashed in alcohol and for nuclei at the end of the isolation procedure. On the other hand, for root nuclei there are large differences between the values for

### Table I

*DNA, RNA and protein per nucleus (N) and per cell (C) from three root segments and from the epicotyl*

<table>
<thead>
<tr>
<th>Root zone (mm. from apex)</th>
<th>0–2</th>
<th>2–4</th>
<th>4–9</th>
<th>Epicotyl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>16</td>
<td>18</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>RNA</td>
<td>15</td>
<td>18</td>
<td>22</td>
<td>39</td>
</tr>
<tr>
<td>Protein</td>
<td>13</td>
<td>469</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>No. of cells</td>
<td>200,000</td>
<td>133,600</td>
<td>89,400</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents g. × 10⁻¹². All values are the means of at least two, and at the most ten, separate sets of determinations. The last line shows the number of cells in each zone of the root.
total dry mass and the values for the combined dry weights of protein, RNA, and DNA. These differences are relatively small for the epicotyl nuclei.

The volumes of nuclei from different regions of the root and from the epicotyl, both in the intact cell and after isolation, are shown in Table III. It is apparent that in the root the volume of the nucleus increases considerably with increasing distance from the apex, and that the volume of the nucleus in

**TABLE II**

_Dry mass (g. × 10⁻¹²) per nucleus from three root segments and from the epicotyl_

Values are given for nuclei released from cells squashed in alcohol (A) and for nuclei obtained by the isolation procedure (B). The last line (C) gives the combined dry weights (g. × 10⁻¹²) of DNA, RNA, and protein taken from Table I. The values in lines A and B are each the mean of 20 determinations, and the standard deviation for each value is shown in parentheses.

<table>
<thead>
<tr>
<th>Root zone (mm. from apex)</th>
<th>0-2</th>
<th>2-4</th>
<th>4-9</th>
<th>Epicotyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>87  (±36)</td>
<td>105  (±32)</td>
<td>93  (±38)</td>
<td>74  (±23)</td>
</tr>
<tr>
<td>B</td>
<td>75  (±28)</td>
<td>81  (±17)</td>
<td>91  (±28)</td>
<td>78  (±24)</td>
</tr>
<tr>
<td>C</td>
<td>44</td>
<td>51</td>
<td>52</td>
<td>66</td>
</tr>
</tbody>
</table>

**TABLE III**

_Volumes (μ³) of nuclei in intact tissues (A) and after isolation (B) from different regions of the root and from the epicotyl._

Each value is the mean of 20 determinations.

<table>
<thead>
<tr>
<th>Root zone (mm. from apex)</th>
<th>0-2</th>
<th>2-4</th>
<th>4-9</th>
<th>Epicotyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>225</td>
<td>521</td>
<td>628</td>
<td>1,026</td>
</tr>
<tr>
<td>B</td>
<td>159</td>
<td>219</td>
<td>327</td>
<td>445</td>
</tr>
</tbody>
</table>

the epicotyl is considerably greater than in the root. This is shown not only by the nuclei in the intact cells but also by those that have been isolated. The values for isolated nuclei are consistently smaller than those for nuclei in intact cells and although this difference may be ascribed partly to the high concentration of sucrose in the medium in which the isolated nuclei were suspended it is also due to the presence of 0.01 M. calcium chloride, which was observed to cause shrinkage of nuclei released into it from pieces of root tissue.

**DISCUSSION**

The experimental data may be considered in relation to (A) the validity of the technique for isolating nuclei, and (B) the extent to which changes occur in the nucleus during cellular development.

A. The isolation procedure was designed to provide preparations from which the properties of the nucleus in the intact cell may be deduced. Accordingly, such preparations are satisfactory only if they are free from cellular components other than nuclei, if the nuclei themselves are not extensively altered
by the isolation procedure, and if the isolated nuclei are a representative sample of the nuclei in the original tissue.

On visual inspection the preparations of isolated nuclei appeared to be free from all but slight contamination with non-nuclear fragments. Undoubtedly some contaminating material was present but it was clearly negligible. The absence of appreciable cytoplasmic contamination was confirmed by the low values for RNA and protein per nucleus in contrast to the high values per cell (Table I). The close correspondence of the DNA values per cell and per nucleus also indicates that these preparations of isolated nuclei were free of DNA fragments, which occur in preparations of nuclei isolated from wheat and pea embryos (Stern, personal communication).

During isolation the nuclei may be altered by mechanical disruption, by loss or accretion of particular components, and by change in physical state. Again inspection indicated that most of the nuclei in the preparations were intact. The extent to which the composition of the nucleus may change during isolation is more difficult to assess. Some change almost certainly occurs. However, the loss of soluble substances of low molecular weight is unlikely to affect the metabolic properties of the nucleus. On the other hand, accretion or loss of substances of high molecular weight would probably have a much more profound effect. The substances most likely to be involved in this connexion are the proteins. Protoplasmic proteins may be adsorbed on to the nucleus from the homogenate after disruption of the cells, or protein may be leached from the nuclei into the medium in which they are suspended. If either of these changes were to occur then the metabolic activity of the nucleus might be altered. The present experimental data do not suggest that there was any significant accretion of material to the nuclei. If this had occurred, it might be expected that the apparent protein content of the nucleus would be greatest where the cytoplasmic protein content was highest in the original cells, that is, in the epicotyl. However, the protein content of nuclei isolated from the epicotyl was certainly not greater than that of nuclei isolated from the root. Furthermore, if accretion were extensive the dry mass of the nucleus might be expected to increase as a result of isolation. In fact the data of Table II show that the dry mass was not appreciably altered.

It has been claimed that protein may be leached from certain animal nuclei when they are isolated in sucrose media (Stern and Mirsky, 1953; Allfrey, Mirsky, and Stern, 1955), although the evidence is not decisive (Roodyn, 1959). One aspect of the present experimental data suggests that this may have occurred with nuclei from the pea root and epicotyl. In the isolated nuclei the ratio of protein to DNA is remarkably low (cf. Table I). Allfrey, Stern, Mirsky, and Saetren (1952) recorded values of this ratio all greater than 2.8 for animal nuclei isolated in non-aqueous media, and Johnston et al. (1957) found a value of 4.4 for wheat-germ nuclei isolated in sucrose media. In the present investigation the highest value found was 1.3 for nuclei from the intermediate root segment. The close correspondence between DNA values for intact cells and for isolated nuclei indicates that the DNA values on which the
ratio is based may be taken as reliable. Thus if the apparent low ratio is an artifact it can be attributed only to a loss of protein from the nucleus. On the other hand, the determinations of total dry mass before and after isolation do not indicate any extensive loss of dry matter during the process. It is significant that a change in the ratio from 3:1 to 1:1, with a constant DNA content, would require the loss of about $40 \times 10^{-12}$ g. of protein from each nucleus. This would involve a reduction in dry mass of the order of 50 per cent., and a change of this magnitude would certainly have been shown by the dry mass determinations. It may therefore be concluded that any loss of protein is negligible, and that a low protein-to-DNA ratio is a characteristic feature of these root and epicotyl nuclei.

This conclusion is, of course, not affected by there being a difference between the dry mass of the nucleus determined directly and the dry mass given by the combined values for protein, RNA, and DNA (Table II). The difference between these two sets of values indicates only that the root nucleus contains relatively large amounts of alcohol-insoluble substances in addition to those (protein, RNA, and DNA) which were measured. It may be noted that in this respect there is an apparent difference between root and epicotyl nuclei, for in the latter the proportion of the total dry mass which is not accounted for is relatively small.

There is some evidence that the physical state of the nucleus changes during isolation. The high concentrations of sucrose in the suspending media help to prevent the disruption of the nuclei and may restrain the leaching of substances from them. On the other hand, the data of Table III show that the volume of the nucleus after isolation is considerably smaller than it is before it. There is clearly a considerable withdrawal of water, which may be partly due to the high osmotic pressures of the sucrose solutions. Simple dehydration of this type is hardly likely to affect catalytic activity, but the hardening which accompanies dehydration when calcium chloride is present may have more profound consequences. All the suspending media contained calcium chloride, which preserves the integrity of the nuclei. When it is omitted they tend to fragment, but in its presence they shrink and become singularly resistant to mechanical disruption. The significance of this change is difficult to assess, for even though it may not affect the gross amounts of substances in the nucleus it may influence metabolic activity.

The possibility that the final preparation of nuclei is not a representative sample is suggested by the low yield obtained in the isolation procedure. The final sample consists of only about 5 per cent. of the nuclei originally present in the tissue, and with such a low proportion it is clearly possible that there may have been some selection during the course of isolation. However, if in fact selection were involved, the mean values per nucleus for DNA content and dry mass might be expected to be different for the isolated nuclei and for the original population of nuclei in the intact tissue. In fact they are both the same (Table I), so that the final preparation of nuclei may be regarded as representative of the nuclei in the tissue.
B. The three successive segments of the root consist of cells in progressively advanced stages of development. In the apical segment most of the cells are in a meristematic non-vacuolated state, in the second segment they are in a phase of active expansion, and in the third vacuolation and expansion are complete in many of the cells. As this succession is traversed the protein and RNA contents of the whole cell increase (Table I). The protein content of the nucleus also increases, but the relative increase is less than it is for the cell as a whole and therefore relatively less than it is for the cytoplasm. It may be noted in this connexion that the ratio between the protein content of the cytoplasm and that of the nucleus can apparently vary quite considerably. While it is about 20:1 in the apical root segment and about 30:1 in the basal segment, in the epicotyl it is about 100:1.

While the RNA content of the cytoplasm is increasing with development it is significant that the RNA content of the nucleus is decreasing (Table I). Moreover the decrease is most pronounced between the meristematic region and the region of active cell expansion. This is the same point at which nuclear protein is doubled (Table I) and corresponds to the point at which Loening (1961) found large changes occurring in the microsomal components of the cells. The data therefore suggest that this may be a critical phase of the cell's development and that, at this stage, the nucleus may exercise control of the process through a discharge of RNA into the cytoplasm. One possibility is that the nucleus carries with it from division a quantity of RNA which is released into the cytoplasm when expansion begins and that it is this single contribution which controls subsequent differentiation. On the other hand, the nucleus may subsequently continue to contribute RNA to the cytoplasm without this being accompanied by accumulation of RNA in the nucleus.

Another striking change during development is the increase in volume and hydration of the nucleus. In the intact cells in water the increase in nuclear volume from the apex to the more basal part of the root is about threefold and in the isolated nuclei about twofold (Table III). This increase is similar to that noted by Sunderland and McLeish (1961). Clearly the large change in the volume of the nucleus in the intact cell cannot be attributed merely to a greater availability of water to the nucleus, because a difference in volume is still apparent after the nuclei from the different root segments and the epicotyl have been isolated and exposed to the same high concentration of sucrose. The increase of nuclear volume in the intact cell must therefore be attributed to a change in the nucleus which results in an absorption and retention of water. This point is further emphasized in the case of the epicotyl nuclei, which, although having approximately the same dry mass and protein content as the root nuclei, are nevertheless considerably larger. As the amounts of both DNA and cytoplasmic protein are greater in the epicotyl than in the root the data also point to a connexion between nuclear volume and increased DNA content, as suggested by Sunderland and McLeish (1961), and increased cytoplasmic mass.

Thus the data presented here, although limited in scope, certainly do not
suggest that the nucleus remains a stable entity during cellular development. On the contrary they indicate that while changes are occurring in the cytoplasm, separate changes are occurring in the composition and metabolic state of the nucleus. Cellular development therefore probably involves a change in the pattern of interaction between the nucleus and the cytoplasm.

ACKNOWLEDGEMENTS

I wish to thank Professor R. Brown, F.R.S., for his constant interest, and for his assistance in writing this paper; and Dr. J. M. Mitchison for advice concerning interference microscopy. This work was supported by a grant from the Nuffield Foundation.

LITERATURE CITED


PLATE I. Preparation of nuclei, stained with iodine, isolated from pea seedling epicotyls. (x 700)

R. F. LYNDON—PLATE I
INTRACELLULAR DISTRIBUTION OF RIBONUCLEASE ACTIVITY IN PEA ROOTS

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(Received May 17th, 1965)

SUMMARY

The distribution of ribonuclease activity between the nuclear, mitochondrial, microsomal, and soluble fractions of pea root cells was measured. All activity appeared to be in the cytoplasm. Basic proteins extracted from nuclear material were inactive. Alkaline ribonuclease activity was found predominantly in the soluble fraction, acidic activity in all cytoplasmic fractions. The approximate percentage activities of the isolated cytoplasmic fractions were: soluble, 75; particulate (mitochondrial), 20; and microsomal, 5.

INTRODUCTION

Increased nucleolytic activity is associated with growth in the root, in germinating seedlings and in many animal cells. Also, when roots are treated with pancreatic ribonuclease (ribonucleate:pyrimidinenucleotido-2'-transferase(cyclizing), EC 2.7.7.16) cell expansion is stimulated, although cell division is inhibited. Interpretation of these observations requires data on the intracellular distribution of ribonuclease.

Kessler and Engelberg showed that in apple leaves microsomal ribonuclease was related positively, and soluble ribonuclease negatively, to growth. Much of the ribonuclease activity of leaf tissue is in the soluble fraction of the cells. In animal tissues most of the ribonuclease activity is found in the mitochondrial, microsomal, or lysosomal fractions but it has also been reported as being present in the nuclei or nuclear components.

Since methods are now available for isolating nuclei from root tissues the present investigation was designed to measure the relative distribution of ribonuclease activity between cytoplasmic and nuclear fractions of pea roots, and also the distribution in the particulate (mitochondrial and microsomal) and soluble fractions of the cytoplasm. Since the yield of nuclei from the pea roots was small, observations were amplified by using preparations of chromatin.

It will be shown that ribonuclease activity appears to be confined to the cyto-
plasm, where it is found mainly in the soluble fraction, and may be due to more than one enzyme.

MATERIALS AND METHODS

Peas (Pisum sativum var. Meteor) were germinated in vermiculite in the dark at 23.5° for 2.5 days, by which time their roots had reached a length of about 3-4 cm.

Isolation of subcellular fractions

Nuclei were isolated from whole roots or the terminal centimetre of the roots, and the numbers of nuclei in the preparations were counted, with techniques described elsewhere. The nuclei were resuspended in about 1.0 ml of water and 0.1- or 0.25-ml aliquots were used for the ribonuclease assays.

For the preparation of chromatin about 50 roots were homogenized, by hand in a glass homogenizer, in 3.0 ml of a medium consisting of 0.5 M sucrose, 0.03 M Tris—HC1 (pH 7.3), 5 mM MgCl₂, and 0.15 M KCl. The homogenate was passed through a nickel filter having a 75-μm mesh, which retained cell-wall material, and the filtrate was centrifuged at 1500 x g for 5 min. The precipitate was resuspended in 0.1 M Tris—citrate buffer (pH 7.3) and was recentrifuged at 1500 x g for 5 min. The resulting sediment, containing much of the DNA of the tissue as well as starch, was designated chromatin.

The other subcellular fractions were isolated from the terminal centimetre of a known number of roots. About 60 root tips were homogenized as described above and the homogenate was centrifuged at 1200 x g for 5 min, the resulting sediment being discarded. Centrifugation of the supernatant at 30 000 x g for 30 min yielded a precipitate which will be called large particles, and which presumably included the mitochondria. The remaining supernatant was recentrifuged at 150 000 x g for 1 h to yield the microsomal and soluble fractions. The microsomal fraction consisted of the “light” (vesicular) and “heavy” (ribosomal) fractions described by Loening but these were mixed together in the present series of experiments, although each fraction has been shown to possess distinct ribonuclease activities. The large particulate and microsomal fractions were each resuspended in 1.5 ml of water and 0.1-ml aliquots were used for the ribonuclease assays. After the volume had been measured the soluble fraction was diluted 10-fold and 0.1-ml aliquots were used for the ribonuclease assays.

In the experiments in which the ribonuclease activities of the nuclei and the tissue homogenate were compared the latter was obtained as the initial step in the preparation of the nuclei and consisted of cellular material mixed with approx. 2.0 M sucrose and 0.01 M CaCl₂ and from which gross debris has been removed by filtration through glass wool. For the ribonuclease assays this homogenate was diluted 10-fold and 0.25- or 0.1-ml aliquots were used.

Determination of DNA

Aliquots of the same nuclear suspensions as were taken for the ribonuclease assays were used without further treatment for the determination of DNA by the method of Burton. Aliquots of the root homogenate were first treated by the addition of sufficient ethanol to bring the ethanol concentration to 70% (v/v). The pre-
precipitate was washed with 70\% ethanol, absolute ethanol, ethanol–ether (1:1, v/v) and ether, and the DNA content of the dry precipitate was then measured.

**Assays for ribonuclease activity**

The various fractions were assayed at two different temperatures and in the presence and absence of NaCl. Two temperatures were used since it was found in preliminary experiments that the position of the pH optimum may vary with temperature, and NaCl was used since LESLIE\(^{11}\) had shown that it stimulated ribonuclease activity.

An aliquot of the material to be tested was mixed in a total volume of 1.0 ml with 0.02 M buffer of the appropriate pH, 0.25\% yeast RNA (sodium salt, supplied by British Drug Houses) and, when required, 0.15 M NaCl. The buffers used were sodium acetate from pH 4.1 to 5.2, Tris–maleate–NaOH from pH 5.8 to 7.4, and Tris–HCl at pH 8.1 and 8.6. The pH's were those of the assay mixtures measured at the temperature of the assay. The assay mixtures were incubated at the required temperature for 1 h. They were then quickly cooled to room temperature and 0.5 ml of a solution of 0.375\% uranyl acetate in 3.75\% trichloroacetic acid was added to each. Measure-

---

**Fig. 1.** Ribonuclease activity of isolated cell fractions, at 60° in the presence of 0.15 M NaCl, as a function of time of incubation. \(\triangle-\triangle\), homogenate; \(\bigcirc-\bigcirc\), soluble fraction; \(\bigcirc-\bigcirc\), large particles; \(\nabla-\nabla\), microsomes; \(\square-\square\), nuclei × 10. Ordinate: increase of absorbance at 260 μν. Abscissa: period of incubation.

**Fig. 2.** Ribonuclease activity of isolated cell fractions as a function of enzyme concentration. Incubation for 1 h at 60° in the presence of 0.15 M NaCl. Symbols as in Fig. 1. Ordinate: increase of absorbance at 260 μν. Abscissa: concentration of cell fraction.

ments confirmed that this reagent completely precipitated the yeast RNA from the solution. The mixtures were allowed to stand for 30 min and the precipitate was then removed by centrifugation. Each supernatant solution was diluted 10-fold with distilled water and its absorbance at 260 nm measured (1 cm light path) with a Unicam spectrophotometer. The absorbance values given in the Tables and Figures are those before dilution and are therefore ten times the measured values. The absorption spectra of the solutions were checked to ensure that the measured absorbances were due to substances having the absorption properties of the components of nucleic acid. In all experiments control mixtures without the tissue extract were included.

The distribution of enzyme activity between the various cell fractions was measured after incubation for 1 h at 60° in the presence of 0.15 M NaCl and with concentrations of cell fractions no greater than the arbitrary values of 0.2 (Fig. 2) as indicated by the absorbance values which were measured. Under these conditions ribonuclease activity in all cell fractions was proportional to the time of incubation and the concentration of enzyme (Figs. 1 and 2).

EXPERIMENTAL RESULTS

Ribonuclease activity of the isolated nuclei

The yields of nuclei from the pea roots were low and their ribonuclease activities were also low. The ribonuclease assays of the nuclei were therefore performed at 60°, which was about the optimum temperature for the assay, rather than 37° so that what activity there was was more easily measured. The ribonuclease activities of different

![Graph](image1.png)

![Graph](image2.png)

Fig. 3. Ribonuclease activity of two preparations of isolated nuclei at 60° in the absence of NaCl. Ordinate: increase of absorbance at 260 nm per aliquot of nuclei. Abscissa: pH of assay.

Fig. 4. Ribonuclease activity of a preparation of isolated nuclei at 60° in the presence of 0.15 M NaCl. Ordinate: increase of absorbance at 260 nm per aliquot of nuclei. Abscissa: pH of assay.
preparations of isolated nuclei in the absence and presence of 0.15 M NaCl at 60° are shown in Figs. 3 and 4 respectively. In the absence of NaCl two peaks of ribonuclease activity were found, at pH 5.0 and at about pH 7. The ribonuclease activity in the pH-7 region is here seen to be quite distinct from that at pH 5.0-5.8. In the presence of NaCl only one peak of ribonuclease activity was apparent and this was clearly at pH 5.0 rather than pH 5.8; the activity above pH 7 was entirely suppressed. The addition of 0.15 M NaCl stimulated the ribonuclease activity of the nuclei at pH 5.0 by 50%, 175%, and 970% in three different experiments.

Ribonuclease activity of the homogenate

The observation that there was detectable, although apparently low, ribonuclease activity in the nuclei raised the question of what proportion this represented of the total activity of the cells. First, the characteristics of the whole-tissue homogenate were examined.

The ribonuclease activities of the homogenate within the pH range 4.1 to 8.6 at 60° were measured and three patterns were observed (Fig. 5). In the absence of NaCl there was substantial activity at pH 5.8 and similar (Fig. 5a) or, in others, that at pH 7.1 was higher (Fig. 5b) but activity at both these pH's was higher than that at pH 5.0. However, in one experiment the activity at pH 5.0 was as great as that at higher pH's (Fig. 5c). On the addition of NaCl, although in two experiments the pattern was not the same (Figs. 5b,c), in both cases the activity at pH 5.0 was stimulated and at pH 5.8 and above was diminished.

In some respects the pattern of ribonuclease activity in the homogenate and in the nuclei differed, mainly in the much greater activity in the homogenate in the pH-6.6 region in the absence of NaCl, and in the more complete suppression by NaCl of activity in the nuclei above pH 7.

However, in both nuclei and homogenate at 60° with 0.15 M NaCl at pH 5.0 ribonuclease activity appeared to be at or near the maximum. The comparison of the relative activities of homogenate and nuclei was therefore made under these conditions.

**Distribution of ribonuclease activity between nuclei and cytoplasm**

The ribonuclease activities of nuclei and of aliquots of the homogenate from which the nuclei were subsequently isolated were measured. Because the yield of nuclei was low, DNA was measured in aliquots of the homogenate and of the nuclear preparations so that the ribonuclease activity attributable to all the nuclei could be assessed. The values obtained (Table I) show that no more than 3% of the activity of the homogenate at pH 5.0 could be ascribed to the nuclei. This suggests that the ribonuclease activity of the pea root is probably entirely cytoplasmic.

The possibility that in the nucleus there was ribonuclease activity specific for nuclear RNA was tested by supplying a preparation of nuclei with pulse-labelled \[^{32}\text{P}\]RNA prepared in this laboratory by Dr. U. E. LOENING. This RNA is apparently synthesised in the nucleus and has DNA-like composition\(^{17,18}\). The ribonuclease activity of the nuclei relative to that of the whole homogenate was very similar whether the DNA-like RNA from peas or yeast RNA was used as the substrate for the assay (Table I). Consequently there is no evidence of activity in the nuclei specific for only a certain type of RNA.

The small amount of ribonuclease activity associated with the nuclei may be presumed to be the result of contamination with cytoplasmic enzymes. This conclusion is supported by the observation that when merely washed (by recentrifugation for

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**Table I**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Substrate</th>
<th>Ribonuclease activity per µg DNA</th>
<th>Percentage of total ribonuclease activity in nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Homogenate</td>
<td>Nuclei</td>
</tr>
<tr>
<td>I</td>
<td>Yeast RNA</td>
<td>8.37</td>
<td>0.108</td>
</tr>
<tr>
<td>II</td>
<td>Yeast RNA</td>
<td>16.52</td>
<td>0.492</td>
</tr>
<tr>
<td>III</td>
<td>Pulse-labelled pea RNA</td>
<td>1468</td>
<td>52</td>
</tr>
</tbody>
</table>

---

7.5 min at 1000 × g in a medium of 1.8 M sucrose containing 0.01 M CaCl₂ the nuclei lost almost half their ribonuclease activity (Table II). Such nuclei still have the same dry mass as nuclei in intact cells\textsuperscript{13}. It was still possible, however, that at least some of the activity of the nuclear preparations did represent that of a nuclear component, perhaps histone, as has been reported for the nuclei of guinea-pig liver\textsuperscript{11,12}.

**Ribonuclease activity of basic proteins of the nuclei**

Isolated nuclei were obtained only in low yield from the pea roots. A larger yield of nuclear material was obtainable as chromatin, in which the ratio of acid-soluble protein to DNA was about 0.8. This acid-soluble protein was basic protein, behaving like histone on electrophoresis. A chromatin preparation containing approx. 4.8 μg of basic protein, when assayed at 60° and in the presence of 0.15 M NaCl, possessed a ribonuclease activity so low that it could not be reliably distinguished from the blank values. It remained possible that the basic proteins might show ribonuclease activity only when isolated from the nucleoprotein. Basic proteins were therefore extracted from chromatin by the action of 0.33 N sulphuric acid at 0° overnight. The sediment was then centrifuged off and the basic proteins precipitated from the clear supernatant by the addition of 3 vol. of ethanol. The precipitate was dried, dissolved in water and its ribonuclease activity measured at pH 5.0 at 60° with 0.15 M NaCl. With aliquots each containing 43 μg of basic proteins the slight activity detectable was equivalent only to 0.0026 μg of pancreatic ribonuclease assayed under the same conditions. Clearly the ribonuclease activity of the chromatin and of the basic proteins isolated from the chromatin was negligible. These observations support the view that the activity of the nuclei was probably a result of contamination with cytoplasmic components.

**Ribonuclease activity of the cytoplasmic fractions**

Some characteristics of the root homogenate have been described above. In Fig. 6 are shown the patterns of ribonuclease activities of the three major fractions of this homogenate (soluble fraction, large particles, and microsomes) in the pH range 4.1 to 8.6, at 37° and 60°, and with or without 0.15 M NaCl. In the particulate frac-

TABLE II

**RIBONUCLEASE ACTIVITY OF NUCLEI BEFORE AND AFTER WASHING IN 1.8 M SUCROSE**

All assays done at 60° with 0.15 M NaCl at pH 5.0.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Increase of absorbance at 260 μm</th>
<th>Number of nuclei</th>
<th>Increase of absorbance per 10⁶ nuclei</th>
<th>Percentage reduction of ribonuclease activity due to washing</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>before washing 5.02</td>
<td>922 500</td>
<td>5.44</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>after washing 1.57</td>
<td>565 000</td>
<td>2.78</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>before washing 5.69</td>
<td>767 500</td>
<td>7.41</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>after washing 1.06</td>
<td>235 000</td>
<td>4.51</td>
<td></td>
</tr>
</tbody>
</table>

*Biochim. Biophys. Acta, 113 (1966) 110-119*
tions most of the activity was below pH 6.6; at higher pH's activity was relatively
low and may have been due to contamination from the soluble fraction, which showed
almost as much activity at pH 8 as at pH 6.

The ribonuclease activity of all three fractions of the homogenate in the pres-
ence of 0.15 M NaCl at 37° and at 60° showed a pH optimum at pH 5.0-5.2 compared
with pH 6.0 at 37° in the absence of NaCl. The pH optimum of the two particulate
fractions, unlike the soluble fraction, was also shifted to pH 5.0 by raising the
temperature from 37° to 60° in the absence of NaCl. A similar effect has been observed
in preparations made from corn seedlings. Maximum activity at pH 5.0 rather than
pH 5.8 (at 60°) has already been noted for the isolated nuclei (Fig. 4). In both partic-
ulate and soluble fractions the maximum amount of ribonuclease activity was hardly
altered by the addition of NaCl at 37°. At 60° the maximum activity of the particulate
fractions, but not of the soluble fraction, was increased 2- to 3-fold by the addition
of NaCl. The effect of NaCl at 60° on the isolated nuclei (a 0.5- to 10-fold stimulation
of maximum activity) again resembled the effect on the particulate fractions.

The distribution of ribonuclease activity between the three fractions of the
homogenate is shown in Table III. Only for the values obtained at 60° in the presence
of 0.15 M NaCl are appropriate kinetic data available (Figs. 1 and 2) and so only these
are strictly comparable. However, the data of Fig. 6, obtained under different condi-
tions of assay, also gave similar values which fell within the range shown as Expt A
in Table III.

The percentage activities of the isolated fractions (expressed as a percentage
of their sum) was approx. 70% or more in the soluble fraction, less than 10% in the
microsomes, and between 10 and 30% in the large particles. The sum of the activities
TABLE III

PERCENTAGE RIBONUCLEASE ACTIVITIES OF THE ISOLATED FRACTIONS OF PEA ROOT HOMOGENATE

Assay at 60° with 0.15 M NaCl for Expts. VI, VII, VIII. Expt. A is a summary of distribution of ribonuclease activities observed for assays done at 37° and 60° without NaCl and at 37° with 0.15 M NaCl.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Soluble fraction</th>
<th>Large particles</th>
<th>Microsomes</th>
<th>Sum of isolated fractions</th>
<th>Unfractionated homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>74</td>
<td>19</td>
<td>7</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>VII</td>
<td>79</td>
<td>15</td>
<td>6</td>
<td>100</td>
<td>129</td>
</tr>
<tr>
<td>VIII</td>
<td>82</td>
<td>15</td>
<td>3</td>
<td>100</td>
<td>137</td>
</tr>
<tr>
<td>A</td>
<td>&gt;69</td>
<td>12–27</td>
<td>&lt;6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of the isolated fractions was only about 75% of the activity of the homogenate from which the fractions had been isolated.

DISCUSSION

The data indicate that there is no ribonuclease activity intrinsic to the nuclei of pea root cells under the conditions of the experiments which have been reported. No measurements could be made on some preparations of isolated nuclei for the activity in them was negligible. The experiments described here necessarily refer only to active preparations. It was noted that the less active or inactive nuclear preparations were those which were freest from microscopically visible contamination. Since the homogenate always showed vigorous ribonuclease activity these observations tend to confirm that the activity of the nuclear preparations was due to contamination from cytoplasmic components.

From other measurements (unpublished data) it can be calculated that in the terminal centimetre of the pea root approx. 8% of the protein of the whole cells is found in the nuclei. (The low values for protein per nucleus reported earlier are now known to be erroneous.) Less than 4% of the ribonuclease activity of the cells was associated with the nuclei (Table I). Therefore on a unit protein basis the activity of the nuclei was less than that of the homogenate of the whole root tip. This again confirms that the activity of the nuclear preparations was probably due to contamination from the cytoplasm. The main source of this contamination appeared to be the particulate fractions, for the ribonuclease activity of the nuclear preparations resembled that of the cytoplasmic particles in that at 60° in the absence of NaCl the pH optimum was at pH 5.0 rather than pH 5.8, and the maximum activity could be increased about 2-fold or more by the addition of 0.15 M NaCl while activity above pH 7 was very low. The nuclei resembled the soluble fraction, however, in showing appreciable activity at pH 7.1 when NaCl was absent. One may therefore conclude that the ribonuclease activity of the nuclear preparations was probably due to differential contamination with particulate and some soluble protein. It remains possible that the activity of the nuclear preparations was at least partly due to ribonuclease of nuclear ribosomes; however, WANG AND WANG have shown that the ribonuclease activity...

activity of thymus nuclear ribosomes is latent unless the ribosomes are disrupted. Loening\textsuperscript{17,18} has shown that in pea roots there is a DNA-like RNA which becomes labelled with \textsuperscript{32}P only minutes after the administration of the isotope to the roots and is synthesized in the nucleus. Since the nucleolytic enzymes of the pea root seem to act on this DNA-like RNA in the same way as they do on yeast RNA one could conclude that the DNA-like RNA would be degraded by the enzymes investigated here only after it had left the nucleus. This would be consistent with the hypothesis that this RNA is in fact a messenger RNA (or its precursor) which may migrate from the nucleus to the cytoplasm and there be broken down after it has fulfilled its role in protein synthesis.

The distribution of ribonuclease activity in the cytoplasm of the cells of the pea root is similar to that already reported for plants in that most of the activity is in the soluble fraction as in pea leaves\textsuperscript{7} and apple leaves\textsuperscript{8}. In the soluble fraction of the root (Fig. 6) and in the nuclear preparations (Fig. 3) the hint of two optima of ribonuclease activity, one at about pH 5 or 6 and the other above pH 7, suggested the possible presence of two enzymes. This suggestion was strengthened by the different effects of 0.15 M NaCl on the activity at the more acid pH's, where it was stimulatory and at the more alkaline pH's, where it was inhibitory. These effects were found with all the subcellular fractions examined so that if they were due to two distinct enzymes, these enzymes were apparently both present in all the cytoplasmic components. However the alkaline ribonuclease seemed to preponderate in the soluble fraction and the acidic ribonuclease in the particulate fractions.

\textbf{ACKNOWLEDGEMENTS}

I wish to thank Professor R. Brown for his continued interest and guidance. Technical assistance was ably given by R. M. O'Brien. This work was made possible by a grant from the Agricultural Research Council.

\textbf{REFERENCES}


THE GROWTH OF THE NUCLEUS IN DIVIDING AND NON-DIVIDING CELLS OF THE PEA ROOT

by

R.F. LYNDON

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The Growth of the Nucleus in Dividing and Non-dividing Cells of the Pea Root

BY

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Department of Botany, University of Edinburgh

ABSTRACT

The growth of the nucleus and the cell in the pea root was followed through the mitotic cycle and subsequently in post-mitotic development by comparing cells and nuclei from the meristem, at different stages of interphase, and cells and nuclei from two regions of the enlarging zone of the root. Measurements of cell and nuclear volumes were made in sections of fixed roots. Measurements of nuclear volume, DNA content, and dry mass were made on isolated nuclei. Growth in the mitotic cycle was characterized by a doubling of DNA and nuclear dry mass and a five-fold increase of nuclear volume. Since cell volume doubled, a differential hydration of cytoplasm and nucleus is inferred. Post-mitotic growth was characterized by a four-fold or greater increase in cell volume, with vacuolation and a continued increase of cytoplasmic constituents, but a cessation of nuclear growth except by uptake of water; the only increase in nuclear dry matter appeared to be in cells becoming endopolyploid. The concentration of dry matter in the nucleus fell as the nuclei enlarged in the mitotic cycle and in post-mitotic growth. The relationships between the measured parameters are examined to see whether they might be indicative of causal relationships.

INTRODUCTION

The changes which occur in the nucleus during cell development in the root tip were, in previous work, assessed by comparing nuclei isolated from cells in the meristem with nuclei isolated from cells in the enlarging and the maturing regions (Lyndon, 1963). The technique was essentially that of using serial sections of the root on the assumption that the temporal sequence of development is reflected in the spatial sequence of cells along the root. In the enlarging region this is probably so, for at any point all the cells are elongating at the same rate per unit length and all are equally distant from the end of their development into mature cells. The cells at any point in the enlarging region of the root may be regarded as a relatively homogeneous population, all having similar characteristics. For the cells of the meristem, however, this is not the case, for in an asynchronous meristem, as in Pisum, they may be at any stage of the mitotic cycle. The temporal sequence of development of cells in such a meristem may be reconstructed by comparing individual cells at different but known points in the mitotic cycle. When this has been done, cellular development through the mitotic cycle can be compared with development after mitotic activity has ceased, as exemplified by cells from successively more basal sections along the root. The development of a single
cell, and its nucleus, through the phases of the mitotic cycle and in its subsequent (post-mitotic) growth by enlargement to its final state as a mature, non-growing cell may then be inferred as a continuous process.

An attempt to follow and compare the development of cells of the root, and especially their nuclei, through the mitotic cycle and in their subsequent growth is reported in this paper. In the meristem the size of each nucleus serves to indicate its position in the mitotic cycle, for the volume of the nucleus increases considerably from telophase to prophase and nuclei of intermediate size may be considered to be at some stage of interphase. Nuclei were isolated from the meristem and the volume, DNA (deoxyribonucleic acid) content, and dry mass of individual nuclei were measured. Since the measurements of DNA and dry mass had to be done on different nuclei, these two sets of values were related through nuclear volume, which served as a reference point indicative of the position of each nucleus in the mitotic cycle. The volumes of individual cells and their nuclei in the meristem were measured in longitudinal sections of fixed and stained roots, and again the volumes of the nuclei served as the point of reference to relate these values to those for nuclear dry mass and DNA content. Measurements of cell volume and nuclear volume in longitudinal sections of roots, and of nuclear volume, DNA, and dry mass in isolated nuclei were also made from two regions of the root basal to the meristem and in which the cells were no longer dividing but were elongating. From these measurements the growth of the nucleus and the cell at two points in their post-mitotic development was followed and was compared with growth through the mitotic cycle.

Other work has suggested that in the meristem there are close correlations between cell volume, nuclear volume, and nuclear DNA content (List, 1963) and different but characteristic correlations between cell and nuclear growth in meristematic and enlarging regions of the root (Trombetta, 1939). The present data have also been examined to see if there are any correlations between the various parameters measured which may point to causal relationships in the growth of the nucleus and the cell.

METHODS

Peas (*Pisum sativum* cultivar Meteor) were germinated for 2.5 days by which time their roots were 3.0 to 3.5 cm long.

Total dry matter and DNA content of individual, isolated nuclei were measured. The roots were cut into sections; the region 0–2 mm from the apex included the meristem, 2–4 mm behind the apex represented the region of rapid elongation, and 4–9 mm behind the apex the region of elongation and maturation. The isolation of nuclei from the root sections and the measurement of nuclear volume and dry mass has been described previously (Lyndon, 1963).

DNA was measured in nuclei which were precipitated on to slides by the addition of absolute ethanol to drops of the nuclear suspensions. This treatment resulted in most of the nuclei sticking to the slide. They were washed
several times with 70 per cent ethanol followed by absolute ethanol and then ether. These ether-dried preparations of nuclei were stained with Feulgen reagent by the method of McLeish and Sunderland (1961). The absorptions of 50 nuclei from each region of the root were measured at 565 m\(\mu\) using a Barr and Stroud integrating microdensitometer. A random selection was made by measuring the first 50 intact nuclei encountered on regular traverses of the slides. The volume of each nucleus was also measured. Ethanol was chosen as the precipitant because it did not alter the size of the isolated nuclei. The values for DNA were converted from arbitrary units into absolute amounts in the following way. The mean DNA values obtained as readings on the microdensitometer for nuclei of the 0–2, 2–4, and 4–9 mm regions of the root were 51, 57, and 61 respectively. The ratios of these values to the mean amounts in \(\mu\text{g DNA per nucleus}—\text{measured in similar preparations of nuclei isolated from these same regions of the root (Lyndon, 1963)}—were 3.2, 3.2, and 2.9, i.e. about 3.1. Dividing the microdensitometric readings by 3.1 then converts them into \(\mu\text{g of DNA}.

In the course of isolation the nuclei were exposed to media which shrank them, but the sizes of these isolated nuclei remained proportional to the sizes of nuclei in intact, living cells (Lyndon, 1963) and in fixed and sectioned cells (Table 1). The size of nuclei after isolation was approximately half that of nuclei before isolation, irrespective of their initial size.

### Table 1

<table>
<thead>
<tr>
<th>Distance from root apex (mm)</th>
<th>Mean volume per cell ((\mu^3))</th>
<th>Mean volume of cells measured in sections ((\mu^3))</th>
<th>Mean volume of nuclei measured in sections ((\mu^3))</th>
<th>Mean volume of isolated nuclei (mean of 150) ((\mu^3))</th>
<th>Mean dry mass per nucleus (\text{(data of Fig. 3)}) ((\mu\text{g}))</th>
<th>Mean DNA per nucleus (\text{(data of Fig. 2)}) ((\mu\text{g}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>5750</td>
<td>3060</td>
<td>304</td>
<td>187</td>
<td>82</td>
<td>16</td>
</tr>
<tr>
<td>2–4</td>
<td>13,010</td>
<td>3820</td>
<td>504</td>
<td>210</td>
<td>85</td>
<td>18</td>
</tr>
<tr>
<td>4–9</td>
<td>62,270</td>
<td>24,300</td>
<td>698</td>
<td>351</td>
<td>87</td>
<td>20</td>
</tr>
</tbody>
</table>

* Mean fresh weight per root segment divided by number of cells per segment, measured by the method of Brown and Rickless (1949), as modified by Brown and Broadbent (1951).

The isolated nuclei appear to be a representative sample of the nuclei in the intact root (Lyndon, 1963) and measurements from the meristem confirm this. In sections, average values of 93 \(\mu^3\) for telophase and 460 \(\mu^3\) for prophase nuclei were found and would correspond to volumes of about 47 \(\mu^3\) and 230 \(\mu^3\) for isolated nuclei. The measured volumes of isolated nuclei with exclusively the 2C and 4C amounts of DNA ranged from 45 \(\mu^3\) to 260 \(\mu^3\). These values agree well with what is expected from the measurements on sections and also show that the preparations of isolated nuclei included all stages of interphase.

The sizes of cells and their nuclei were measured in median longitudinal sections, 10 \(\mu\) thick, of roots fixed in formalin-acetic acid-ethanol (FAA) or chromic-acetic acid-formalin (CRAF) and stained with haematoxylin and
fast green. In the region of the root where the cells were highly vacuolate there was some shrinkage of the tissue on dehydration. Only those cells were measured which showed no wrinkling of their walls. For the calculation of cell volumes the cells were assumed to be cylinders. Nuclear volumes were calculated as $\frac{4}{3}\pi \left(\frac{\text{length} + \text{breadth}}{4}\right)^3$. All measurements were made with an ocular micrometer. Inevitably some nuclei would be cut in the sectioning, and to reduce the error due to measuring tangential sections of nuclei only the larger nuclei or those with clearly defined nucleoli (suggesting the nuclei were cut more or less medianly) were measured. At each of successive 0.2 mm intervals behind the root apex three cortical, three procambial (or inner cortical), and three central stelar cells and their nuclei were measured. Further back than 4.0 mm three cells were measured at each interval. Since equal numbers of cells from cortex, procambium, and central stele were measured and it is improbable that there are equal numbers of cells in each of these tissues in the intact root, and also since cells of some tissues (e.g. epidermis) were not measured at all, the values obtained are not an exact description of the whole root. They are, however, related to the values for the whole root; this is apparent from a comparison of columns 2 and 3 in Table 1. The smaller mean size of cells measured in sections was partly because it was much more difficult, especially in the more basal parts of the root, to find undamaged large cells than smaller ones.

In the terminal 2 mm of the pea root only about 50 per cent of the cells appear to be meristematic (Brown, 1951), so that nuclei isolated from this region of the root are a mixed population of approximately equal numbers of nuclei in stages of the mitotic cycle and in post-mitotic growth. The characteristics of the mitotic nuclei may therefore be expected to be obscured to some extent. The results have therefore been supplemented, as described in the text, by observations on nuclei isolated from the region 0.5 to 1.5 mm from the root apex, which corresponds more closely to the meristem.

**Results**

**Nuclear Volume**

**Mitotic cycle**

The average nucleus increased five-fold in volume from 93 $\mu^3$ at telophase to 460 $\mu^3$ at prophase. These values are the means of 20 and 10 nuclei respectively, measured in sections. Allowing for 50 per cent shrinkage during isolation, corresponding volumes for isolated nuclei would be 47 and 230 $\mu^3$. Nuclei of these sizes were in fact found to have exclusively the 2C and 4C amounts of DNA (see below and Fig. 2).

**Post-mitotic growth**

In the region 2–4 mm behind the root apex the average size of a nucleus was about the same as that of the prophase nuclei in the meristem, but in the 4–9 mm region the nuclei had become larger than this (Table 1). That the
growth of the nucleus was continuous as the cells enlarged and were displaced from the root tip is shown in Fig. 1. The anticipated average volume of nuclei in the mitotic cycle would be approximately midway between telophase and prophase values, i.e. 275 μ³, which is the measured value for nuclei just behind the promeristem and in a region of great mitotic activity (Fig. 1). The continued increase in nuclear volume in the basal part of the 0–2 mm region probably reflects the increasing number of post-mitotic cells, having the characteristics of enlarging rather than dividing cells, in this part of the root.

At the tip of the root cap, where the nuclei have ceased division, they have enlarged very little above the size of telophase nuclei (Fig. 1). The greater average volume of nuclei in the root cap closer to the promeristem reflects the proportion of dividing nuclei which are found there.

**DNA**

**Mitotic cycle**

From the measured DNA values of nuclei isolated from the terminal 9 mm of the root was constructed a histogram which showed two distinct peaks (Fig. 2). These were confirmed as representing the 2C and 4C values for DNA.
by measuring the absorption of telophase and anaphase (2C) and prophase and metaphase (4C) nuclei in a Feulgen-stained macerate of ethanol-fixed root meristems. The values obtained in this way were $2C = 9.5 \mu g$ (mean of 12) and $4C = 19 \mu g$ (mean of 4)—a doubling of DNA in the mitotic cycle—and are indicated by the arrows in Fig. 2.

In the 0–2 mm region there is considerable overlap in the sizes of nuclei of different DNA classes (Fig. 2) so that nuclear volumes between 70 and 250 $\mu m^3$ may represent either 2C or 4C nuclei. This shows that there is no particular nuclear size at which DNA synthesis occurs, but rather a range of sizes. If nuclear size were indicative of the position of the nucleus in interphase, as in *Vicia* (Woodard, Rasch, and Swift, 1961), then in these *Pisum* roots DNA synthesis took place at different points in the mitotic cycle in different nuclei. Alternatively, and this seems more probable, DNA synthesis may occur at a different volume in different nuclei. Fig. 2 may then be interpreted as a series of similar F-shaped curves (each representing a relatively small number of nuclei) displaced from one another on the x-axis. In this case it should be, and in fact it is, possible to find telophase and prophase nuclei not of a standard size but showing a range of sizes. Prophase nuclei have been measured in sections within the range 260 to 860 $\mu m^3$—a difference of 3·5-fold. A greater range in size has been found for telophase nuclei, from 30 to 300 $\mu m^3$, so that the largest are about the size of the smallest prophase nuclei.

Nuclear volume apparently varied independently of DNA content within each DNA class. This is compatible with the growth of the nucleus occurring...
predominantly in the G1 and G2 phases (Van’t Hof, 1963), that is, before and after the period (S) of DNA synthesis. Furthermore, if DNA synthesis occurs about half-way through interphase it follows that the average volume of nuclei in G2 (i.e. with the 4C amount of DNA) will be about twice that of nuclei in G1 (with the 2C amount of DNA) and this is what is found (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Distance from root apex (mm)</th>
<th>Mean nuclear volume ($\mu^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2C*</td>
</tr>
<tr>
<td>0–2</td>
<td>122</td>
</tr>
<tr>
<td>2–4</td>
<td>100</td>
</tr>
<tr>
<td>4–9</td>
<td>160</td>
</tr>
<tr>
<td>0–9</td>
<td>125</td>
</tr>
</tbody>
</table>

* DNA values < 15 $\mu$g.
† DNA values > 15, < 25 $\mu$g.
‡ DNA values > 25 $\mu$g.

**Post-mitotic growth**

The mean amount of DNA per nucleus increased as the cells enlarged and passed from the 2–4 to the 4–9 mm region (Table 1). This increase was very similar to that shown from chemical estimations of DNA in whole cells (Heyes, 1960). Inspection of Fig. 2 shows that this is due to an increasing proportion of 4C and 8C nuclei as the cells become further removed from the root apex. The regions of the root more than 2 mm from the tip are virtually free of cell divisions and this increased number of nuclei with greater amounts of DNA is ascribed to DNA synthesis to bring the nuclei to the G2 stage (4C), but without subsequent division, and to the occurrence of endopolyploidy in the regions of the root behind the meristem. The fact that nuclei with the 2C amount of DNA are still present more than 4 mm behind the root apex shows that some nuclei, however, have not synthesized any more DNA after their last division. In the 2–4 and 4–9 mm regions of the root the 4C nuclei are twice the size and 8C nuclei four times the size of 2C nuclei (Table 2). This is compatible with the growth of nuclei after their last mitosis and also during endopolyploidization occurring mainly before and after DNA synthesis, in the same way as in the mitotic nuclei.

**Nuclear Dry Mass**

**Mitotic cycle**

The isolated nuclei used for measurements of their dry mass were from similar suspensions to those used for the measurement of DNA values and it is therefore probable that nuclei with a volume of 40–80 $\mu^3$ have only or predominantly the 2C amount of DNA and those with a volume of 250–260 $\mu^3$ the 4C amount. These nuclei are indicated by the arrows in Fig. 3 and, as
Lyndon—Nuclear Growth in the Pea Root

**FIG. 3.** Dry mass of isolated nuclei in relation to nuclear volume in the regions of the root (a) 0–2 mm, (b) 2–4 mm, and (c) 4–9 mm from the apex.
shown earlier (p. 136), probably represent nuclei just after telophase and at prophase. Dry mass therefore approximately doubles during interphase of the mitotic cycle, and appears to be a function of nuclear volume. For nuclei up to a volume of $260 \mu^3$ (Fig. 3a) the correlation coefficient for nuclear dry mass and volume was 0.69, and in the 0.5–1.5 mm region of the root, containing a higher proportion of meristematic cells, 0.73. This is consistent with there being a continuous accumulation of substances other than DNA (and which comprise most of the dry mass) in the nucleus during growth in interphase, as represented by the increasing nuclear volume, in contrast to the discontinuous increase in DNA.

**Post-mitotic growth**

The mean dry mass of nuclei isolated from the 4–9 mm region of the root was virtually the same as that of nuclei from the 2–4 mm region (Table 1) showing that increase in nuclear total dry matter is not maintained as the nuclei grow after their last mitosis. This is illustrated in Fig. 3 which shows that with increasing distance from the root apex the values for nuclear volumes become displaced to the higher values but that there is virtually no change in the distribution of values on the dry-mass axis. The appearance of a few values greater than 160 $\mu g$ in the 4–9 mm region may represent nuclei which have become endopolyploid, for they are the same proportion (about 10 per cent) as have the 8C amount of DNA (cf. Fig. 2).

The tendency of nuclei to enlarge without a corresponding increase in their dry mass may be shown in another way, by relating the concentration of dry matter to nuclear volume (Fig. 4). It is clear that as the nuclei enlarge the concentration of dry matter in them decreases and that the relationship of concentration to volume is similar for nuclei in all parts of the root. Therefore the greater hydration of nuclei in the enlarging regions of the root (Lyndon, 1963) is not a feature peculiar to enlarging cells but is only a reflection of the greater mean volume of the nuclei in these cells.

**Cell Volume**

**Mitotic cycle**

The mean volume (measured in sections) of 10 double telophase cells was 2940 $\mu^3$, so that the mean volume of the resulting daughter cells embarking on interphase would be 1470 $\mu^3$. Yet the mean value of 10 prophase cells measured in the same root was only 1780 $\mu^3$, so that the difference in mean cell volumes of telophase and prophase cells was much less than the twofold expected. There was indeed no correlation between nuclear volume and cell volume for telophase and prophase cells and there was a wide range of cell volumes associated with nuclei at the same stage of mitosis (Table 3). A comparison of cell volumes with nuclear volumes during interphase in the 0–2 mm region of the root as a whole similarly showed that there was no correlation of cell and nuclear volumes.
The discrepancy was resolved when cells having a common recent origin, in other words cells in the same file, were compared (Table 3). It becomes apparent that the size of the cells showing mitotic figures was much more constant for cells of the same file than for cells of different files, even in the same tissue. The similar sizes of the double telophase cells and prophase cells in the same file confirms that doubling of cell size occurs during the mitotic cycle.

![Graph](image)

**Fig. 4.** Concentration of dry matter in isolated nuclei in relation to nuclear volume in the regions of the root 0–2 mm (Θ), 2–4 mm (△), and 4–9 mm (□) from the apex.

**Post-mitotic growth**

In their passage from 2–4 to 4–9 mm behind the root apex the cells increased in mean volume by fourfold or more (Table 1). As in the root cap, this increase was not accompanied by a comparable increase in nuclear size (Fig. 1). There was no obvious correlation between cell size and nuclear size ($r \leq 0.5$ in all parts of the root).

**Discussion**

In mitotic growth in the pea root DNA and nuclear dry mass double during interphase. The volume of the nucleus increases fivefold so that its degree of hydration increases by more than 100 per cent, but the volume of the cell doubles from telophase to prophase, so that the presumed doubling of
**Table 3**

Sizes of telophase and prophase nuclei and of cells with mitotic figures in the same and in different files in the same root

<table>
<thead>
<tr>
<th>Tissue</th>
<th>File number*</th>
<th>Stage of mitosis†</th>
<th>Distance from root apex (mm)</th>
<th>Cell volume (μ³)</th>
<th>Nuclear volume (μ³)</th>
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<tr>
<td></td>
<td></td>
<td>M</td>
<td>0.8</td>
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<td>..</td>
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<tr>
<td>Cortex</td>
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<td>T</td>
<td>1.2</td>
<td>5100</td>
<td>310</td>
</tr>
<tr>
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<td></td>
<td>M</td>
<td></td>
<td>5150</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td></td>
<td>4340</td>
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<td></td>
<td>P</td>
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<td>4750</td>
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<tr>
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<td>430</td>
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<td></td>
<td></td>
<td>M</td>
<td>0.8</td>
<td>2000</td>
<td>..</td>
</tr>
</tbody>
</table>

Brackets indicate adjacent cells.

* File numbers are for identification only and do not imply location in the root.
† *P* = prophase (chromosomes visible).
* M = metaphase (chromosomes arranged equatorially).
* A = anaphase (chromosomes separated into two groups, no cell plate).
* T = telophase (new nuclei reforming, separated by a cell plate which extends only part way across the cell). Cell size is that of the binucleate unit.
the dry mass of the cytoplasm is accompanied by approximate doubling of its volume. The cytoplasm therefore probably changes in concentration very little during the mitotic cycle and does not hydrate as the nucleus does.

In post-mitotic growth the fourfold increase in cell volume from the 2–4 to the 4–9 mm region of the root is accompanied by an increase of 60 per cent or more in cellular protein and RNA (ribonucleic acid) (Heyes, 1960; Lyndon, 1963) but growth of the nucleus is by uptake of water and not by any significant increase of dry matter. Post-mitotic growth is therefore characterized by the continuation of the growth of the cell and cytoplasm by increase of dry matter, as well as by vacuolation, but virtual cessation of nuclear growth except for some uptake of water. The cessation of nuclear growth does not necessarily imply that synthetic activity in the nucleus stops, for the products of nuclear metabolism may be exported to the cytoplasm. This may well be the case for RNA, as the RNA which continues to accumulate in the cytoplasm is presumably synthesized in the nucleus. It may be that protein synthesis also continues in the nucleus but that the protein passes out of the nucleus into the cytoplasm. In whatever way the metabolism of the nucleus is changed in post-mitotic growth it seems unlikely that it is connected with the degree of hydration of the nucleus, for this increases to a similar extent in all parts of the root as the nuclei enlarge and is not a feature peculiar to either dividing or expanding cells.

In the developing metaxylem of several species of plants List (1963) found that cell volume was related to nuclear volume and that the growth of both cell and nucleus appeared to be step-wise and related to the DNA content, which in some cases reached as high as 64C. It has been shown above, when considering DNA content and nuclear volume, that a relationship of this sort is to be expected if DNA synthesis is somewhere about the middle of interphase (Table 2), and will be found even though there is no correlation of DNA content with nuclear volume for individual nuclei (Fig. 2). Relating cell volume, nuclear volume, and DNA content necessarily results in the finding of two classes of cells (or more in endopolyploid tissues such as metaxylem), one with DNA, and nuclear and cell volumes twice that of the other. This is in accordance with List's findings for the metaxylem, but it does not provide evidence for a causal connexion between DNA content, nuclear volume, and cell volume. On the contrary, the values in Table 3 show that the correlation between cell and nuclear volumes may be different in different cell files (files 4 and 7, telophase cells; files 4 and 6, prophase cells) as may also be the correlation between cell volume and DNA (cf. cell volumes of double telophase cells, all with twice the 2C amount of DNA, in files 2 and 6).

Although there appeared to be no general relationship between cell and nuclear volumes it was possible that there was a relationship between the heterogonic, or allometric, growth-rates as suggested by Trombetta (1939). However, an attempt to assess allometric growth constants for different parts of the root by comparing the sizes of cells and nuclei within these regions as Trombetta did, may give values which express more the range of nuclear size
in relation to cell size than actual growth-rates. Trombetta found an allometric growth constant \( \frac{\Delta \log \text{nuclear volume}}{\Delta \log \text{cell volume}} \) of 0.7 in the meristem of pea roots, a value which implies that a 100 per cent increase of cell volume is accompanied by only 60 per cent increase in nuclear volume, whereas it actually increases by 500 per cent, so that the true value for \( \frac{\Delta \log \text{nuclear volume}}{\Delta \log \text{cell volume}} \) in the mitotic cycle is 0.7/0.3 or about 2.3. A decrease in this ratio from 0.7 in the meristem to 0.52 in the enlarging regions of the root was found by Trombetta and could also be calculated from the present data, being 2.5, 1.4, and 1.0 in the 0-2, 2-4, and 4-9 mm regions of the root respectively. This decrease reflects the greater increase in cell size than nuclear size during cell enlargement. The relationships of the growth of cell and nucleus are best examined in the growing region itself and not in cells that have ceased growth. When the ratio \( \frac{\Delta \log \text{nuclear volume}}{\Delta \log \text{cell volume}} \) is calculated from the curves of Fig. 1 it is found to be 0.4 at 1-2 mm behind the promeristem decreasing to 0.3 at 3-5 mm behind it. If, as seems likely, this ratio declines continuously as the cells grow, the concept of a growth ‘constant’ becomes meaningless. It is probable that the relationship of the growth of the cell to that of the nucleus cannot be expressed by a simple mathematical relationship. On the data available up to now it may be best to regard the size of the cell and the size of the nucleus during cell elongation as independent variables.

The lack of correlations between the various parameters measured in the growth of the cells, and the differences between cells of different files, suggests that the highly ordered growth of the root as a unit does not derive from integrated growth at the subcellular level. It seems that the growth of the cells may vary within limits which are imposed by their being part of an organ in which the control of growth is exercised at the supracellular rather than the cellular level.

**Acknowledgements**

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**Literature Cited**


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I. INTRODUCTION

The nucleus is the largest cell organelle and it is therefore hardly surprising to find that its structure is complex and that it performs many different functions. The very size of the nucleus has proved an obstacle to understanding its ultrastructure and to isolating it without damage from the cell for analysis and experimentation. The nuclei of both plants and animals appear to have the same structure and functions and in many cases much more work has been done using animal nuclei. Although it is therefore inappropriate to consider the plant nucleus alone, in this chapter the emphasis will be on work which has been done on plant nuclei.

II. STRUCTURE

A. NUCLEAR ENVELOPE

The most characteristic feature of the ultrastructure of the nucleus is the limiting envelope which is a double membrane consisting of two unit mem-
membranes each 80 Å thick separated by a space, the perinuclear space, about 50 Å wide and perforated by pores of about 800 Å in diameter. The pores are places in the nuclear envelope where the inner and outer membranes are fused together at their edges (Fig. 1). The structure of the pores has been seen in more detail in nuclear envelopes isolated from cells of the onion (Franke, 1966). The border of the pore is an annulus showing 8-fold sym-

Fig. 1 Electron micrograph of part of a cell of the root meristem of a seedling of *Sinapis alba* showing the structure of the nucleus. NE, nuclear envelope with pore, P; C, chromatin, consisting of the extended chromosomes; G, granular and F, fibrillar regions of the nucleolus; CYT, cytoplasm. The line represents 1μ. Fixed in glutaraldehyde followed by osmium tetroxide. The section was stained with uranyl acetate followed by lead citrate. Photograph by courtesy of Mr. A. J. Tulett, Department of Botany, University of Edinburgh.

metry, similar to the octagonal pores in the nuclear envelope of the amphibian egg (Gall, 1967). The technique of freeze etching has also been used to obtain surface views of the nuclear envelope in yeast (Moor and Muhlethaler, 1963) and in onion roots (Branton and Moor, 1964) so that the number and distribution of pores can be seen. Approximately 8% or more of the area of the nuclear envelope may be occupied by pores. This means there are about 200 pores per nucleus for a small nucleus (diameter 2μ), such as in yeast, or about 3000 pores per nucleus in a higher plant, such as the onion, having nuclei about 8μ in diameter.
In many lower plants, especially in the fungi, the nuclear envelope remains intact during nuclear division but it divides and is partitioned equally to the daughter nuclei (Hawker, 1965). In higher plants the nuclear envelope is ruptured at the end of prophase and disintegrates into pieces which become indistinguishable from the endoplasmic reticulum which is distributed more or less equally to the two poles (Porter and Machado, 1960). The nuclear envelope is reformed in telophase from pieces of the endoplasmic reticulum which come to lie close to the chromosomes. These pieces of membrane join up in some way and eventually become continuous to form the new nuclear envelope around the telephase chromosomes (Porter and Machado, 1960; Lafontaine and Chouinard, 1963). Whether or not the new nuclear envelope is formed from pieces of the old envelope which have retained their identity through division is perhaps a meaningless question, for the outer membrane of the nuclear envelope is continuous with the endoplasmic reticulum and they are, therefore, really a single system of membranes (Marinos, 1960). The envelope of fusion nuclei in the embryo sac is derived equally from the two nuclei which fuse together (Jensen, 1964).

Since the endoplasmic reticulum is continuous through the plasmodesmata from one cell to the next, it is very probable that the perinuclear spaces of nuclei in adjacent cells are in direct continuity and that substances could therefore pass through the lumen of the endoplasmic reticulum from one nucleus to another without having contact with any other cytoplasmic components. It is often believed that the endoplasmic reticulum is also continuous with the plasma membrane. Were this so, there could be direct continuity between the perinuclear space and the external environment of the cell. However, this belief has only the most slender evidence to support it. To the writer's knowledge there is only one instance of an electron micrograph showing continuity of the plasma membrane and the endoplasmic reticulum in plant cells (Buvat, 1963) and two examples in animal cells (see Fawcett, 1964). We must surely agree that "... if communication between the lumen of the reticulum and the extracellular space does exist, it is relatively rare and probably quite transient" (Fawcett, 1964).

B. CHROMOSOMES

Chromosomes appear in electron micrographs as a tangle of fine threads 100-140 Å in diameter which are sometimes resolvable into two or four separate strands each 20-50 Å in diameter. Such fibrils form the fundamental structure of the chromosomes of plants (as of animals) during both division and interphase (Bopp-Hassenkamp, 1959; Peveling, 1961; Rossner, 1961; Setterfield, 1961; Ris, 1961, 1966; Chadard, 1962; Albersheim and Killias, 1963; Resch and Peveling, 1964; Hyde, 1964; Brandham and Godward, 1965). Since the diameter of the deoxyribonucleohistone helix is about 30 Å (Zubay,
2. STRUCTURE, FUNCTION, DEVELOPMENT OF THE NUCLEUS

(Seconded by Doty, 1959), the fine strands are probably nucleohistone helices. The 30 Å strands may in turn make up fibres about 200 Å thick (Ris, 1961) which, in turn may make up thicker strands. The sensitivity of the fibres to deoxyribonuclease and proteinases shows them to be deoxyribonucleoprotein (Setterfield, 1961; Callan, 1963; Wolfe, 1965).

How is this hierarchy of fibres related to the structure of the chromosome? The 30 Å fibrils have been interpreted as the individual chromatids, coiled on themselves to form the 100 Å fibrils and these being themselves coiled and so on until the coiled coil is the dimensions of the chromosome (Hyde, 1964). In this view the anaphase chromosome consists basically of a single, extremely long, strand of nucleohistone. An alternative view is that the chromosome consists of many strands coiled together like a rope, so that the 100 Å fibre consists of two 30 Å fibrils twisted around each other and the 200 Å fibre is two 100 Å fibres twisted together (Ris, 1961, 1966).

The discovery that plant chromosomes (Taylor et al., 1957) and DNA (Filner, 1965) replicated in a semi-conservative fashion as expected of the DNA helix, suggested that the basis of the anaphase chromosome was one long double helix of DNA, coiled in a complex manner. This is consistent with the genetic and mutational evidence that the functional unit is the chromatid. On the other hand, there is considerable evidence from classical cytology (see Sharp, 1943; Swanson, 1958; Mazia, 1961) and from X-ray and other evidence (Ris, 1961; Peacock, 1965) that the anaphase and telephase chromosomes are multistranded. Most electron micrographs can be interpreted on either hypothesis. However, in electron micrographs of prophase nuclei of Tradescantia the ends of the chromosomes were often found abutting in the nuclear membrane and at this point each chromatid could sometimes be seen to consist of several distinct strands about 3000 Å thick (Sparvoli et al., 1965). Models reconstructed from serial sections showed that there were at least five, and probably about eight, strands in each prophase chromatid. These strands are an order of magnitude larger than the 100 Å and 200 Å fibres and what the relation is of one to the other is not known. The number of such strands per chromatid has been estimated, by different procedures, to be 4 in Vicia (Trosko and Wolff, 1965) and 8 in Tradescantia (Ris, 1961).

The linear continuity of either a single stranded (unieme) or a multistranded (polyneme) chromosome would presumably be due to the DNA molecules stretching from one end of the chromosome to the other but there is evidence that the linear continuity of the chromosome may not be due entirely to DNA. Mirsky and Ris (1951) first showed that the continuity of isolated chromatin was not affected by the removal, by enzyme action, of histone but that the chromatin fell apart as soon as the DNA or the non-histone proteins were attacked. Wolff (1965) has recently made very similar
observations on *Vicia* chromosomes. He has also found that when chromosomes were broken by radiation they could rejoin and that the rejoining was prevented by puromycin (an inhibitor of protein synthesis) but not by fluorodeoxyuridine (an inhibitor of DNA synthesis). A model, proposed by U (1965), for the structure of the chromosome demonstrates that both multi-strandedness and the participation of proteins in the linear continuity could be compatible with the observed behaviour of the chromosomes in mitosis, meiosis and crossing over. The essential point is that multiple strands of DNA must behave as a unit. It is equally difficult to understand how a mutation would occur in all the copies of a gene which undoubtedly exist (Birnstiel *et al.*, 1967), whether they are arranged linearly, in a uniene chromosome, or side by side, in a polyneme chromosome.

Apart from the demonstration that plant chromosomes, like those of animals, consist partly of RNA (Kaufmann *et al.*, 1948; La Cour, 1963) and proteins other than histones (Wolff, 1965) and may contain calcium (Steffensen and Bergeron, 1959), little is known of their chemical structure. The only components of which we have a little more than passing knowledge are the DNA and the histones. The DNAs of plants are characterized by having a relatively high content of 5-methylcytosine (Thomas and Sherratt, 1956). Histones isolated from plant sources have an amino acid composition similar to comparable fractions from calf thymus, the classical animal source. The histones of wheat embryos, pea buds and *Chlorella* cells, in contrast to those of most animal cells examined, consist predominantly of the lysine-rich histone fractions (Johns and Butler, 1962; Iwai, 1964) which in pea buds comprise three quarters of the histone of the nucleus (Huang and Bonner, 1965). The histone fractions of rice embryos are, however, very similar to those of calf thymus (Iwai, 1964). As would be expected of lysine-rich histones, the major fraction from wheat embryos does not aggregate above pH5 as arginine-rich histone does (Cruft *et al.*, 1957). An interesting qualitative difference between the lysine-rich histones of animals and wheat embryos (the only plant source for which data are available) is that in the plant these histones have alanine as the principal N-terminal amino acid rather than proline. This is usual a characteristic of arginine-rich histones (Johns and Butler, 1962). Not the DNA of the chromosome may be complexed with histone; probably on about 80% of it is complexed at any one time (Bonner, 1965).

In meiotic chromosomes of both plants and animals a consistent feature is the occurrence of paired strands of dense material, about 300 Å wide and about 1500 Å apart, which have been called the synaptonemal complex because they are found only during zygotene and pachytene, i.e. during synapsis. Since the complex is not found in organisms in which the chromosomes pair but crossing over does not take place, it is almost certainly of some special significance in the formation of chiasmata (Moses, 1964). The synaptonemal complex is not found in organisms in which the chromosomes pair but crossing over does not take place, it is almost certainly of some special significance in the formation of chiasmata (Moses, 1964). The synaptonemal complex is not found in organisms in which the chromosomes pair but crossing over does not take place, it is almost certainly of some special significance in the formation of chiasmata (Moses, 1964).
Giant chromosomes, like those of Dipteran salivary glands, are also found in some specialized plant cells (references in Stange, 1965) and emphasize the similarity of the chromosomes of animals and plants. We are still some way from being able to say what is the fundamental organization which is common to interphase, mitotic, meiotic, lampbrush and giant chromosomes. The numbers of different models for chromosome structure which have been proposed are really only cloaks for our ignorance. The difficulties in the electron microscopy may be simply that the chromosome is a relatively large body made up of very fine fibrils coiled in three dimensions. If this is so then the structure of the chromosome at this level may yet be resolved by someone with a sufficient fund of patience to obtain sufficient serial sections.

C. NUCLEOLUS

The nucleolus has no membrane and is therefore in direct continuity with the rest of the nucleus. It consists of a network of fibres about 100 Å in diameter which in turn consist of fibrils about 20 Å in diameter. The fibres stain for protein but unlike the chromosomes are destroyed by RNase and not by DNase, so that they are ribonucleoprotein (Hyde et al., 1965; La Cour, 1966). In parts of the nucleolus there are also granules about 150 Å in diameter which probably represent tightly packed fibres (Hyde et al., 1965; Lafontaine and Chouinard, 1963). The granules, which are almost certainly the precursors of ribosomes, are often found around the periphery of the nucleolus so that the centre is virtually free of them and consists of fibrils in an amorphous protein matrix (Fig. 1). These two parts of the nucleolus, the fibrillar and granular regions, can with the appropriate stains be made visible in the light microscope (Chouinard, 1966). The granular region often appears to be organized into strands about 1000 Å thick which have been called the nucleolonema. Structures probably representing the chromatin of the nucleolar organizer can be derived from the central part of the nucleolus by treatment with detergent (La Cour, 1966) and seem to be analogous to nucleoli of amphibian eggs which can be made to form ring nucleoli in the same way (Callan, 1966). The association of the chromatin of the nucleolar organizer with the nucleolus has been most clearly seen in electron micrographs of \textit{pirogryra} (Godward and Jordan, 1965).

The nucleolus may therefore be regarded as a collection of gene products having sufficient morphological structure to remain associated with the loci...
at which they were produced. This implies of course that the nucleolus had DNA associated with it. Plant nucleoli in fact contain about 10–20% of total DNA of the nucleus (Birnstiel et al., 1964; McLeish, 1964) but this DNA does not stain with Feulgen (McLeish, 1964). In Blastocladiella the DNA of the nucleus could be fractionated into two components \( \alpha \) and \( \beta \) which were found in the ratio of \( 3\alpha : 2\beta \) in the chromosomes but only the \( \alpha \) component was present in the nucleoli (Comb et al., 1964). The DNA in the pea nucleolus is perhaps localized in the knots which were seen in the fibrous network, for some unknotting occurred when the nucleoli were treated with DNase (Hyde et al., 1965).

In dividing cells the nucleolus typically disappears at the end of prophase and is reformed at telophase. Electron microscopy has shown that in late prophase the nucleolus becomes more loosely organized until the 100 Å fibrils merge with the surrounding nucleoplasm and the 150 Å granules become dispersed throughout the nucleoplasm (Lafontaine and Chouinard, 1963). The formation of new nucleoli in telophase has long been recognized to be a function of specific parts of certain chromosomes (the nucleolar organizers) so that initially the number of new nucleoli is the same as the number of organizers although later the nucleoli may fuse to form a single nucleolus. In telophase when the new nucleoli are first observed the chromosomes are seen to be coated with a material that is fibrillar-granular in texture (Albersheim and Killias, 1963; Lafontaine and Chouinard, 1963; Sparviero et al., 1965). This material disappears in late telophase and some have considered that it represented nucleolar material synthesized at various sites on the chromosomes and then assembled by the nucleolar organizer into the nucleolus. Further work is necessary before the significance of the material coating the chromosomes is known. The nucleolus may well be formed when it is assembled, i.e. at the nucleolar organizer.

**D. MACROMOLECULAR COMPOSITION OF THE NUCLEUS**

The structural and staining evidence indicates that the chromatin consists primarily of deoxyribonucleohistone and the nucleolus of ribonucleoprotein which includes particles looking like ribosomes. To what extent can chemical analyses of isolated and fractionated nuclei clarify this picture? The DNA content of the nucleus can vary widely (Martin, 1966) from 0.48 μg in *Marchantia* (Ishida, 1961) to more than 300 μg in tetraploid *Lilium* (Sundland and McLeish, 1961). This variation is related to chromosome size rather than chromosome number for closely related species within the same family and having the same number of chromosomes can have widely different amounts of DNA per chromosome (Rothfels et al., 1966).

Measurements on isolated nuclei have shown that DNA may represent anything from 8–40% of the dry mass of the nucleus. The amount of RNA...
-21 %) is usually somewhat less than that of DNA and the bulk of the nucleus (50-80 %) consists of protein (Table I). The nucleus also presumably contains small amounts of lipids. The techniques used for isolation of the nuclei would allow the loss of soluble constituents which are small molecules.

Table I

<table>
<thead>
<tr>
<th>Amounts of DNA, RNA and protein in the nucleus.</th>
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</tbody>
</table>

Determined indirectly.


Isolated by a non-aqueous procedure.

but macromolecules such as protein are not lost during these isolation procedures. The dry mass of isolated pea root nuclei was the same as that before isolation (Lyndon, 1963) and the same as nuclei isolated from fixed pea roots (McLeish, 1963).

Measurements on isolated nucleoli (Tables II and III) show that 20-40% of the nuclear dry matter is in the nucleolus. The volume of the nucleolus is usually less than 10% of that of the nucleus so that the concentration of material in the nucleolus is two or three times as great as in the rest of the nucleus. From 10 to 20% of the DNA of the nucleus is found associated with the nucleolus and about a third of the RNA.

A more detailed analysis of the composition of pea nuclei and nucleoli has been made by Birnstiel et al. (1964). Probably something like half of the ribosomal type of particles, and of the ribosomal RNA and also half of the transfer RNA of the nucleus, is located in the nucleolus. Their data make possible a comparison of the relative concentrations of ribosomal particles
in the nucleus and the cytoplasm. The nucleoli of their preparations were about 6% of the volume of the nucleus and in cells of the pea root tip the cytoplasm is about four times the volume of the nucleus (Lyndon, 1967) so that the relative volumes of nucleolus : rest of nucleus : cytoplasm are approximately 1 : 16 : 64. The cytoplasm contains about 10 times as much RNA as the nucleus (Lyndon, 1963). Since 80% of cytoplasmic RNA are

### Table II

**Amounts of DNA, RNA and protein in the nucleolus.**

<table>
<thead>
<tr>
<th>Plant</th>
<th>DNA (µg)</th>
<th>RNA %</th>
<th>Protein %</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea seedlings</td>
<td>25*</td>
<td>1.5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Pea embryos</td>
<td>-</td>
<td>&lt;= 1</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Broad bean root tips</td>
<td>28</td>
<td>10.5</td>
<td>37</td>
<td>11</td>
</tr>
</tbody>
</table>

* Determined indirectly.
† See Table I.

### Table III

**Proportion of nuclear material in the nucleolus.**

<table>
<thead>
<tr>
<th>Plant</th>
<th>DNA (µg)</th>
<th>RNA %</th>
<th>Protein %</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea seedlings</td>
<td>36</td>
<td>15</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>Pea embryos</td>
<td>8</td>
<td>1</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Broad bean root tips</td>
<td>19</td>
<td>18</td>
<td>41</td>
<td>11</td>
</tr>
</tbody>
</table>

* See Table I.

50% of the nuclear RNA is ribosomal, half of this being in the nucleolus (Chipchase and Birnstiel, 1963b), the ratio of the number of ribosomes in the nucleolus: rest of nucleus : cytoplasm is about 1 : 1 : 32. The relative concentrations of ribosomes in nucleolus : rest of nucleus : cytoplasm are therefore approximately 16 : 1 : 8. This compares with what is seen in electron micrographs (Fig. 1) where the concentration of granules in the nucleolus is about twice that found in the cytoplasm and there is a much lower concentration in the rest of the nucleus.

There are not sufficient data available to be able to say how the variations in the composition of the nucleus may be related to the developmental and metabolic status of the cell. Such overall data are in any case of limited value. It will be necessary to find out what changes may occur in the different...
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The structure of the nucleus and to correlate this with the ultrastructural changes which they reflect.

III. FUNCTION

A. SEQUESTRATION OF THE CHROMOSOMES

In a meristematic cell the nuclear envelope serves as a bag to keep the chromosomes together from one division to the next. This may be particularly important in large dividing cells such as those of the cambium. In addition we like to think that the nuclear envelope can in some way regulate the passage of molecules between nucleus and cytoplasm so that these two parts of the cell could interact with each other during development in a way which would be impossible if they were a continuum. This idea implies that the pores in the nuclear envelope may sometimes be open and sometimes occluded. Electron micrographs usually show the pores as being open but can always be argued that the occluding substances when present are not electron-dense with the procedures used. Attempts have been made with animal cells to relate the electrical resistance across the nuclear envelope to the structure and metabolic properties of the envelope but without any real access. In salivary gland nuclei there was a high resistance across the envelope but this had the same structure as that of the envelope of the nucleus of the dog oocyte which showed no resistance (Loewenstein et al., 1966) despite being semi-permeable (Harding and Feldherr, 1959). However, it is tantalizing to find that in salivary glands the resistance of the envelope changed in a regular fashion during development and could be altered in a predictable way when the cells were treated with hormones which affected development (Loewenstein et al., 1966).

Another suggested function of the nuclear envelope is that mitochondria and plastids originate from it by evagination during oogenesis (Bell and Muhlethaler, 1964; Bell et al., 1966). It is difficult to be sure since evaginations from the nuclear envelope cannot be classed as mitochondria and free organelles have not been shown with certainty to have been continuous with the nucleus. Although evaginations from the nuclear envelope have often been seen during oogenesis, in animals as well as plants, it can be argued even more convincingly that the organelles show continuity throughout oogenesis and do not arise from the nuclear envelope (Diers, 1966). Studies of oogenesis in other plants have likewise provided no evidence for a nuclear origin of cytoplasmic organelles (Jensen, 1965; Israel and Sagawa, 1964).

B. REPLICATION OF THE CHROMOSOMES

The synthesis of the DNA of the chromosomes takes place usually about the middle of interphase and may be initiated simultaneously at several sites
on a chromosome (Evans, 1964). Synthesis is completed sooner at some loci than at others (Wimber, 1961; Evans and Rees, 1966). The last parts of the chromosomes to finish DNA replication are the heterochromatin (Evans, 1964) and those parts which show the greatest frequency of chiasmata (Rees and Evans, 1966). The rate of synthesis of DNA may not be constant but may change, being less in the middle of the S-period than at the beginning and end (Howard and Dewey, 1961; Kusanagi, 1966). The maturation of the seed apparently results in an abrupt suspension of metabolism, including DNA synthesis, for nuclei could be found in dry wheat embryos which had amounts of DNA intermediate between telophase and prophase values, suggesting that chromosomes could be suspended in the process of replicating their DNA while the embryo was in the dry fruit and could resume synthesis on germination (Avanzi et al., 1963). The histones are probably replicated at the same time as the DNA (McLeish, 1959; Rasch and Woodard, 1957; Woodard et al., 1961; De, 1961). Some of the chromosomal protein may be renewed completely after each division of the nucleus. $^3$H-arginine incorporated into chromosomes of Vicia faba roots during or just after DNA synthesis was found, by radioautography, to be present on the chromosomes when they reached metaphase but by the next division most chromosomes had lost this protein for they were unlabelled (Prensky and Smith, 1964).

In meiosis, after the main period of DNA synthesis is completed in the microspore mother cell, there is a later synthesis of DNA during the zygotene and pachytene stages of prophase (Hotta et al., 1966). The amount of DNA synthesized at this time is very small, being only 0.3% of the total. This late synthesis has also been found in animal cells (Wimber and Prensky, 1966) and may be concerned with the reformation of DNA molecules that are broken during crossing over which occurs at this time. Before the chromosomes enter the meiotic prophase, there is synthesized a characteristic histone component which is not found in any of the somatic cells of the plant (Sheridan and Stern, 1967). This histone has been found in both lily and tulip but its significance is unknown. When the microspores have been formed and are maturing there may be changes in the relative amounts of the different histone fractions. This is long after DNA synthesis has ceased but it is consistent with the finding that synthesis of histones can continue when DNA synthesis is inhibited (Flamm and Birnstiel, 1964a).

In plant cells the replication of the chromosomes occurs not only prior to division but also in cells which have completed division. As the cells of the root pass out of the meristem into the more basal regions where they do not divide, they nevertheless synthesize DNA (Jensen et al., 1960) so that the proportion of nuclei with the 4C amount of DNA increases (McLeish and Sunderland, 1961). In addition some of these cells undergo endomitosis and further DNA synthesis so that they become polyploid. This may be looked
2. STRUCTURE, FUNCTION, DEVELOPMENT OF THE NUCLEUS

on as a type of cellular differentiation (Partanen, 1965) and it is more often
and in dicotyledons than in monocotyledons (Deeley et al., 1957; McLeish
Sundeland, 1961).

C. DIVISION OF THE CHROMOSOMES:
FORMATION OF THE SPINDLE

The formation of spindle material by the nucleus has been followed in a
es of elegant experiments by Bajer and his colleagues using the cells of
osperm. During prophase the mass of the chromosomes increased. Then
ate prophase the nuclear volume decreased slightly and, while the nuclear
velope was still intact, the dry mass of the nucleus began to fall (Ambrose
Bajer, 1961). The loss of nuclear dry matter coincided with the formation
outside the nucleus of the clear zone which contained birefringent
erial and microtubules and which later formed the spindle (Inoue and
er, 1959; Pickett-Heaps and Northcote, 1966). While the clear zone was
ng formed, and the mass of the chromosomes was falling, the mass of the
ceoplasm remained constant. The inference was that the material which
accumulated earlier on the chromosomes was now being secreted from
m to pass out of the nucleus into the clear zone. Material continued to be
th through the nuclear membrane until this broke down, marking the end
prophase. At this point the nuclear sap became birefringent, like the clear
e with which it became mixed, and formed the spindle (Ambrose and
er, 1961). Each spindle fibre consists of a bundle of fine filaments (Bajer
Allen, 1966). These are probably the microtubules each about 200 Å
diameter which are seen in electron micrographs (Ledbetter and Porter,
3; Manton, 1964; Harris and Bajer, 1965; Pickett-Heaps and Northcote,
6).

D. TRANSCRIPTION OF THE GENES: RNA SYNTHESIS

A vast amount of work has made it clear that the nucleus is the main site
RNA synthesis in the cell although some of the cytoplasmic organelles
synthesize RNA in their own right. Most, if not all, RNA synthesis is
A-dependent and hence the nucleus, as the main site of localization of
A in the cell, is the main site of RNA synthesis (Zalokar, 1960; Mitchison,
6; Perry, 1965). Although RNA synthesis is not restricted to any one
t of the nucleus, it is perhaps a reflection of the fact that 85% of the DNA
in the extranucleolar chromatin that this is where most of the RNA is
thesized.

Isolated plant nuclei will synthesize RNA to a limited extent (Rho and
phase, 1962; Bandurski and Maheshwari, 1962). In intact cells (Woods,
0), as in nuclei, RNA may be synthesized by the chromatin and the nucleolus
the RNA synthesized by the chromatin may move to the nucleolus and
accumulate there (Rho and Bonner, 1961). The RNA appears to be synthesized on the chromatin while this is in an expanded state, although it may later contract (Kemp, 1966). In the nucleolus newly synthesized RNA is first detected in the central, fibrillar region (La Cour and Crawley, 1965) and only later in the outer granular region (Karasaki, 1965).

1. **Ribosomal RNA**

A ribosome contains three molecules of RNA, one each of the 28S, 18S and 5S components. The sedimentation coefficients of ribosomal RNAs from plants may in fact be slightly lower. There is lots of evidence, nearly all from animal cells, that the 28S and 18S RNAs are synthesized in the nucleolus (Perry, 1965; Birnstiel, 1967). The clearest evidence that the nucleolus is the site of synthesis is that the mutant of the toad *Xenopus*, which lacks a nucleolus, cannot synthesize ribosomal RNA (Brown and Gurdon, 1964). The nucleus of the anucleolate mutant also lacks the 150 Å particles which resemble cytoplasmic ribosomes and which are found in the nucleus of the wild type (Jones, 1965).

The nucleolus of the pea has been shown to contain RNA having the same sedimentation coefficients, 28S and 18S, as the RNA of cytoplasmic ribosomes (Birnstiel *et al*., 1963a). This RNA was extracted from isolated pea nucleoli and was annealed with pea DNA to form a hybrid RNA–DNA complex. RNA extracted from cytoplasmic ribosomes also annealed with DNA and competed with the nucleolar RNA for the same sites on the DNA, showing that the nucleolar RNAs were identical in base sequence with the ribosomal RNAs (Chipchase and Birnstiel, 1963b). The 5S RNA, which is thought to link the two subunits of the ribosome, has also recently been shown to be synthesized in the nucleolus in *Blastocladiella* (Comb and Katz, 1964; Comb and Zehavi-Willner, 1967).

2. **Transfer RNA**

Transfer RNA is synthesized in the nucleus but it is not yet clear in what part of the nucleus. The transfer RNA is distinguished experimentally from other RNA by its ability to combine with amino acids in the presence of activating enzymes. About 30% of the RNA of the pea nucleus is transfer RNA and it can be synthesized by isolated nuclei (Chipchase and Birnstiel, 1963a). In the pea about 50% of the nuclear transfer RNA is found in the nucleolus (Chipchase and Birnstiel, 1963b). The nucleolus of *Blastocladiella* also contains transfer RNA (Comb and Katz, 1964). This is consistent with the synthesis of transfer RNA occurring in the nucleolus, as it apparently does in Dipteran salivary glands (Sirlin *et al*., 1965). On the other hand, in *Vicia faba* roots the nucleoli seem to contain no transfer RNA and experimental evidence points to the extranucleolar chromatin as the exclusive site of
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Transfer RNA synthesis (Woods and Zubay, 1965). The incorporation of nucleotides into transfer RNA in the cytoplasm was thought to represent the addition there of the cytidine-cytidine-adenine end of the molecule. The concentration of methylating enzymes in the nucleolus of the pea (Birnstiel et al., 1963b) does not necessarily point to the nucleolus as the site of synthesis of transfer RNA; it could be other RNAs, including ribosomal RNA, which are methylated there (Greenberg and Penman, 1966).

Messenger RNA

The DNA-like RNA which has been isolated from root tissues has been found in association with ribosomes (Loening, 1965) and polyribosomes (Lin et al., 1966) and therefore possesses one of the qualifications of messenger RNA. This RNA is also rapidly labelled and in excised soybean hypocotyl as a half life of only 2 hr (Ingle et al., 1965). Since only 20% of the DNA-like RNA was associated with 75% of the ribosomes of the tissue, one might guess that most of the other 80% of this RNA would be found in the nucleus here it is presumably synthesized (Lin et al., 1966). Much of this RNA may indeed never leave the nucleus (see Mitchison, 1966) and some of it could be involved in protein synthesis within the nucleus.

E. TRANSLATION OF THE GENIC INFORMATION: PROTEIN SYNTHESIS

When cells are supplied with radioactive amino acids the nucleus is labelled quickly (Mattingly, 1963) but, at least in some cases, this could represent accumulation rather than synthesis of protein for it is now known that there are proteins which can shuttle to and fro between the cytoplasm and the nucleus (Prescott, 1964). Unequivocal evidence that nuclei can synthesize protein can be obtained by the use of nuclei in vitro. Nuclei isolated from pea and tobacco cells have been shown to incorporate amino acids into their protein (Birnstiel et al., 1962a; Flamm et al., 1963). Labelling kinetics pointed to the nucleolus as the principal site of protein synthesis in the nucleus (Birnstiel et al., 1961; Flamm and Birnstiel, 1964b). When nuclei were fractionated and the ability of the isolated fractions to incorporate amino acids was examined, the isolated nucleoli were found to be more active than the chromatin. The nucleolar proteins which eventually became most highly labelled were the residual proteins, the amino acid composition of which was similar to that of ribosomes (Birnstiel and Hyde, 1963). It was not clear whether these were ribosomal proteins which had been synthesized in the nucleolus or whether the newly synthesized protein was still associated with ribosomes on which it had been synthesized. There is, however, considerable evidence that the nucleolus is in fact the site of synthesis of ribosomes (Birnstiel, 1967). Ribonucleoprotein particles, which look like ribosomes, are very similar in size (Hyde et al., 1965) and have a composition and
sedimentation characteristics like those of ribosomes (Birnstiel et al., 1963) are concentrated in the nucleolus in pea cells. These ribosome-like particles differed from cytoplasmic ribosomes in their labelling kinetics and the proteins also became labelled more slowly than the other proteins of the nucleolus (Flamm and Birnstiel, 1964b). The relative inactivity of these particles in protein synthesis, together with the observation that sometimes they occurred mainly as 60S (rather than 78S) particles, makes it probable that they are in fact precursors of the cytoplasmic ribosomes and that they are synthesized in the nucleolus (Birnstiel et al., 1963a). The preponderance of 60S particles in the plant nucleus also suggests that, as in animal cells (Penman, 1966), the 39S subunit of the ribosomes is released from the nucleus into the cytoplasm before the 60S subunit.

**F. FORMATION AND RELEASE OF MORPHOGENETIC SUBSTANCES**

The unicellular alga *Acetabularia* can be grown for long periods after removal of the single nucleus. This has made possible the direct study of nuclear function by observing the effects of enucleation. Such experiments and also those in which nuclei have been transferred from one strain to another, have shown that the nucleus is responsible for the formation of morphogenetic substances, almost certainly RNA, which are released into the cytoplasm and there determine the structure of the cap which is formed (Haemmerling, 1963; Werz, 1965; Brachet, 1967). The chloroplasts too are in some way dependent on the nucleus for the rate of division of the chloroplasts is less when the nucleus is absent (Shephard, 1965).

Experiments with *Micrasterias* have shown that the nucleus synthesizes material which is necessary for the ordered growth of the cell. This material again is almost certainly RNA (Kallio, 1963; Selman, 1966). By inactivating the nucleus at particular points in the cell cycle, specific blockages or changes at specific points in the subsequent growth of the cell can be induced. In this way the time at which the message, determining the growth of a particular arm or lobe of the cytoplasm, is formed in the nucleus can be measured quite precisely (Selman, 1966).

**IV. DEVELOPMENT**

**A. DEVELOPMENT OF THE NUCLEUS**

The obvious development of the nucleus is during the mitotic cycle. The nucleus and nucleolus grow throughout interphase by accumulation of protein and nucleic acid until they reach maximum size at the end of prophase. Accumulation of most nuclear protein and RNA occurs more or less linearly throughout interphase (Woodard et al., 1961; Lyndon, 1967) although there is some evidence that RNA synthesis is twice as fast in G2 as in G1 (Van...
If so this might imply that the rate of RNA synthesis is a function of the amount of DNA present. The synthesis of histones occurs at the same time as DNA synthesis, about the middle of interphase (Rasch and Woodard, 1959), and the spindle proteins seem to be synthesized in prophase (Ambrose and Bajer, 1961). While the chromosomes in mitosis are attached to the spindle, synthesis of RNA drops to a low level or stops (Taylor, 1953;arris and La Cour, 1963; Van't Hof, 1963; Davidson, 1964; Das, 1963; Das et al., 1965). In meiosis the rate of RNA synthesis fluctuates and is inversely related to the degree of contraction of the chromosomes (Taylor, 1958; Hotta and Stern, 1963).

Much of the RNA and protein which is synthesized in the nucleus during interphase and about half of what accumulates in the nucleus will be in the

| TABLE IV |
| Changes in the composition of nucleus and cytoplasm during cellular development in roots of Pisum. |

<table>
<thead>
<tr>
<th>mm from root apex</th>
<th>Nucleus</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>RNA</td>
<td>15</td>
<td>64</td>
</tr>
<tr>
<td>Protein</td>
<td>45</td>
<td>242</td>
</tr>
<tr>
<td>Sum</td>
<td>76</td>
<td>242</td>
</tr>
<tr>
<td>Dry mass (measured by interferometry)</td>
<td>82</td>
<td>413</td>
</tr>
</tbody>
</table>

The increase of all the nuclear components slows down considerably as cells in the root mature (Table IV). In enlarging cells of the root, cytoplasmic protein and RNA continue to increase rapidly but nuclear protein and RNA
increase little if at all. This may be either because synthesis in the nucleus stops or because it continues but the products are broken down or exported to the cytoplasm. The continued accumulation of RNA in the cytoplasm during cell enlargement suggests that synthesis in the nucleus in fact continues but that RNA (and perhaps protein, too) passes into the cytoplasm and does not contribute to the growth of the nucleus. The increased incorporation of protein and RNA precursors into the cytoplasm but not into the nucleus of enlarging cells is consistent with this view (Jensen, 1957). The formation of large cells by endopolyploidy, especially those of the developing vascular elements (List, 1963), is probably related to the increase in ribosome content which accompanies doubling of the chromosomes (Li and Anderson, 1967; Painter and Biesele, 1966). In microsporogenesis synthesis of RNA in the nucleolus and on the chromosomes falls off during meiotic prophase but the decrease in nucleolar synthesis is much more rapid (Das, 1965). When the pollen has been released and germinated, the nuclei of the pollen tube of Z. mays possess no nucleoli. Synthesis of RNA is resumed but it is not ribosomal RNA which is made (Steffensen, 1966). The ribosomes in the pollen tube are apparently made earlier during the formation of the pollen. It would be interesting to know to what extent the limited growth of the pollen tube is a result of the apparent inability of its nuclei to synthesize ribosomes.

Another case which may prove of interest in this respect is the difference which may be found between the nuclei of the root and shoot meristems of the same plant. The cells of the shoot meristem divide more slowly than those in the root. Prophase nuclei in the shoot meristem are only about half the size of those in the root and their dry mass is correspondingly smaller. Their DNA content, as expected, is the same (Table V). The increases of nuclear

<p>| Table V |
| Comparison of early prophase nuclei in root and shoot meristems of <em>Pisum.</em> |</p>
<table>
<thead>
<tr>
<th></th>
<th>Root</th>
<th>Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear volume $\mu^3$</td>
<td>460</td>
<td>310</td>
</tr>
<tr>
<td>Nuclear dry mass $\mu$g</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>DNA per nucleus $\mu$g</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Nucleolar volume $\mu^3$</td>
<td>25</td>
<td>8</td>
</tr>
</tbody>
</table>

protein and RNA during interphase are therefore only about half the corresponding increases during interphase in the root. The growth of the nucleolus is also very much less during interphase in the shoot, which might point to a synthesis of fewer ribosomes than during interphase in the root.

The growth of the nucleus during interphase is also due to intake of water. The dry mass of the nucleus approximately doubles but the volume increases.
2. STRUCTURE, FUNCTION, DEVELOPMENT OF THE NUCLEUS

Fold (Lyndon, 1967) and the nuclear envelope therefore doubles in area. This may be by stretching for the diameter of the pores may increase as the nucleus enlarges (Tamiya, 1964; Branton and Moor, 1964). Alternatively, the nuclear envelope might grow at least partly by addition of new material and the pores could become redistributed by closure of old pores and opening of new ones as was found in aged yeast cells (Moor and Muhlethaler, 1963). The number of pores in the envelope may also change during development. Large discontinuities in the envelope are characteristic of nuclei in the meristem but as the cells mature these holes are no longer found and the envelope is thus has fewer connections with the endoplasmic reticulum (Whaley et al., 1960). When formed in telophase, the nuclear envelope at first has no pores (Porter and Machado, 1960; Chadard, 1962). They must be formed soon afterwards. At the end of interphase, the pores close up for just before the envelope is ruptured at the end of prophase it is once more devoid of pores (Porter and Machado, 1960; Israel and Sagawa, 1965). An uneven distribution of the pores could possibly result in a concentration difference, between the end of the cell and the other, of nuclear products such as RNA which pass out into the cytoplasm and might thus contribute to polarity within the cell.

B. REGULATION OF GENE ACTION

In whatever ways the activity of the nucleus changes, this must ultimately be by the repression or derepression of the activity of the genes. Histones have been the obvious candidates as the actual gene repressors. Huang and Sonner (1962) first showed that histones could act as inhibitors of DNA-primed synthesis of RNA. It was later shown that deoxyribonucleoprotein (DNP) isolated from dormant potato buds was much less effective in supporting RNA synthesis than DNP from buds released from dormancy (Tuan and Sonner, 1964). These and similar experiments were criticized on several scores. It was pointed out that the reconstituted DNP was likely to be inactive simply because of the electrostatic aggregation of the DNA with a polycation. This is probably why histones can inhibit the growth of plant tissues (Kaufmann and Das, 1955; Fellenberg, 1965; Fellenberg and Bopp, 1966; McLaren and Bradfute, 1966). The observation that the recombination of histone with DNA in reconstituted DNP was nonspecific confirmed this view (Barr and Butler, 1963). Another criticism was that although DNP was a poor primer for RNA synthesis its priming ability could be increased when it was broken into small fragments by sonication without altering the ratio of histone to DNA. The lesser ability of DNP than DNA to act as a primer or RNA synthesis could therefore be due to the precipitation and aggregation of the DNA when the histone was added (Sonneberg and Zubay, 1965). A difficulty with all these experiments was that they concerned only
quantitative effects of histone on the priming ability of DNA. If the histones really are the agents of genetic repression then it needs to be demonstrated that in native DNP some genes are repressed and others are not and that the genes which are repressed are specific to a particular type of cell or tissue. This problem was anticipated by Bonner et al. (1963) who got some evidence to show that the DNP from pea cotyledons could direct the synthesis of globulin but that the DNP from buds and roots (in which globulin does not occur in any quantity) could not, although the DNA from all sources was effective. Recently experiments have been carried out which have vindicated the idea that specific stretches of the DNA are free of histone in native DNP. Paul and Gilmour (1966) used sonicated and unsonicated DNP from calf thymus as templates for the synthesis of RNA. They then took the RNA which was synthesized and annealed it with calf thymus DNA. The RNA could anneal with only 5–10% of the sites on the DNA showing that in the DNP it was specific sites on the DNA which were not complexed with histone. Had the histone been removed unspecifically from the DNP, the RNA which was synthesized on the DNP would have been expected to correspond to all the sites on the DNA, since different parts of the DNP would have had different stretches of DNA exposed. Similar results have also been obtained by Georgiev et al. (1966). It is important to note that these experiments demonstrate that histone complexed with DNA reduces the ability of the DNA to prime RNA synthesis but they do not demonstrate that the histone complexes in a specific way with the DNA. What they do show is that the removal of histone from DNP may be specific.

If the histones are the gene repressors and act specifically, one would expect that there ought to be as many species of histones as there are genes to be repressed. This is almost certainly not the case, for whereas there are probably many thousands of genes in an organism, it is doubtful if there will prove to be, at the most, more than a hundred or so histones. Indeed in bacteria, for which the concepts of gene repression and activation were formulated, almost all the DNA is free of histone (Wilkins and Zubay, 1959; Leaver and Cruft, 1966). Also, in the ctenophores (simple, multicellular differentiated animals) more than 99% of the DNA is free of histone (Cruft, 1966). It has been suggested that the specificity of the histones would be increased if they were combined with molecules which did show specificity such as RNA. Huang and Bonner (1965) have been able to isolate from pea buds a complex of histone and an RNA characterized by a high dihydrouridylic content. A similar RNA-histone complex has been isolated from mammalian cells (Benjamin et al., 1966) but its existence in mouse cells is denied (Commerford and Delihas, 1966).

The evidence which we have so far does not allow us to say with certainty what the role of the histones is. It may be purely structural, it may be as the
pressor of gene activity. Some histones may perform one function, some
be other. Whatever it is which does repress the genes it must ultimately be
governed by substances from elsewhere in the cell. The nucleus by its pro-
ucts regulates the activity of the cytoplasm but in turn the cytoplasm
regulates the activity of the nucleus. Nuclear transplantation experiments
ith amphibia have shown that the synthetic activity of the nucleus depends
on the cytoplasmic \textit{milieu} in which it finds itself (Gurdon, 1966). Whether or
ot the nucleus synthesizes DNA and RNA depends on the developmental
age not of the nucleus but of the cytoplasm which surrounds it (Gurdon
nd Brown, 1965; Graham et al., 1966).

In plants the activity of the nucleus is presumably affected by the growth
stances which are produced in the apices of the axis. When kinetin is
plied to onion roots, within less than 30 minutes there is a very rapid and
arked increase of RNA in the cells (Jensen et al., 1964). The RNA content
f the nuclei, especially the chromatin, is very much increased (Guttman,
57) and it is probably due to an increased rate of synthesis of RNA in the
ucleus (Olszewska, 1959). We may anticipate that work now in progress in
veral laboratories will point to a direct effect of growth substances on the
ate of RNA synthesis by the nucleus and we may hope that this will provide
pointer to the mechanism of the regulation of gene action.

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2. STRUCTURE, FUNCTION, DEVELOPMENT OF THE NUCLEUS


2. STRUCTURE, FUNCTION, DEVELOPMENT OF THE NUCLEUS


CHAPTER 3

The Structure and Possible Function of the Vacuole

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I. Structure and Origin

II. Ion Transport

III. Function

I. Structure and Origin

Vacuoles are an obvious and characteristic feature of plant cells. In the older, pre-electron microscope literature the size of vacuoles, their distribution among various types of cells and various types of plants, the nature of the contents and inclusions and the pH etc. have been described and discussed, although most of the discussion of function has been and still is almost purely speculation. Some further observations have been made in recent years (Voeller, 1964; de Robertis et al., 1965); these have added something, although not a very great deal, to our total knowledge.

All cells contain what might be called a vacuolar system represented by the endoplasmic reticulum, the Golgi complex and the nuclear envelope. In plant cells this vacuolar system has added to it the plant vacuole(s) to which we usually reserve the name vacuole. All these vacuoles, vesicles, cisternae and so on, seem to be essentially "aqueous inclusions" in the cytoplasm bounded by phospholipid membranes. On the whole, as judged by the evidence gained from electron microscope pictures, these phospholipid membranes and the plasmalemma, are very similar so-called unit membranes. It is true that some of them may appear fractionally wider than others and some may be "rough" and others "smooth". It may be, too, that some are of the Danielli type of bimolecular layer of lipid, while in others the membrane may be made up of subunits, micelles of lipid coated with protein. However, at present, we would say that they are all basically similar phospholipid membranes. The special name of tonoplast is given to the membrane bounding the large central vacuole of mature plant cells.

There has, of course, been some speculation as to the origin of the characteristic vacuoles of plant cells. Some think of them as originating by be
ACRYLAMIDE GEL ELECTROPHORESIS OF SOLUBLE PLANT PROTEINS: A STUDY ON PEA SEEDLINGS IN RELATION TO DEVELOPMENT

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Laboratory for Cell Physiology, Growth and Development, Cornell University, Ithaca, New York

ABSTRACT
The application of the technique of acrylamide gel electrophoresis to the separation of components of the soluble proteins of plants is described. The reproducibility of the results is demonstrated with reference to Neurospora proteins, and suitable procedures to document the results are described. These techniques have been applied to the soluble protein complement of segments along the axis of the root of Pisum sativum and of other parts of the seedling. The observations are interpreted in the light of current views on protein synthesis and cellular development.

The growing root is a most convenient experimental object for the study of the development of cells from their meristematic to mature state. Successive transverse segments, in a basipetal sequence from the root tip, comprise a series of cell populations which represent, in space, a developmental series in time. Cells pass from the meristematic undifferentiated state near the root tip to the mature differentiated state in the more basal parts of the root. As the cells mature in this way, their enzyme patterns change, and this has been ascribed to changes in the relative amounts of the various proteins rather than to the presence and absence of stimulators and inhibitors of enzyme action (Robinson and Brown, 1952). It was postulated that cell growth and development is characterized by a succession of metabolic states, which are the result of a succession of enzymic states (Brown and Robinson, 1955).

Observations of a change in the protein complement of cells as they developed would support this view of the nature of cellular development. Although electrophoresis is perhaps potentially the most useful tool for observing a spectrum of proteins, the electrophoretic techniques available until recently have not given adequate separation of the protein fractions of plants. However, by the use of electrophoresis using polyacrylamide gel as the supporting medium (disc electrophoresis) (Ornstein and Davis, 1961), more successful separations of the soluble proteins of Neurospora (Chang, Srb, and Steward, 1962), cultured carrot tissue (Steward and Chang, 1963) and other plant materials have been achieved (unpublished work of the Cornell laboratory). The observations now to be reported were made on protein preparations obtained from successive segments of pea roots and from other organs of the seedling. They show that there are graded differences in the protein pattern between the meristematic and mature cells of the root, and between the various organs of the seedling.

EXPERIMENTAL MATERIALS AND METHODS—
Growth of material and preparation of extracts—Surface-sterilized peas (Pisum sativum var. 'W. R. Alaska') were germinated in Vermiculite for about 3 days. The roots were by this time about 3-4 cm long and the epicotyls about 1 cm long. After washing the roots, successive millimeter segments were cut from the terminal 6 mm of several hundred roots (successive segments are numbered 1-6 in Fig. 1). The rest of the root (sample 7 in Fig. 1) was severed just below the hypocotyl. The epicotyl (sample 8 in Fig. 1) and plumule (sample 9 in Fig. 1) were cut from each other at the plumular hook. The testa was removed and pieces of cotyledon were taken (sample 10 in Fig. 1).

These samples of pea seedlings were homogenized in tris-glycine buffer (0.1 M; pH 8.3) in the cold and the resulting homogenates were centrifuged at 3,000 × g for 2 hr at 0 C. The resulting supernatants were then dialyzed overnight at 0 C against the tris-glycine buffer and concentrated by evaporation from dialysis bags at room temperature. Evaporation was continued in a stream of air until the concentration of protein in the various samples was about equal.

The samples were then dialyzed again and their protein content determined by Lowry's Folin test (Lowry et al., 1951). The volume of each sample necessary to give approximately 300 μg of protein for electrophoresis was calculated.

Preparation of the gel and electrophoresis
(1) The stock solutions—The stock solutions were prepared as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>30.0 g</td>
</tr>
<tr>
<td>Crystallized N,N'-methylened bisacrylamide (Bis)</td>
<td>0.8 g</td>
</tr>
<tr>
<td>Deionized water to</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

1 Received for publication June 8, 1964.
2 This collaboration resulted from a visit of Dr. R. F. Lyndon, of the Department of Botany, University of Edinburgh, Edinburgh, Scotland, to the Cornell Laboratory.
3 The acrylamide monomers were obtained from American Cyanamid Company, Wayne, New Jersey.
The resultant solution was filtered through Fisher Qualitative Filter Paper No. 9–795.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Components</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>b.</td>
<td>1 N Hydrochloric acid Tris (hydroxyethylamino)methane (Tris)</td>
<td></td>
<td>24.0 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.85 g</td>
<td>Deionized water</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 ml</td>
</tr>
<tr>
<td>c.</td>
<td>Riboflavin</td>
<td></td>
<td>0.32 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 ppm</td>
</tr>
<tr>
<td>d.</td>
<td>1 N Hydrochloric acid Tris</td>
<td></td>
<td>48.0 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.98 g</td>
<td>Deionized water</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 ml</td>
</tr>
<tr>
<td>e.</td>
<td>Acrylamide</td>
<td></td>
<td>10.5 g</td>
</tr>
<tr>
<td></td>
<td>Bis</td>
<td></td>
<td>2.5 g</td>
</tr>
<tr>
<td></td>
<td>Deionized water</td>
<td></td>
<td>100 ml</td>
</tr>
<tr>
<td>f.</td>
<td>Riboflavin</td>
<td></td>
<td>40 ppm</td>
</tr>
</tbody>
</table>

Stock solutions were stored in brown bottles in the cold for several months without appreciable deterioration.

(2) The working solutions—The working solutions were prepared as follows:

For the lower gel: 1 part a 1 part b 2 parts c

The lower gel solution was freshly prepared immediately prior to use. Solutions a and b were mixed and after bringing them to room temperature they were "de-gassed" using an aspirator. Solution c was added to, and well mixed with, the de-aerated solution.

For the upper gel: 1 part d 2 parts e 1 part f 4 parts deionized water

Solutions d, e, and f were mixed and stored in the cold and the water added immediately before use. The resultant solution was "de-gassed" using an aspirator.

For the sample gel: This was prepared in the same way as the upper gel except that the protein solution was substituted for the water and "de-gassing" was omitted.

(3) Polymerization of the gel—Twelve open-ended glass tubes (5 mm ID X 63 mm long) were stoppered at their bases and placed upright in holes bored in a polyethylene block. The lower gel solution was pipetted into each tube to a depth of 45 mm, taking care to avoid the trapping of air bubbles. Five mm of deionized water was placed on top of the gel solution using a medicine dropper fitted with a fine thread. The tip of the thread was placed just above the surface of the gel solution and water was added slowly so as to avoid mixing. A sharp refractive boundary was visible between the gel solution and water. This step was found to be critical in obtaining high resolution. A second polyethylene block, also

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*Fig. 1. Diagram of 3-day-old pea seedling to show regions from which soluble proteins were extracted and separated by acrylamide gel electrophoresis (see Fig. 2 [1–10]).*
drilled with holes to correspond to the tubes, was fitted over the tubes and the whole placed between 2 fluorescent lamps and left for 15 min. The purpose of the polyethylene polymerization rack was to act as a light diffuser so that each tube was evenly illuminated, and so that the gels would polymerize evenly, both within a given gel and in comparison with each other.

After 15 min of light polymerization, the water-layer was shaken from the top of each tube and the last drops carefully removed with strips of filter paper. The upper gel solution was now added to each tube to a depth of 7-8 mm and again a 5-mm layer of water was added. After a further 30 min in front of the fluorescent lamps, the water layer was shaken off and the inside of the tubes dried with filter paper. The gel containing the sample was polymerized in a similar manner, except that the protein solution was substituted for the water in the upper gel solution and no additional layer of water was used.

4) The electrophoresis—The apparatus used for electrophoresis consisted of 2 polyethylene dishes (9 cm deep X 16.5 cm diam.) placed one above the other; these acted as containers for the buffer solutions. A platinum electrode was placed vertically in the center of each container. Around the base of the upper container, 12 holes were cut equidistant from the electrode. Each hole was fitted with a gasket made from 2 cm of tygon tubing; this permitted a watertight seal when the tubes containing the gels were inserted. Since the power supply delivered 600-1,000 v, the upper vessel was fitted with a micro-switch which disconnected the circuit whenever the lid was removed.

After the gels containing the samples had been polymerized, the stopper from the base of each tube was removed in such a way that suction did not displace the gel from the wall of the tube. Air bubbles trapped at the bases of the gels were removed and the resultant spaces filled with tris-glycine buffer (0.1 M; pH 8.3). The upper ends of the tubes were then inserted into the tygon fittings of the upper electrode vessel. Tris-glycine buffer (0.1 M; pH 8.3) was pipetted into each tube until the level reached the top of the tygon fittings. This was done with care to avoid disturbing the solution containing the sample in the event that it had not completely polymerized. The upper and lower electrode vessels were each filled with 1 liter of chilled tris-glycine buffer (0.1 M; pH 8.3), a few drops of bromophenol blue were added to the upper buffer solution and the positive terminal of the power supply (an Electrophoresis Constant Rate Source, Model 1,400, Canaco, Bethesda, Md.) was connected to the electrode in the lower container. The upper electrode vessel was so placed that the lower ends of the sample tubes were just immersed in the buffer solution in the lower vessel. The negative terminal of the power supply was connected to the electrode in the upper container which was then covered by a tightly fitting lid. The current was regulated to 4 ma/tube, thus giving the appropriate current density, and electrophoresis lasted approximately 35 min, or until the tracking dye had moved approximately 3 cm into the lower gel.

5) Staining and destaining the gels—At the end of the electrophoresis the gel columns were loosened from their tubes by gently “rimming” them at their lower ends with a blunted BD-22 long hypodermic needle through which water was flowing. This operation is critical, as scarring the gels must be avoided since any marks affect the densitometric traces which are to be made later. The gels were fixed and stained immediately on their removal from the tubes, using 0.7% (w/v) amido black in 7% (v/v) acetic acid for not less than 1 hr. After staining, as much as possible of the excess dye was removed from the surface of the gels by gently blotting them with filter paper.

The destaining of the gels was achieved electrolytically in an apparatus which consisted of a rectangular electrode compartment (12 cm X 32 cm) in which the gels were held vertically in a rack between 2 platinum electrodes. A current of 160 ma removed excess dye from the gel columns to the anode. The destaining solution of 7% (v/v) acetic acid was circulated by means of a pump which passed the stained solution through a bottle packed with layers of glass beads, asbestos wool and charcoal; the cleaned destaining solution then returned to the electrode compartment and was re-circulated. By use of this system the time necessary for destaining was reduced to approximately 3 hr. When part of the gel which contained no protein was completely clear of dye, the gels were removed and placed in glass tubes in 7% (v/v) acetic acid for permanent storage, photography and densitometric analysis.

6) The recording of the results—The gels were analyzed densitometrically using a Model E microdensitometer (Canaco, Bethesda, Md.). It was found that irregularities in, or on the gel, such as dust and scratches on the surface of the gel, gave rise to considerable “noise” on the traces but that the insertion of a ground-glass diffuser in front of the tungsten light source, and of a Wratten G Filter No. 15 series IV in the provided filter holder, eliminated or minimized this “noise” without any appreciable loss in the sensitivity of the densitometer.

During the electrophoresis, it was difficult to arrange that each sample ran to exactly the same length, hence, the chart speed of the densitometer was so adjusted that the trace obtained for each gel was of a standard length (30 cm) and was thus directly comparable with any other. The peaks on each trace which corresponded to visible bands in the appropriate gel were marked with arrows.

The gels were then photographed against a diffuse white background, illuminated from the
rear, using a lens of long focal length (the distance from the lens to a group of gels being about 4 ft). Proceeding in this way, each band appears as a single line on the photographs (see Fig. 2, 4). Prints made from the negatives were enlarged to the standard length which corresponded exactly to that of the densitometer traces. Therefore, when the corresponding trace and photograph were in juxtaposition, each visible band on the photograph could be related to a peak on the trace.

Even so, some of the very fine and faint bands in the gels did not show well on either the densitometric traces or photographs. Therefore, a diagrammatic interpretation (Fig. 2) was made

Fig. 2. The electrophoretic separations on acrylamide gel of the soluble proteins of 3-day-old pea seedlings extracted by tris-glycine buffer (0.1 M, pH 8.3): Root tip (1), successive mm segments of the roots (2–6), hypocotyl (7), epicotyl (8), plumule (9) and cotyledons (10). A is a densitometric trace of the gel; B is a photograph of the gel; and C is a diagrammatic interpretation of the gel.
for each gel, taking account of all 3 types of observation, i.e., the visual, the photographic and the densitometric. These diagrams were also made the same size as the traces and photographs so that they could be mounted side by side when it is evident that the interpretative diagrams accurately summarize all the observations made on the gels. Finally, the diagrammatic interpretations (Fig. 3) obtained from all the gels in a given series could be again mounted side by side and the protein constituents of the various samples directly compared. If the visible band had a "beard" to the fore and a "tail" to the rear, only the dense portion of it was shown on the diagram. Three shades of stippling were used to indicate dense, less dense and weak bands. However, it should be stressed that it was only upon the cumulative evidence of visual examinations,
Fig. 2 (continued)
photographs and densitometric traces that the diagrammatic interpretations were made. A relative scale (movement of the front = 100) has been added to the diagrams so that individual bands may be referred to by numbers which represent their movement relative to the front; these numbers are therefore comparable to the Rf values commonly used in chromatography (Chang et al., 1962).

RESULTS—From Fig. 3 it can be seen that apart from the cotyledons, all the samples had 2 strong bands (Rf values 28 and 45, respectively)

Fig. 3. Diagrammatic interpretations of the electrophoretic separations on acrylamide gel of the soluble proteins of 3-day-old pea seedlings extracted by tris-glycine buffer (0.1 M, pH 8.3): Successive mm sections from the terminal part of the root (1–6), hypocotyl (7), epicotyl (8), plumule (9) and cotyledons (10).
as well as the mobile material which constituted the front. In addition, there were other bands which varied in number, position and relative intensity depending on the sample from which they came. Particular bands appeared in different organs (e.g., bands at Rf 15 in samples 6 and 8) or in non-adjacent root segments (e.g. bands at Rf 38 in samples 4 and 6). The possibility is still open, however, that bands which have the same Rf values may contain different proteins and that the technique may not be sufficiently sensitive to resolve them. However, where bands appear consistently in each sample or in serially successive root segments, it seems to be more probable that they contain the same or similar proteins.

The protein patterns of the epicotyl (sample 8) and plumule (sample 9) appear to be distinctive and, although the general pattern of the epicotyl is similar to that of the base of the root (sample 7), there are marked differences in the number and intensity of the bands observed. The cotyledons are, however, quite different from any other part of the seedling for, as one might expect of a storage tissue, they show few but intensely stained bands which presumably represent storage proteins.

Figure 3 suggests that there is a decrease in the number of protein bands from the meristematic region of the root (16 bands in sample 1) to the enlarging region (10–13 bands in samples 2–6) and to the region of mature cells at the base of the root (9 bands in sample 7).

The successive individual root segments, though relatively small, nevertheless covered a substantial developmental range. It is not surprising, therefore, that a given type of protein or assemblage of proteins may appear in one segment, increase in intensity in the next, and then seem to disappear in the subsequent segments. This could be due in part to a change in their concentration as well as to the appearance, or disappearance, of a given protein or group of proteins. As an example, reference may be made to the band having an Rf value of 91 which is absent in samples 1 and 2, which varied in intensity in samples 3–5 and was absent from sample 6.

Some protein bands were remarkably constant from sample to sample, e.g., bands with Rf values of 28 and 45. Bands which appear in certain other parts, e.g., Rf values 54–59 and 65–70 in samples 2–6, show orderly behavior. For example, in samples 2–6, one set of bands seemed to be displaced first toward the origin and then away again in subsequent samples while another set was displaced first away from the origin and then towards it. On the evidence available, one cannot say whether these bands in such close proximity represent different protein moieties or whether they are very closely related substances which are subject to only very minor changes from sample to sample. However, the contrast between the constancy of bands at Rf 28 and 45 and the orderly behavior of those referred to will merit further attention.

**DISCUSSION**—Although the acrylamide gel electrophoresis technique is relatively new, it has been shown to be both sensitive and reproducible when applied to plant material (Fig. 4, cf. Steward and Barber, 1964).

The reproducibility of the acrylamide gel technique is best demonstrated with reference to *Neurospora* because it is easier with this organism to eliminate variations otherwise due to effects of genetic constitution, conditions of culture and the stage of development of the material.

The data in this paper were assembled by the use of amido black as a general, convenient and arbitrarily selected protein stain. Differences in the patterns so revealed therefore reflect changes in the proteins in question. However, it is also known that different protein stains (e.g., those now being used to detect enzymes on the gels) would often have produced different band patterns; thus, any one of these stains might have been used to detect and trace the changes in protein that occur during development.

The use of a large number of seedlings and many successive segments along the axes of roots reduced sampling errors in this investigation to a minimum. This being so, any observed differences in the Rf values of the protein bands and in their relative intensities should reflect differences in the properties of the extracted soluble proteins. The gradations of proteins along the root axis should be more meaningful since all the comparisons were made and the conclusions drawn...
from observations made on the same roots. The fact that some bands (especially the two at Rf values 28 and 45, respectively) occur in all, or almost all, the samples examined suggests that differences, where they occur, are also real.

The interpretation of the results is, therefore, that different proteins are characteristic not only of different organs of the pea seedling, i.e., root and shoot, but also of different stages of development within the same organ; this is shown by the behavior of serial segments cut from the roots. Superimposed upon a pattern which consists of certain bands that appear to be typical of the pea seedling (notably 2 bands with Rf values of 28 and 45 which occurred in all the samples with the exception of the cotyledons), there are others which vary from organ to organ and root segment to root segment. Though both of the conspicuous bands (at Rf 28 and 45) appear to be single, each may well consist of a group of proteins all of which had the same mobility under the electrophoretic conditions used. To resolve such proteins it would be necessary to elute them from the gel and to submit them again to electrophoresis on another gel of different pore-size or pH. It is recognized that the number of distinctive bands shown in these gels is small relative to the potential number of soluble proteins which the tissue contained. If all the cells required a given complement of enzymes to function, they might be expected to appear in parts of the protein "spectrum" which are common to all samples. Later work may well show that many of the common enzymes do in fact occur in such regions, but, conversely, however, the proteins of the main complement of enzymes which are present in every cell may occur in such low concentrations that they are not detected by these methods. This being so, the proteins which occur consistently in all samples may merely be storage or non-metabolic proteins, while those proteins which occur only in specific organs or locations may be those that give the particular tissue its distinctive characteristics. In short, it is hardly to be expected that morphogenesis will be determined by the presence or absence of an enzyme which is absolutely essential for the most basic reactions of life. Further elucidation of these points must await the use of specific enzyme tests on the unstained gels or the elution of each protein band to determine its enzymological or chemical characteristics. From our later work on Neurospora proteins, it is clear that some single bands as revealed by amido black show activity in more than one enzyme test.

Robinson and Brown (1952) postulated a change in the protein complement of cells as they developed. This conclusion was based on the changing activities of several enzymes relative to each other, in samples containing progressively more mature cells of the bean root. They were able to show that certain enzymes (e.g., phosphatase) showed reasonably constant activity along the axis of the root whereas others (e.g., invertase and a dipeptidase) showed peaks of activity at different distances from the root tip. Thus those enzymes which, according to Robinson and Brown (1952), showed constant activity along the root axis may be homologized with those bands which occurred in each of the pea root samples. By contrast, the enzymes which, according to Robinson and Brown (1952), were more localized could correspond to those protein bands on the gels which appeared only in particular pea root segments. When such bands first appeared they tended to be faint but then increased in intensity and later disappeared from the subsequent segments (cf. band at Rf 91 in samples 3-5 in Fig. 3). Wright (1963) showed that the changes in the amounts of specific proteins during the development of the oat coleoptile could also be measured quantitatively by antigenic techniques and also that certain proteins were characteristic of different organs of the oat seedling. In fact, the changes in the amounts of protein comprising the various bands (Fig. 2) are of the same general order of magnitude as those observed by Wright (1963) for specific proteins and by Robinson and Brown (1952) for enzyme activities. Therefore, the results obtained from the electrophoresis of the soluble proteins of the pea seedling provide further evidence that the protein complement of cells may change as they develop.

Changes in the protein complement of cells during their development may imply differences in the rates of synthesis of their individual component proteins. Insofar as these syntheses of proteins depend upon the DNA of the nucleus, in the way postulated by Jacob and Monod (1961), the acquisition of a particular protein complement by a cell should be associated with the formation of specific "messenger" RNAs. An RNA fraction which may fulfill the requirements of a "messenger" RNA has been claimed by Loening (1962, 1965) to occur in pea seedling roots. However, the task of demonstrating a range of "messenger" RNA species to correspond to the observed protein species is hardly yet begun. The implications for cellular differentiation of the now standard views of protein synthesis have been discussed by Brown (1963).

The data, therefore, show that the different parts of the pea seedling all contain different protein complements in spite of the fact that all must be presumed to have the same genetic constitution and therefore the same DNA encoded information in their chromosomes. If, therefore, all cells contain the information necessary for the manufacture of all the different types of proteins found in the pea plant, what is it that controls the expression of this information? How does gene activity result in one protein complement in one part of the plant and an entirely different complement in another part? What is it that controls the gene activity so that the same organ can exhibit different protein
complements at different developmental stages? These are salient problems to be solved.

Huang and Bonner (1962) have shown that removal of histone from pea embryo chromatin may result in a 5-fold increase in the rate of chromosomal RNA synthesis. Also Bonner, Huang, and Gilden (1963) claimed that the removal of histone from pea bud chromatin, in which the genes for globulin synthesis are repressed, yields DNA which will support globulin synthesis. Approximately 80% of the DNA of pea embryo chromatin seems to be bound in the form of nucleohistone, which is inactive in supporting DNA-dependent RNA synthesis, while the remaining 20% of the DNA not being complexed with histone is active in supporting DNA-dependent RNA synthesis (Bonner and Huang, 1963).

This work suggests that the complexing of DNA with histone may turn the genes "on or off," and also that there are degrees of regulatory gene control caused by the amount of these complexes which are formed. If, however, the histone component of chromosomes is one agent of genetic repression, there still remains the question of what factors motivate the behavior of the histone causing it to complex with DNA to such different degrees in different organs of the same plant or of the same organ at different developmental stages.

The technique of gel electrophoresis can at least describe the protein complement, show how it changes during development and morphogenesis from organ to organ and thus present the facts which require explanation.

LITERATURE CITED


Changes in Volume and Cell Number in the Different Regions of the Shoot Apex of Pisum during a Single Plastochron

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Changes in Volume and Cell Number in the Different Regions of the Shoot Apex of *Pisum* during a Single Plastochron

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Abstract

The length of the ninth plastochron in shoot apices of *Pisum sativum* was measured and found to be almost 46 h. This single plastochron was divided into 11 morphologically recognizable stages and the time taken to reach each stage was measured. The cell number and cell volume of five regions of the apex was measured at each stage of the plastochron. Although the apex as a whole grew exponentially, growth during the first 30 h of the plastochron was predominantly in the primordium and the adjacent tissues, whereas in the last 16 h growth was mainly in the apical dome. Since the mean cell volume remained constant, different rates of growth were due to different rates of cell division. The data suggested that the apex probably grows by the formation of growth centres on alternate sides of the apex, the beginning of each new growth centre being apparent as an increased rate of growth in the apical dome 16 h before the beginning of the next plastochron. The inception of a new primordium may therefore precede its appearance as a bump by about 16 h, and precede the first periclinal division in the tunica by 26 h. A central zone of larger cells with lightly-staining nuclei was found at the extreme apex. This central zone became reduced in size or disappeared at the time at which a new primordium was about to become visible.

Introduction

It is characteristic of the meristem of the shoot apex that leaf primordia are initiated and formed by a process which occurs repeatedly and regularly, in the same orderly manner, so that successive primordia arise in predictable positions on the apical dome. Although considerable work, both anatomical and histological, has been done, little is yet known of the cellular events in the apex which must be a necessary preliminary to the formation of a primordium. Once it has been formed the primordium grows much faster than the axis (Sunderland and Brown, 1956) although its rate of respiration is lower (Sunderland, Heyes, and Brown, 1957). This work of Brown and his colleagues was concerned primarily with the changes in the growth pattern and metabolism of the shoot apex in successive plastochrons. The present paper is a sequel to this and concerns the changes which occur during a single plastochron.

To begin to understand why a primordium is formed in the first place, one must consider what is happening in the apex during the course of a single plastochron. The changes in the shoot apex which are a necessary prelude to
lymphinit presumably occur before any anatomical change is visible. Even at the anatomical level, however, the pattern of cell division during the plastochron has never been fully described and the first quantitative study of changes in cell number has been made only recently (Denne, 1966). The significance of any histochemical changes which may be found can be realized only if one first understands the characteristics of the growth of the apex, and the way in which its changing form is a result of differential rates of cell growth and division. As a preliminary to an investigation of the cellular metabolic changes which occur in the shoot apex it is therefore necessary to describe growth in anatomical terms. One needs to know how cell numbers change in different parts of the apex; the place of origin of these cells; the relative rates of cell division and cell expansion throughout the apex; and the planes of cell division and growth.

The present paper is concerned with the changes in cell number and volume which occur in the different parts of the shoot apex during the course of a single plastochron, between the initiation of one leaf primordium and the next.

**Methods**

The plant chosen for this work was *Pisum sativum* (cultivar Lincoln), for it possesses a number of desirable properties as experimental material. The plants show strict apical dominance, with no branching; and the first flower to develop is usually that in the axil of the seventeenth or eighteenth leaf. The leaf arrangement is distichous, so that the position and plane of new primordia can be accurately predicted; this also facilitates the orientation of the material for sectioning. Nearly all plants are at the same stage, with five primordia, in the seed. Subsequent growth is regular in that plants of the same plastochron age are closely comparable and the youngest primordium always grows out to a length of 60 µ before the next primordium appears. Finally the pea shoot apex is apparently amenable to experimental manipulation (Gulline and Walker, 1957) and data on the cellular composition, growth, and metabolism of the pea are available (see, for example, Heyes and Brown, 1965).

Observations are restricted to the ninth plastochron. Any variation which there might be from one plastochron to another is thereby eliminated. The ninth plastochron is typical of subsequent plastochrons in being of the same length as these, about 2 days (Fig. 1). The ninth primordium is the fourth new primordium to be formed as the plants grow after germination.

**Preparation of the Apices**

Seeds were sown in wet sand and grown in a controlled environment chamber at a constant temperature of 22.5° C. The plants were kept in a 24-h cycle of 12 h light, 12 h dark. The light intensity was 850 f.c. Relative humidity was 60 per cent. Eight to ten days after sowing (according to the plastochron stage required) the plants were taken and the terminal centimetre or so of the stem was cut off. The first and second leaves (discounting the
two epicotylary leaves) and their stipules were removed. The third leaf was also removed but its stipules left on the stem to facilitate orientation later. These stem tips were fixed for 1 h in ethanol:acetic acid (3:1), then dehydrated in ethanol and embedded in paraffin wax. Serial longitudinal sections were cut in the plane of the primordia, which was the same plane as that of the stipules by which the material was orientated. Sections were cut 10 μ thick and were stained with Feulgen by the method of McLeish and Sunderland (1961). A comparison of the dimensions of living apices, measured under the dissecting microscope, and of the stained sections showed that shrinkage of the latter was negligible.

**Division of the Apex into Regions**

The apex was divided into five regions (Fig. 5 (a)). The limit of the apex is defined as the line drawn between the axils of the seventh and eighth primordia (a–b in Fig. 5 (a)). The ninth primordium is defined as the tissue external to the line drawn from the axil of the seventh leaf to the point of inflection of the curve of the apical dome (b–c in Fig. 5 (a)). The line a–c represents the limit of the apex in the tenth plastochron so that at the end of the ninth plastochron the tissue bounded by the line a–b–c, here called the axis, plus the ninth primordium is the net amount of tissue which is formed during the ninth plastochron. The central zone is the area at the apex of the dome in which the boundaries of the individual nuclei are indistinct because they are large and overlap in these sections although it is still possible to
estimate the numbers of nuclei in this region. The parts of the apical dome where the tenth primordium ($I_1$) and the eleventh primordium ($I_2$) will be formed are separated by a line perpendicularly bisecting the line $a-c$. Growth of the axillary bud does not begin until the primordium is four plastochrons old. The region where the axillary bud will arise is therefore not demarcated. This division of the apex is admittedly arbitrary to a large extent and different ways of dividing the apex would be equally valid, for any divisions are arbitrary in what is really a continuous system. It is nevertheless useful to divide the apex into manageable parts for analysis.

**Stages of the Plastochron**

In order to analyse the growth of the different parts of the shoot apex during the course of a single plastochron one must first define distinctive morphological stages during the plastochron, then measure the duration of each stage, and finally measure the numbers of cells and their volumes in the different parts of the apex at the different stages.

1. **Morphological definition of the stages of the plastochron**

Only those apices in the ninth plastochron were used for the measurement of cell number. The apices could always be assigned to the correct plastochron since the largest intact leaf in each section was the fourth. The different stages in the development of the apex are defined in terms of the length of the ninth primordium measured perpendicular to the line $b-c$ (Fig. 5 (a)). It has been found that a primordium can only be measured as a distinctive entity when it has reached a length of $12 \mu$. When a primordium has reached this length the next oldest in the series is generally $72 \mu$ long; in a very few instances it is sometimes a little larger than this. It is therefore assumed that the increase in length of a primordium from its formation to the end of that same plastochron is $60 \mu$. In this investigation the development of the apex throughout a plastochron has been divided into 10 stages, each stage being marked by an increment of $6 \mu$ in the length of the primordium. The time of emergence of the ninth primordium is designated $9-0$. The first stage involving a growth of $6 \mu$ is designated $9-1$, the second as $9-2$ when a further increment of $6 \mu$ is achieved in the length of the ninth primordium, and so on until the stage of $10-0$ is reached, when the tenth primordium is being formed. The stage of $9-0$ is identified when the eighth primordium has reached a length of $60 \mu$, and $9-1$ when it is $66 \mu$. Thereafter further developmental stages are determined from the length of the ninth primordium itself.

2. **Durations of the stages of the plastochron**

In principle there are three ways in which the durations of the different stages of a plastochron might be measured. First, when the over-all length of the plastochron is known the durations of the stages might be determined from the relative frequencies of the different stages in a sample of plants.
Second, the apex may be exposed and observed directly. Third, the succession of stages in the plastochron may be related to the growth, with time, of a particular leaf. The first method cannot be used since it is only applicable when the formation of primordia is strictly asynchronous and for various reasons this may not be the case. The second method is unsatisfactory since exposure of the apex and observation of it may affect its growth and development.

The third method is the one adopted here. In principle the procedure involves identifying the different stages of the plastochron with particular lengths of a chosen leaf. When the rate of growth of a leaf is measured the intervals between the different stages in the development of the apex can then be calculated. This whole procedure cannot be applied to a single plant since it is impossible to observe the apex and measure the growth of the chosen leaf simultaneously. The procedure therefore has to be adapted to a sampling technique. The whole method can then only be applied if the growth-rate of the chosen leaf is constant and if the relationship between leaf length and the stage of development in the apex is also constant. Evidence is presented below showing that both these assumptions are justified.
The indicator leaf which has been chosen is the fourth from the base, i.e. the fourth oldest. The relation between length and developmental stage in the apex is then determined by simple measurement of the eighth or ninth primordium and the leaf. More than a hundred plants were dissected and on each the length of the fourth leaf from the point of insertion on the stem to the tip of the central tendril or leaflet and the length of the ninth primordium were measured. Data for the length of the fourth leaf in relation to the

![Graph showing growth in length of the fourth oldest leaf in situ. Each line describes the growth of a single leaf.](image)

Fig. 3. Growth in length of the fourth oldest leaf in situ. Each line describes the growth of a single leaf.

stages of the ninth plastochron gave a curve expressing this relationship. This curve was statistically indistinguishable \( p = 0.05 \) from similar curves showing the relationship between leaf length and the stage of the plastochron for leaves of the same developmental age but in plants of a different age (i.e. the third and fifth leaves in the eighth and tenth plastochrons respectively). These data were therefore pooled to give the curve in Fig. 2 showing the length of the \( n \)th oldest leaf in plastochron \( n+5 \). Similar curves were obtained for leaves of other developmental ages (plastochrons \( n+3 \) and \( n+4 \)). All these data were combined in Fig. 2 which shows that the relationship between leaf length and the stage of the plastochron was also the same from one plastochron to the next as the leaf aged. Since the curves for the three different plastochron ages were statistically indistinguishable from each other, all the data of Fig. 2 could be expressed as a single curve, shown as \( a \) in Fig. 4,
which expresses more accurately than could otherwise have been achieved the length of the fourth leaf as a function of the stage of the ninth plastochron which had been reached.

The growth of the fourth leaf was measured throughout a period of 3 days on a sample of 19 plants. On the eighth day after sowing one stipule of the third leaf was cut off to expose the fourth leaf to view and the first measurement of its length was made. Removal of this stipule did not affect the growth of the plants. The leaves were measured on a further twelve occasions during the ninth and tenth days of growth. The measurements were made with an ocular micrometer in a dissecting microscope, in the growth room in which the plants were growing so that there was no alteration in their environment during the measurements. The change in length of the leaf with time for the
different plants is shown in Fig. 3. The origin of the lines is in each case
different since the initial sizes of the leaves at the beginning of measurement
were different. It is clear, however, that the slope of the lines is constant and
an average rate of growth can therefore be calculated from the data. The
intervals of time taken to achieve a given increment of leaf length can then be
determined from the regression line showing the relationship between the
increase of leaf length and time (line b in Fig. 4).

The curve showing leaf length as a function of the stage of the plastochron
(line a in Fig. 4) can be superimposed on the line showing the time taken to
achieve a given increment in the length of the leaf (line b in Fig. 4) since in
both cases the increase in the length of the leaf during the plastochron is of
course the same. The increase in the length of a leaf during the course of a
plastochron is 2.53-fold (increase in log length of 0.403 in line a, Fig. 4) and
the time taken to achieve this, i.e. the length of the plastochron, is 45.7 h
(line b, Fig. 4). The intervals between the different stages of the plastochron
can then be read from Fig. 4 and are given in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Plastochron stage</th>
<th>Hours</th>
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<tr>
<td>9.0</td>
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<tr>
<td>9.1</td>
<td>4.5±10.4</td>
</tr>
<tr>
<td>9.2</td>
<td>10.0</td>
</tr>
<tr>
<td>9.3</td>
<td>18.5</td>
</tr>
<tr>
<td>9.4</td>
<td>25.0</td>
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<td>30.0</td>
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</tr>
<tr>
<td>9.8</td>
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</tr>
<tr>
<td>9.9</td>
<td>39.0</td>
</tr>
<tr>
<td>10.0</td>
<td>45.7</td>
</tr>
</tbody>
</table>

3. Cell numbers and volumes

Cell counts were made by tracing the outline of each section with the aid
of a camera lucida and on the diagram scoring those nuclei which were
judged to be whole or more than half present; smaller fragments were ignored.
All the sections comprising each apex were scored so that the total number
of nuclei, and hence cells, in each apex was obtained directly. The use of all
the sections comprising the apex is valuable for only in this way can the cell
number and volume of irregularly shaped regions be determined accurately.
It can be appreciated from Fig. 5 (b) that measurements restricted to median
sections would, for example, tend to underestimate the size of \( I_2 \) and exaggerate
the size of the central zone.

The volume of the regions of the apex was obtained by tracing the outlines
of the camera lucida drawings on to paper of uniform thickness and by
weighing the pieces of paper corresponding to each region. The weights were then easily converted into volumes ($\mu^3$) since the weight of a standard area of paper and the magnification and thickness of the sections were known. The volume of each region divided by the number of cells in it gave the mean cell volume for that region.

**Fig. 5.** Regions of the shoot apex as seen in (a) longitudinal section and (b) from above. $C$ = central zone; $P$ = primordium (9th); $I_1$ = region where the next (10th) primordium will be formed; $I_2$ = region where the next primordium but one (11th) will be formed; $A$ = axis

All measurements were made in triplicate, the values presented each being the mean of three different apices.

**Results**

**Cell Number**

The total number of cells in the whole apex increased logarithmically with time, the increase in the number of cells being almost threefold during the plastochron (Fig. 6). The number of cells in the primordium increased logarithmically with time once it had reached a size of about 100 cells (Fig. 7). The growth of the apex was due to a continuous increase in cell number.
throughout the plastochron in all regions except the central zone (Figs. 7 and 8). However, in the lower part of the apex (the axis plus the primordium) the increase in the number of cells was less in the second half than in the first half of the plastochron (Fig. 6). This was entirely due to the smaller increase in cell number in the axis in the second half of the plastochron (Fig. 7). The complementary situation was found in the apical dome, i.e. $C+I_1+I_2$,

![Graph showing changes in cell number over plastochron stages.](image)

**Fig. 6.** Changes in cell number in the whole apex (Total), the apical dome ($C+I_1+I_2$), and in the primordium plus axis ($P+A$)

in which the increase in cell number was predominantly in the second half of the plastochron (Fig. 6). This increase was entirely due to an increase in $I_1$ and $I_2$ (Fig. 8); the slightly lower values for $I_2$ reflect the fact that the central zone encroached on $I_2$ more than $I_1$. This was because the central zone was always at the vertical apex and $I_2$ as defined here is more apical than $I_1$ (Fig. 5). The increase in cell number during the plastochron was 2.8-fold in both the apical dome and in the axis plus primordium.

The net number of cells added during the course of the plastochron (see Fig. 5a) is represented by the final size of the axis plus primordium, i.e. about 1,600 cells. The new apex for the tenth plastochron corresponds to the central zone $+I_1+I_2$ of the ninth plastochron and consists of about 1,000 cells (Fig. 6). This is slightly larger than the total number of cells, about 900, in the apex at the beginning of the ninth plastochron and may indicate that the apex is slowly enlarging with age. Observation of older plants suggests that this may be so, and certainly this does occur in the lupin (Sunderland, 1961).

The central zone remained the same size, about 120 cells, throughout the plastochron except at the beginning and the end. At the time of the appearance of a new leaf, i.e. at plastochron stages 9.0 and 10.0, the central zone...
could not be distinguished in several apices and this is reflected in the low mean values for cell number in this region at stages 9·0 and 10·0 (Fig. 8). This zone had not regained its former size until stage 9·2 and began shrinking again after stage 9·8. So for about 19 h (9·0 to 9·2 and 9·8 to 10·0) the central zone was in a state of flux. The change in size of this region of course does not necessarily imply any movement of cells away from the apical region, but might be a change in state of the cells in the central zone so that they are no longer recognizable as such and therefore become temporarily included in \( I_1 \) and \( I_2 \). The number of cells in \( I_1 \) and \( I_2 \) is sufficiently large for a relatively small increase due to the inclusion of cells from the central zone at the beginning and end of the plastochron not to be noticed.

**FIG. 7.** Changes in cell number in the axis (\( A \)) and the primordium (\( P \))
Cell Volume

The mean cell volume in each region showed no regular variation throughout the plastochron. Values for changes in the volumes of the regions of the apex throughout the plastochron consequently give similar graphs as for cell numbers. Cell size did not differ significantly from one region to another, except that cells of the central zone usually seemed to be larger than in the other regions (Table 2). It seemed possible that this was an artefact due to underestimation of the numbers of nuclei, the central zone being characterized by the indistinct boundaries of its nuclei. The volumes of the cells were therefore checked by direct measurement. The mean diameters of 100 cells in each region were measured from camera lucida drawings of the cells in median longitudinal sections of apices at plastochron stages 9.1 to 9.8 which had been fixed in chromic : acetic acid : formalin and stained with Heidenhain's haematoxylin and orange G. The values obtained for cell volume by cubing the mean diameters (Table 3) agree fairly well with the values in Table 2 and show that the cells of the central zone do seem to be larger than the cells in the rest of the apical dome and in the primordium. From these direct measurements (Table 3) the cells of the axis appeared to be unusually large. This was because the measurements were made mainly on cells in the centre of the axis, which are beginning to enlarge, whereas the
### Table 2

*Mean cell volumes (μ³) in the regions of the apex throughout the plastochron*

<table>
<thead>
<tr>
<th>Region</th>
<th>9.0</th>
<th>9.1</th>
<th>9.2</th>
<th>9.3</th>
<th>9.4</th>
<th>9.5</th>
<th>9.6</th>
<th>9.7</th>
<th>9.8</th>
<th>9.9</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central zone</td>
<td>1,036</td>
<td>1,111</td>
<td>996</td>
<td>983</td>
<td>1,036</td>
<td>1,162</td>
<td>1,062</td>
<td>1,218</td>
<td>980</td>
<td>1,032</td>
<td>1,430</td>
</tr>
<tr>
<td>$I_1$</td>
<td>882</td>
<td>948</td>
<td>814</td>
<td>732</td>
<td>868</td>
<td>860</td>
<td>840</td>
<td>957</td>
<td>854</td>
<td>865</td>
<td>1,016</td>
</tr>
<tr>
<td>$I_2$</td>
<td>873</td>
<td>1,066</td>
<td>875</td>
<td>799</td>
<td>991</td>
<td>941</td>
<td>962</td>
<td>992</td>
<td>888</td>
<td>932</td>
<td>1,053</td>
</tr>
<tr>
<td>Primordium</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>Axis</td>
<td>839</td>
<td>1,042</td>
<td>833</td>
<td>795</td>
<td>827</td>
<td>840</td>
<td>862</td>
<td>918</td>
<td>869</td>
<td>839</td>
<td>1,042</td>
</tr>
</tbody>
</table>

Mean value for all apices: 1,067, 889, 954, 822, 891
values in Table 2 include the many smaller cells which make up the bulk of the axis.

**Table 3**

*Cell volume obtained by direct measurement of 100 cells in each region of the apex*

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean cell diameter (μ)</th>
<th>Mean cell volume (diameter³) (μ³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central zone</td>
<td>9.89</td>
<td>967</td>
</tr>
<tr>
<td>I₁</td>
<td>8.95</td>
<td>717</td>
</tr>
<tr>
<td>I₂</td>
<td>9.56</td>
<td>874</td>
</tr>
<tr>
<td>Primordium</td>
<td>9.21</td>
<td>781</td>
</tr>
<tr>
<td>Axis</td>
<td>9.98</td>
<td>994</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Although the shoot apex as a whole is an exponentially growing system the different parts of the apex grow at different and changing rates. Indeed, it is somewhat surprising to find that the sum of the varying increases in cell number in the different parts of the apex is the exponential increase shown by the apex as a whole. It would be curious if this were merely fortuitous. When more is known about the apex it may be found that the over-all growth-rate of the apex is determined by the supply of some factors from the older parts of the plant which are increasing exponentially. There would still remain to be explained the precise control of the growth which does occur at the apex and the factors which in turn control the exponential increase of the older parts.

The distribution of growth in the apex is far from uniform. The fact that a primordium arises at all shows that the rate of growth in this part of the apex is greater than elsewhere. The primordium, once it has attained a size of about 100 cells, grows exponentially at a rate which is considerably greater than the average for the whole apex (cf. Figs. 6 and 7). Since cell volume remains constant and does not differ much from one region to another, differences in rates of growth are in fact differences in the rates of cell division. The different rates of growth in different parts of the apex are illustrated by the different mean cell generation times for the regions. On the assumption that all the cells are dividing the mean cell generation time for the whole apex is 28 h, the time it takes for cell number to double (Fig. 6). In the axis the generation time in the first half of the plastochron is similar to this but in the second half of the plastochron it increases to about 80 h (Fig. 7). In the primordium, which it should be noted is adjacent to the axis, the generation time in the second half of the plastochron is only 12 h. These values may well be modified when it is known to what extent cells are displaced from one region to another, but it is clear that the growth-rates of these adjacent tissues may differ considerably, perhaps by as much as sevenfold. The difference in growth-rates between the primordium and I₂ in the first half of the plastochron may even be as great as twelvefold. There would
therefore seem to be steep gradients in the rates of cell division and growth within the shoot apex. The probable gradient of average rates of cell division which all the cells in the apex pass through is illustrated in Fig. 9, where the shorter the mean cell generation time the faster the rate of division.

![Diagram](image)

**Fig. 9.** Changes in the mean cell generation time (in hours) of a single cell as it changes position during the growth of the apex. The average rates of cell division are inversely proportional to these values. *P* = primordium; *A* = axis

The mean cell generation time for the apex as a whole is 28 h, and this compares with 3 days, more than twice as long, for the lupin apex (Sunderland and Brown, 1956) although the plastochron was the same, 2 days, for both species. This difference in growth-rates reflects the fact that in the pea an apical dome (central zone +I₁+I₂) of 500 cells in the middle of one plastochron gives rise to an apex of about 1,700 cells, more than three times as many, in the next whereas in the lupin, although the dome is larger—about 3,500 cells—the apex (apical unit) to which it gives rise by the middle of the next plastochron consists of 5,800 cells, an increase of only 1.7 times (Sunderland and Brown, 1956). These differences are related to the different morphology of the pea and lupin apices. In the pea the primordia are much larger in relation to the dome, in the lupin, which characteristically has a large dome with relatively small primordia.

A major feature of growth during the plastochron is the shift in emphasis from growth of the axis in the first half of the plastochron to growth of the apical dome (central zone +I₁+I₂) during the second half (Fig. 6). Since the I₁ of one plastochron becomes the primordium and axis of the next, it might be truer to say that the growth of the apical dome is greatly increased about half-way through the plastochron and that this is continued as growth of the primordium, and of the axis adjacent to the primordium, in the next. The rate of growth of the primordium is in fact very similar to the rate of growth of I₁ at the end of the previous plastochron (Figs. 7 and 8). Since the diameter of the axis remains the same throughout the plastochron we can consider growth here to be mainly in the vertical plane. By definition the axis does not grow at the surface adjacent to I₁. The growth of the axis may therefore be envisaged as such that the thickness of the wedge of tissue comprising it increases so that the height of the surface adjacent to the primordium (the distance b–c in Fig. 5 (a)) increases. In other words, the apical dome is lifted as a lid hinged at I₁ (Fig. 10). This is perhaps better shown by direct measurements of the linear dimensions of the apex (Fig. 11). These show that the height of the axis (the distance b–c in Fig. 5 (a)) increases by
about 40 μ in the first 30 h of the plastochron. Since the cells are approximately 10 μ in diameter this is equivalent to the addition of another four layers of cells. The time taken for these cells to be formed, 30 h, is the same as the mean cell generation time for the axis at this stage of its growth (plastochron $x+2.2$ in Fig. 9). The formation of these four cell layers in this part of the axis is therefore probably due to a single transverse division in each of four of the original layers of cells abutting on the primordium.

![Diagrams of shoot apex growth](image)

**Fig. 10.** Possible interpretations of the growth of the shoot apex. Double-headed arrows indicate principal directions and places of growth.

There are two ways of envisaging the growth of the apical dome in the second half of the plastochron. It could be similar to that already suggested for the axis; an increase in the surface of $I_1$ (but not of $I_2$) would result in the growth of the apical dome as a hinged lid, the hinge being at the junction of $I_2$ and the axis (Fig. 10 (a)). In this case the growth of the dome, mostly due to growth of $I_1$, in one plastochron would be continuous with the growth of the axis ($I_1$ becoming most of the axis) in the next. At first sight this interpretation may not seem possible, for the rate of growth of $I_2$ increases at the same time and to the same extent as that of $I_1$ (Fig. 8). However, this could well be because of the way $I_1$ and $I_2$ are defined rather than because of an intrinsic similarity between them. As long as the apical dome remains, as it does, symmetrical as it grows then $I_2$ must increase to some extent if $I_1$ increases, and this is shown in Fig. 10 (a). The rate of growth of $I_1$ is maintained in the next plastochron in the growth of the primordium to which it gives rise. At the same time $I_2$ becomes $I_1$ and the rate of growth at the transition from one plastochron to the next apparently falls abruptly (Fig. 8, cf. plastochron stages 10.0 and 9.0). This seems unlikely and it is thought that in fact the rapid rate of increase of $I_2$ in the second half of the plastochron is due mainly to the associated increase in $I_1$, as shown in Fig. 10 (a). What probably happens is
that the negligible increase in the number of cells in \( I_2 \) in the first 32 h of the plastochron (Figs. 8 and 9) grades into a slightly greater rate of increase of cell number in the next 16 h which in turn grades into the slow increase shown by \( I_1 \) at the beginning of the next plastochron.

![Graph](image-url)

**Fig. 11.** Dimensions (\( \mu \)) of the apex as seen in median longitudinal section; \( a \), length of the surface of the dome (surface from \( a \) to \( c \) in Fig. 5 (a)); \( b \), height of the axis adjacent to the primordium (\( b-c \) in Fig. 5 (a)); \( c \), height of the dome (maximum height measured from, and perpendicular to, \( a-c \) in Fig. 5 (a))

The increase in length of the surface of the apical dome as seen in median longitudinal section, in other words the increase in length of each layer of the tunica (the surface from \( a \) to \( c \) in Fig. 5), during the second half of the
plastochron is about 80 or 90 \( \mu \) in 16 h (Fig. 11). The mean cell generation time for the dome at this time is about 12 h (plastochron \( x+1.7 \) in Fig. 9). The increase in cell number in the tunica is therefore probably due to a single anticlinal division in each of eight or nine cells in each layer of the tunica. If these divisions occur only in \( I_1 \) then, since the surface of \( I_1 \) as seen in median section consists of about eight cells by the middle of the plastochron, the implication would be that every cell in the tunica layers of \( I_1 \) divides once, anticlinally, during the last 16 h of the plastochron. In the central part of the dome there would be a single horizontal division in each of four cell layers, to give the increase in height of the dome which amounted to about 45 \( \mu \) in the second half of the plastochron (Fig. 11). In \( I_2 \) there would be, on this interpretation, no cells dividing so that there would be a gradient from no divisions at this side of the apex, to division in four or five cell layers in the centre of the apex, to division in eight or nine cell layers in \( I_1 \).

If growth occurs in this way (Fig. 10 (a)) then growth of the apex would be essentially the result of an alternation of growth centres from one side of the apex to the other, each new growth centre being initiated in \( I_1 \) half-way through a plastochron and continuing as the growth of the primordium in the next. It seems probable that this is so, for this was also suggested by the data of Denne (1966), who provided evidence for the rates of cell division in the different regions of the shoot apex of *Trifolium*. Growth of the apex by such an alternation of growth centres would presumably be superimposed on a more general, continuous, growth throughout the apex.

The other way of envisaging the growth of the apex is to assume that the dome grows not principally in \( I_1 \) as suggested above but that cells are in fact formed at the same rate in both \( I_1 \) and \( I_2 \) (as Fig. 8 suggests) so that the dome is lifted evenly (Fig. 10 (b)). If this were so, the dividing cells on the surface of the apical dome would be evenly distributed so that there would be about four cells which each divided once during the last 16 h of the plastochron in each of the tunica layers of both \( I_1 \) and \( I_2 \). In the centre of the dome there would be, as calculated above, also division in four layers. The growth of the dome as shown in Fig. 10 (b) could then be visualized as due to a single horizontal division in each of four cell layers. The growth of the apex would therefore be by a rather general growth of the dome in the second half of a plastochron which would be continued at the beginning of the next plastochron predominantly only on that side of the apex where the axis grows and the primordium is formed. However, if growth were to occur mainly in this way then the mean cell generation time for \( I_2 \) would decrease drastically in the second half of the plastochron and would become the same as that for \( I_1 \) at this time. This would mean that in the scheme shown in Fig. 9 there would have to be interpolated at plastochron \( x+0.7 \) a value of 12 for the mean cell generation time in \( I_2 \).

To be certain of the way in which the apex grows data are needed on the rates of displacement of cells in different parts of the apex and work is in progress to provide this information. The main significance of the first, and
most probable, interpretation is that the inception of the primordium could be regarded as the point at which the growth-rate of $I_1$ begins to increase, i.e. about 16 h before the appearance of the primordium. The position of the formation of the next leaf, $I_2$, would thus be fixed by half-way through the plastochron. This is similar to what has been found for the lupin apex, in which $I_1$ is determined but $I_2$ is not (Snow and Snow, 1933). The inception of the leaf primordium would then precede considerably what is usually accepted as the first visible anatomical change in the apex, the first periclinal division in the inner layer of the tunica, for this does not occur until plastochron stage 9-2.

The growth of the apical dome may also be affected to a considerable extent by cell divisions in the central zone for, although this region does not alter in size during most of the plastochron, this could be because cells are produced there but are displaced into $I_1$ and $I_2$. Again, data on rates of cell displacement are needed to decide this. A notable feature of the plastochron is the marked reduction in size, or the disappearance, of the central zone at the end of the plastochron, coincident with the appearance of a new primordium. What the significance of this change is, it is difficult to say. More needs to be known about the central zone itself. The fact that it can be distinguished at all is because the nuclei are large in relation to cell size and stain diffusely with Feulgen. They also stain lightly with haematoxylin, unlike the other nuclei, which stain darkly. This region appears to be homologous with the central zone as described by other workers (for references see Philipson, 1949; Gifford, 1954).

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Rates of Cell Division in the Shoot Apical Meristem of *Pisum*
AND
Planes of Cell Division and Growth in the Shoot Apex of *Pisum*
BY
R. F. LYNDON

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Rates of Cell Division in the Shoot Apical Meristem of *Pisum*

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Date accepted: 29 May 1969

ABSTRACT

The relative rates of cell division in different regions of the pea shoot apical meristem were obtained by measuring the increase in the numbers of metaphases following application of colchicine to the plants. Absolute values for the rates of cell division could be calculated since the average rate of cell division for the whole apex was known. Measurements of the rates of cell division were obtained at defined intervals during the course of a single plastochron. Within each region of the apex the rate of cell division did not change more than about two-fold throughout the plastochron. There was very little or no increase in the rate of cell division associated with leaf initiation. The formation of a leaf primordium and the subsequent growth of the apical dome apparently result from changes in the direction of growth rather than changes in the rates of growth. Three main regions were discernible within the apical meristem: a region with a slow rate of cell division in the apical dome, a region of a faster rate of cell division at the base of the apical dome and at the site of initiation of procambial strands, and a region of an intermediate rate of cell division in the newly initiated leaf primordium and the adjacent part of the shoot axis.

INTRODUCTION

THE numbers of cells in the different regions of the pea shoot apex change as the apex grows during a single plastochron (Lyndon, 1968). The exponential increase in cell number in the apex as a whole was brought about primarily by an increase of cell number in the incipient primordium and its associated tissues during the first half of the plastochron and by an increase of cell number in the apical dome during the second half. Two possible interpretations were put forward of the way the apical dome grew. On the first, the inception of the primordium could be recognized as the increase in the rate of cell accumulation in I₁ 16 h before the primordium appeared as a bump. On the second the origin of the primordium coincided with its appearance as a bump. For a more precise interpretation of the growth of the apex further data are necessary. One must know the actual rates of division and the planes of growth within the apex during the plastochron.

This paper is concerned with the measurement of the rates of cell division throughout the shoot apex during the course of a single plastochron. Since the rates of increase of cell numbers are known (Lyndon, 1968) the values for the rates of division can be used to assess the degree of cell displacement from
one region of the apex to another and a more complete description of the
growth of the apex during a plastochron can be obtained.

**Methods**

The most direct way of measuring the rate of cell division is to measure the
rate of formation of new cell plates or the rate at which the cells enter a
specific part of the mitotic cycle. Such measurements do not presuppose a
knowledge of the number of cells which are dividing, and do not depend on
divisions being asynchronous. The duration of the experiments must be
sufficiently short to allow several measurements to be made during the course
of a single plastochron so that valid measurements of rates of division may
be obtained even if cells are in transition from regions where the mitotic
cycle is long to regions where it is short. The method of choice is therefore
one such as that using colchicine to bring about accumulation of metaphases,
under conditions in which the rate of entry into metaphase is not affected but
exit from metaphase is blocked.

Plants of *Pisum sativum* (cultivar Lincoln) were grown and serial sections
of the apices were prepared in the ways previously described (Lyndon, 1968).
Colchicine was applied to the apices as a 0.5 per cent aqueous solution. A
drop of the solution was placed, by means of a syringe, between the stipules
enclosing each apex. At the time of treatment the plants had grown for 8,
9, or 10 days after sowing and included plants at all stages of the ninth plasto-
chron. Colchicine was used at 0.5 per cent because lower concentrations did
not result in the complete elimination of anaphases and telophases. In the
presence of colchicine almost all metaphases were of the 'ball' type described
by Barber and Callan (1943).

Ideally the accumulation of metaphases in the presence of colchicine should
be measured while the accumulation is linear with time and is occurring at
the same rate as the entry into metaphase in an untreated plant. In a meristem
which is in a steady state and in which divisions are asynchronous the rate of
entry of cells into metaphase is equal to the rate of cell division. In the pea
shoot apex the average rate of division was found to be 2.5 per cent cells per
hour (Lyndon, 1968). When shoot apices were treated with 0.5 per cent
colchicine the rate of accumulation of metaphases was not linear and even
during the period of most rapid accumulation (4 to 6 h after the application
of the colchicine) it is doubtful whether the rate reached 2.5 per cent per hour
(Fig. 1).

For the first 2 h after application the colchicine appeared to have no effect
on the shoot apices (Fig. 1). This may have been because the colchicine had
not reached the apex, for the observation that apices in which metaphases
had accumulated were often dry when dissected out, even though the surround-
ning leaves and stipules were wetted with the colchicine solution, suggests that
the colchicine probably entered by the young leaves and stipules and was
translocated to the shoot apex. However, it is clear the colchicine inhibited
entry into metaphase after 8 h of treatment (Fig. 1). The low maximum
The accumulation of metaphases in the pea shoot apices in the presence of colchicine is therefore not an absolute measure of the rate of entry into metaphase (and hence the rate of division) in an untreated plant. It can, however, be a relative measure if the percentage inhibition of entry into metaphase due to colchicine can be shown to be the same for all cells irrespective of their rates of division in the untreated apex. In the one case in which it can be tested, a comparison of the epidermis and the rest of the apex, it can in fact be shown that the accumulation of metaphases is a true measure of the relative rates of cell division. Divisions in the epidermis are all anticlinal so that they result in an increase in the number of cells only in the epidermis. If it is assumed that the flux of epidermal cells across the boundary of the apex with the rest of the shoot is negligible then the rate of increase of cell number in the epidermis is a direct measure of the rate of cell division. The number of cells in the whole of the epidermis of the apex (defined as in Lyndon, 1968) increased from 280 cells at the beginning of the plastochron to 700 at the end. Since the increase was exponential the rate of increase was 2.0 per cent per hour. The average rate of division for all the cells of the apex was 2.5 per cent per hour (Lyndon, 1968). The ratio of the rate of
cell division in the epidermis to that in the whole apex is therefore 0.8. When apices were treated with 0.5 per cent colchicine for 8 h the accumulation of metaphases was 5.4 per cent in the epidermis and 6.5 per cent in the whole apex. The ratio of the percentage of metaphases accumulated in the presence of colchicine in the epidermis to that in the whole apex was therefore also 0.8. Thus, the accumulations of metaphases in the epidermis and the whole apex are directly proportional to the known rates of division in these regions.

The distribution of metaphases between the different regions of the apex remained constant during accumulation of metaphases in the presence of colchicine and also when accumulation was finally inhibited after 8 h. The distribution of metaphases was also the same in apices in which the rate of accumulation was lower (about 1 per cent per hour) because of the use of other samples of colchicine. This shows that the relative rates of accumulation of metaphases in different regions of the apex remain the same throughout the period of accumulation. The extent of inhibition of entry into metaphase does not therefore change differentially in the different regions of the apex during metaphase accumulation. It also follows that metaphase accumulation in all parts of the apex continues for the same time; no part of the apex continues to accumulate metaphases while another part has stopped doing so, for then the distribution would have changed. Apices treated with colchicine can therefore be taken at any time for measurement of the accumulation of metaphases. Since it is convenient to have a reasonably large number of metaphases, samples were taken 8 h after the application of the colchicine when the number of metaphases was near the maximum. Taking samples when accumulation of metaphases has virtually ceased has the added advantage that the exact time of sampling is not so critical as it is when metaphases are accumulating.

Plants were therefore treated with colchicine for 8 h and were then fixed and sectioned. The stages of the plastochron were defined and the regions of the apex were delineated in the ways previously described (Lyndon, 1968). The numbers and distribution of metaphases in each region of the apex in plants treated with colchicine and in untreated plants were scored. All the serial sections comprising each apex were used. The values presented are the means of those for three colchicine-treated apices and six untreated apices at each stage of the plastochron.

Results

For untreated apices the number of metaphases as a percentage of the total number of cells present in each region is shown for all stages of the plastochron in Fig. 2. The regions into which the apex is divided for analysis are shown in Fig. 7c. In apices which had been treated with colchicine the number of metaphases was scored as a percentage of the total number of cells in each region throughout the plastochron. From the values so obtained were subtracted the corresponding values for the untreated apices. The
resultant values, shown in Fig. 3, represent the accumulation of metaphases which occurred subsequent to the application of colchicine, and are a measure of the relative rates of accumulation of metaphases and hence the relative rates of cell division. The actual numbers of metaphases recorded in the apices treated with colchicine were the sums of the values shown in Figs 2 and 3.
Fig. 3. Increase in the number of metaphases (as percentage of total number of cells) at each stage of the plastochron in shoot apices treated for 8 h with 0.5 per cent colchicine
In the whole apex the rate of accumulation of metaphases was more or less constant throughout the whole plastochron (Fig. 3). In the central zone it was a low but positive value throughout most of the plastochron, but at the very beginning and at the very end the rate of accumulation of metaphases was increased somewhat. There was also an increase at 30 h, the significance of which is not certain. In the I₂ region the accumulation of metaphases was more or less constant at about 5 per cent for the first 35 h or so, but then increased to about 8 per cent at the end of the plastochron. The rate of accumulation of metaphases in the I₁ region was essentially constant throughout the whole plastochron. In the primordium the rate of metaphase accumulation was higher in the first half of the plastochron than towards the end of the plastochron. In the axis the rate of accumulation of metaphases was constant throughout the plastochron.

The pattern of cell division would be seen more clearly if three-dimensional models of the apex were constructed. This can be done using the present data since the position of every metaphase (potential division) in every section of each serially sectioned apex has been mapped. Three-dimensional models can then be constructed from the component sections. Alternatively, and...
Fig. 5. Rates of cell division in 40 \( \mu \) thick sections perpendicular to the plane of the primordia. The plane of (a) and (b) is illustrated in (e) and is parallel to the primordium-axis boundary. The plane of (d) and (e) is parallel to the boundary of the axis with the rest of the shoot and is illustrated in (f). (a) and (d) each represent plastochron stages 9.0 to 9.4, and (b) and (e) each represent plastochron stages 9.5 to 10.0. P = position of ninth primordium. Each point represents a colchicine metaphase. The density of points is proportional to the rate of division.

This is done here, sections of any desired thickness in any plane can be reconstructed.

The distribution of divisions in median sections of apices in the plane through the primordium is shown in Fig. 4. As the plastochron progresses
Lyndon—Rates of Cell Division in the Shoot Apex of Pisum

FIG. 6. Rates of cell division over the surface of the apical dome during plastochron stages 9·0 to 9·4 (a), and 9·5 to 10·0 (b). The diagrams represent zenithal equal area projections, centred on the boundary between I₁ and I₂. Lateral as well as median parts of the apical dome are included. Each point represents a colchicine metaphase. The density of points is proportional to the rate of division. P = position of the ninth primordium. The distribution of divisions over the surface of the apical dome was recorded in the following way. The apical dome was assumed to approximate to a hemisphere and a zenithal, equal area projection was constructed centred on the perpendicular bisector of the dome in the median section. This plan was then divided into 14 or 15 strips corresponding to the sections into which the dome had been cut by microtome, and each metaphase in the epidermis was recorded in its appropriate position on the plan of the apical dome using the data from the longitudinal sections of apices which had been treated with colchicine.

a band of faster-dividing cells appears across the base of the apical dome between the axils of the eighth and ninth primordia. It first becomes apparent at plastochron stages 9·2/9·3/9·4 (Fig. 4b) and is quite clear by the end of the plastochron. A section perpendicular to the plane of Fig. 4 through the
Lyndon—Rates of Cell Division in the Shoot Apex of Pisum

centre of the apex (Fig. 5b) shows that this zone of more frequent division is continuous with similar zones which extend from it down the sides of the axis. A transverse section of the base of the apex (Fig. 5e) shows the V-shaped nature of this zone of faster division, the arms of the V being towards the primordium. The apex at the end of the plastochron therefore consists of 3 main regions in terms of rate of division:

1. a bowl-shaped region of slower division at the tip of the apex and including the central zone and the upper parts of I₁ and I₂,
2. the primordium, the axis adjacent to it and the centre of the axis, the frequency of division in this region being intermediate, and
3. a region of faster division as a plate across the apex from the axil of the 8th to the axil of the 9th primordium with extensions down the sides of the axis, and this region separating the other two regions of slower division. In the transverse section of the base of the apex (Fig. 5e) the pattern of divisions in this region is seen to match the pattern of the procambium lower down the stem and in fact is continuous with the procambium.

This pattern of 3 regions of cell divisions can just be discerned first at plastochron stages 9-2/9-3/9-4 (Figs. 4b, 5a).

The distribution of the rates of division over the surface of the apical dome reflects the pattern of divisions within the dome (Fig. 6). In the first part of the plastochron the greater frequency of division at the surface of I₁ is a consequence of these cells being sited at the end of the band of more frequent division which reached to the axil of the eighth leaf at the end of the previous plastochron (see Fig. 4). In the second part of the plastochron the distribution of divisions over the dome becomes symmetrical. The lower frequency of division in I₂ in the first half of the plastochron reflects the lower value found then for I₂ as a whole (Fig. 3).

Discussion

The main feature of the data is the relative constancy of the rate of metaphase accumulation, and hence the rate of division, throughout the plastochron (Fig. 3). Apart from the central zone, there is no more than a two-fold difference between the fastest and slowest rates of accumulation in any of the regions of the apex. Similarly, in Trifolium Denne (1966) found only a three-fold difference in the rates of cell division throughout the shoot apex. This is in contrast to the wide differences in the rates of accumulation of cells in some regions of the apex in the first and second parts of the plastochron (Lyndon, 1968). In the apical dome the rate of cell accumulation in I₁ and I₂ increased by about seven-fold, and in the axis the rate of cell accumulation fell more than three-fold to a very low rate (Figs. 7 and 8 in Lyndon, 1968). The rates of cell division and cell accumulation in the different regions of the apex are compared in Table 1. The transition between the first and second parts of the plastochron is placed at 30 h, between plastochron stages 9-4 and 9-5, for it was at 30 h that the sharp changes in the rates of accumulation of cells in the apical dome occurred (Lyndon, 1968). The values for the rates
of cell division are derived from Fig. 3. It is known that in the whole apex the rate of cell division is 2.5 per cent cells per hour (Lyndon, 1968). It has been shown (see Methods) that the accumulations of metaphases in the presence of colchicine are a measure of the relative rates of division. The mean accumulation of metaphases throughout the plastochron, 6.5 per cent, therefore represents a rate of division of 2.5 per cent cells per hour and the rates of division in the various regions of the apex are the same proportion of 2.5 per cent as the corresponding values for metaphase accumulations are of 6.5 per cent. The rates of cell division derived in this way are given in Table 1. The rates of cell accumulation were obtained from the data of Lyndon (1968) by reading off from the appropriate graph the increase per hour in the logarithm of cell number and then expressing this as the increase, or accumulation, in per cent per hour (Table 1).

**Table 1**

**Mean rates of cell accumulation and division (per cent per h) in the first (0–30 h.) and second (30–46 h) parts of the plastochron**

<table>
<thead>
<tr>
<th>Region</th>
<th>Accumulation</th>
<th>Division</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole apex</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Central zone</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>I₃</td>
<td>0.5</td>
<td>1.9</td>
</tr>
<tr>
<td>I₁</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Primordium</td>
<td>7.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Axis</td>
<td>2.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>Accumulation</th>
<th>Division</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Comparison of the rates of cell division and accumulation in each region of the apex during the same periods (Table 1) reveals that there are wide discrepancies between the two sets of values. In I₁ and I₂ during the first 30 h of the plastochron the rate of cell division is faster than the rate of cell accumulation whereas after 30 h the converse is true, for the rate at which cell number increases is then much greater than can be accounted for by the rate of cell division. In the axis the rate of cell division is more than adequate to account for the increase of cell number, particularly in the second part of the plastochron. In the primordium at no time is the rate of cell division sufficient to account for the numbers of cells which accumulate.

These discrepancies between the rates of cell division and cell accumulation may be accounted for by the displacement of cells from one region to another. A measure of the degree of cell displacement can be obtained by comparing the absolute number of cell divisions in a region and the absolute number of cells which accumulate in that region in the same time. The difference between these two sets of values gives the number of cells displaced into or out of that region. When the number of cells which have accumulated in a region is subtracted from the number of cell divisions in that region at the same time a value is obtained which, if positive, indicates that there has been a surplus
of cell divisions and, if negative, a deficit compared with the number of cells accumulated. A surplus of cell divisions over the number of cells accumulated implies an export of cells to other regions and conversely, a deficit of cell divisions implies an import of cells from other regions. The number of cell divisions can be derived from the values for the rates of division given in Table 1 when the number of cells in each region at the beginning of the first and second parts of the plastochron is known (Figs. 7 and 8 in Lyndon, 1968) and the values so obtained are given in Table 2. The number of cells accumulated in each region of the apex in the first and second parts of the plastochron may be read off directly from the graphs already published (Figs. 7 and 8, in Lyndon, 1968) and these values are also given in Table 2.

It is clear that in the apical dome during the first part of the plastochron there is a surplus of cell divisions with a consequent export of cells to the axis and during the second part of the plastochron there is a deficit of cell divisions so that cells are imported from the axis (Table 2). In the primordium there is a constant deficiency of divisions to account for the cells which are accumulated so that there is a constant displacement of cells from the axis to the primordium during the whole of the first plastochron of its growth. In the axis there is a surplus of cell divisions during the whole plastochron. In the first part of the plastochron the axis receives cells from the dome, but exports even more to the primordium so that there is a flow of cells from the dome through the axis to the primordium. During the second part of the plastochron the axis is exporting cells to both the primordium and the dome.

There is no net change in the size of the central zone over the period of a plastochron although there may be fluctuations in size within this period (Lyndon, 1968). The import of 60 cells in the first part of the plastochron (Table 2) is a reflection of the increase in size of the central zone at the expense of $I_1$ and $I_2$ at the beginning of the plastochron. Similarly, the export of 100 cells at the end of the plastochron reflects in part the decrease in size of the central zone at this time. However, the net result is that over the whole plastochron there are 40 cell divisions in the central zone and that the cells so formed are exported to $I_1$ and $I_2$. The displacement of cells between the regions of the apex which are given in Table 2 are illustrated in Fig. 7.

The extent of cell displacement is put into perspective when it is expressed as the numbers of cell layers which are displaced. The boundary of the axis with the apical dome is about 14 cells in diameter so that the area of this boundary is about 150 cells. The export of 60 cells from the dome to the axis in the first half of the plastochron therefore represents a displacement equivalent to about half a cell layer, and the displacement of 300 cells from the axis to the dome in the second part of the plastochron represents a bulging equivalent to two layers of cells.

How do we interpret these data in terms of the growth of the apex? In the first part of the plastochron the displacement of cells from the dome into the axis and the displacement of cells from the axis into the primordium means that there is a net displacement of cells from the dome to the primordium via
**Table 2**

Cell displacement during the first (0–30 h) and second (30–46 h) parts of the plastochron and during the whole plastochron (0–46 h)

An excess of cell divisions implies export of cells from a region, a deficit of cell divisions implies import of cells into a region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of cell divisions</th>
<th>Number of cells accumulated</th>
<th>Number of divisions in excess(+) or deficient(−)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–30 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central zone</td>
<td>20</td>
<td>80</td>
<td>−60 +55 +60</td>
</tr>
<tr>
<td>Apical dome</td>
<td>95</td>
<td>40</td>
<td>+65</td>
</tr>
<tr>
<td>I₁</td>
<td>165</td>
<td>100</td>
<td>+190 −60</td>
</tr>
<tr>
<td>Primordium</td>
<td>60</td>
<td>250</td>
<td>−190 −60</td>
</tr>
<tr>
<td>Axis</td>
<td>600</td>
<td>470</td>
<td>+130</td>
</tr>
<tr>
<td></td>
<td>30–46 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central zone</td>
<td>20</td>
<td>−80</td>
<td>+100 −215 −300</td>
</tr>
<tr>
<td>Apical dome</td>
<td>105</td>
<td>320</td>
<td>+135 +215 −185</td>
</tr>
<tr>
<td>I₁</td>
<td>135</td>
<td>320</td>
<td>+190 −145 −335</td>
</tr>
<tr>
<td>Primordium</td>
<td>105</td>
<td>250</td>
<td>+300 −335 +75</td>
</tr>
<tr>
<td>Axis</td>
<td>515</td>
<td>70</td>
<td>+445 +575 +240</td>
</tr>
<tr>
<td></td>
<td>0–46 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central zone</td>
<td>20</td>
<td>−80</td>
<td>+100 −215 −300</td>
</tr>
<tr>
<td>Apical dome</td>
<td>105</td>
<td>320</td>
<td>+135 +215 −185</td>
</tr>
<tr>
<td>I₁</td>
<td>135</td>
<td>320</td>
<td>+190 −145 −335</td>
</tr>
<tr>
<td>Primordium</td>
<td>105</td>
<td>250</td>
<td>+300 −335 +75</td>
</tr>
<tr>
<td>Axis</td>
<td>515</td>
<td>70</td>
<td>+445 +575 +240</td>
</tr>
</tbody>
</table>
the axis (Fig. 7). At this time the primordium protrudes no more than three cell widths and it is essentially tunica in character although evidence of periclinal divisions may be found. The displacement of cells will therefore be predominantly parallel to the surface of the emerging primordium so that the primordium, the corner of the axis, and $I_2$, can be regarded as a single growth zone which is expanding between the tip of the primordium and the tip of the dome. In fact, the point of inflection of the dome which is used to mark the end of the dome-axis boundary is not particularly clear during the first part of the plastochron. One can regard this boundary as having been fixed an arbitrary distance from the tip of the dome. Since the tip of the dome moves further away from the tip of the primordium as the region between them grows, the dome-axis boundary can be visualized as moving across this growth zone away from the axil of the seventh leaf. The increase in the height of the base of the primordium during the first part of the plastochron and the increase in size of the axis at this time (Lyndon, 1968) are now seen to be partly (but not entirely) a consequence of the way in which the apex is divided into regions for analysis.

At the beginning of the second part of the plastochron the limit of the apical dome becomes clear. Now the axil of the ninth leaf can be recognized.
as the point of inflection of the apical dome which subsequently remains at a constant distance from the axil of the seventh leaf (Fig. 11, in Lyndon, 1968). This also defines the axis as a region of constant size from this point onwards. Cell division in the axis now contributes mainly to the growth of the dome. In the dome itself the rate of division is very similar in I₁ and I₂ (Table 1) and growth is probably fairly evenly distributed. Presumably in I₁ the events are now set in train for the formation of the next growth zone and the emergence of the next primordium. In this sense the visible determination of the site of the axil of the youngest primordium half-way through the plastochron marks the time of inception of the next primordium. There is, however, no change in the rate of division in I₁ at this time. It is only in the primordium when it is actually emerging that the rate of cell division increases slightly (Fig. 3 and Table 1).

Throughout the apex the relative constancy of the rates of cell division coupled with the great changes in the rates of cell accumulation have led to the interpretation that the main change which occurs in the growth of the apex during the formation of a primordium is not a change in the rates of growth but in the directions of cell displacement, that is, a change in the directions of growth. In the first part of the plastochron a bulging produces the primordium and in the second part of the plastochron the main direction of growth is changed so that bulging of the axis now results in the growth of the apical dome. On the emergence of the primordium, during the first part of the plastochron, we have already seen that the tip of the primordium and the tip of the apical dome become separated by the growth of the tissues between them so that the direction of growth becomes increasingly parallel to the lower boundary of the axis which marks off the apex from the rest of the shoot. One might expect that the orientation of the mitotic figures in I₁ in the second part of the plastochron would change in anticipation of the change in the direction of growth in this region at the beginning of the next plastochron. Observations which have been made, and which are given in the following paper, show that this in fact happens. This confirms the correctness of the interpretation of the growth of the shoot apex that has been made here on the basis of the rates of cell division and accumulation.

Although the formation of primordia may be a result of a change in the planes of growth and not changes in the rates of growth there are nevertheless quite distinct differences in the rates of cell division in the apex, but these are found in the regions other than that in which the inception of a primordium is about to occur. The main difference is between the central zone and the flanks of the apex. The rate of division in the central zone is about a third of that in the rest of the apex (Table 1). As cells are displaced from the central zone into I₁ and I₂ their rate of division increases. This transition is illustrated in Figs. 4 and 5. Most of the cell divisions in I₁ and I₂ occur in the lower parts of these regions near the boundary with the axis where the plate of cells with more frequent divisions becomes established (Fig. 4). This plate of cells is probably involved in the rapid growth of the apical dome which occurs
in the second part of the plastochron, and may be the counterpart in the pea of the cambium-like zone described in other plants (Popham and Chan, 1950). Many of the divisions in the axis which contribute to this growth of the dome will occur near the axis-dome boundary.

The region of the apical dome in which divisions are sparse (Fig. 4) is larger than the central zone (Fig. 7C) and the rates of division within and just outside the central zone appear to be similar. The visible difference between the central zone and the rest of the apical dome is therefore presumably a result of a physiological state which is independent of the rate of division.

The data which have been presented here can also be used to make some estimates of the lengths of mitosis and interphase in the different regions of the shoot apex. Since there are apparently no regions of non-dividing cells in the apex, the length of the mitotic cycle can be found. One must note, however, that some of the changes in the rates of accumulation of metaphases within a region during the plastochron, such as the fluctuation in the central zone (Fig. 3), could be due to the synchronous division of groups of cells. While this does not affect the measurements of the rates of division as given in this paper, it would invalidate measurements of the length of the mitotic cycle which depend on the assumption that cell division throughout the apex is asynchronous. With this reservation in mind the data can be used to give some indication of the average length of the mitotic cycle in the shoot apex and the time taken from entry into metaphase to the formation of a cell plate.

Where the rate of division is $D$ per cent per hour then the length of the mitotic cycle, $T$ (in hours), is given by

$$T = \frac{100 \log_e 2}{D}$$

which is the same as equation (2) of Evans, Neary, and Tonkinson (1957), when it is assumed that all cells are meristematic. The values of $D$ and corresponding values for $T$ are given in Table 3. The time spent in metaphase, anaphase, and telophase can be calculated in the following way. In an untreated apex the percentage of metaphases in each region of the apex remains essentially constant, at least for periods of a few hours (Fig. 2). The rates of entry into and exit from metaphase are therefore equal. When exit from metaphase is blocked (by colchicine) then the time taken to accumulate the percentage of metaphases which is found in an untreated apex (i.e. to double the number of metaphases originally present) is equal to the time normally spent in metaphase. The length of metaphase is therefore found when the percentage of metaphases in the untreated apex is divided by the rate of cell division (obtained from Table 1) for this latter is the same as the rate of accumulation of metaphases would be in an apex treated with colchicine having no other effect than blocking exit from metaphase. The values for the lengths of metaphase are given in Table 3. The durations of metaphase, anaphase, and telophase are proportional to the relative frequencies of these
Lyndon—Rates of Cell Division in the Shoot Apex of Pisum stages in untreated apices and, the duration of metaphase having been found, the duration of ana-telephase can also be found (Table 3).

The time taken for a cell to progress from entry into metaphase to cell division is found to be remarkably similar in all parts of the apex, being of the order of 1 h (Table 3). Variations in the length of the mitotic cycle are therefore almost entirely due to fluctuations in the length of interphase plus

<table>
<thead>
<tr>
<th>Region</th>
<th>D</th>
<th>M</th>
<th>MAT</th>
<th>Hours in metaphase (M/D)</th>
<th>Hours in metaphase plus ana-telephase (MAT × h in metaphase)</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central zone</td>
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<td>1.7</td>
<td>0.70</td>
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<td>69</td>
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<tr>
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<td>0.83</td>
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<td>30</td>
</tr>
<tr>
<td>I₂</td>
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<td>1.9</td>
<td>0.68</td>
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<td>28</td>
</tr>
<tr>
<td>Primordium</td>
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<td>1.9</td>
<td>1.7</td>
<td>0.79</td>
<td>1.4</td>
<td>29</td>
</tr>
<tr>
<td>Axis</td>
<td>2.7</td>
<td>1.6</td>
<td>1.8</td>
<td>0.59</td>
<td>1.1</td>
<td>26</td>
</tr>
<tr>
<td>Whole apex</td>
<td>2.5</td>
<td>1.6</td>
<td>1.7</td>
<td>0.64</td>
<td>1.1</td>
<td>28</td>
</tr>
</tbody>
</table>

D = Rate of division (divisions per cent cells per hour), which is also the rate of accumulation of metaphases per cent cells per hour (data of Table I).

M = Metaphases per cent cells in an untreated apex (data of Fig. 2).

MAT = Frequency of metaphases plus ana-telephases in an untreated apex as a multiple of the frequency of metaphases alone (per cent cells).

T = Length of the mitotic cycle (hours).

prophase in the pea shoot apex grown under the constant conditions used here. The values for mean cell generation times which were estimated earlier (Lyndon, 1968) are now seen to be wrong, for they were based on the erroneous assumption that cell displacement between the regions of the apex was negligible.

Acknowledgements

I wish to thank Professor R. Brown, F.R.S., for his continuing interest and helpful criticism. I am indebted to Mr. E. S. Robertson for his skilful preparation of the sections. This work was made possible by a grant from the Agricultural Research Council.

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Planes of Cell Division and Growth in the Shoot Apex of *Pisum*

BY

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*Department of Botany, University of Edinburgh*

With one Plate

Date accepted: 29 May 1968

**Abstract**

The planes of cell division and growth were examined in the course of a single plastochron in the shoot apical meristem by observing the orientations of mitotic spindles. In the I₁ region of the apical dome, cell divisions were at first anticlinal but 30 h before a leaf primordium emerged at this site 20 per cent of the cell divisions became periclinal. These periclinal divisions were found only in the corpus. Periclinal divisions in the tunica were coincident with the appearance of the primordium as a bulge. The change in the direction of growth in I₁ at the site of the incipient leaf primordium occurred without any change in the rate of growth in this region of the meristem.

**Introduction**

During the course of a single plastochron the growth of the apical meristem of the shoot is in two phases. During the first half of the plastochron the primordium and associated internodal tissue grows. In the second half of the plastochron growth of the apical dome predominates. This was shown by measuring changes in cell number in different regions of the apex and by direct measurements of the linear dimensions of the apex (Lyndon, 1968). When the rates of division in the different parts of the apex were measured by the use of colchicine it was discovered that the rate of division changed very little throughout the plastochron (Lyndon, 1970). This was interpreted as showing that the formation of the primordium resulted primarily from a change in the plane of growth rather than a change in the rate of growth.

If this interpretation is correct then it should be possible to observe directly the changes in the planes of growth as exemplified by changes in the orientations of the mitotic spindles. The orientation of the mitotic spindle is an indication of the orientation of the plane of growth if the growth of the cell, subsequent to division, continues primarily in the same plane as the axis of the spindle. A comparison of anaphase, telophase, or recently divided cells with the surrounding interphase cells shows that this is the case in the pea shoot apex. By recording the orientation of the spindle in cells in anaphase and telophase throughout the whole apex at all stages of the plastochron, the frequencies of division in different planes can then be found and the planes of growth can be inferred.

METHODS

Longitudinal 10 μm sections of the shoot apex were prepared from pea plants (Pisum sativum, cultivar Lincoln), and the plastochron between the emergence of the 9th and 10th primordia was divided into 11 morphologically recognizable stages (9·0, 9·1 ... to 9·9, 10·0) in the ways previously described (Lyndon, 1968). The orientations of the spindle in all anaphase and telophase cells in six apices at each stage of the plastochron were recorded. Metaphases were not recorded for although metaphase plates seen in side view could be unequivocally assigned an orientation, those seen in surface view could be confused with pro-metaphase plates which show no orientation. Accurate values could not therefore be obtained for the frequency of orientation of metaphase spindles in different directions.

The orientation of the spindle was determined as follows. When the two chromosome masses of the mitotic figure were separated by a clear space and both chromosome masses could be brought into focus at the same time the spindle was scored as being in the plane of the section. When the two chromosome masses were vertically superimposed upon each other, as shown by focusing up and down through the section, or were in different sections, the orientation of the spindle was recorded as being perpendicular to the section. When the two chromosome masses were not superimposed on each other but focusing up and down through a distance of more than 4 μ was necessary, the spindle was scored as being at an angle to the section. The direction of slope of a spindle at an angle could be recorded by noting which chromosome mass appeared to be uppermost to the observer.

Because of the distichous arrangement of the primordia and leaves in Pisum interpretation of the results would be facilitated if only the median section in the plane of the primordia was considered. In order to obtain enough observations, in effect a broad median section was used. The eight most median sections were used and the three or four most lateral sections on each side were ignored for the present purposes. Since the apex is 14 or 15 sections thick this means that just over half the apex was used for scoring the orientations as recorded here. The data for a given plastochron stage were all plotted on to a single diagram of the sort shown in Fig. 2. The division of the apex for purposes of analysis was as follows. Since the delimitation of the central zone was somewhat subjective this was ignored in the present analysis. The division of the apical dome into I₁ and I₂, and the delimitation of the primordium and the axis (Fig. 1a) were done as previously described (Lyndon, 1968). The regions of the apical dome I₁ and I₂ were each further subdivided into the more proximal or flank regions (I₁₁, I₂₁) and the more distal regions (I₁₂, I₂₂) by lines at 45° to the perpendicular bisector in each case. The axis was divided into three regions of equal width by lines parallel to that delimiting the primordium (Fig. 1a).

The orientation of a spindle in space can be described with reference to two planes; first the plane of the section and second the plane of the nearest surface of the apical meristem. Each spindle can then be recorded as being orientated
in the plane of the section, perpendicular to the section or at an angle to the section, and secondly, periclinal, anticlinal, or intermediate. It must be noted that a periclinal spindle corresponds to a subsequent anticlinal cell division and vice versa. In each region of the apex the number of spindles in each of

The six orientations can then be recorded and the frequency of the spindles orientated in these planes is then given by a set of six values.

**Results**

For analysis the apex is divided into two parts, stages 9.0 to 9.4 and 9.5 to 10.0. It is between plastochron stages 9.4 and 9.5 that there is a marked change in the rate of cell accumulation in the apical dome (Lyndon, 1968).
Orientation of spindles parallel to and perpendicular to the surface of the apex

Periclinal spindles are those in the apical dome and the primordium lying parallel with the nearest surface of the apex, and in the axis those lying parallel with the axis-primordium boundary. Anticlinal spindles are those perpendicular to these reference planes. Throughout the apex all spindles perpendicular to the plane of the section are scored as periclinal.

Table I

Numbers of spindles periclinal and antiphonal to the surface of the apex in the first and second parts of the plastochron

<table>
<thead>
<tr>
<th>Region</th>
<th>Plastochron stages 9.0 to 9.4</th>
<th></th>
<th>Plastochron stages 9.5 to 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>X</td>
<td>A</td>
</tr>
<tr>
<td>Apical dome</td>
<td>Ia</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ia</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ia</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Ia</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>81</td>
<td>5</td>
</tr>
<tr>
<td>Primordium</td>
<td></td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>Ap</td>
<td></td>
<td>38</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>Ac</td>
<td></td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>Ai</td>
<td></td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>87</td>
<td>13</td>
</tr>
</tbody>
</table>

The orientation of spindles in the shoot apex in the first and second part of the plastochron is shown in Table I. The proportion of anticlinal spindles in the primordium and the adjacent part of the axis (Ap) increases by 5 per cent in the second part of the plastochron. This increase, and its corollary of an increased frequency of periclinal cell divisions, is associated with the increasing size of the primordium and the consequent increase in the proportion of corpus cells in it. The high proportion of spindles of intermediate orientation in the axis adjacent to the primordium (Ap) is due to spindles which are parallel to the surface near the axils of the 7th and 9th primordia. These spindles in the axis are classed as intermediate because in the axis the reference plane of the surface of the apex is the tip of the primordium which is parallel to the primordium-axis boundary and at an angle to the surface at the leaf axil.

The greatest increase in the proportion of anticlinal spindles is in the I₁ region of the apical dome, an increase of 18 per cent in the second part of the plastochron. In the axis adjacent to I₁ (Ai) the proportion of anticlinal spindles may also increase, though only by 5 per cent (Table I). The few spindles
which are found in the tunica of I are all periclinal (divisions being anticlinal) so that the change in orientation of the spindle is confined to the corpus. More detailed data (Table 2) show that the change in spindle orientation occurs quite sharply at plastochron stage 9.5. There was only one anticlinal spindle in I in plastochron stages 9.0 to 9.4 but from plastochron stage 9.5 onwards there were at least two anticlinal spindles at each plastochron stage. The change in spindle orientation occurs to the same extent in both the proximal (I_{1a}) and distal (I_{1b}) parts of I.

**Table 2**

Numbers of periclinal and anticlinal spindles in the I region of the apical dome throughout the plastochron

<table>
<thead>
<tr>
<th>Plastochron stage</th>
<th>I_{1a}</th>
<th>I_{1b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P  X  A</td>
<td>P  X  A</td>
</tr>
<tr>
<td>9.0</td>
<td>8 3 0</td>
<td>7 0 0</td>
</tr>
<tr>
<td>9.1</td>
<td>7 0 1</td>
<td>6 0 0</td>
</tr>
<tr>
<td>9.2</td>
<td>7 0 0</td>
<td>4 0 0</td>
</tr>
<tr>
<td>9.3</td>
<td>8 0 0</td>
<td>2 0 0</td>
</tr>
<tr>
<td>9.4</td>
<td>7 0 0</td>
<td>4 1 0</td>
</tr>
<tr>
<td>9.5</td>
<td>7 0 2</td>
<td>7 0 0</td>
</tr>
<tr>
<td>9.6</td>
<td>3 0 2</td>
<td>5 1 2</td>
</tr>
<tr>
<td>9.7</td>
<td>5 0 2</td>
<td>6 0 4</td>
</tr>
<tr>
<td>9.8</td>
<td>4 2 0</td>
<td>6 0 2</td>
</tr>
<tr>
<td>9.9</td>
<td>12 0 3</td>
<td>8 1 2</td>
</tr>
<tr>
<td>10.0</td>
<td>8 3 3</td>
<td>7 4 1</td>
</tr>
</tbody>
</table>

The data for the I_2 region of the apical dome suggest that there might be a possible increase in the frequency of anticlinal spindles in I_{2b}, which is adjacent I_{1b}, in which an increase was found and a possible decrease in I_{2a} (Table 1), but there are too few observations to be certain. There is a decrease in the proportion of anticlinal spindles in the central part of the axis (Ac) in the second part of the plastochron (Table 1) when cell enlargement becomes important in this region of the apex.

There are therefore two regions of the apex in which the frequency of anticlinal spindles increases in the second part of the plastochron. The main increase is in I_1 and its adjacent tissues and the other increase is in the primordium and the adjacent part of the axis. The increase in the proportion of anticlinal spindles in the primordium is associated with the increased contribution of the corpus to the primordium as it grows and changes shape. On the contrary, the increase in the frequency of anticlinal spindles in the second part of the plastochron in I_1 is associated with neither a change in the proportions of tunica and corpus nor with any apparent change in the direction of growth in I_1 at this time. It is not until the beginning of the next plastochron when I_1 gives rise to a new primordium that the direction of growth changes.
Orientation of spindles in the plane of and perpendicular to the sections

The frequency of orientation of spindles in the plane of and perpendicular to the sections in the first and second parts of the plastochron is shown in Fig. 1. The total number of spindles recorded in each region was the same as the totals for Table 1, for of course Table 1 and Fig. 1 simply represent the same data but analysed with respect to a different pair of planes.

Throughout the plastochron there is a predominance of spindles perpendicular to the section in $I_{2b}$, the tip of the apex, and a gradation down the flanks of the apex to a preponderance of spindles in the plane of the section in $I_{1a}$ and the primordium. This gradation is mirrored in the axis (Fig. 1). The apex therefore consists on this analysis of three main regions; two of these are the opposite sides of the apex, where primordia arise, and in which the spindles are orientated primarily in the plane of the section. They are separated by the third, the central region (extending from the tip of the apex to the centre of the axis) in which the mitotic spindles are orientated primarily perpendicular to the section. This is illustrated in Fig. 2.

In $I_{2b}$ and $I_{1b}$, the most distal parts of the apical dome, the relative frequency of spindles in the two planes remains unchanged throughout the plastochron (Fig. 1). On the flanks of the dome, however, there are changes during the course of the plastochron. During the first part of the plastochron $I_{2a}$ resembles $I_{2b}$ in having spindles mostly perpendicular to the section but in the second part of the plastochron most spindles are in the plane of the section so that $I_{2a}$ comes to resemble the region which it will become, $I_{1a}$, in the first part of the next plastochron. In $I_{1a}$ and the adjacent tissue of the axis, $A_i$, in the second part of the plastochron there is an increased proportion of
FIG. 3. Orientation of mitotic spindles over the surface of the apical dome during plastochron stages 9·0 to 9·4 (a), and 9·5 to 10·0 (b). The diagrams represent zenithal equal area projections centred on the boundary between $I_1$ and $I_3$. Lateral as well as median parts of the apical dome are included. P is the position of the 9th primordium.

spindles perpendicular to the section. In the primordium and the rest of the axis there is no change throughout the plastochron.

**Orientation of the spindles on the surface of the apical dome**

The orientation of spindles over the surface of the apical dome in the first and second parts of the plastochron is shown in Fig 3. These diagrams represent
zenithal equal area projections constructed as previously described (Lyndon, 1970). In I_{1a} the frequency of spindles perpendicular to the plane of the section increases in the second half of the plastochron and in I_{1b} and in I_{2b} there is no change throughout the plastochron (Fig. 3 and Table 3). This resembles what has already been found for all the cells as shown in Fig. 1. In I_{2a} there is similarly no change during the plastochron. This contrasts with what was found for I_{2a} when all the cells, including the underlying tissues, were examined.

**Table 3**

Percentage frequencies of mitotic spindles in three orientations on the surface of the apical dome during plastochron stages 9.0 to 9.4 (a), and 9.5 to 10.0 (b)

These values represent the data shown diagrammatically in Fig. 3. Spindles are classed as being orientated in the plane of the sections (⊥), at right angles to the plane of the sections (|), or of intermediate orientation (×). These orientations correspond respectively to the horizontal, vertical, and slanted lines in Fig. 3.

<table>
<thead>
<tr>
<th>Region</th>
<th>Orientation of spindles</th>
<th>I_{1a}</th>
<th>I_{1b}</th>
<th>I_{1b}</th>
<th>I_{1a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>⊥</td>
<td>33</td>
<td>22</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td>(b)</td>
<td>⊥</td>
<td>30</td>
<td>26</td>
<td>44</td>
<td>21</td>
</tr>
</tbody>
</table>

This means that the changeover in the orientation of the spindles in I_{2a} from primarily perpendicular to the section in the first half of the plastochron to predominantly in the plane of the section during the second half of the plastochron is probably confined to the underlying tissues, that is, the corpus and possibly the inner layer of the tunica.

**Discussion**

On the basis of a comparison of the rates of cell division and cell accumulation within the shoot apex it was concluded that the formation of a primordium resulted from a change in the plane, rather than the rate, of growth in the apical dome (Lyndon, 1970). The observed increase in the frequency of anticlinal spindles in the I_{1} region of the dome in the second part of the plastochron is consistent with this interpretation of the growth of the apex. This change in the orientation of the mitotic spindles in I_{1} takes place about 30 h before the emergence of the primordium at this site and is the earliest cellular event which has so far been found in the position of I_{1} which can be interpreted as the beginning of the inception of a primordium. The fact that the change in orientation of the spindles from periclinal to anticlinal occurs within the corpus means that the morphogenetic centre of the new primordium is seated rather deeply in the apex and is not a surface phenomenon.

Since the length of the plastochron is 46 h (Lyndon, 1968) this change in the plane of growth occurs just more than half a plastochron before the
appearance of the primordium. This parallels the observation of Snow and Snow (1933), from their surgical experiments with apices of *Lupinus*, that determination of the position of the primordium occurs probably about the middle of the I₁ stage.

It was anticipated that the apical dome would show radial growth with the origin in the central region. However, this does not seem to be so, although the number of spindles seen on the surface of the dome in the central region is low and no valid conclusions can be drawn from them. In the I₁s region the mitotic spindles are predominantly in the plane of the section during the first part of the plastochron. Since this is also the region of the dome where cell division is fastest at this time (Lyndon, 1968) it is probable that what growth of the dome occurs in the first part of the plastochron is primarily by periclinal growth in the plane of the section in this proximal region. In I₁b and I₂b the orientation of spindles throughout the whole plastochron is mainly perpendicular to the plane of the section. Because the rates of division are greater on the flanks of the dome than at the tip (Lyndon, 1970) this orientation of growth will be related particularly to the formation of the stipules. During the second part of the plastochron, when the growth of the dome is much more rapid and the rate of division in the distal part of the dome increases, I₁b and I₂b will also contribute to the growth in height of the dome on the flanks where primordia do not arise. The spindles are in a predominantly vertical orientation down the sides of the dome in I₁b and I₂b (Figs. 3a and b) and this is reflected in the vertical files of cells which are a characteristic feature of grazing sections of the sides of the apex (Plate 1).

In the proximal region of the dome next to the primordium, I₂a, there is a marked change in the second part of the plastochron to an orientation primarily in the plane of the section (Fig. 1). This anticipates the transformation of I₂a into I₁s at the beginning of the next plastochron. However, on the surface of I₂a the orientation of spindles seems to remain unchanged, being predominantly perpendicular to the plane of the median section throughout the plastochron (Fig. 3). The change in orientation of the spindles in I₂a therefore probably occurs in the underlying cells and only later (when it becomes I₁s) on the surface. This again points to the inner layers of the meristem as the regions where the primary morphogenetic events are occurring.

The details of the interpretation presented here of the growth of the apex are open to test by placing marks at known points on the apex and observing their movement. The data are, however, sufficiently clear to allow a consistent model to be envisaged of the way the shoot apex grows during the course of a plastochron and from one plastochron to the next. The formation of a primordium results from a change in the plane of growth but not a change in the rate of growth. In I₁, the point where a primordium is about to arise, the rate of cell division and growth remains constant (Lyndon, 1970). In the establishment of the growth centres which give rise to primordia the primary event seems to be a change in polarity of the cells. An important task would now seem to be to find whether chemical agents such as growth substances, which
we presume control the formation of primordia, can affect the plane of growth in a tissue without necessarily affecting its rate.

**Acknowledgements**

I am grateful to Professor R. Brown, F.R.S., for his continued and helpful interest, and to Mr. E. S. Robertson for his skilled assistance. This work was made possible by a grant from the Agricultural Research Council.

**Literature Cited**


**Explanation of Plate**

Longitudinal section in the plane of the primordia of a pea shoot apex showing a grazing section of the apical dome so that the vertical files of epidermal cells at the side of the apex are seen in surface view. The large primordium to the right is the 8th. The 9th primordium is the slight bulge on the left of the apex which was at plastochron stage 9:1. The section was stained with galloxyanin after treatment with ribonuclease. The line represents 20 μ.
PLATE 1
DNA, RNA, and Protein in the Pea Shoot Apex in Relation to Leaf Initiation

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DNA, RNA, and Protein in the Pea Shoot Apex in Relation to Leaf Initiation

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ABSTRACT

The average amounts of DNA, RNA, and protein per cell measured histochemically in each region of the shoot apical meristem remained unchanged during the course of a plastochron and during the early development of the leaf primordium. The average content of DNA, RNA, and protein per cell was the same in all regions of the shoot apical meristem.

INTRODUCTION

Although the apical meristem of the shoot consists of undifferentiated cells there are discernible differences between the cells in different parts of the meristem. There are morphological differences, which allow the central zone (Fig. 1), the procambium, and the vacuolating cells of the incipient pith meristem to be distinguished. There are physiological differences, for the cells in the shoot apex divide and grow at different rates (Lyndon, 1970a). Cells in different parts of the shoot apex also differ from each other in their position in the developmental sequence from a promeristematic cell (at the summit of the apex) to a differentiating cell such as that in the leaf primordium. The I₁ and I₂ regions do not appear to differ morphologically or physiologically and are recognized solely by their positions in the apical dome.

Since the cells of the shoot apex differ from each other morphologically, physiologically, and developmentally we might anticipate that they should also differ from each other biochemically. In order to measure the biochemical characteristics of its different regions the meristem must either be dissected, which, with such a small object, is very difficult, especially if damage to the cells is to be avoided, or sectioned. In the latter case observations can be made by the appropriate histochemical procedures. Here measurements have been made in sections of fixed shoot apices of the DNA, RNA, and protein content of cells in the different regions of the apex as it grows between the inception of two successive leaf primordia in the course of a single plastochron.

MATERIALS AND METHODS

Peas (Pisum sativum c.v. Lincoln) were grown and serial longitudinal 10 μm sections, in the plane of the primordia, of shoot apices at all stages of the ninth plastochron were prepared.
DNA (deoxyribonucleic acid) was stained by the Feulgen technique as described by McLeish and Sunderland (1961), and by the galloevanin technique as described by Mitchell (1968).

Total nucleic acid (DNA + RNA) was stained with galloevanin by the technique of Mitchell (1968). Some sections were treated with ribonuclease before staining and then stained for DNA alone. RNA (ribonucleic acid) was measured as the difference between the value (expressed as a multiple of C, see below) for total nucleic acid per apex for each region, and the

corresponding value (as a multiple of C) for DNA (stained with galloevanin after treatment with ribonuclease).

Protein was stained with dinitrofluorobenzene by the technique of Mitchell (1967). These sections were also stained with Feulgen to render them more easily visible, but measurements of the Feulgen stain were not made.

Measurements of the amounts of stain in the various parts of the sections were made with a Barr and Stroud integrating microdensitometer. Ideally one would like to measure the composition of individual cells, but since it is extremely difficult to separate one cell from its neighbour optically and since some of the cells may be cut in the sectioning, measurements must necessarily be done on groups of cells and mean values obtained. A standard circular aperture was used corresponding to a diameter of 25 μm on the section. The positions in the sections at which measurements were made are shown in Fig. 1. A standard area of 491 μm² (and since the sections were 10 μm thick, a standard volume of 4910 μm³) was measured for each region of the apex in each of the three most median sections of each apex (i.e. 15 × 10³ μm³ per apex). For each stage of the plastochron three apices were measured making a total of nine sections for each area. Each value in the tables is therefore based on nine measurements, representing three apices.

Since the staining by the Feulgen or galloevanin methods may differ between batches of sections processed at different times it was necessary to bring all values to a common basis for comparison. This was done by using mitotic figures as an internal standard. These were selected from the more basal parts of the section where the cells had undergone enlargement so that the nuclei of adjacent cells were well separated and were therefore more easily measured. Measurements of prophase or metaphase nuclei gave the absorption value corresponding to the 4C amount of DNA (1C being the amount of DNA in a gamete). When these measurements were
made on galloceyanin-stained mitotic figures only those sections which had been treated with ribonuclease before staining were used. Thus, in the different regions of the apex, the values for DNA per unit area obtained by both staining techniques could be expressed as multiples of C. In this way a valid comparison could be made between different batches of sections even when they had stained to different extents. Since the stoichiometry of the binding of galloceyanin to DNA and RNA has been shown to be essentially identical (Kiefer, Kiefer, and Sandritter, 1967) the absorption values obtained for total nucleic acid were also converted into multiples of C for convenience of comparison between batches of sections. Sections stained for DNA only and from which the 4C value for nucleic acid was obtained were always processed in parallel with sections stained for total nucleic acid. In justification of this approach to the standardization of the various measurements it may be noted that the 4C values obtained from mitotic figures on different slides which were treated at the same time in the same batch of stain were always closely comparable. Ten mitotic figures were measured on each slide.

In expressing the results it is more meaningful to convert the C values into absolute amounts of nucleic acid. This can be done, for it is known that in *Pisum* the 1C amount of DNA is about 5 pg (Lyndon, 1967). The amounts of DNA and RNA expressed as C values were therefore converted to the absolute amounts given in the tables by multiplying them by 5.

For each of the standard areas in which DNA, RNA, and protein were measured, the mean number of cells was estimated independently from camera lucida drawings of the sections which had been stained with Feulgen (for DNA) and with Feulgen and dinitrofluorobenzene (for protein). An aperture, equivalent on the drawing to the standard area of 491 μm² on the section, was placed over the appropriate part of the drawing and the number of nuclei appearing in this area was noted. Only nuclei were scored which were judged to be whole or more than half present; smaller fragments were ignored. The values in Table 4 are therefore the mean of values for six apices.

**RESULTS**

The values (per standard volume of tissue) for the amounts of DNA and RNA and for the relative absorptions due to protein are given in Tables 1, 2, and 3 respectively. Within each region of the apex (Fig. 1) the values remained essentially constant throughout the plastochron. The values for DNA measured by the two different staining procedures appeared to be comparable (Table 1).

Since the number of cells in equivalent volumes of tissue within each region of the apex did not change throughout the plastochron (Table 4) then the DNA, RNA, and protein per cell also remained constant throughout the plastochron.

To obtain the mean amounts of DNA, RNA, and protein per cell in the different regions of the apex, the means of all the values for each region were used (Table 5). In all regions of the apex the amounts of DNA or RNA or protein per cell were similar. There is no evidence for any difference in the amounts of DNA, RNA, and protein in cells in different parts of the pea shoot apical meristem nor for any changes throughout the plastochron. Changes in the composition of individual cells as they progress through the mitotic cycle are obscured because the mean values represent groups of cells which are presumably dividing asynchronously.

When the amounts of DNA are expressed as multiples of the 1C amount, it is found that all parts of the apex have approximately the 2·5C amount of DNA per cell (final column of Table 1). There appears to be no difference between the central zone in which the cells are dividing slowly (Lyndon, 1970a) and the regions of the flanks of the apex (the primordium and I₁) in which cell division is about three times as fast as in the central zone.

The ratio of RNA to DNA in all parts of the apex (Table 5) is less than 1·0 and this has been confirmed by chemical measurements on excised pea shoot a pieces
Table 1. Amount of DNA (pg) in the measured volume (15 × 10³ µm³) per apex throughout the plastochron

For each region of the apex, the upper value represents sections stained with Feulgen reagent, the lower value represents sections stained with galloceyanin after they had been treated with ribonuclease. The values as multiples of the 1C amount of DNA per apex were one-fifth of the values shown below. S.D. = standard deviation of the values shown; S.E. = standard error of the mean value for all stages.

<table>
<thead>
<tr>
<th>Region</th>
<th>Plastochron stage</th>
<th>S.D. Mean value for all stages</th>
<th>S.E. Mean amount of DNA per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9-0</td>
<td>9-1</td>
<td>9-2</td>
</tr>
<tr>
<td>Central zone</td>
<td>245</td>
<td>205</td>
<td>225</td>
</tr>
<tr>
<td>I₀</td>
<td>190</td>
<td>205</td>
<td>220</td>
</tr>
<tr>
<td>I₁</td>
<td>260</td>
<td>230</td>
<td>275</td>
</tr>
<tr>
<td>I₂</td>
<td>220</td>
<td>235</td>
<td>250</td>
</tr>
<tr>
<td>Primordium</td>
<td>250</td>
<td>205</td>
<td>240</td>
</tr>
<tr>
<td>Primordium</td>
<td>210</td>
<td>205</td>
<td>210</td>
</tr>
<tr>
<td>A(P)</td>
<td>200</td>
<td>205</td>
<td>220</td>
</tr>
<tr>
<td>A(I₁)</td>
<td>250</td>
<td>215</td>
<td>235</td>
</tr>
</tbody>
</table>

Table 2. Amount of RNA (pg) in the measured volume (15 × 10³ µm³) per apex throughout the plastochron

The values as multiples of the 1C amount of DNA per apex were one-fifth of the values shown below and were obtained as the difference between the C values for total nucleic acid and DNA. S.D. = standard deviation of the values shown; S.E. = standard error of the mean value for all stages.

<table>
<thead>
<tr>
<th>Region</th>
<th>Plastochron stage</th>
<th>S.D. Mean value for all stages</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9-0</td>
<td>9-1</td>
<td>9-2</td>
</tr>
<tr>
<td>Central zone</td>
<td>155</td>
<td>140</td>
<td>160</td>
</tr>
<tr>
<td>I₀</td>
<td>185</td>
<td>185</td>
<td>220</td>
</tr>
<tr>
<td>I₁</td>
<td>165</td>
<td>165</td>
<td>165</td>
</tr>
<tr>
<td>Primordium</td>
<td>200</td>
<td>205</td>
<td>220</td>
</tr>
<tr>
<td>Primordium</td>
<td>185</td>
<td>185</td>
<td>210</td>
</tr>
<tr>
<td>A(P)</td>
<td>200</td>
<td>195</td>
<td>205</td>
</tr>
<tr>
<td>A(I₁)</td>
<td>200</td>
<td>195</td>
<td>205</td>
</tr>
</tbody>
</table>

Table 3. Relative absorption due to protein in the measured volume (15 × 10³ µm³) per apex throughout the plastochron

S.D. = standard deviation of the values shown; S.E. = standard error of the mean value for all stages.

<table>
<thead>
<tr>
<th>Region</th>
<th>Plastochron stage</th>
<th>S.D. Mean value for all stages</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9-0</td>
<td>9-1</td>
<td>9-2</td>
</tr>
<tr>
<td>Central zone</td>
<td>173</td>
<td>172</td>
<td>188</td>
</tr>
<tr>
<td>I₀</td>
<td>186</td>
<td>183</td>
<td>197</td>
</tr>
<tr>
<td>I₁</td>
<td>176</td>
<td>173</td>
<td>187</td>
</tr>
<tr>
<td>Primordium</td>
<td>182</td>
<td>188</td>
<td>208</td>
</tr>
<tr>
<td>Primordium</td>
<td>186</td>
<td>175</td>
<td>179</td>
</tr>
<tr>
<td>A(P)</td>
<td>194</td>
<td>182</td>
<td>188</td>
</tr>
<tr>
<td>A(I₁)</td>
<td>194</td>
<td>182</td>
<td>188</td>
</tr>
</tbody>
</table>
Table 4. *Number of cells in the measured volume (15 x 10^3 μm^3) per apex*

<table>
<thead>
<tr>
<th>Region</th>
<th>Plastochn stage</th>
<th>S.D.</th>
<th>Mean value for all stages</th>
<th>S.E.</th>
<th>Mean cell volume (μm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9-0</td>
<td>9-1</td>
<td>9-2</td>
<td>9-3</td>
<td>9-4</td>
</tr>
<tr>
<td>Central zone</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>I_1</td>
<td>21</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>I_2</td>
<td>20</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Primordium</td>
<td>21</td>
<td>21</td>
<td>24</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>Axis (A,P)</td>
<td>22</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Axis (A,I)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>21</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 5. *Mean amounts of DNA, RNA, and protein per cell*

<table>
<thead>
<tr>
<th>Region</th>
<th>DNA (pg)</th>
<th>RNA (pg)</th>
<th>Protein (relative absorption)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central zone</td>
<td>12:8</td>
<td>8:5</td>
<td>10:6</td>
</tr>
<tr>
<td>I_1</td>
<td>12:4</td>
<td>9:5</td>
<td>9:6</td>
</tr>
<tr>
<td>I_2</td>
<td>13:1</td>
<td>9:7</td>
<td>11:1</td>
</tr>
<tr>
<td>Primordium</td>
<td>11:8</td>
<td>9:1</td>
<td>8:9</td>
</tr>
<tr>
<td>Axis (A,I)</td>
<td>10:5</td>
<td>8:5</td>
<td>8:8</td>
</tr>
</tbody>
</table>

Similar low RNA/DNA ratios have been found for the meristems, particularly the meristem of both shoots and roots of other plants. RNA/DNA ratios of 1:0 or less occur in the shoot apex of *Lolium* (Rijven and Evans, 1967), and in young wheat leaves (Williams and Rijven, 1965). In the meristem region of the roots RNA/DNA ratios of 1:4 and 1:6 have been found in bean and pea respectively (Jensen, 1956; Heyes, 1960).

Chemical measurements made in this laboratory on excised pea shoot apices have indicated a protein/DNA ratio of approximately 6. The mean composition of the cells of the shoot meristem is therefore approximately 12 pg DNA, 9 pg RNA, and 70 pg protein. (A relative absorption value of 10 (Tables 3 and 5) represents about 70 pg of protein.)

The calculated values for cell volume (Table 4) suggest that the cells in the central zone and I_2 are the largest in the apex, the cells in the primordium are the smallest, and the cells in I_1 and the axis intermediate in size. This agrees with previous measurements of cell numbers that were not restricted to the median sections (Lyndon, 1968).

DISCUSSION

There are apparently no changes in the gross composition of the cells in the apical meristem throughout the course of a plastochron, and no differences in the average DNA, RNA, and protein content per cell in different parts of the meristem even though the cells differ from each other morphologically, physiologically, and
developmentally. The biochemical differences which presumably exist are too subtle to have been detected by the methods used here.

The amounts of RNA per cell, and hence the number of ribosomes per cell, is the same in the slowly dividing and faster dividing regions of the apex (Table 5). This suggests that in these cells, which are probably synthesizing protein at different rates, it is not the numbers of ribosomes but the degree of their association into polyribosomes that is related to the rate of protein synthesis. The amounts of protein per cell are similar throughout the apex but protein could also be distributed in different ways between the subcellular organelles in different parts of the apex.

Clearly further data are needed on the submicroscopic structure and composition of the cells of the apex and on the cellular morphology of the apex so that the events which lead to the changes in the polarity of growth (Lyndon, 1970b) and to the initiation of leaf primordia may be more precisely pinpointed.

ACKNOWLEDGEMENTS
I am indebted to Mr. E. S. Robertson for invaluable assistance in preparing the sections. This work was made possible by a grant from the Agricultural Research Council to Professor R. Brown, F.R.S.

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—— 1968. Changes in volume and cell number in the different regions of the shoot apex of Pisum during a single plastochron. Ibid. 32, 371–90.


Growth of the Surface and Inner Parts of the Pea Shoot Apical Meristem during Leaf Initiation

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Department of Botany, University of Edinburgh

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Growth of the Surface and Inner Parts of the Pea Shoot Apical Meristem during Leaf Initiation

R. F. LYNDON

Department of Botany, University of Edinburgh

Date received: 2 July 1970

ABSTRACT

The rate of cell division and the rate of increase in cell number were compared in the epidermis and in the underlying cells of the apical dome, the incipient primordium, and the axis of the pea shoot apex. These rates did not coincide in any part of the apex, but in the primordium and the apical dome there was a closer correspondence in the epidermis than in the underlying cells. This is interpreted as showing that the changing shape of the apex, during growth of the primordium and the apical dome, is associated with a tendency to local changes in the rate of growth in the epidermis but to a tendency to changes in the direction of growth in the underlying cells.

INTRODUCTION

The initiation of leaf primordia and the growth of the apical dome in the pea shoot apex are apparently the result of changes in the directions, rather than in the rates, of growth. The apex first bulges to produce a primordium and then bulges in a different direction to give growth of the apical dome (Lyndon, 1970a). This interpretation is supported by the evidence that the direction of growth in the I₁ region (Fig. 1) of the apical dome changes, as predicted, before the appearance of the leaf primordium (Lyndon, 1970b).

The bulging of the apex was apparently associated with the displacement of cells across the arbitrary boundaries by which it was marked out for analysis (the continuous lines in Fig. 1). Cell displacement was inferred mainly because, in the second part of the plastochron, in the axis cell division continued although increase in cell numbers did not, and conversely the rates of cell division in the primordium and the apical dome were insufficient to account for their increase in cell numbers. It was assumed that the axis had in fact grown from the size, enclosed by the dotted lines, of that in Fig. 1a to that in Fig. 1b. However, the arbitrary boundaries separating the regions of the apex were those shown by the continuous lines, and so the growth of the axis appeared as a displacement of cells into the primordium and the apical dome.

This interpretation of the growth of the apical meristem applies to the apex as a whole. The growth of the apex can be examined more closely by comparing the growth of the surface and the inner parts of the meristem. This is possible because the epidermis is a distinct tissue. It does not contribute cells to the tissues internal to it for all divisions in the epidermis are anticlinal. Nor is there any evidence that the internal tissues contribute cells to the epidermis. The diagrams (Fig. 1a and b) show how cell
displacement is thought to be involved in the growth of the apex as a whole. If cell displacement from the axis into the primordium and the apical dome occurred in the epidermis as well as in the inner parts of the meristem then Fig. 1a and b would also represent the behaviour of the surface cells. The surface of the axis (which is a triangular area at each side of the apex) would have grown from the size shown in Fig. 1a to that shown by the dotted lines in Fig. 1b. If there was no cell displacement in the epidermis then the growth of the surface of the axis would have been from the size shown in Fig. 1a to the size enclosed by the continuous lines in Fig. 1b.

![Diagram](image)

**Fig. 1.** Longitudinal sections of an apex (a) in the first part and (b) in the second part of the initial plastochron in the growth of the primordium. P = primordium; A = axis; ApD = apical dome. \( I_1 \) = region of the apical dome where the next leaf primordium will appear. Further explanation in the text.

The degree of cell displacement can be estimated by comparing the rates of cell division and increase in cell number in each region of the apex (Lyndon, 1970a). This has been done here for the epidermal and subepidermal cells of the apical meristem during leaf initiation. The subepidermal cells include all cells of the apex other than the epidermal cells.

**METHODS**

All observations were made on pea plants (*Pisum sativum*, cultivar *Lincoln*) during the plastochron between the initiation of the ninth and tenth leaves (Lyndon, 1968). Cell numbers were counted in the same sections previously used for measurement of total cell numbers (Lyndon, 1968).

Rates of cell division were measured by the method of accumulation of metaphases in the presence of colchicine. The increase in the numbers of metaphases in apices treated with colchicine, compared with untreated apices, was measured in the same sections which were previously used for measurements of the rates of cell division in the apex as a whole (Lyndon, 1970a). The increase in the numbers of metaphases gave a measure of the relative rates of cell division. These were converted into absolute rates in the same way as was done before (Lyndon, 1970a). The true rate of cell division in the epidermis as a whole is the same as the rate of cell accumulation which was 1.9 per cent cells per hour (Fig. 2). This corresponded to a mean accumulation of metaphases of 4.9 per cent (Fig. 4). The rates of cell division for the various regions of the epidermis are therefore 1.9/4.9 of the values for the percentage increase of
metaphases. Similarly the rate of accumulation of cells in the subepidermal cells as a whole was 2.8 per cent per hour (Fig. 3) and the mean increase of metaphases was 7.3 per cent (Fig. 4) so that the rates of division in the subepidermal cells in the different regions of the apex were 2.8/7.3 of the values for the percentage increase of metaphases.

Each section was divided for analysis into apical dome, primordium, and axis by straight lines joining the axils of the primordia (Fig. 1a and b). All the serial sections comprising the whole of each apex were analysed.

| Table 1. Mean rates of cell accumulation (Acc) and division (Div) (per cent per h) in the epidermis (E) and the subepidermal cells (S) in the first (0–30 h) and second (30–46 h) parts of the plastochron |
|---|---|---|---|---|---|---|
|   | o–30 h |   |   | 30–46 h |   |   |
|   | Acc | Div | Ratio Acc/Div | Acc | Div | Ratio Acc/Div |
| Whole apex | E | 1.9 | 1.9 | 1.0 | 1.9 | 1.9 | 1.0 |
|           | S | 2.8 | 2.8 | 1.0 | 2.8 | 2.8 | 1.0 |
| Apical dome | E | 0.9 | 1.6 | 0.6 | 2.9 | 2.0 | 1.5 |
|           | S | 1.8 | 2.4 | 0.8 | 6.5 | 2.6 | 2.5 |
| Primordium | E | 4.2 | 3.1 | 1.4 | 4.2 | 2.0 | 2.1 |
|           | S | 8.2 | 3.4 | 2.4 | 8.2 | 2.0 | 4.1 |
| Axis      | E | 1.4 | 1.7 | 0.8 | 0.0 | 1.2 | 0.0 |
|           | S | 2.7 | 2.9 | 0.9 | 0.5 | 3.0 | 0.2 |

RESULTS

The increase in cell numbers in the different regions of the apex is shown for the epidermis in Fig. 2 and for the subepidermal cells in Fig. 3. From these data the rates of cell accumulation (Table 1) have been derived in the ways already described (Lyndon, 1970a).

The rates of cell division (Table 1) were derived from the data of Fig. 4, which shows the percentage increase of colchicine-metaphases in the different regions of the apex throughout the plastochron.

Throughout the apex the rates of cell accumulation were consistently lower in the epidermis than in the subepidermal cells of the same region (Table 1). This was paralleled by lower rates of cell division in the epidermis than in the subepidermal cells except in the primordium where the rates of division were essentially the same. In neither the epidermis nor the subepidermal cells, in any of the three regions of the apex, was the rate of cell accumulation the same as the rate of cell division. The number of cells accumulated in a particular region was therefore not equivalent to the number of cell divisions in that same region. In the first part of the plastochron (0–30 h), in both the epidermal and the subepidermal cells, the rate of division was in excess of that required to account for the rate of cell accumulation in the apical dome.
(and to a lesser extent in the axis) and there was a deficiency in the rate of division in the primordium. This implies a displacement of cells, in both the epidermis and the subepidermal cells, from the apical dome and axis into the primordium. In the second part of the plastochron (30-46 h), in both the epidermis and the subepidermal cells, there was a deficiency in the rate of division in the apical dome as well as in the primordium. Only in the axis was the rate of division greater than required to account for the rate of cell accumulation, and presumably epidermal and subepidermal cells were now being displaced from the axis into both the primordium and the apical dome.

A measure of the degree of cell displacement into or out of a region is given by the ratio of the rate of cell accumulation (Acc)/rate of cell division (Div) (Table 1). When there is no net displacement the value of the ratio is 1. Values of > 1 indicate displacement of cells into a region, values of < 1 indicate displacement out. The further the value of the ratio is from 1, the greater is the extent of cell displacement.
comparison of the values of this ratio for the epidermis and the subepidermal cells of the primordium, in the same part of the plastochron, shows that while there was displacement of cells into the primordium throughout the plastochron, the degree of displacement in the epidermis was only about half that in the subepidermal cells (Table 1).

This is because the rates of cell division and accumulation in the epidermis, although not the same, were more similar than they were in the subepidermal cells. Similarly, in the second part of the plastochron when the apical dome increases in size (Lyndon, 1968), the rates of cell division and accumulation in the apical dome were less divergent in the epidermis than in the subepidermal cells (Table 1) and therefore the ratio

Fig. 3. Changes in cell number in all the subepidermal cells (total) and in subepidermal cells in the different regions of the apex. For the first 5 h or so the primordium consists only of epidermal cells.
Fig. 4. Increase in the number of metaphases (as percentage of the total number of cells) in the epidermis (—) and subepidermal cells (○—○) at each stage of the plastochron in the various regions of shoot apices treated for 8 h with 0.5 per cent colchicine. These values are the differences between the measured frequencies of metaphases after treatment with colchicine and the frequencies of metaphases in the apices before treatment, and are proportional to the rates of cell division (Lyndon, 1970a).
Acc/Div and the degree of cell displacement into the apical dome is very much less in the epidermis than in the subepidermal cells. It is doubtful if there was any real increase in the number of cells in the epidermis of the axis (Fig. 2). Whether there was or not, cell division continued and cells were therefore displaced from the epidermis of the axis to the epidermis of the primordium and the apical dome.

**DISCUSSION**

When the apex bulges, to give the growth of the primordium, and of the apical dome in the second part of the plastochron, there is less displacement of the epidermal than of the subepidermal cells (Table 1). The growth of the epidermis seems to be a result of local divisions to a greater extent than is the case for the subepidermal cells. Conversely, since there appears to be more cell displacement in the inner parts of the meristem, the bulging of the apex seems to be predominantly an internal phenomenon. If the sort of analysis done here could be extended so that the relative extents of cell displacement in successive layers of cells deeper and deeper within the meristem could be estimated, one might perhaps find a gradation of an increasing degree of cell displacement with increasing distance from the surface of the apex.

The number of cells in the epidermis of the axis in the second part of the plastochron remained constant, even though there were cell divisions in it (Table 1). This may be partly related to the lateral extension of the primordium round the sides of the apex to form the stipules, the primordium commandeering parts of the sides of the apex that might otherwise have been recorded as axis. This is seen as a displacement of cells from the axis to the primordium. Cell displacement also occurs in the apical dome. In the first part of the plastochron the excess of divisions over cells accumulated implies displacement of cells out of the apical dome, to about the same extent in the epidermis as in the subepidermal cells (Table 1). This displacement is associated with the formation of the zone of growth which separates the tip of the incipient primordium and the summit of the apical dome (Lyndon, 1970a).

The bulging of the shoot apex to give the incipient primordium and the growth of the apical dome can now be interpreted as being the result of changes in the direction of growth in the inner part of the meristem (Lyndon, 1970b) so that it bulges, accompanied by a more localized growth of the epidermis. This suggests that it is the inner part of the meristem that is the main site of morphogenetic activity, and that the epidermis may be to a large extent growing locally in response to the bulging of the tissues which it encloses.

Whether the shoot apices of other plants grow in the same way remains to be seen. The only apex for which information is available is that of the tomato, in which the rate of cell division in the incipient primordium was less in the epidermis than in the underlying cells (Hussey and Turner, 1969). This contrasts with what has been found for the pea, in which the rate of cell division in the epidermis of the primordium was the same as in the underlying cells (Table 1). It may be pointed out that Hussey and Turner used median sections whereas the values for the pea represent the whole primordium. The values for the rates of division for the median and lateral parts of the pea primordium are given in Table 2. These show that in the median parts of the
primordium the rates of cell division are the same in the epidermis and the subepidermal cells when the primordium has just been formed. Only after about 30 h of growth does the rate of division in the epidermis fall below that of the subepidermal cells as is the case in the tomato primordium. The differences between the growth of the pea and tomato apices might possibly be related to the different sizes of the primordia relative to the apical dome and to the different shapes of the growing primordia. In the lateral parts of the pea primordium the rates of division in the epidermis and the subepidermal cells remain the same even though the over-all rate falls in the second part of the plastochron (Table 2). It is the lateral parts of the pea primordium which form stipules at this time whereas, of course, the tomato primordium does not form stipules at all.

There have been several attempts to find out whether or not the surface of the shoot apex, where leaves are about to be formed, is under tension. The technique has been to make cuts in the surface of the apex and to see whether they gape, as would be expected if the cells were under tension (Snow and Snow, 1947). The results have been conflicting, but it is of particular interest to note that cuts made in tomato apices gaped (Hussey and Turner, 1969) whereas cuts made in pea apices did not (Gulline and Walker, 1957). This is correlated in the way that might be expected with the observations that in the incipient primordium the rate of cell division in the epidermis is slower than in the underlying cells in the tomato, whereas in the pea it is as fast. It would be interesting to know to what extent the gaping or otherwise of cuts made in the apices reflects differences in the experimental procedures of the different groups of investigators and to what extent it may reflect real differences in the behaviour or degree of tension of the surface cells, relative to the inner cells, of apices with differently shaped primordia.

**Table 2. Mean rates of cell division (per cent per h) in the epidermis (E) and the subepidermal cells (S) of the median and lateral parts of the primordium in the first (0–30 h) and second (30–46 h) parts of the plastochron**

<table>
<thead>
<tr>
<th></th>
<th>0–30 h</th>
<th>30–46 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median 30 μm of the primordium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>3.6</td>
<td>1.4</td>
</tr>
<tr>
<td>S</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Lateral parts of the primordium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2.9</td>
<td>2.1</td>
</tr>
<tr>
<td>S</td>
<td>2.9</td>
<td>2.1</td>
</tr>
</tbody>
</table>

LITERATURE CITED


Leaf formation and growth at the shoot apical meristem

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Department of Botany, University of Edinburgh, Mayfield Road, Edinburgh, EH 9 3 JH
(Manuscrit reçu le 6 janvier 1972)

SUMMARY

A simple mathematical model shows that, when the apical meristem and the leaf primordia formed by it are all growing exponentially, the final form of the shoot could be essentially the same whether the primordia are growing faster or slower than the apical dome. The growth of the pea shoot apex is interpreted as being mainly due to changes in the directions of growth, rather than in the rates of growth. When a leaf primordium is about to be formed, changes are first seen in the planes of cell division in the I, region of the apical dome. In the cells beneath the epidermis, the orientation of the mitotic spindles temporarily becomes random. The different rates of cell division and growth which can be demonstrated within the apical meristem may be paralleled by differences in the mitotic index, when the time in mitosis is constant, but are not paralleled by differences in the frequency of cells labelled by radioactive thymidine. The differences in concentrations of RNA within the shoot apex are shown by a simple method to be a function of differences in cell size in some plants but of different amounts of RNA per cell in others. The characteristics of the slowly dividing cells in the shoot apex appear to be different from those of slowly dividing cells in the root.

RÉSUMÉ

Un modèle mathématique simple montre que dans le cas où le méristème apical et les primordia foliaires qu'il forme croîtraient tous exponentiellement, la forme définitive de la tige pourrait être essentiellement la même, que les primordia foliaires croissent plus vite, ou plus lentement que le dôme apical.

L'interprétation de la croissance du sommet de la tige de pois est la suivante. Cette croissance dépend des changements de direction de la croissance plutôt que du taux d'accroissement. Quand un primordium foliaire est sur le point d'être formé, les premiers changements se manifestent dans les plans de division cellulaire dans la région I, du dôme apical. Dans les cellules situées sous l'épiderme, les fuseaux mitotiques s'orientent temporairement au hasard. Les taux différents de division et de croissance cellulaires sont vraisemblablement

en corrélation avec les différences de l'index mitotique (quand la durée des mitoses est constante). Mais ils ne montrent pas de corrélation avec la fréquence de marquage des cellules par la thymidine radioactive.

Les différences de teneurs en ARN dans le sommet de la tige, révélées par une méthode simple, sont fonction des différences de volume cellulaire dans certaines plantes. Dans d'autres, elles sont fonction essentiellement des quantités d'ARN par cellule. Les cellules qui se divisent lentement au sommet de la tige semblent posséder des caractéristiques différentes des cellules qui se divisent lentement dans la racine.

INTRODUCTION

The first visible sign that a leaf is being formed is the appearance of a bump on the flanks of the apical dome. This bump then grows into a small protuberance and later assumes the shape of a leaf. The first stage in the formation of a leaf therefore involves a change in shape of the apex. How is this change in shape brought about?

RATES OF GROWTH AT THE SHOOT APEX

It has sometimes been thought that it is necessary to postulate the existence of a growth centre in which the rate of growth is greater than in the rest of the apex. The incipient leaf could be thought of as arising from a region of faster growth which, after forming a nodule of cells, would then become sufficiently organised to assume a definite shape. But is it necessary to assume that a leaf is formed as the result of a localised increase in growth rate? On first sight this seems a reasonable assumption, for the apical dome is so obviously small, and remains small, whereas the leaves grow rapidly and soon overtop it.

The shoot meristem is large and in the broadest sense includes not only the shoot apex and the youngest primordia but also most of the bud, including all the young unfolded leaves. The growth of young leaves, measured by volume, length or cell number, is usually exponential with time (Sunderland and Brown, 1956; Sunderland, 1960; Williams, 1960; Williams and Bouma, 1970; Lyndon, 1970a; Evans and Berg, 1971). The growth of a leaf during the first half of its development can therefore be expressed by the equation for exponential growth,

\[ y = ae^{kt} \]

where \( y \) = final number of cells
\( a \) = original number of cells
\( e \) = base of natural logarithms
\( k \) = \( f \) (growth rate) = \( d \ln y / dt \)
\( t \) = time (in plastochrons).
From what we know of the growth of the apical dome it is probable that this is exponential too, as would be expected of a meristem in which all the cells (or a constant proportion of the cells) divide (Denne, 1966; Lyndon, 1970a; Berg and Cutter, 1969; Evans and Berg, 1971).

Let us postulate that the growth rate in the apical dome and the developing primordia is the same (i.e. \( k \) is the same), and then let us take as an example a plant in which the youngest primordium (at the time of initiation of the next primordium) has the same number of cells as the apical dome, so that at the beginning of a plastochron \( a \) is also the same for both the apical dome and the primordium. In the course of this plastochron (i.e. \( t = 1 \) for both dome and primordium) \( a, e, k \) and \( t \) would then be the same for both the primordium and the apical dome and therefore the final number of cells, \( y \), would also be the same.

For the next and each subsequent plastochron the apical dome is redefined, since another primordium will have been initiated on its flanks. For the apical dome \( a \) therefore reverts to its original value, \( t \) can never be greater than 1, and therefore \( y \) is always the same at the end of each plastochron. But exponential growth of the primordium may continue through 6 or 7 plastochrons. In this case \( a \) would be the original number of cells in the primordium and \( t \) would be greater than 1, and so \( y \) could finally become very large. The primordium would soon grow large compared to the dome even if the growth rates of the primordia and the apical dome differed greatly. Even if we suppose \( k \) for the apical dome to be 3 times as great as that for the primordia (and with \( a \) the same for both), then for the dome after one plastochron,

\[
y_{\text{dome}} = ae^{kt}
\]

and for the primordium,

\[
y_{\text{prim}} = ae^{k't}
\]

and the primordium would be smaller than the apical dome (since \( k' = k/3 \)). But after 3 plastochrons of growth

\[
y_{\text{prim}} = ae^{3k't}
\]

and the primordium would have reached the same size as the dome as its maximum size. In successive plastochrons the primordium would become much bigger than the dome even though its growth rate was so much less. This example is deliberately an extreme one and is unlikely in a vegetative apex. In most plants during the vegetative phase \( k \) for the dome is probably about the same or less than it is for the young primordia. The point to be noted is that there can be considerable variations in the relative growth rates of the apical dome and the young leaf primordia, and in their relative initial sizes, and yet the result would still be the shoot apex with which we are familiar and in which the young leaf primordia appear to be growing more rapidly than the apical dome. When an inflorescence is initiated the characteristically rapid growth of the apex could be due to either an increase in \( k \) in the apical dome relative to the primordia, or to the reduction of \( a \) for the primordia (and a corresponding increase in \( a \) for the apical dome) or to both of these, as may be happening in the growth of the Composite capitulum (Schwabe, 1959).

It is clear that the growth rates of the different parts of the shoot apical meristem and its derivatives can be found reliably only by direct quantitative measurements, which so far have only been begun, and for only a few plants.
In order to follow the process of leaf initiation it is necessary to obtain a series of plants of successive developmental stages within a single plastochron, i.e. between the initiation of one leaf and the next, and this is done most easily by sorting the plants according to the size of the youngest primordium (Lyndon, 1968; Berg, 1970).

The size of the apical dome has long been known to fluctuate during the plastochron, from minimal area when a new primordium has just been initiated, to maximal area just before the emergence of the next primordium. Growth of the apex between minimal and maximal area phases is not necessarily at a constant rate. The rate of growth of the apical dome could be measured precisely in the pea because the developmental stages during a single plastochron could be expressed as a function of time (Lyndon, 1968). During the first part of the plastochron the base of the primordium broadened as the primordium grew (the distance between Y' and Z in fig. 2a increased) but there was very little change in the size of the apical dome. In the second part of the plastochron the axial tissue adjacent to the primordium no longer increased in volume (the distance between Y' and Z in fig. 2b no longer increased), but the apical dome grew rapidly. Measurements of changes in cell numbers confirmed the simple linear measurements which were made on the sections and showed that the growth of the apex is biphasic.

Is this biphasic growth a result of changing rates of cell growth and division? When the rates of cell division were measured in the pea apex they were found to be surprisingly constant at the time and in the place that a new primordium was initiated (Lyndon, 1970a). How then can one reconcile the apparently conflicting data from the linear measurements and the cell numbers on the one hand, which demonstrate a biphasic growth, and from the rates of cell division on the other hand, which indicated an almost constant growth rate?

Let us digress for a moment and consider the sort of growth phenomenon we are studying. In fig. 1a is depicted a cylinder of tissue which is elongating only, and is not increasing in breadth. At the beginning of a period of growth it is placed with one end on the line X-X' and a line Y-Y' is noted which is halfway along the cylinder. The numbers of cells in the two halves of the cylinder, A and B, are equal and the rates of cell division are also equal. After the cylinder has been allowed to grow for some time it is measured again, its end being placed against X-X' and the line Y-Y' being the same distance from X-X' as it was originally. The regions A and B are now different sizes (fig. 1b). The shape of A and the cell number in A is the same as before but the shape of B has changed and the cell number in it has increased, even though the rate of cell division is still the same A and B. We find that although there have been cell divisions recorded in A, it has not increased in cell number. Conversely, the cell number in B has increased far more than could be accounted for by the numbers of cell divisions which have occurred in B alone. It is obvious that this apparent paradox has come about because when measuring the tissue we have arbitrarily fixed the position of
X-X' with respect to the tissue, and Y-Y' with respect to X-X'. In this way A has been defined arbitrarily as a piece of tissue of fixed size. In fact the original tissue in A has grown so that if we had marked particular cells in the tissue we would have found them to have been displaced across the arbitrary boundary Y-Y'. Displacement of cells in this sense does not of course imply any movement of cells with respect to each other, it only implies movement of cells past a fixed and arbitrary boundary as a result of the growth or bulging of the tissue.

Let us now apply these ideas to the growth of the shoot apex. In the first part of the plastochron (fig. 2 a) the apical dome is growing at about the same rate as the rest of the apex as shown by the occurrence of cell divisions within it (LYNDON, 1970 a). This growth is not apparent because it occurs by cell displacement, or bulging, across the arbitrary boundary Y-Y' which is drawn across the base of the apical dome. The shape of the apical dome remains unchanged and the cell divisions recorded within it are not matched by an increase in the cell number of the dome itself (compare A in the cylinder of tissue). Conversely, the region B of the apex (as in B in the cylinder of tissue) changes in shape and increases in size and cell number to a greater extent than could be accounted for by the number of cell divisions occurring within it. B gains cells from A (the apical dome) by displacement, or bulging to the extent of about two cell layers (LYNDON, 1970 a). The lack of change in the shape and size of the apical dome in this first part of the plastochron can be ascribed to a restraint of some sort on the growth of the surface of the dome.

In the second part of the plastochron (fig. 2 b) the restraint on the growth of the apical dome is lifted and so it now begins to grow. Now it is the shape and the cell number of that part of B within the boundary Y-Y'-Z which does not change, and the growth of this region, which still continues as shown by the numbers of cell divisions recorded within it, is seen as a displacement of cells into A (the apical dome) and the primordium.
This interpretation of the growth of the pea apex leads to the conclusion that it is changes in the directions of growth which result in the changing shape of the apex and not changes in the rates of growth. This being so, it was predicted that the formation of the next primordium would follow from a change in the direction of growth at the $I_1$ position (fig. 2 b) so that the axis of growth would become perpendicular to the surface of $I_1$ instead of parallel to it. It was argued that before the cells grew out in this changed direction the plane of cell division would have changed and that this would be observable as a change in the orientation of anaphase and telophase mitotic spindles in the $I_1$ region. Such a change was looked for and found (Lyndon, 1970 b).

In the first part of the plastochron only 1 out of 65 mitotic spindles in the $I_1$ region (including both tunica and corpus) was perpendicular to the surface. But in the second part of the plastochron in the $I_1$ region (where the next primordium was to be formed) 22 out of 111 spindles, i.e. 20 %, were perpendicular to the surface of the apical dome (Lyndon, 1970 b). For each mitotic spindle its position in the apex and its orientation was recorded on drawings and so it is possible to re-examine these data more closely. In the epidermis all the mitotic spindles are always parallel to the surface of the apex, and all divisions are anticlinal. If we therefore exclude the epidermis and examine only the non-epidermal cells, we find that the number of mitotic spindles perpendicular to the surface of the apex in

<table>
<thead>
<tr>
<th>Region of apical dome</th>
<th>Orientation of mitotic spindles</th>
<th>Plastochron stages 9.5 - 9.7</th>
<th>Plastochron stages 9.8 - 10.0</th>
<th>Total : Plastochron stages 9.5 - 10.0</th>
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</thead>
<tbody>
<tr>
<td>$I_{1a}$</td>
<td>a</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>5</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>int</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$I_{1b}$</td>
<td>a</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>6</td>
<td>5</td>
<td>11</td>
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<tr>
<td></td>
<td>int</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>$I_{2b}$</td>
<td>a</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>5</td>
<td>13</td>
<td>18</td>
</tr>
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<td></td>
<td>c</td>
<td>2</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>int</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Table I
Orientation of mitotic spindles in the apical dome of the pea shoot apex in the second part of the plastochron.

The regions of the apical dome are those shown in fig. 3. The orientations of the mitotic spindles (a, b, and c) correspond to those shown in fig. 4. Int - spindles of intermediate orientation. The plastochron is divided into stages as described by Lyndon (1968).
the more basal part of the $I_1$ region ($I_{1a}$ in figs 3 a and 3 b) is 11 out of a total of 36, i.e. 31% or very nearly one third. Of the remaining two thirds, 11 (nearly one third) are parallel to the surface of the apex and also in the plane of the sections, and 12 (one third) are parallel to the surface of the apex but perpendicular to the plane of the sections. Of particular interest is the fact that these proportions remain unchanged throughout the course of the second part of the plastochron (table I). Only the $I_{1a}$ region of the apex showed these features. The proportion of spindles perpendicular to the surface of the apex decreased with increasing distance round the apical dome away from the $I_{1a}$ region, and the proportions of spindles in the other two planes also changed (table I and Lyndon, 1970 b).

What mechanism could bring about, and maintain, this 1:1:1 proportion of spindle orientations in the $I_1$ region? The spindles perpendicular to the surface of the apex are not all found together; they seem to be distributed throughout the $I_{1a}$ and lower $I_{1b}$ regions (figs. 3 a and 3 b). If some stimulus were acting on

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**Fig. 3.** — The positions in the apical dome of the pea of those anaphase and telophase mitotic spindles which were perpendicular to the surface of the apex. (a) Plastochron stages 9.5-9.7, (b) plastochron stages 9.8-10.0, as described by Lyndon (1968). Each line represents the long axis of a mitotic spindle. The epidermis, and in the apical dome the subepidermal (tunica) cell layer, are indicated. Each diagram represents the collected observations from the 8 most median 10 μm sections from each of 18 apices (i.e. a total of 144 sections).

**Fig. 4.** — Possible orientations of mitotic spindles parallel to the faces of cubical cells. The double-headed arrows represent the mitotic spindles, the arrowheads being at the poles. The shaded face of each cell is the face parallel to, and nearest, the surface of the $I_1$ region of the apex. The plane of the section is represented by the plane of the page.
the cells to promote the orientation of spindles perpendicular to the surface of the apex, one might expect the proportion of such spindles to increase locally with time, but this does not happen.

The 1:1:1 ratio of spindles would, however, be consistent with the maintenance of a random distribution of spindles between each of the three defined orientations. If the cells are considered as essentially cubes, and the mitotic spindle can be orientated only parallel to the faces of the cube, then there would be three possible orientations for the mitotic spindle, as shown in fig. 4. In this case a random orientation of mitotic spindles would result in one third being in the plane of the section and parallel to the surface (fig. 4 a), one third parallel to the surface of the apex but perpendicular to the plane of the sections (fig. 4 b), and one third perpendicular to the surface of the apex (fig. 4 c). This is what is found (table I).

The interpretation of the events which precede leaf formation in the I1a region of the pea apex is therefore as follows. In the first part of the plastochron, when the apical dome does not increase very much in size or change in shape (LYNDON, 1968), the planes of the mitotic spindles within it are restricted to those parallel to the surface of the apex, so that only anticlinal divisions occur. The growth of the apical dome is by bulging downwards, so that it contributes to the growth giving the primordial bulge. Halfway through the plastochron the restriction on the plane of the mitotic spindles is removed and spindle orientation becomes random between the three possible planes so that one third of the divisions become periclinal. On this view the primary event involved in the change of shape of the apex, which results in the inception of a leaf primordium, is a randomisation of the plane of cell growth and division, localised in space to the I1 region (mostly the basal or I1a part), and localised in time to the period from half a plastochron before the emergence of the primordium to the time at which the primordium has become established with the dorsiventral thickness of the subsequent leaf lamina. The release of the constraint on the plane of growth in the I1 region occurs in both the corpus and the tunica (figs. 3 a and 3 b).

The primary event in the formation of a leaf primordium in the pea therefore seems to be change in the direction of growth, without an accompanying change in the rate of growth. In other plants, possibly in most, there may be increases in the rate of growth too, as there is in the tomato (HUSSEY, 1971). However, the fact that primordium formation in the pea does not seem to be preceded by an increase in growth rate suggests that, even though this might occur in many plants, it is not an essential part of the process by which the apex changes its shape to form a leaf primordium.

RATES OF CELL DIVISION IN THE SHOOT APEX

Although there may be very little change in the rate of cell division in relation to leaf initiation in the pea, there are of course differences in the rates of growth and cell division in different parts of the apical meristem. These are most clearly seen from the distribution of accumulated metaphases (frustrated
divisions) after application of colchicine. Three main regions can be distinguished in the pea shoot apex (figs. 5a and 5b): (1) the slowly dividing cells in the apical dome, (2) the fast-dividing plate of cells at the base of the apical dome (perhaps corresponding to a cambium-like zone (Popham and Chan, 1950) or meristeme medullaire (Buvat, 1955)), and (3) the cells with an intermediate rate of division in the primordium and its associated tissues.

![Diagram](image)

**Fig. 5.** — Median 30 μm sections of pea shoot apices.  
(a) and (b). Relative rates of cell division, as shown by the distribution of metaphases accumulated in the presence of colchicine.  
(c) and (d). Relative mitotic indices, as shown by the distribution of metaphase, anaphase and telophase mitotic figures.  
(e) and (f). Relative proportions of the cell cycle spent in S, as shown by the distribution of labelled nuclei after application of [3H]-thymidine for 2 hours.  
(a), (c) and (e) — just after initiation of a leaf primordium.  
(b), (d) and (f) — just before initiation of the next leaf primordium.  
Each diagram represents observations from several apices.

Direct measurements of the rates of cell division, by methods such as that which entails the use of colchicine, are the only reliable measurements, and even these must take account of the undesirable side effects of the experimental treatment. The distribution of mitotic figures in the pea (figs. 5c and 5d) gives much the same picture as the distribution of colchicine metaphases. This is because the length of time spent in mitosis is essentially the same in all parts of the pea apex (Lyndon, 1970a). In other plants in which the rates of cell division and the mitotic index have been compared, they have been found to be correlated (Denne, 1966; Corson, 1969). This implies that the length of time spent in mitosis is essentially constant in all parts of these shoot apices, too, and this may well be the case for many other plants.

The frequency of occurrence of labelled nuclei after application of labelled thymidine has also been used as an indicator of the relative rates of cell division in the shoot apex. This is not valid, except where the length of S remains essentially constant and the length of the mitotic cycle is a function of the length of G1 + G2
mitosis. This seems to be the case in the root (Clowes, 1965) but not in the shoot. The figs. 5 e and 5 f show the distribution of labelled nuclei in the pea apex after application of radioactive thymidine for 2 hours. When they are compared with figs. 5 a and 5 b (which show the relative rates of cell division) it is clear that the distribution of labelled nuclei is not an indicator of relative rates of cell division. The relatively uniform frequency of labelled nuclei (except for the incipient pith) indicates that the proportion of the cell cycle occupied by S is rather similar in most parts of the apex, and that the length of S in hours is therefore a function of the rate of division. Measurements in the pea apex show that this is so, and that in the slowly dividing cells of the central zone, where the length of the mitotic cycle is 69 hours (compared to about 28 hours elsewhere in the apex), S is also longer, being 11 hours (compared to about 7 hours elsewhere in the apex) (Lyndon, 1972).

GROWTH AT THE CELLULAR LEVEL

When shoot apices are sectioned and stained, for instance with pyronin (which stains RNA) or haematoxylin (which stains DNA and protein as well), it is usually observed that the central zone at the summit of the meristem stains lightly and the incipient primordia stain deeply. These differences in staining intensity imply differences in the concentrations of nucleic acids and protein in different parts of the apex. What is the significance of these differences in concentration which show up as zonation patterns? Do they imply that the primordia are growing rapidly? In the pea this does not seem to be so. In the pea, as in many other plants, there is a greater concentration of RNA and protein in the primordia than in the central zone. But when the amounts of RNA and protein per cell were calculated it was found that the amounts (per cell) were the same in all the cells throughout the apex (Lyndon, 1970 c). This meant that RNA and protein were more concentrated in the primordia than in the central zone only because there were more cells per unit volume in the primordia. This being so, the pattern of cell density should be the same as the pattern of stain density, and this is what is found (figs. 6 a and 6 b). Here is a method, not requiring the use of a microdensitometer, which makes possible a comparison of the differences in amounts per cell of a constituent throughout the whole of a section of an apex. In the vegetative shoot apex of Viscaria the pattern as shown by pyronin staining and the pattern of cell density resemble each other fairly.

Fig. 6. — Shoot apices of (a) (b) Pisum sativum; (c) (d) Viscaria cardinalis; (e) (f) Helianthus tuberosus; (g) (h) Chrysanthemum uliginosum.
(a) (c) (e) (g) Sections stained with pyronin.
(b) (d) (f) (h) Cell densities. The intensity of shading is proportional to the number of cells per unit area. The position of each cell in the median and adjacent sections of the apex was noted on drawings made using a camera lucida. The resulting drawings for an apex were superimposed and the number of cells recorded in each square of a grid laid over the drawing.
closely (figs. 6c and 6d), so that *Viscaria* is like *Pisum* in having a relatively constant amount of RNA per cell. This type of apex, in which the differences in RNA concentration are a reflection of differences in cell size, may be less common than the other type of apex, in which the differences in RNA concentration are a reflection of differences in the amount of RNA per cell. An example of the latter type is *Chrysanthemum*, in which the staining pattern shows that the concentration of RNA is highest in the primordia and least in the central zone (fig. 6g), but in which the cell density pattern is the reverse of this, i.e. cell density is lowest (and the cells are largest) in the primordia and highest in the central zone (where the cells are smaller) (fig. 6h). In *Chrysanthemum* the amount of RNA per cell is therefore higher in the primordia than in the central zone. Similarly in *Helianthus* the RNA concentration in the primordia is higher, but cell density is lower, than in the central zone (figs. 6e and 6f) and therefore the amount of RNA per cell again is higher in the primordia than in the central zone. The zonation pattern as revealed by pyronin, which is usually very much the same in the vegetative shoot apices of most plants, may nevertheless in different plants have a different basis at the cellular level, as these examples show.

Neither the differences in concentrations of RNA nor in the amounts of RNA per cell give us by themselves any information about rates of RNA synthesis. It may be possible to infer that the rate of net increase in amount (or accumulation) of RNA is less in the central zone, but this is only because we can assume that the amount of RNA per cell in the central zone is likely to be less (or no greater) than elsewhere in the apex and that rate of cell division and growth is also likely to be less. Since the rate of increase of RNA (and other cellular constituents) is the resultant of the rates of synthesis and breakdown, we cannot necessarily infer differences in the rates of synthesis of RNA in different parts of the apex in the absence of direct measurements. There is some evidence that in fact the central zone may show surprisingly high rates of incorporation of RNA precursor molecules even though the rate of net increase of RNA may be slow (Lyndon, 1972), but whether this represents a high rate of RNA synthesis is not yet clear.

**GROWTH IN THE CENTRAL ZONE**

The cells of the central zone at the summit of the apex are often morphologically distinct. These cells usually stain lightly and are sometimes more vacuolated than the cells on the flanks of the apex, as in *Perilla* (Nougarede, 1967). Conversely the cytoplasm may be free of vacuoles but the nuclei may be larger and more hydrated, as seems to be the case in the pea. As shown in fig. 6, the cells of the central zone may be either larger (*Pisum* and *Viscaria*), smaller (*Chrysanthemum*), or about the same size (*Helianthus*) as the cells in the rest of the apex.

The length of the component parts of the mitotic cycle has been measured for only one shoot apex, that of *Rudbeckia* (Jacqard, 1970). In the central zone both S and G2 were longer than in the rest of the apex (G1 could not be measured). The length of S in the central zone in *Pisum* is also longer than elsewhere in the apex (Lyndon, 1972). This evidence points to a lengthening of at least S and G2.
in the central zone. G1 is almost certainly lengthened too, as shown by the observation that many of the nuclei in the central zone (in Helianthus) have the 2C content of DNA (Steeves et al., 1969). In Pisum individual nuclei were not measured but the average amount of DNA per cell was the same as elsewhere in the apex, implying the same proportion of 2C and 4C cells in the central zone as in the faster dividing cells of the apex (Lyndon, 1970c).

**Table II**

*Comparison of the slowly dividing cells of the promeristems of the shoot and the root.*

<table>
<thead>
<tr>
<th></th>
<th>Central zone of shoot</th>
<th>Quiescent centre of root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of cell division compared with fastest dividing cells of meristem</td>
<td>1 or less</td>
<td>1 or less</td>
</tr>
<tr>
<td>The part of interphase which is extended</td>
<td>G1, S, G2</td>
<td>G1</td>
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<tr>
<td>Predominant DNA content of cells</td>
<td>2C, 4C</td>
<td>2C</td>
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<tr>
<td>Incorporation of RNA precursors</td>
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<td>Slow</td>
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</tbody>
</table>

The cells of the central zone of the shoot apex may in fact be relatively active cells, although having a low rate of division, for incorporation of RNA precursors can be as rapid in the central zone as elsewhere in the apex (Lyndon, 1972). This is unlike the root, in which incorporation of RNA precursors is slowest in the quiescent centre (Clowes, 1956; Barlow, 1970). The differences between the slowly dividing cells of the shoot and root meristems, which are summarized in table II, suggest that the control of the rate of cell division may involve different events in the mitotic cycle in the shoot and the root.

**CONCLUSION**

The growth of the shoot apex and the formation of leaves involve changes in shape which depend on changes in the directions of growth. There may also be changes in the rates of growth within the apex but these are probably secondary phenomena. It has become clear that only direct measurements can give reliable information about rates of growth and cell division in the shoot apex. So far almost all work has been directed to understanding the growth of the apex and the formation of leaves when the apex has reached a steady state of leaf production. But ultimately we shall need to understand how the first foliage leaf, and cotyledons, are formed, when the whole process is occurring for the first time in the life of the seedling. We might then get some insight into the fundamental physiological processes we presume to underlie the rhythmic production of leaves which results in the beautifully ordered arrangements we see in the mature shoot.
REFERENCES


4 to 14 days exposure at 1 °C the autoradiographs were developed in Ilford ID 19 developer, fixed in Hypam and washed. The sections were then stained in methyl green/pyronin (Casselman, 1959) and mounted in Canada balsam.

Sections labelled with $[^{3}H]$-uridine

Comparison of the silver grain density in different parts of a section by counting grains was almost impossible except in apices where the grain density was very low and label was evenly spread over the cells. In cells which had accumulated appreciable label the labelling was non-uniform, for grain density in nuclei and nucleoli was usually greater than in the cytoplasm and the relative amount of label in the nucleus and nucleolus differed from cell to cell. In order to compare grain densities in different parts of the apex it was necessary to use a method by which the grain density over a relatively large area, including several cells, could be measured quickly. It proved possible to use a Barr and Stroud integrating microdensitometer for this purpose. The absorption spectrum of sections stained in methyl green/pyronin showed a region of minimum absorption from 400 to 450 nm. All measurements were therefore made at 420 nm. With the ×100 objective it was possible to measure a sufficiently small area of section so that the number of silver grains in it could be counted. With the instrument set at Extinction = 1.0, the recorded absorption was a direct function of grain density except at extremely high grain densities where half or more of the field was obscured (Fig. 1). Having established this, it was then possible to use a lower power objective (×45) and a larger field to obtain a measure of relative grain densities in different parts of the section. A standard area of 420 $\mu$m$^2$ was measured. Most measurements gave the same absorption with Extinction = 0.75 and = 1.0, showing that there was no underestimation of the values for regions of high grain density. In the few cases where the measurement at Extinction = 1.0 was higher.
than at Extinction = 0.75, the former value was used. Measurements were made at the positions shown in Fig. 2. Each value is the mean from 6 sections i.e. 3 sections from each of 2 apices at each sampling time.

Fig. 2. Areas of the longitudinal section of the pea apex in which silver grain densities were measured microdensitometrically

1 = summit of apex (central zone) in which the rate of cell division is slow
2 = site of next but one leaf primordium (I₂)
3 = site of next leaf primordium (I₁)
4 = developing leaf primordium
5 = region of fastest dividing cells
6, 7, 8 = incipient pith and axial tissues

Sections labelled with [³H]-thymidine

The positions of labelled nuclei in alternate serial sections right through each of 6 apices were recorded on tracings with the aid of a camera lucida. The use of alternate sections reduced the possibility of recording the same (cut) nucleus in adjacent sections. Cell counts were made on the same sections, the tracings of the sections being divided for this purpose into the regions of central zone, I₁, I₂, primordium and axis as described by Lyndon (1968).

RESULTS

RNA synthesis

In all plants the incorporation of [³H]-uridine into RNA, after a 2.5 h application, was surprisingly uniform throughout the whole apex (Fig. 3), and microdensitometric measurements of relative silver grain densities in different regions of the apex confirmed this (Table 1). Contrary to expectation, there was as much incorporation of [³H]-uridine at the summit of the apex, where the cells are known to be dividing slowest, and increasing in RNA content slowest, as in the faster dividing cells further down the apical dome. Since the concentration of RNA is lowest at the summit of the apex, the specific activity of the RNA after supplying [³H]-uridine is in fact highest in these slowly dividing summit cells (Table 1). After 6 h of labelling the total amount of [³H]-uridine incorporation into
RNA had increased (as shown by the doubling of the grain count per unit area of section even though the exposure time of the autoradiographs was cut by two thirds) but the pattern of labelling was almost identical with that after 2.5 h (Table 1).

The distribution of label within the cells, with the nucleoli being the most intensely labelled, did not appear to differ appreciably after labelling for 2.5 or 6 h.

**DNA synthesis**

The percentages of cells with labelled nuclei, after 2 h labelling with $[^3]H$-thymidine (Fig. 4), in the different regions of the apex are shown in Table 2, and these values represent the percentages of the mitotic cycle which the cells spend in the S phase. The lengths of the mitotic cycles are also given and hence the length of S in hours in the different regions of the apex can be calculated (final column of Table 2). The length of S is about the same (6 to 8 h) in all parts of the apex except for the slowly dividing cells at the summit where it is longer (11 h). Since nuclei with only the 2C and 4C amounts of DNA have been found in the pea shoot.
Table 1

Relative amounts of [³H]-uridine incorporated into the different areas of the pea shoot apex

<table>
<thead>
<tr>
<th>Area of apex</th>
<th>Relative amount of [³H]-uridine incorporation</th>
<th>Relative concentrations of RNA</th>
<th>Relative specific activity of RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 h</td>
<td>6 h</td>
<td>2.5 h</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>99</td>
<td>101</td>
<td>114</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>109</td>
<td>131</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>103</td>
<td>138</td>
</tr>
<tr>
<td>5</td>
<td>78</td>
<td>93</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>94</td>
<td>114</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>89</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>99</td>
<td>117</td>
</tr>
</tbody>
</table>

* As shown in Fig. 2.
** Measured by microdensitometry.
+ Data from Lyndon (1970b).

Fig. 4. Autoradiograph of L. S. of a pea shoot apex after labelling with [³H]-thymidine for 2 h
Table 2

Incorporation of \[^{3}H\]-thymidine into nuclei in the pea shoot apex

<table>
<thead>
<tr>
<th>Area of apex</th>
<th>Percentage of labelled cells</th>
<th>Mitotic cycle (h)**</th>
<th>DNA S-period (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>69</td>
<td>11·0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>30</td>
<td>7·5</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>28</td>
<td>7·8</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>29</td>
<td>7·5</td>
</tr>
<tr>
<td>6, 7</td>
<td>23</td>
<td>26</td>
<td>6·0</td>
</tr>
<tr>
<td>and 8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* As shown in Fig. 2. The areas examined included the cells adjacent to the numbered areas and corresponded with the regions of central zone (1), I\(_2\) (2), I\(_1\) (3), primordium (4), and axis (6, 7, 8), for which measurements of the lengths of the mitotic cycle were made (Lyndon, 1970a).

** Data from Lyndon (1970a).

apex (by microdensitometry after Feulgen staining) (Lyndon, unpublished), it follows that during the S period of the mitotic cycle all cells synthesize the same amount of DNA, the 2C amount which is approximately 9·5 pg (Lyndon, 1967). The rate of DNA synthesis is therefore slower in the slowly dividing cells at the summit of the apex than in the cells elsewhere in the apex.

DISCUSSION

The fairly even distribution of label shows that the rate of incorporation of \[^{3}H\]-uridine into RNA in these experiments is not a function of the rates of cell division and growth which, in the central zone at the summit of the apex, are half or less of the rates of division and growth elsewhere in the apex (Lyndon, 1970a, b). On the contrary, incorporation of the RNA precursor was as rapid in the summit cells as in cells elsewhere in the apical dome and in the developing primordia. This is the same as has been found for the vegetative shoot apices of Lolium (Knox and Evans, 1968), Brachychiton (West and Gunckel, 1968), and Sinapis (Bernier and Bronchart, 1970).

In the pea the rate of net increase of RNA is a function of the rate of cell division and so will be slower in the summit cells than in the other cells. Since the rate of synthesis is the same or greater in the summit than in the other parts of the apex, but the rate of net increase (or accumulation) in the summit is only half of that elsewhere, it follows that the rate of RNA breakdown is greater in the summit than elsewhere in the apex.

On this interpretation the slowly dividing cells of the summit of the pea shoot apex show a more rapid turnover of RNA than the more rapidly dividing cells of the apex. Another characteristic of these summit (or central zone) cells is that the volume of their nuclei is 30 to 40 per cent greater than that of nuclei with the same DNA content elsewhere in the
apex (Lyndon, unpublished). In having a rapid rate of RNA turnover and having enlarged nuclei these cells at the summit of the apex resemble the slowly dividing or non-dividing cells in the more mature regions of the root which have large nuclei (Lyndon, 1967) and a rapid rate of RNA turnover (Jensen, 1961). Enlargement of the nucleus is also associated with an increased rate of RNA synthesis when nuclei are reactivated (Gurdon and Brown, 1965), and Harris (1967) has shown that in reactivated erythrocyte nuclei there is a direct relation between the volume of the nucleus and the rate of RNA synthesis. This raises the interesting possibility that the disappearance of the central zone in the pea apex when a new leaf is initiated (Lyndon, 1968) is a reflection of changes in nuclear size and synthetic activity.

There is no evidence for increased labelling of either the sites of initiation of new leaf primordia (I1 and I2 — areas 3 and 2 in Fig. 2) or the young primordium itself (Table 1 and Fig. 3). This is consistent with the view that leaf initiation results from changes in the direction of growth rather than changes in the rates of growth in the apex (Lyndon, 1970 a, c).

The interpretation of the data which has been given depends on the rate of incorporation of [3H]-uridine being a measure of the rate of synthesis of RNA. However, the possibility cannot be ruled out that this is not so. One cannot be certain until one knows that the synthesis of RNA itself is the rate-limiting step in the incorporation of label in all parts of the apex and that the specific activity of the uridine at the site of incorporation into RNA is the same throughout the apex. It could also be argued that the cells at the summit of the apex have been stimulated into activity, including RNA synthesis, by mechanical damage during the defoliation and application of the radioactive solution. This is unlikely, because the apices of non-defoliated plants and of excised shoot tips immersed in radioactive solution, although only lightly labelled, showed the same pattern of relatively uniform distribution of label. Also, some apices which could be seen to be damaged showed much less incorporation of label in the damaged region.

The rate of DNA synthesis in the summit cells, unlike RNA synthesis, is slower than elsewhere in the apex and takes about 50 per cent longer (Table 2). This is almost the same as Jacquemard (1970) found for Rudbeckia, the only other shoot apex in which the length of S has been measured. Jacquemard's data suggest that the lengths of G1 and G2 are also extended in the slowly dividing cells at the summit of the apex. Taken together with the observation that the proportion of cells with the 2C and 4C DNA content is similar at the summit and elsewhere in the apex (Steeves et al. 1969; Lyndon, 1970b), this points to the whole of interphase being extended in the slowly dividing cells of the shoot meristem.

The slowly dividing cells in the root meristem have, however, different characteristics. It is only the G1 phase of the mitotic cycle which is extended. The length of S, and hence the rate of DNA synthesis, is the same as elsewhere in the root (Clowes, 1965; Thompson and Clowes, 1968). Also the rate of RNA synthesis in these cells is low, and RNA turnover will be correspondingly low (Clowes, 1956; Jensen, 1961; Barlow, 1970). Whatever interpretation is placed on the labelling data it is clear that the slowly dividing cells of the shoot apex are readily labelled with nucleic acid precursors.
whereas those cells in the root are not. These differences allow us to speculate that the mechanism of controlling the rate of cell division may be different in the root and shoot meristems.

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THE CELL CYCLE IN THE SHOOT APEX

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Almost all our knowledge of the cell cycle in the apical meristems of higher plants has been gained from the use of seedling roots. It is not hard to see why. The root apex is easily seen, actively growing roots can easily be obtained and manipulated in large numbers, and they readily absorb radioactive substances from solutions. In contrast, the shoot apex has been neglected. It is small, usually about 0.1 or 0.2 mm in diameter, it is enshrouded in the young leaves, and it is difficult to label with radioisotopes. Nor does the shoot grow in a steady state, for it changes its shape as it initiates new leaves at regular intervals, each interval being a plastochron.

MEAN CELL GENERATION TIMES FOR THE WHOLE APEX

The production of new leaves at the shoot apex has the advantage that it provides a series of markers which represent a time scale against which to measure growth. The first estimates of the mean cell generation time (MCGT) appear to be those of Richards (1951) for the apices of Dryopteris and Lupinus. The cell doubling times were assumed to be the same as the volume doubling times which were calculated from the rates of radial displacement of leaf primordia at the shoot apex. The MCGT's estimated by this method range from 47 h for Triticum to 48 days for Elaeis, the oil palm (Table 1).

Direct determinations of the MCGT have been made by measuring the number of cells in the apical dome and the total number of cells produced by it in the course of a plastochron, i.e. the number of cells in the apical dome, the youngest primordium and its associated axial tissue. The MCGT in plastochrons for the cells of the apical dome can then be found. Since the rate of leaf initiation (the plastochron) is easily measured, the MCGT in hours can be calculated. The values obtained in this way are all one or more days (Table 1), and indicate that the cell cycle tends to be longer than in the root apex where it may be as short as 6 or 8 h (Barlow, this symposium). Only in Vicia, excised and grown in culture, has a MCGT as short as 8 h been recorded for the shoot (Ball & Soma, 1965).
Table 1. Mean cell generation time (MCGT) in vegetative shoot meristems

<table>
<thead>
<tr>
<th>Plant</th>
<th>MCGT (h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elaeis*</td>
<td>1150</td>
<td>Rees (1964)</td>
</tr>
<tr>
<td>Dryopteris*</td>
<td>480</td>
<td>Richards (1951)</td>
</tr>
<tr>
<td>Lupinus*</td>
<td>120</td>
<td>Richards (1951)</td>
</tr>
<tr>
<td>Chrysanthemum*</td>
<td>60</td>
<td>Schwabe (1971)</td>
</tr>
<tr>
<td>Chrysanthemum*</td>
<td>50</td>
<td>Berg &amp; Cutter (1969)</td>
</tr>
<tr>
<td>Triticum*</td>
<td>47</td>
<td>Evans &amp; Berg (1971)</td>
</tr>
<tr>
<td>Tradescantia</td>
<td>96</td>
<td>Denne (1966b)</td>
</tr>
<tr>
<td>Lupinus</td>
<td>72</td>
<td>Sunderland &amp; Brown (1956)</td>
</tr>
<tr>
<td>Trifolium</td>
<td>64</td>
<td>Denne (1966a)</td>
</tr>
<tr>
<td>Secale</td>
<td>48</td>
<td>Sunderland (1961)</td>
</tr>
<tr>
<td>Lonicera</td>
<td>45</td>
<td>Edgar (1961)</td>
</tr>
<tr>
<td>Lupinus</td>
<td>32</td>
<td>Sunderland (1961)</td>
</tr>
<tr>
<td>Pisum</td>
<td>28</td>
<td>Lyndon (1968a)</td>
</tr>
</tbody>
</table>

An asterisk denotes those plants in which the MCGT was calculated from the rate of radial expansion at the apex. In the others, the MCGT was calculated from the rate of increase of cell number.

Values are, of course, averages for the whole meristem and take no account of the variations in the length of the cell cycle which we may reasonably expect there to be in different parts of the apex.

**Cycle Lengths in Different Regions of the Apex**

It has for long been thought that the cells at the extreme summit of the shoot apex are dividing slowly, except when the apex becomes floral, but the evidence for this has until recently been rather circumstantial. Much of it has depended on the assumption that the mitotic index is proportional to the rate of cell division (which is probably usually correct, see below), and that the metabolic activity of the cells could be inferred from their staining characteristics. The cells of the central zone, at the summit of the apical dome, characteristically stain more lightly and have a mitotic index about 50% lower than the cells further down on the flanks of the apex where the leaves are initiated (Nougarède, 1967).

The first direct measurement of the rates of cell division in the different regions of the shoot apex was made by Denne (1966a) who used the method of accumulation of colchicine-metaphases to measure rates of cell division in *Trifolium*. Although the accumulation of metaphases with colchicine gives a measure only of the number of cells dividing per unit time, the length of the cell cycle in the different regions of the apex can be calculated if we know what proportion of cells in each region is meristematic. What
evidence we have suggests that all the cells in the shoot apex are meristematic. This is thought to be so because (at least in *Pisum*) there are no regions from which cell divisions are absent (Lyndon, 1970a), DNA is synthesised in all parts of the apex (Lyndon, 1972b), and all cells appear to be metabolically active in that they synthesise RNA (Lyndon, 1972a).

Measurements have also been made of the length of the cell cycle in the various regions of the apices of three other species by the method of metaphase accumulation, and these are summarised in Table 2. Cycle times for the subapical meristem and the region of the incipient pith have not been included because the cells in this region soon start to differentiate and the rate of division may slow down. Estimates of cycle times in this region are therefore liable to vary widely from species to species, according to the detailed structure of the apex (Denne, 1966a; Lyndon, 1970a).

**Table 2. Length of the cell cycle (hours) at the summit (central zone) and on the flanks (I₁ and I₂)* of the shoot meristem as measured from the accumulation of colchicine-metaphases**

Except where shown, all apices were vegetative

<table>
<thead>
<tr>
<th>Region of apex</th>
<th>Central zone</th>
<th>I₁</th>
<th>I₂</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trifolium</em></td>
<td>108</td>
<td>87</td>
<td>69</td>
<td>Denne (1966a)</td>
</tr>
<tr>
<td><em>Pisum</em></td>
<td>69</td>
<td>30</td>
<td>28</td>
<td>Lyndon (1970a)</td>
</tr>
<tr>
<td><em>Chrysanthemum</em></td>
<td>140</td>
<td>70</td>
<td></td>
<td>Berg (quoted in Gifford &amp; Corson, 1971)</td>
</tr>
<tr>
<td><em>Datura</em></td>
<td>76</td>
<td>36</td>
<td>26</td>
<td>Corson (1969)</td>
</tr>
<tr>
<td><em>Datura</em> (floral)</td>
<td>46</td>
<td>26</td>
<td></td>
<td>Corson (1969)</td>
</tr>
</tbody>
</table>

* Those parts of the apical dome where the next leaf primordium will be formed (I₁), and where the next one after that will be formed (I₂).

Although the cells of the central zone have a longer cycle than the cells on the flanks of these apices, it is only about twice as long (Table 2). This is not a very marked difference when we consider that in the root the difference in cycle times between the slowly dividing and faster dividing regions of the meristem is often sixfold or more (Barlow, this symposium). The cycle times for the pea apex were shorter than for the other plants, the flank cells (I₁ and I₂) having a cycle time of 28 to 30 h. It was noted that in the pea apex, in the presence of 0.5% colchicine, metaphases accumulated at a maximum rate of only about 1% per hour (Lyndon, 1970a). This was not sufficient to account for the known rate of cell division in the apex as a whole which was 2.5% per hour which had been calculated
from the MCGT obtained from the rate of increase of cell number. The colchicine-metaphase data were therefore corrected to give values for the rates of division which correspond to the cycle times given in Table 2. It was concluded that the colchicine, at the concentration of 0.5% which was necessary to eliminate anaphases and telophases, was inhibiting entry of the cells into metaphase as well as exit from it. Corson (1969) also used 0.5% colchicine on the *Datura* apex but there was no independent check on the MCGT so it is possible that the values for *Datura* represent apices which have been inhibited to some extent by the colchicine. In the cases of *Trifolium* and *Chrysanthemum*, independent estimates gave MCGTs somewhat shorter than the cell cycles calculated from the rate of accumulation of colchicine-metaphases. The MCGT (from cell counts) was 64 h in *Trifolium*, whereas the shortest cycle time measured with colchicine was 69 h (Denne, 1966a). In *Chrysanthemum* the MCGT (volume doubling time) was 50 h but the shortest cycle time measured with colchicine was 70 h (Berg, quoted in Gifford & Corson, 1971).

Even if some of the values in Table 2 err on the side of too long a cell cycle, it seems probable that the shortest cycle in the shoot apex is usually not much less than a day. Whatever their accuracy these values are almost certainly correct in giving the relative lengths of the cell cycle in the slowly dividing and faster dividing cells of the apices, for it was shown for the pea that whatever their rates of accumulation, the relative numbers of colchicine-metaphases in different regions of the apex remained the same (Lyndon, 1970a).

In any measurements of the absolute length of the cell cycle or its phases by methods which involve treating the plant with chemicals or with radioisotopes, or in any way which could conceivably alter the rate of growth, it is obviously desirable, indeed essential, to have an independent check on the MCGT by measuring the rate of increase of cell number directly by counting cells.

The only phase of the cell cycle which could be measured in these experiments was mitosis, which proved to be remarkably constant for a given species. The length of time spent in mitosis was, in each plant, the same for all cells of an apex irrespective of the length of the cell cycle. Within an apex the mitotic index therefore appears to be proportional to the rate of cell division and inversely proportional to the length of the cell cycle when the plant is vegetative and growing under constant conditions. This means that most of the inferences that have been made about the relative rates of cell division in vegetative apices on the basis of mitotic index are fortuitously correct (Nougarède, 1967). However, such inferences are no substitute for direct measurements of rates of division or cycle times.
Fig. 1. Rates of cell division and length of the cell cycle in a median longitudinal section of the shoot apex of *Pisum*. Each point represents a colchicine-metaphase and the density of points is proportional to the rate of cell division (Lyndon, 1970a). The values are the approximate lengths of the cell cycle (h) for cells lying on the lines.

Values such as those in Table 2, which group together all the cells in a particular region of the apex, obscure the more detailed variation in cycle lengths which there is within an apex. These variations can be mapped by plotting the positions of colchicine-metaphases, since the density of the accumulated metaphases is proportional to the rates of cell division (Fig. 1). Average values for the density of colchicine-metaphases can be converted into the corresponding values for the lengths of the cell cycle. These have been superimposed as contours on the diagram of the pea apex (Fig. 1).

We can see that the average values for the cell cycle (Table 2) do not do justice to the gradations in cycle length which there are within the apex, and that the fastest cell cycles may be as short as 15 h (this was at 23 °C).

In the shoot apex cells are continually being formed at the summit as growth occurs and so cells become displaced down the apical dome and eventually into the leaf primordia. Fig. 2 illustrates how a cell would have its cell cycle accelerated as it was displaced down the apical dome and into a leaf primordium in successive plastochrons. A cell would go through a period when each cycle was shorter than the last. In fact, apart from the cells at the extreme summit of the apex it seems unlikely that many cells are ever in a state in which the length of the cell cycle is constant for even a single cycle. Since it seems that all phases of the cell cycle (except mitosis) may be extended or shortened when the cycle length changes (see later) we may presume that the cells, in their transition from one region to another...
where the cycle time is shorter, will adjust by a general speeding up of their progress through all the phases of the cycle, except mitosis itself.

We ought to bear in mind that in long-term labelling experiments, which last for about a plastochron or more, a cell which is labelled in one part of the apex will be observed later in some quite different region.

**SYNTHESIS DURING THE CELL CYCLE**

The average composition of cells in all parts of the pea shoot apex is the same and is approximately 12 pg DNA, 9 pg RNA and 70 pg protein (Lyndon, 1970b). The composition of comparable cells in the pea root tip is 16 pg DNA, 79 pg RNA and 287 pg protein (Lyndon, 1968b). The cells
of the shoot meristem have to accumulate much less RNA and protein and yet their cell cycle is almost certainly longer than that of the root meristem cells. The greater length of the cell cycle in the shoot apex is therefore not because more material has to be accumulated—on the contrary. The presumed slowness of RNA synthesis in the shoot apex is reflected in the slowness with which the nucleoli increase in size over the cell cycle and their much smaller size at prophase than in the root (Lyndon, 1968b). The slower rate of nucleolar growth in the shoot apex cannot be ascribed to there being fewer nucleolar (ribosomal) genes per nucleus in the shoot than in the root, for such differences do not seem to exist (Ingle & Sinclair, 1972). The cells of the central zone, with a long cell cycle, increase their RNA and protein content at a slower rate than the cells on the flanks of the apex with a shorter cycle. But this does not necessarily imply a lower metabolic activity of the central zone cells. The uptake of precursors into RNA and protein can be as fast or faster than in the cells with a shorter cycle (Lyndon, 1972a) but it is not yet clear whether this implies a rapid rate of synthesis of RNA and protein (with a concomitant faster turnover). It may be merely that the central zone cells are more efficient at concentrating exogenous precursor molecules in the first place, or have smaller precursor pools which reach a higher specific activity than elsewhere in the apex.

**LENGTHS OF THE PHASES OF THE CELL CYCLE**

All the measurements considered so far have been of the whole cell cycle. Measurement of the lengths of the component phases of the cell cycle in the shoot apex, by the use of radioisotopes, has just been started. The reason for the lack of data is at least partly due to the difficulty of doing labelling experiments with the shoot apex. Firstly, it is difficult to get label in at all. Whereas the root is an absorbing organ which readily takes up solutes, this is not the case for the shoot. Often substances applied to the intact shoot apex hardly find their way into the plant. Clowes (1959) tried to overcome the problem by using aquatic plants which could be immersed in the radioactive solution, but, even so, the plants had to be immersed in the solution for one or more days in order to label the nuclei of the apex. Gifford, Kupila & Yamaguchi (1963) tried a number of ways of labelling the apex but, even with the most effective method, application of the radioactive solution directly onto the apical dome, the plants had to be exposed to the radioisotope for 24 h or longer to get labelled nuclei. Bernier & Bronchart (1963) also compared many methods of applying labelled compounds to shoot apices and they found the only effective method to be
partial defoliation before applying the label. Nuclei then become labelled within 0.5 h. Autoradiographs show that the radioactive substances are probably taken up via the stumps of the excised leaves. Although this technique is successful it suffers from the disadvantage that the amount of mutilation to which the plant is necessarily subjected might well be producing a wound reaction in the form of an alteration of the length of the cell cycle and its various phases in the shoot meristem under investigation. The need to dissect every plant before application of label also seriously limits the number of plants that can be used in any experiment. This can be a serious limitation if it is necessary to score the percentage of mitotic figures which become labelled, since the mitotic index in the shoot apex is characteristically very low, of the order of one or two per cent.

A further difficulty is that the long cycle times in the shoot apex mean that experiments often have to go on for several days, which raises the possibility of radiation damage and consequent alterations of the cell cycle times. All these difficulties are compounded when we come to look at what may be some of the most interesting cells in the shoot apex, the slowly dividing cells of the central zone. Since the central zone may consist of only about 100 cells (e.g. in the pea) and since the mitotic index is least in this region, about 1%, this means that there is, on average, only one mitotic figure per plant in this region. Therefore, in order to get any idea of the percentage of mitotic figures labelled, a large number of plants is needed, which as we have already seen is itself difficult.

In view of these difficulties it is perhaps hardly surprising that so far there are only two sets of measurements of the lengths of the component phases of the cell cycle in the shoot apex. The first of these illustrates some of the difficulties. Michaux (1969) measured the lengths of the component phases of the cell cycle in the water plant *Isoetes*, and her results are summarised in Table 3. The length of the whole cell cycle in the lateral zone (where leaves are initiated) was measured by supplying [3H]thymidine continuously by immersion of the plants in the solution. The length of the whole cell cycle could be measured only by the time taken to reach a maximum number (93%) of labelled nuclei. The pulse-labelling method was tried but had to be abandoned because there were so few mitoses. The time spent in DNA synthesis (S) had to be estimated from the percentage of nuclei labelled 6 h after the label was introduced, for this was the earliest that labelled nuclei could be detected. The time spent in mitosis (M) was estimated from the mitotic index. $G_2 + M$ was taken as the time for the first labelled telophases to appear. $G_1$ was obtained by difference. Measurements of cycle phases in the central zone were much more restricted because only 12% of the nuclei had become labelled after 36 h (so that the
length of the whole cycle could not be found) and after 36 h necrosis of the apex set in and no more nuclei became labelled. Only $G_2 + M$ could be estimated, from the appearance of the first labelled anaphase, as 34 h, twice as long as in the lateral regions. Even assuming that the lengths of the other phases of the cell cycle in the central zone were no longer than in the lateral regions, the cycle time in the central zone would be 52 h, and was probably much more.

Table 4. Duration (hours) of the cell cycle and its component phases in four regions of the apical bud of Rudbeckia bicolor (from Jacqmard, 1970)

<table>
<thead>
<tr>
<th>Region</th>
<th>Whole cycle</th>
<th>$G_1 + M/2$</th>
<th>S</th>
<th>$G_2 + M/2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral zone</td>
<td>30.1</td>
<td>9.0</td>
<td>11.6</td>
<td>9.5</td>
</tr>
<tr>
<td>Central zone</td>
<td>–</td>
<td>–</td>
<td>19.2</td>
<td>14.8</td>
</tr>
<tr>
<td>Pith-rib meristem</td>
<td>30.5</td>
<td>6.8</td>
<td>15.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Subapical pith</td>
<td>32.9</td>
<td>13.1</td>
<td>13.0</td>
<td>6.8</td>
</tr>
</tbody>
</table>

More complete measurements were made by Jacqmard (1970) on the apex of *Rudbeckia*. The apices were supplied for 30 min with $[^3]$H]thymidine which was then washed off with water. The percentage of labelled mitotic figures was scored at intervals during the subsequent 40 h and the values which were obtained for the lengths of the phases of the cell cycle are given in Table 4. Again, the length of the cell cycle and most of its phases in the central zone could not be obtained because of the long cycle time. Except for a lengthening of $G_1$ in the subapical pith, the length of the cell cycle and its phases was similar in all parts of the apex except the central zone where both $S$ and $G_2$ were extended. The only other measurements of the length of a phase of the cycle is for the pea, in which $S$ was estimated (from the proportion of nuclei labelled after 2 h) as 11 h in the central zone as opposed to 7 to 8 h elsewhere in the apex (Lyndon, 1972a). These meagre data suggest that $S$ and $G_2$ in the central zone are both longer than in the peripheral regions of the apex, possibly twice as long. Since the whole cycle in the central zone is about double what it is elsewhere in the apex (Table 2) then $G_1$ would be expected to be about twice as long in the central zone as on the flanks of the apex.
Phases of the cell cycle in Pisum

In an attempt to get some idea of the lengths of the component phases of the cell cycle in the central zone as well as in the rest of the meristem an analysis of the pea shoot apex was made by the method of Mak (1965). This consists essentially of labelling the apex with $[^{3}\text{H}]$thymidine so that the nuclei in S become labelled. The apices are then sectioned, stained with Feulgen, and exposed to autoradiographic emulsion. The unlabelled nuclei are those in G$_1$ and G$_2$ and can be distinguished by measurement of their DNA content with a microdensitometer so that the proportions of G$_1$ and G$_2$ nuclei can be found. The proportion of cells in M is given by the mitotic index, and in S by the proportion of labelled nuclei. The relative lengths of the component phases of the cell cycle, which are related to the number of cells in each phase, can then be calculated. When the length of the cell cycle is known then the absolute lengths of the phases can also be found.

For analysis each section was divided in the ways already described (Lyndon, 1968a) into (i) the central zone, distinguished by its larger nuclei which are therefore less intensely stained; (2) the regions on the flanks of the apical dome, i.e. I$_1$ which will form the next leaf primordium and I$_2$ which will form the next primordium after that; and (3) the primordium of the youngest leaf.

The DNA values of the unlabelled nuclei fell into two distinct groups representing 2 C (up to 35 units of DNA) and 4 C nuclei (36 and more units of DNA) (Fig. 3). The proportions of 2 C and 4 C nuclei in each of the regions of the apex were similar to those of all the nuclei shown in Fig. 3. It seemed possible that these might not be the true proportions of 2 C and 4 C nuclei since not all nuclei could be measured and underestimation of the numbers of 4 C nuclei could have occurred since these, being larger, might be more likely to have been cut or to have overlapped other nuclei. A check on the proportions of 2 C and 4 C nuclei was possible because the nuclear diameters of 2 C and 4 C nuclei were sufficiently different to allow the number of 2 C nuclei to be estimated from the frequency of nuclei with diameters below the median for 2 C nuclei (Fig. 4). The diameters of all nuclei in the sections were therefore measured (including cut and overlapping nuclei) and their frequency distribution is shown in Fig. 5. It is at once clear that the proportion of 2 C nuclei was not less, and may perhaps be greater, than in the sample for which DNA was also measured (Fig. 4). When the frequency distributions of nuclear diameters shown in Figs. 4 and 5, and also for the separate regions of the apex, were plotted as probability curves it was equally apparent that the proportions of 2 C
Fig. 3. The frequency of nuclei with different DNA contents in the shoot apex of *Pisum*. The arrows indicate the values for the DNA content of telophase nuclei (2 C; mean of 13) and prophase plus metaphase nuclei (4 C; mean of 41). The apices were fixed in 70% ethanol, which contracted the nuclei, making measurement of individual nuclei easier. The DNA contents of all unlabelled nuclei which were uncut, did not overlap other nuclei, and could be optically isolated, were measured (using a Barr and Stroud integrating microdensitometer) in a total of 8 median and adjacent longitudinal sections (12 for the central zone) taken from 4 plants. Sections were 10 μm thick.

and 4 C nuclei which were found in the sample in which DNA was also measured could be accepted and used in the calculations that follow.

The percentages of nuclei which were labelled in these sections are shown in Table 5, together with the proportions of nuclei in G₁, G₂ and M. The proportion of the whole cell cycle spent in each of these phases can be calculated using the formulae given by Nachtwey & Cameron (1968) which correct for the age gradient in a population of cells whose number is increasing exponentially. Using the values for the length of the cell cycle given by Lyndon (1970a) the lengths of the phases of the cycle can be calculated (columns A, Table 6). Before considering the significance of these values, it is worth comparing them with a further set of values (columns B, Table 6),
Fig. 4. The frequency of 2C (clear) and 4C (shaded) nuclei with different diameters in the sample of nuclei in which DNA was measured (shown in Fig. 3). Values for the central zone are shown separately because these nuclei appear to be larger than nuclei with the same amount of DNA in the rest of the apex.

which have been obtained by using published values for the mean DNA content per nucleus as a basis for calculating the proportions of $G_1$ and $G_2$ nuclei. Mean amounts of DNA per nucleus in the different regions of the pea apex were measured and expressed as multiples of the C amount (Lyndon, 1970b). Using the values for the percentages of nuclei in S and M given in Table 5, the proportions of $G_1$ and $G_2$ nuclei can be calculated as follows.
Fig. 5. The frequency of nuclei with different diameters when all unlabelled nuclei were measured in (a) all regions of the apex except the central zone, and (b) the central zone. The proportion of 2 C nuclei is at least as high as in the smaller sample in which DNA was measured (shown in Fig. 4).

Where $c$ is the mean C value for DNA per cell, let $s$, $m$, and $g$ be the fractions of nuclei that are in S, M and G$_1$ respectively. The DNA content of nuclei in M will be 4 C, in S is assumed to be on average 3 C, in G$_1$ will be 2 C and in G$_2$ will be 4 C. Then,

$$c = 3s + 4m + 2g + 4(1-s-m-g),$$

and this reduces to

$$g = \frac{4-s-c}{2},$$

and so gives the fraction of the total number of nuclei in G$_1$, from which the fraction in G$_2$ can also be calculated.

Applying this formula to the data of Lyndon (1970b) the percentages of cells in the phases of the cell cycle were obtained and the lengths of the phases calculated (columns B, Table 6). They turn out to be very similar to those in columns A, Table 6. If anything, G$_2$ is a little shorter by this second method, which is consistent with the possibility that the percentage of nuclei in G$_2$ may have been overestimated by the first method.

The values in Table 6 show that in the slowly dividing cells of the central zone of the pea all the phases of the cell cycle (except M) have been
Table 5. Proportions of cells in the different phases of the cell cycle in four regions of the shoot apex of Pisum

<table>
<thead>
<tr>
<th>Region</th>
<th>G_1</th>
<th>S*</th>
<th>G_2</th>
<th>M†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central zone</td>
<td>63</td>
<td>17</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>I_2</td>
<td>53</td>
<td>23</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>I_1</td>
<td>62</td>
<td>24</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Leaf primordium</td>
<td>55</td>
<td>29</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

* The percentage of labelled nuclei in the sections. The nuclei were labelled for 1 h by the method of Lyndon (1972a). Although the sections were 10 μm thick, labelled nuclei and nuclear fragments at all levels in the section were recorded. Presumably this was because during the exposure the section and emulsion were desiccated so that all parts of the section were within 3 μm of the emulsion. On rehydration the sections regained their former thickness of 10 μm which they retained when mounted.

† Data from Lyndon (1970a).

Table 6. Lengths of the phases of the cell cycle (hours) in four regions of the shoot apex of Pisum

<table>
<thead>
<tr>
<th>Region</th>
<th>Whole cycle</th>
<th>G_1</th>
<th>S</th>
<th>G_2</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Central zone</td>
<td>69</td>
<td>38</td>
<td>37</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>I_2</td>
<td>30</td>
<td>13</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>I_1</td>
<td>28</td>
<td>15</td>
<td>15</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Leaf primordium</td>
<td>29</td>
<td>14</td>
<td>16</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

Columns A: data obtained by the method of Mak (1965). Columns B: values derived from published data (see text).

extended, S by about 50%, G_1 by about 250%, and G_2 by 250% or more. This is the first direct evidence that in the slowly dividing cells at the summit of the shoot apex both G_1 and G_2 are extended. These values, together with those for *Rudbeckia* (Jacqmard, 1970), indicate that in the shoot apex the cells which are dividing more slowly than the rest tend to have the whole of interphase extended rather than a single phase as tends to be the case in the root (Barlow, this symposium). This may suggest that the control of the rate of cell division and of progress through the cell cycle is effected in different ways in the root and the shoot meristems. The situation may be different when the growth of the whole apex is inhibited, for in *Tradescantia* axillary buds which were inhibited by auxin nearly all the nuclei were held at the 2 C level (Naylor, 1958). Before we can extend our speculations we need information about the levels of growth substances.
in the root and the shoot, and how these might affect the different phases of the cell cycle.

To get information about the cell cycle in the shoot it should be a relatively straightforward matter to examine the meristems of different species, measure the average DNA content per nucleus by the methods already used (Lyndon, 1970b), find the percentage of nuclei labelled with a terminal label of \[^{3}\text{H}]\text{thymidine}, and measure the mitotic index. From these data, even if the length of the cell cycle is not known, it should be possible to estimate and compare the relative lengths of the phases of the cell cycle in different parts of the apex. Although measuring the phases of the cell cycle by labelling experiments alone has its difficulties, it may be easier if new methods were devised which relied on smaller numbers of plants and used terminal labelling where possible.

**THE TRANSITION FROM VEGETATIVE TO FLORAL GROWTH**

The cell cycle becomes speeded up during floral induction and evocation. Although this has been inferred mostly from the uncertain evidence of changes in the mitotic index (Nougarède, 1967) there is also some direct evidence that this happens. In lupin and in vernalised rye cell counts showed that the cell cycle became faster when the apices underwent transition from vegetative to floral growth, whereas in continued vegetative growth the cell cycle lengthened (Sunderland, 1961). In *Datura* the cell cycle in all parts of the apex was shortened to about 60 to 70% of that in the vegetative apex (Corson, 1969) but the differential between the cells at the summit of the apex and the cells on the flanks was maintained (Table 2). A very similar situation has been found in *Sinapis* (M. Bodson, personal communication). In addition to a speeding up of the cell cycle there may also be some synchronisation of the cells during floral induction, soon after the floral stimulus reaches the apex (Bernier, 1971). This is inferred from the slight increase in the mitotic index at this time and in *Sinapis* from the marked increase in the proportion of cells with the 2C amount of DNA (Jacqmard & Miksche, 1971).

In *Rudbeckia* flowering is brought about by long days or applications of gibberellic acid (Jacqmard, 1965). In both cases the mitotic index and the percentage of nuclei which incorporated \[^{3}\text{H}]\text{thymidine increased in the central zone and the subapical meristems, indicating that both M and S occupied a greater part of the cell cycle in the induced plants and hence G\(_1\) or G\(_2\) or both were reduced in proportion. In several other plants too, gibberellic acid treatment resulted in an increase in the proportions of
cells in S and M even though the plants did not flower (Bernier, Bronchart, Jacqmard & Sylvestre, 1967).

The evidence we have so far suggests that changing levels of growth substances in the apex could bring about changes in the lengths of the cell cycle and in the rate of progress from one phase of the cycle to the next, but we do not yet know whether these changes in the cell cycle are essential or merely incidental to the morphogenetic processes which occur at the shoot apex.

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The Quantitative Ultrastructure of the Pea Shoot Apex in Relation to Leaf Initiation

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With 8 Figures

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Summary

The ultrastructure of the pea shoot apical meristem was examined quantitatively in longitudinal sections. Photographs were taken at eleven defined positions in the apex, at six developmental stages within a single plastochron. The only change in ultrastructure during the period of a single plastochron was the increase in the proportion of plastids with starch in the central regions of the apex and in the young leaf axils. This increase occurred midway in time between the emergence of successive leaves, at precisely the time that the orientation of growth changes in the region where a new leaf is to emerge. There were quantitative changes in ultrastructure associated with cell differentiation. In the sequence of cell development from the summit of the apex (central zone) to the incipient pith, cell enlargement was accompanied by an increase in the volume of endoplasmic reticulum, dictyosomes, microbodies and vacuoles per cell, an increase in the number of mitochondria, microbodies and vacuoles per cell, and an increase in the volume, but not the number, of plastids per cell. In the sequence of axillary development (before the axillary bud begins to grow) the number of mitochondria per cell decreased as cell volume decreased but the number of plastids per cell remained constant. The number of plastids per cell increased only in the developmental sequence leading to leaf development, in which the number of mitochondria and dictyosomes per cell also increased. There appeared to be no features of ultrastructure, qualitative or quantitative, which could be correlated with the different rates of cell division in different regions of the meristem. The differences in ultrastructure throughout the apex were mainly quantitative and seemed to be associated with cellular differentiation rather than with the plastochronic functioning of the apex during leaf initiation.

1. Introduction

The immediate event in the pea shoot apex which produces the change in shape of the apex and which results in the formation of a new leaf primordium is apparently a change in the direction, or orientation, of growth.

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rather than a change in the rate of growth (Lyndon 1970a, b, 1972). Apart from a reorientation of one third of the mitotic spindles (Lyndon 1972) there are no obvious differences in structure, as seen by light microscopy, which distinguish the cells at the point of leaf initiation from the cells on the opposite flank of the apical dome where a leaf is not initiated until one plastochron later. The different rates of cell division within the apex (Lyndon 1970a) are also not related to obvious differences in structure visible with the light microscope. Differences in the amounts of DNA, RNA, and protein in the cells of the pea shoot apex have been looked for but, irrespective of their position and stage of development within the apex, or their rate of division, all the cells examined had a similar gross chemical composition as measured by quantitative histochemistry (Lyndon 1970c). If there are differences between the cells in different parts of the apex which are at different stages in the sequence towards leaf development or between cells with different rates of growth and division then these are presumably at the ultrastructural or molecular levels.

In order to get an accurate measure of any differences there may be in the ultrastructural composition of cells in different parts of the shoot apex it is necessary that the survey be quantitative and that samples be taken in a defined and objective manner. This can be done for the pea shoot apex by an extension of the technique which has been used successfully for light microscopy (Lyndon 1968). The development of quantitative stereological methods (Weibel 1969) allows the calculation of the relative volumes occupied by different organelles seen on electron micrographs. When the shapes of the organelles are known and the number of profiles is also counted then the numbers of organelles can be calculated. Quantitative measurements can therefore be made not only of the relative volumes of organelles within the tissues but also of the numbers of organelles per unit volume and the numbers of organelles per cell. These methods were used in the present investigation to obtain detailed measurements of the volumes and numbers of organelles in different parts of the pea shoot apex in relation to the development of the cells, and to measure any changes in ultrastructure which might occur during the course of a single plastochron, and which would be related to the timing of leaf initiation.

2. Materials and Methods

Peas (Pisum sativum, cultivar Lincoln) were sown in wet sand and were grown in a controlled environment room at 23°C in a 12 hours light/12 hours dark cycle with a light intensity of 30 W.m⁻². When the peas were 8, 9, or 10 days old the shoot tips were excised and fixed in freshly-made 2.0 percent potassium permanganate for 2 hours. They were washed in water, then dehydrated through an ethanol series and were embedded in araldite. Sections were cut on an LKB ultratome and those of a thickness of 60 to 70 nm (light gold) were taken and mounted. Fixation in glutaraldehyde was attempted but the image was poor. Fixation in permanganate, although perhaps less desirable, did result in a clearer
Fig. 1. Low power electron micrograph of a longitudinal section of a pea shoot apex showing the positions at which photographs were taken. C = central zone, cells at the summit of the apex. P = youngest leaf primordium, the 9th to be formed above the two reduced epicotylary leaves (LYNDON 1968). I1, I2 = cells which will give rise to the 10th and 11th leaf primordia respectively. DAB = cells at the base of the apical dome, on the dome/axis boundary as defined by a line joining the axils of the 8th and 9th leaf primordia. A(P), A(C), A(I1) = regions of the stem axis near the 9th primordium, the centre of the pith, and the I1 region respectively. AxP9, AxP8, AxP7 = axils of the 9th, 8th, and 7th oldest leaves respectively. These were the only positions in which epidermal cells were included in the areas photographed. The lines at the left of the section are folds or wrinkles in the section.

delineation of the organelles even though ribosomes and microtubules were lost. For the present purposes permanganate proved quite satisfactory and allowed easy identification of the organelles present in the cells.

Longitudinal sections were cut in the plane which passes through the insertions of all the leaves and also cuts medially the positions of all future leaf primordia. Three sections were cut from each apex; the first was 10 μm from the midline, the second was median, and the third was 10 μm further into the apex. Since the cells are approximately 10 μm in diameter each of these three sections would tend to cut through different cells.
The sections were mounted on formvar/carbon films. Their ultrastructure was not examined before photography. Eleven photographs were taken of each section at the predetermined positions shown in Fig. 1. All photographs were taken on an AEI EM6 microscope at the same instrument magnification of $4,000 \times$ on the plate. A twelfth photograph was taken. This was a low power view of the whole section obtained with the microscope used in the diffraction mode. Although this produced a very low contrast image it was possible to discern the outlines of the cells and subsequently to locate the positions of the other eleven pictures on the section of the whole apex (Fig. 1). This served as a check that the eleven photographs had been taken at the correct positions.

The eleven positions were chosen so that several aspects of cell development within the apex could be followed (Table 1). The sequence central zone/DAB/A (C) represents the sequence of cell enlargement and maturation followed by the cells which become the pith. The sequence central zone/I 2/I 1/primordium represents the successive developmental stages of a cell which becomes incorporated into a young leaf primordium. The sequence AxP 9/AxP 8/AxP 7 represents the sequence of development of cells which are committed to the axillary position of the young primordium. A comparison of cells in the central zone, primordium and DAB is a comparison of cells with slow, intermediate and rapid rates of cell division respectively (LYNDON 1970a). The lengths of the cell cycle have been shown to be approximately 69 hours in the central zone, 29 hours in the primordium, and 20 hours in the DAB region (LYNDON 1970a, 1973).

Quantitative analysis of the ultrastructure of cells in these eleven positions in the apex at a given point in time gives information about any changes there might be in the cells as they progress along these developmental sequences, assuming the apex to be growing in a steady state. By repeating this analysis at six developmental stages during a single plastochron, defined as described elsewhere (LYNDON 1968), it becomes possible to analyse the changes in ultrastructure at each of the positions in the apex as a function of time, and particularly in relation to the timing of the events leading to the initiation of a new leaf primordium. It is already known that the change in direction of growth occurs quite abruptly about 16 hours before the new primordium appears as a bump (LYNDON 1970b).

The electron micrographs were all enlarged and printed at a standard magnification giving a linear magnification of $9,350 \times$ and representing an area of 400 $\mu$m$^2$. A grid of 100 points arranged as described by WEIBEL et al. (1966) was placed over each photograph and the type of organelle occurring under each point was noted (Fig. 2). The organelles were classified as one of ten types. These were: 1. nucleus, 2. cytoplasm (in which no ultra-

Table 1. Sequences of Cell Development Represented by Positions in the Shoot Apex

<table>
<thead>
<tr>
<th>Developmental sequence</th>
<th>Positions shown in Fig. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell enlargement in developing pith cells</td>
<td>C/DAB/A (C)</td>
</tr>
<tr>
<td>Leaf formation: successive positions of cells displaced down the apical dome</td>
<td>C/I 2/I 1/P</td>
</tr>
<tr>
<td>Axillary development</td>
<td>AxP 9/AxP 8/AxP 7</td>
</tr>
<tr>
<td>Rates of cell division: cells with slow, intermediate and fast rates of division</td>
<td>C/P/DAB</td>
</tr>
</tbody>
</table>
Fig. 2. Electron micrograph of I1 region showing its ultrastructure and the positions of 25 of the 100 points which were placed in a grid over the photograph for the measurement of the relative volumes occupied by the various organelles. N = nucleus; M = mitochondrion; P = plastid; Mb = microbody; D = dictyosome; ER = endoplasmic reticulum; W = wall; C = clear cytoplasm

structure could be seen), 3. cell wall, 4. plastids, 5. mitochondria, 6. dictyosomes, 7. endoplasmic reticulum, 8. microbodies, 9. vacuoles, and 10. unidentified particles. It was always possible to distinguish plastids from mitochondria, not only by the presence or absence of clearly defined cristae but also by the darker staining of the outer membrane of the plastids. The plasma membrane was included with the endoplasmic reticulum. Plasmodesmata were included with the wall. The nuclear envelope was included with the nucleus but extensions of the nuclear envelope into the cytoplasm were classified as endoplasmic reticulum. Since there were three sections for each apex and two apices for each developmental stage there were six replicates of each photograph and these were grouped together and a mean value was obtained. The values for the relative volumes of the organelles were calculated as described by Weibel et al. (1966).
The numbers of profiles of cells, plastids, mitochondria, microbodies, vacuoles, dictyosomes and plastid profiles with starch granules were counted on each photograph. The absolute numbers of organelles were derived from the formula:

\[ N = \frac{n^{3/4}}{\beta \cdot \varphi} \]

as described by Weibel and Gomez (1962), where \( N \) = number of organelles per unit volume, \( n \) = number of transections per unit area of cut surface, \( \beta \) = coefficient of configuration, and \( \varphi \) = fraction of volume occupied by that organelle. \( \beta \) was obtained by making some assumptions about the shape of each organelle, based upon the shapes of the profiles which were seen in the photographs. The cells were assumed to be isodiametric (\( \beta = 1.375 \)). The mitochondria, plastids and microbodies were assumed to be ellipses with diameter = 0.66 \times length (\( \beta = 1.6 \)). The dictyosomes were assumed to be cylinders with diameter = 1.3 \times length (\( \beta = 1.5 \)). The vacuoles were assigned the value of \( \beta = 1.5 \). The numbers of cells per unit volume were calculated by taking \( \varphi = 1 \).

These calculations depend upon the arrangement of the organelles within the sections being random, and they also depend upon the sections themselves being random with respect to the tissue. Since there were approximately seven or so cell profiles in each photograph and each section represented different cells it was assumed that the micrographs represented essentially random cuts through the cells. It was also assumed that the arrangement of the organelles was random with respect to the plane of the section even though this was the same with respect to the positions at which leaves were initiated.

The sizes of individual organelles were obtained by dividing the volume of organelles per unit volume of tissue by the number of organelles per unit volume of tissue.

3. Results

3.1. Cellular Structure and Stage of the Plastochron

There appear to be no consistent changes in the relative volumes of the subcellular components as a function of the stage of the plastochron (Table 2). When the volume of organelles per cell, the number of organelles per cell, and the sizes of organelles were calculated, there were no clear differences between one stage of the plastochron and another in any region of the apex. In each case the data were therefore collected together and expressed as means for the whole plastochron in Figs. 5-7. There was only one clear change in ultrastructure related to plastochron stage and this was an increase in the percentage of plastids with starch. This was greatest in the regions AxP 9, A (P), and DAB (Fig. 3) and occurred between plastochron stages 9.4 and 9.65 (Fig. 4). This is the point in the plastochron when the axil of the youngest leaf (i.e., AxP 9) becomes a distinct morphological entity, when periclinal divisions begin in the I 1 region of the apex at the onset of leaf initiation (Lyndon 1970 b, 1972), and when the apical dome begins to enlarge (Lyndon 1968). This sudden increase in the number of plastids containing starch is not likely to be associated with the onset of photosynthesis in these plastids, since the membrane systems are not highly developed (Fig. 8). The increase in starch is probably due to an increase in the rate of synthesis from existing
Table 2. Percentage of the Total Tissue Volume Occupied by Each Organelle at Each Stage of the Plastochron

<table>
<thead>
<tr>
<th>Plastochron stage</th>
<th>Nucleus</th>
<th>Cytoplasm</th>
<th>Wall</th>
<th>Plastids</th>
<th>Mitochondria</th>
<th>Endoplasmic reticulum</th>
<th>Dictyosomes</th>
<th>Microbodies</th>
<th>Vacuoles</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.0</td>
<td>36.91</td>
<td>34.87</td>
<td>8.91</td>
<td>5.09</td>
<td>5.32</td>
<td>6.87</td>
<td>1.21</td>
<td>0.15</td>
<td>0.36</td>
<td>0.30</td>
</tr>
<tr>
<td>9.2</td>
<td>36.74</td>
<td>32.96</td>
<td>10.20</td>
<td>5.98</td>
<td>4.55</td>
<td>7.85</td>
<td>1.09</td>
<td>0.08</td>
<td>0.45</td>
<td>0.15</td>
</tr>
<tr>
<td>9.4</td>
<td>36.82</td>
<td>35.74</td>
<td>8.59</td>
<td>4.84</td>
<td>4.55</td>
<td>6.84</td>
<td>1.73</td>
<td>0.19</td>
<td>0.60</td>
<td>0.15</td>
</tr>
<tr>
<td>9.65</td>
<td>34.92</td>
<td>35.24</td>
<td>11.05</td>
<td>5.28</td>
<td>5.05</td>
<td>5.98</td>
<td>1.25</td>
<td>0.16</td>
<td>1.03</td>
<td>0.15</td>
</tr>
<tr>
<td>9.85</td>
<td>35.83</td>
<td>32.61</td>
<td>10.84</td>
<td>6.65</td>
<td>5.03</td>
<td>6.39</td>
<td>1.58</td>
<td>0.26</td>
<td>0.70</td>
<td>0.14</td>
</tr>
<tr>
<td>10.0</td>
<td>35.70</td>
<td>33.00</td>
<td>11.44</td>
<td>6.04</td>
<td>4.75</td>
<td>6.39</td>
<td>1.19</td>
<td>0.15</td>
<td>0.81</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Least significant difference between means (p = 0.05)

3.2. Developmental Sequences Within the Apex

Although there are no other marked changes which can be associated with the periodic functioning of the apex in the initiation of leaves, there are changes in the cells as they progress along various developmental pathways in the apex.

3.2.1. Cell Enlargement: Incipient Pith

Along the developmental sequence leading to cell maturation and enlargement [C/DAB/A (C)] (Table 1) the relative volume per cell of all organelles...
Fig. 3. Percentage of plastids with starch in the different regions of the apex a) during the first part of the plastochron (●), from plastochron stages 9.0 to 9.4 (LYNDON 1968) or 0 to 30 hours after the first appearance of the 9th leaf primordium; and b) during the second part of the plastochron (■), from plastochron stages 9.5 to 10.0 (LYNDON 1968) or 30 to 46 hours after the first appearance of the 9th leaf primordium.

Fig. 4. Percentage of plastids with starch in the DAB, A(P) and AxP9 regions of the apex.
Fig. 5. Total volume (μm³) per cell occupied by different classes of organelles. LSD = Least significant difference (p = 0.05). This is not available for microbodies and vacuoles because of zero values in the original data. Since there is only one nucleus per cell the total volume per cell occupied by the nucleus is the same as the volume per nucleus, which is shown in Fig. 7.
increases (Fig. 5), with the exception of the nucleus (Fig. 7). This is consistent with the continuing synthesis of cytoplasmic components as the cells enlarge but the cessation of nuclear growth as the cells pass out of division. The onset of vacuolation is shown by the increase in vacuolar volume (Fig. 5) and in vacuolar numbers (Fig. 6) and size (Fig. 7). There is also an increase in the numbers of mitochondria, microbodies, and possibly dictyosomes, but no increase in the number of plastids (Fig. 6), although the plastids increase in size (Fig. 7). With the exception of the plastids the increase in numbers of organelles implies that development along this sequence entails replication of organelles at a faster rate than replication of cells, whereas plastid division merely keeps pace with cell division.

3.2.2. Leaf Initiation

In the developmental sequence leading to leaf initiation (C/I 2/I 1/P) there is an increase in the volume of endoplasmic reticulum and vacuoles per cell.
Fig. 7. Sizes of cells and of individual organelles. Volume per cell and nucleus in $\mu$m$^3$.

Volume per mitochondrion, dictyosome, plastid, microbody and vacuole in $\frac{\mu m^3}{100} \cdot LSD = 100$.

Least significant difference ($p = 0.05$). This is not available for microbodies and vacuoles because of zero values in the original data.
(Fig. 5), although the cells are not enlarging (Fig. 7). There is also an increase in the number of all organelles including plastids (Fig. 6). The increase in the numbers of mitochondria per cell is accompanied by a decrease in their size (Fig. 7) but no decrease in the volume of mitochondrial material per cell (Fig. 5). This implies that the replication of mitochondrial material goes on at the same rate as the replication of cellular material as a whole but the rate of division of the mitochondria outstrips the rate of cell division. Plastid number per cell also increases (Fig. 6), but plastid size remains the same (Fig. 7). In this developmental sequence, therefore, the rate of plastid replication and the synthesis of plastid constituents outstrips the rate of cell replication so that there are more plastids per cell in the incipient primordium than there are in the cells of the apical dome. The number of plastids in the cells of the apical dome of the pea is the same (about 12) as in the cells of the shoot apex of the spinach (Cran and Possingham 1972) in which the number of plastids per cell has been shown to increase during leaf development (Possingham and Saurer 1969). The increase in the number of plastids, as well as of all other organelles (Fig. 6), in the developmental sequence leading to leaf initiation in the pea shoot apex makes it different from the other developmental sequences and suggests that a release of the constraints on plastid replication is one of the first events in the differentiation of the leaf primordium.
3.2.3. Axillary Development

Along the sequence AxP 9/AxP 8/AxP 7 (the development of the axillary cells), the cells become smaller (Fig. 7) and more densely packed with organelles, shown by the decrease in the volume per cell of clear cytoplasm (Fig. 5). There are fewer mitochondria per cell (Fig. 6) and less endoplasmic reticulum (Fig. 5) as the axil develops, but the number of vacuoles tends to increase (Fig. 6). This suggests that these cells may be ceasing division and entering a more or less quiescent phase before the axillary bud begins to grow out, which it does about one or two plastochrons later, corresponding to AxP 6 or AxP 5. Along this developmental sequence the number of mitochondria decreases and the number of plastids remains about the same. This implies that during the decrease in the cell volume (Fig. 7) the replication of plastids keeps pace with the replication of cells whereas the rate of replication of the mitochondria decreases faster than the replication of the axillary cells in which they are found.

3.2.4. Cell Division Rate

The one developmental sequence along which there seems to be no change at all is the sequence representing a threefold increase in the rate of cell division (C/P/DAB). There is no obvious change along this sequence in the volume of organelles per cell (Fig. 5), or in the numbers or sizes of organelles per cell (Figs. 6 and 7).

4. Discussion

The plastochronic functioning of the apex and the initiation of individual leaves is marked by no obvious changes in the ultrastructure of the apical cells, except for the increase in the proportion of plastids with starch when the orientation of growth in the I 1 region changes at plastochron stage 9.5. The accumulation of starch in association with morphogenesis has been noted on several occasions, in callus tissue on the initiation of shoots (Thorpe and Murashige 1968) and in the shoot apex on transition to flowering (Sadik and Ozbun 1967, Yeung and Peterson 1972, Molder and Owens 1973). Starch accumulation is stimulated in the presence of auxin (Sunderland and Wells 1968, Wozny et al. 1973) and cytokinin (Usciati et al. 1972, Hadacova et al. 1973) but it can be prevented, as can organ formation in callus, by gibberellin (Thorpe and Murashige 1968). This suggests the possibility that the sudden increase in the number of plastids with starch at plastochron stage 9.5 may well be indicative of a sudden change in the concentration of a growth substance in the apex at this time.
One might have expected there to be changes in the central zone correlated with its disappearance and reappearance as an obvious region, at the time a new leaf primordium emerges (Lyndon 1968), but no such changes were found.

The main changes in the ultrastructure of the cells seem to be related more to cell differentiation as seen in the development of cells along the sequences leading to development of pith cells [C/DAB/A (C)], leaf cells (C/1 2/I 1/P), and axillary cells (AxP 9, AxP 8, AxP 7). The lack of ultrastructural changes associated with the event of leaf initiation itself suggests that the morphogenetic changes in the apex which result in leaf initiation depend on changes at the molecular level rather than the ultrastructural level. The lack of any obvious correlation between ultrastructure and cell division rate in the regions of the central zone, primordium, and DAB, which encompass a threefold difference in division rate, again suggests that the differences which occur are at the molecular level.

In the developmental sequence from the central zone to the incipient pith [C/DAB/A (C)], cell enlargement is accompanied by an increase in the number of mitochondria per cell and in the development of the leaf axil (AxP 9/AxP 8/AxP 7) the number of mitochondria decreases as cell size decreases. In both cases the number of plastids remains constant (Fig. 5). The replication of plastids must therefore keep pace exactly with the replication of the cells whereas the rate of replication of the mitochondria in the enlarging cells is faster than that of the cells and in the axillary cells is slower. Plastid replication in these instances is tied to cell replication but mitochondrial replication is not. A different situation is found in the developmental sequence leading to leaf initiation (C/I 2/I 1/P). Here the numbers of both mitochondria and plastids per cell increase to almost the same extent (Fig. 5), so that neither mitochondrial nor plastid replication is tied to cell replication. The control of the replication of mitochondria and plastids is therefore not under the same control in different regions of the apex and neither are necessarily under the same control as cell replication.

The growth and enlargement of the cells of the incipient pith, as shown by the developmental sequence C/DAB/A (C), may be compared with the development of the enlarging cells of the root cap in maize (Juniper and Clowes 1965). In both types of cells the amount of endoplasmic reticulum per cell and the numbers of mitochondria and dictyosomes per cell increased as the cells enlarged but the number of plastids per cell remained unchanged, or nearly so, although in both cases the plastids enlarged as the cells enlarged. Except for the lack of vacuolation in the maize root cap cells, the quantitative ultrastructural changes during cell enlargement and maturation appear to be similar in the root cap cells of the maize and the incipient pith cells of the pea shoot apex.

In the central zone of the pea shoot apex there were 11 plastids, 58 mito-
chondria, and 15 dictyosomes per cell (Fig. 6). These are very similar to the numbers of organelles in the Epilobium shoot meristem in which there are 11 plastids, 60 mitochondria, and 24 dictyosomes in each telophase daughter cell (ANTON-LAMPRECHT 1967). The maize root cap initial cells were similar in having 15 plastids and 20 dictyosomes per cell but had many more mitochondria, about 220 per cell (JUNIPER and CLOWES 1965).

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**References**


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8. The Shoot Apex

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I. INTRODUCTION

The outstanding feature of the shoot apex* is the initiation of new organs—leaves when vegetative and floral parts when reproductive. These processes depend on continued growth and in so far as cell division is a component of growth it is therefore an essential component.

* Sometimes the term shoot apex is used to include the whole of the terminal bud. At the other extreme is its restriction to mean only that part of the meristem distal to the youngest leaf primordium. Here it will be used in a looser sense to include not only the apical dome (equivalent to the shoot “apex” as defined by Abbé and Phinney, 1951), but also the youngest leaf primordia, and the associated axial tissue, with no specific lower limit.
of morphogenesis at the shoot apex. The fact that new organs grow out in directions which are different from the direction of growth of the axis from which they have sprung implies that the direction, or plane, of growth alters and presumably the plane of cell division alters too when the leaves and floral parts are initiated and begin to grow.

The positioning of new leaves on the apex and the consequent leaf arrangement, or phyllotaxis, depends on the interrelations of the relative growth rate, the shape of the apex, and the size of the leaf primordia on initiation (Richards, 1951). At least the first two of these, if not all three, are a function of the rates or directions of cell growth and division. An understanding of the processes of leaf initiation and the siting of new leaves therefore requires an exact knowledge of the rates and planes of division in the shoot apex and how these may change with time in relation to the processes of morphogenesis and development with which they are inextricably bound up.

II. RATES OF CELL DIVISION IN THE VEGETATIVE SHOOT APEX

A. The Apex as a Whole

The shoot meristem originates in the embryo at the base of the cleft between the two cotyledons, in a dicotyledon, or at the side of the embryo in a monocotyledon. The changes in the growth rate of the shoot meristem in the developing embryo have been measured in the maize (Abbé and Stein, 1954). Since average cell size remained constant the changes in the rate of cell division could be measured as changes in the relative growth rate, which slowed down exponentially as successive leaves were formed and eventually became zero when five or six leaf primordia had been formed, and the embryo was mature.

On germination of a seedling, cell division is resumed, and the developing primordia and leaves continue division so that the size of the meristem increases to include not only the shoot apex but all the young unfolded leaves (Sunderland, 1961; Sunderland and Brown, 1956). The meristematic region therefore expands until the plant reaches a steady state of growth and at this stage the shoot meristem in its broadest sense encompasses most of the terminal bud, and consists of tens of millions of cells, all probably dividing at very much the same rate. In contrast to the root, in which the meristem extends back only 1–2 mm from the tip, in a shoot it may extend for 1 or even 2 cm down from the apex. The immense size of the shoot meristem compared with the root meristem means that the shoot can increase in size relatively rapidly even if the relative growth rate is low, because of the large number of dividing cells which it contains.
The mean cell generation time (MCGT), or mean cell doubling time for the whole apex, is a measurement of limited value as it is an average which includes all cells however different their division rates may be. It can, however, be useful in allowing a general comparison of shoot apices of different species and a comparison with the MCGTs of roots of the same species. While it is difficult to make exact comparisons, the MCGTs of shoots seem to be on the whole longer (and cell division is therefore slower) than in the roots of the same species under comparable conditions (Lyndon, 1973). A comparison of the shoot apices of different species shows that the MCGTs differ considerably but are often of the order of 1–3 days. The longest recorded is the oil palm, with a cell dividing on average only once every 7 weeks.

The MCGT of young leaf primordia is in most cases probably about the same as that of the cells in the apex itself (see Chapter 9). The apex, primordia and associated axial tissues can be regarded as all parts of a single system growing exponentially (Schuepp, 1938), the growth of which can be expressed by the equation

\[ y = ae^{rt} \]

where \( y \) is the final number of cells, \( a \) the original number, \( r \) the relative growth rate and \( t \) is time. When this equation is applied to the growth of the apical dome then \( t \) can never be greater than one plastochron because the apical dome is re-defined when each new primordium is initiated. However, the growth of the primordia may continue for as many as six or more plastochrons, so that even when the relative growth rate \((r)\) for the primordia is the same as, or even lower than, that for the apical dome, the primordia soon become much larger than the dome and overtop it (Lyndon, 1972a). The apparently rapid growth of the leaf primordia compared to the apical dome is therefore a consequence of following the growth of the primordia as distinct entities for a much longer time than is done for the dome, the growth of which is of necessity followed only for a single plastochron.

Some plants may reach a steady state of growth so that the MCGT of the apex remains constant for long periods of time. This may well be the case with plants having an absolute photoperiodic requirement and which are kept in non-inductive conditions. There is evidence that in some plants the apex does not reach a steady state. In maize the MCGT shortens, so that the relative growth rate of the apical dome steadily accelerates, during growth of the shoot (Abbé et al., 1951). In the lupin and the rye, the opposite occurs, the MCGT in the apex steadily lengthening during vegetative growth and shortening transiently only during the transition to flowering (Sunderland, 1961).
Detailed measurements of the growth of the apical region of the lupin during vegetative growth reveal that the relative growth rates of the apical dome, the primordia and the axial tissue are all different and all decrease, but at different rates. The data are consistent with the rates of decrease themselves being exponential (Brown, Chapter 1).

B. Cell Division in Different Regions of the Apex

1. Rates of Cell Division

Until about 10 years ago, the conclusions drawn about the relative rates of division within the shoot apex depended on indirect and circumstantial evidence. The cells at the summit of the vegetative shoot apex were believed to divide less rapidly than the cells on the flanks of the apex (where the leaves are initiated) because the summit cells characteristically had a lower frequency of mitotic figures than elsewhere in the apex and the cytological appearance of the summit cells was thought to resemble that of cells with low metabolic activity (Nougarède, 1967). Another point of view was that all the cells of the apex were dividing relatively rapidly. Some of the principal evidence advanced in support of this view depended on the measurement of rates of displacement of cells from the summit of the apex to the flanks (Ball, 1960; Soma and Ball, 1963; Ball, 1972). By analogy with the root meristem, in which rapidly dividing cells are rapidly labelled with $^3$H-thymidine and slowly dividing cells are slowly labelled, it was expected that the rapidity and extent of labelling with DNA precursors would give an estimate of relative division rates. In the apex of some species the summit cells became labelled, but in other plants they did not (see review by Nougarède, 1967).

Much of the discussion has centred around the validity of the concept of the “méristème d’attente” propounded by Buvat (1952) and other workers, which requires that the cells at the summit of the apex do not divide rapidly enough during vegetative growth to allow cells to be displaced into the flanks of the apex, which is occupied by the “anneau initial”, a self-perpetuating ring of rapidly dividing cells in which the leaves are initiated. Only at the onset of flowering would the cells of the “méristème d’attente” begin to divide rapidly and result in the formation of the flower or inflorescence.

The controversy has continued mainly because of a lack of reliable measurements of the rates of division within the shoot apex. Measurements of the frequency of mitotic figures cannot, in the absence of other information, be used as indications of the rates of cell division. It has been repeatedly pointed out (e.g. Brown, 1951) that the mitotic index
8. The Shoot Apex

is a measure only of the proportion of cells in the act of mitosis at the time of observation, and in a completely asynchronous meristem indicates the proportion of the whole cell cycle which is spent in mitosis. If, and only if, the absolute length of time spent in mitosis remains constant, and changes in the length of the cell cycle are due entirely to changes in the length of interphase, is the mitotic index proportional to the rate of division. There are, however, many instances in which this is not so. In Zea roots (Clowes, 1961) the mitotic index was shown not to be proportional to the length of the cell cycle. In Helianthus roots the mitotic index remained constant despite differences in the length of the cell cycle of more than 7-fold (Burholt and Van’t Hof, 1971), and conversely, in Pisum roots, large variations in mitotic index occurred without changes in the length of the cell cycle (Van’t Hof, 1965b). This was because, in both cases, the length of time spent in mitosis altered.

Labelling indices, based on the proportion of nuclei which become labelled after the application of radioactive DNA precursors (usually \( ^3H \)-thymidine), can sometimes be even more misleading. The percentage of the cells which becomes labelled after supplying \( ^3H \)-thymidine is proportional to the number of cells in S which, in an asynchronously dividing tissue, is a function of the proportion of the whole cell cycle spent in S. Like the mitotic index, the labelling index would be a function of the rate of division only if the length of S remained constant, irrespective of the length of the cell cycle. It has been shown that this is not the case for the shoot apices where it has been measured. The length of S varied with the length of the cell cycle in Rudbeckia (Jacqmard, 1970) and Pisum (Lyndon, 1973) so that the proportion of cells in S remained relatively constant in the different parts of the apex. It is quite clear in these plants that the labelling index does not give an estimate of division rates in the shoot apex. This can be demonstrated diagrammatically by comparing a map of the rates of division with a map of the labelling index (Fig. 1). Cells which are undergoing endomitotic duplication of their DNA will also become labelled and (as discussed later) this invalidates many of the conclusions previously drawn about the rates of division in pteridophyte apical meristems.

There are, however, some plants where the labelling index is informative and these are plants in which there is a region of the apex which does not label at all with \( ^3H \)-thymidine whereas other regions do. The central zone at the centre of the Helianthus apex did not become labelled after 24 h or 48 h exposure to the label whereas cells on the flanks of the apex did become labelled (Steeves et al., 1969). This was interpreted as showing that the central zone cells did not synthesize
DNA during the course of the experiment and therefore, if they were dividing at all, were almost certainly dividing much more slowly than the peripheral cells. A convincing demonstration that the central zone cells at the summit of the tobacco (*Nicotiana*) apex do not divide during the growth of the apex has been provided by a series of experiments with excised, cultured apices which initiated leaves at a steady rate (Sussex and Rosenthal, 1973). The peripheral cells all became labelled over a 72 h period of application of $^3$H-thymidine, but the central zone cells remained unlabelled. It was possible to do the converse experiment and show that when the central zone cells were labelled, the label remained in them. Apices were cut into four, longitudinally, and supplied with $^3$H-thymidine which was incorporated into all the cells as a new apex was regenerated. Once this was achieved and the apex was placed on unlabelled medium, the label became dissipated from the peripheral cells by the growth and division of these cells, but the cells of the central zone retained their label, indicating that they were not dividing.

The first direct measurements of the rates of division in different regions of the shoot apex were made on *Trifolium* by the use of colchicine (Denne, 1966a). If the applied colchicine results only in the inhibition of exit from metaphase, and has no other effects, then the rate of accumulation of colchicine-metaphases equals the rate of division (Evans *et al.*, 1957). If the cells are all exposed to colchicine for the same time, then the percentages of colchicine-metaphases in different regions of the apex will be proportional to the rates of division in these regions. In *Trifolium* the rate of division of the summit cells measured by this method was about one-half of that of the cells in the flank regions of the apex where leaves are initiated. This same method was used to measure in detail the rates of division in the pea apex, with very similar results (Lyndon, 1970a). Both these plants, *Trifolium* and *Pisum*, had the advantage of having a distichous leaf arrangement (i.e. the leaves arranged alternately, in two ranks on opposite sides of the stem) so that longitudinal median sections cut through not only the existing leaves and primordia but also the sites at which future leaf primordia would arise. Rates of division have also been measured by the colchicine method in *Datura* (Corson, 1969), *Chrysanthemum* (Berg, quoted in Gifford and Corson, 1971), *Solanum* (Leshem and Clowes, 1972) *Sinapis* (Bodson, 1975) and *Coleus* (Saint-Côme, 1973). In all cases, the rate of division in the cells at the summit of the apex was about half or a third of that on the flanks (Table I).

The lengths of the cell cycle in the different regions of the shoot apex have also been measured by labelling techniques, though these are more
difficult to apply to shoot than to root apices. Firstly, there is difficulty in getting the label taken up by the apex. Secondly, the characteristically low mitotic index in shoot apices means that methods which depend on the scoring of the percentage of labelled mitotic figures require many apices, but because of the necessity of dissecting out each apex before application of the label this is not a requirement that can be met easily, if at all. In these circumstances it is not surprising that there have been only a few measurements made by labelling techniques of the cell cycle length in the shoot apex. In fact the only one to employ the technique of scoring the percentage of labelled mitotic figures was that for *Rudbeckia* (Jacqmard, 1970). This showed that the cell cycle was longer for the central zone (at the summit of the apex) than for the flanks of the apex (Table I), but an exact determination could not be made because the cell cycle in the central zone was longer than the length of the experiment (40 h). In *Isoetes* labelling was also used, but again the length of the cell cycle in the summit cells was longer than that of the cells of the flanks and longer than the period of the experiment (Michaux, 1969). To measure the length of the cell cycle in the summit cells of *Polytrichum* and *Coleus*, continuous labelling experiments had to extend over more than 2 weeks (Hallet, 1969; Saint-Côme, 1969).

Table I. Length of the cell cycle (hours) at the summit (central zone) and on the flanks (the region of leaf initiation) of vegetative shoot apical meristems

<table>
<thead>
<tr>
<th>Region of apex</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Summit</strong></td>
<td><strong>Flanks</strong></td>
</tr>
<tr>
<td><em>Pisum</em></td>
<td>70</td>
</tr>
<tr>
<td><em>Rudbeckia</em></td>
<td>&gt;40</td>
</tr>
<tr>
<td><em>Datura</em></td>
<td>76</td>
</tr>
<tr>
<td><em>Isoetes</em></td>
<td>&gt;53</td>
</tr>
<tr>
<td><em>Trifolium</em></td>
<td>108</td>
</tr>
<tr>
<td><em>Chrysanthemum</em></td>
<td>140</td>
</tr>
<tr>
<td><em>Solanum</em></td>
<td>117</td>
</tr>
<tr>
<td><em>Polytrichum</em></td>
<td>360&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Coleus</em></td>
<td>237</td>
</tr>
<tr>
<td><em>Sinapis</em></td>
<td>288</td>
</tr>
</tbody>
</table>

<sup>a</sup> Apical cell.
presumably growing in a steady state, is proportional to the rate of division. This is because the time spent in mitosis remains constant, and changes in the length of the cell cycle as a cell is displaced down the apex are almost entirely due to changes in the duration of interphase (Denne, 1966a; Corson, 1969; Lyndon, 1973). The estimates of relative division rates based on the mitotic index are therefore, luckily, probably correct. Whether it is safe to assume that this is always so in the shoot apex is more doubtful. Until it is known in each case how the mitotic index is related to the cell cycle it is unsafe (as the data from the root meristem have shown) to assume that it is an indicator of the cell division rate.

It is also unsafe to use cytological characteristics as indicators of division rates. The central zone in the pea consists of cells at the summit of the apex which can be distinguished by their staining characteristics, by their slightly larger size compared to cells in the rest of the apical dome and by their larger nuclei (Lyndon, 1973), and it corresponds to the region which has been thought of as having a slow division rate (Nougarède, 1967). It may be argued that perhaps the central zone of the pea is not so well marked as that in some plants. Nevertheless the region of low division rate is much more extensive than the central zone (Lyndon, 1970a). In this plant at least, the cytological characteristics do not correspond to the region of low division rate. In the pea, the central zone and the cytological characteristics which typify it are not a function of division rate but indicative of some physiological state of which we so far know nothing. There is no reason to think it would be any more valid in other plants to infer relative division rates from cytological characteristics than it is in the pea.

The clearest indication of relative division rates seems to be given by the colchicine method. It has the further advantage that, since the number of metaphases observed is usually much greater than mitotic figures in an untreated plant, it is easier to get more detailed information about the distribution of division rates in the shoot apex. By superimposing data from several apices of the same developmental age it is possible to construct maps showing the distribution of division rates in sections or on the surface of the apex (Fig. 1a). Using this method on the pea apex, with the apices sorted into developmental stages within a single plastochron according to the length of the youngest primordium, maps showing the distribution of rates of division throughout the apex (using all the sections of the serially sectioned apices) were made (Lyndon, 1970a). These showed that as the apex grew between the initiation of one leaf and the next a plate of rapidly dividing cells became established between the axil of the newly formed primordium
8. The Shoot Apex

and the next oldest primordium. That this was in fact a plate of cells could be shown by building up from the data sections in other planes than the median. Just before the next primordium appeared as a bump (at the maximal area phase of the plastochron), the structure of the apex in terms of rates of division was that depicted in Fig. 1a. There is a bowl-shaped area with a low division rate at the summit of the apex, indeed extending to most of the apical dome. The incipient primordium and the axial tissue subtending it have an intermediate rate of division and separating these two zones is the plate of faster dividing cells. This plate is extended downwards at the sides of the apex and links up with the

procambium which is only easily visible in sections a little lower down the stem. Although the position of the incipient procambium (which is hardly visible at this level) is readily indicated by regions of rapid divisions at the sides of the apex where the stipules will be formed, the procambium which can be seen differentiating in the youngest primordium does not seem to consist of cells having this high rate of division. It seems to have the same rate of division as the other cells around about it. The analysis was not extended into the developing nodes and internodes; it would be interesting to see whether the plate of rapidly dividing cells disappears as the node develops or whether it persists as a nodal plate. Is the development of the nodal structure in fact associated with a higher division rate than the cells around it? This seems quite possible, but as yet we have no data on this point.

All the data point to the existence in all or most shoot apices of a gradient in the rate of cell division and growth from a minimum at

![Fig. 1. Median longitudinal sections of *Pisum sativum* shoot apices. (a) Relative rates of division, proportional to the density of the points, each of which represents a colchicine-metaphase (Lyndon, 1970a). (b) Distribution of labelled cells after supplying $^3$H-thymidine for 2 h (Lyndon, 1972a).]
the summit of the apex to a maximum in the region of leaf initiation. This raises the questions of how the rate of division is controlled, whether the gradient in division rates is essential to the functioning of the apex, and what the significance of this gradient may be.

2. Cell Division and the Displacement of Cells from the Summit of the Apex

If there are groups of cells in the shoot apex which do not divide, or divide very slowly, one would expect them to be found somewhere near the summit, just as the quiescent centre in the root is located at the tip of the promeristem, otherwise they would soon be carried out of the meristem by the growth and division of the cells distal to them. Experiments on lupins have shown that when individual cells at the summit of the shoot apex were killed by being punctured with a fine needle they were invariably displaced down the apical dome (Soma and Ball, 1963). It may be argued that the puncturing stimulated in the summit cells divisions that would not otherwise have occurred and that this resulted in the displacement of the punctured cell. When a carbon particle was placed on the apex without apparently damaging the cells, in some plants the particle was still at the summit after 6 weeks, indicating that the surface cells had not been displaced and that division and growth were therefore very slow at this point in the apex.

The carbon particles which were displaced (and this was most of them) took a minimum of 28 days to move off the apical dome, a distance (judged from the illustrations in Soma and Ball, 1963) of less than 20 cell diameters. A movement of 20 cell diameters could be achieved in 4–5 cell generations if all the cells were dividing and giving rise to new cells only in the longitudinal direction. Since divisions give rise to cells laterally as well as longitudinally the rate of division to give this degree of longitudinal displacement would have to be about twice as great, or about 8–10 cell generations in the 4 weeks. This would be equivalent to a cell generation time of the order of 3 days, which is similar to the values obtained by direct measurements (Sunderland and Brown, 1956; Sunderland, 1961). In the apices in which a cell was punctured, the wounds moved off the apical dome in a minimum of about 3 weeks, again equivalent to a mean cell generation time of about 3 days. The occurrence of cell displacement in the apex of *Lupinus* shows that the absence of labelling by $^3$H-thymidine in the cells at the summit of the *Lupinus* apex (Nougarède, 1967) is not a reliable indication of the histogenic function of these cells. It is worth noting (Fig. 2) that even if the rate of division of all the cells in the surface of the apical dome were exactly the same a cell would be displaced with a velocity which would accelerate exponentially with increasing distance.
from the summit. The observation that cells are displaced less rapidly at the summit of an apex than further down the flanks (Loiseau, 1962) does not in itself allow us to infer that the rate of cell division at the summit is also less.

In the lupin about nine new leaves were initiated during the time taken for a cell to be displaced down the apical dome and into a leaf primordium (Soma and Ball, 1963). In plants which have a long vegetative phase there would be a greater possibility that any cell at the summit of the apex could be displaced off the dome and into the leaf primordia. Plants which have an absolute photoperiodic requirement in order to flower can be kept vegetative for long periods. Such a plant is Silene, a long-day plant which has been maintained vegetative for more than 6 months during which time more than 40 pairs of leaves were initiated at a steady rate (Lyndon, unpublished data). A cell would have to be displaced about 14–20 cell diameters' distance from the summit to be incorporated into the leaf primordia.
and this would be achieved in about 8 cell generations. Even if the cells of the apical dome were dividing on average only about once every 3 weeks this would be sufficient to result in displacement of cells down from the summit of the dome and into leaf primordia. In fact the cells in the *Silene* apex divide on average once a day (Miller and Lyndon, 1975), so that even if the cells at the summit were dividing at only about one-twentieth of this rate they could still be displaced down the apex and incorporated into leaf primordia. The fact that the cells at the summit of the apex may be dividing slowly compared to cells in the rest of the apex is therefore not a barrier to their being functional as initials which can contribute cells to the leaves. Although extremely low rates of division in the cells at the summit of the apex may not be detected by histological examination of apices they can be demonstrated by the use of chimaeras, which show that these summit cells, even when they divide infrequently, may in fact play an important role in the growth of the vegetative plant (Stewart and Dermen, 1970).

The concept of the "méristème d’attente" is therefore not likely to be of general application. It may be descriptive of the situation in plants such as *Helianthus* and *Nicotiana* in which the cells of the central zone do not appear to divide in the apices which have been examined. It may also have a use in describing the situation in plants such as *Zea*, *Pisum* and *Lupinus* in which the apical dome grows during the vegetative phase. If the vegetative phase is restricted and the plant fairly quickly makes the transition to flowering then it could well be that the cell divisions in the apical dome could give rise only to growth of the dome itself, during vegetative growth. Such a situation has not yet been quantitatively and rigorously demonstrated, but there seems no reason why it should not occur in some plants.

The displacement of cells from the summit of the apical dome has also been observed directly (Newman, 1956; Ball, 1960; Ball and Soma, 1965). *Tropaeolum* apices were observed by reflected light, under a dissecting microscope, tracings were made (with a camera lucida) of the outlines and positions of the apical cells, and the displacement and division of cells at the summit were observed (Newman, 1956). More complete records were obtained of growth and division of cells in the *Vicia* apex (Ball, 1960). The displacement of cells from the apical summit and into the developing primordia was clearly recorded. In Newman's experiments the apices began to dry out between observations and in Ball's experiments the apices were excised and cultured on media which included coconut milk and gibberellin. It has been argued that these apices may therefore not have been behaving in the same way as apices in the intact plant (Nougarède, 1967). However,
they were, as far as could be judged, quite normal and comparable to apices in intact plants.

Once a cell leaves the summit of the apex it becomes displaced into regions where the rate of division is faster and the cell cycle is shorter. However, it is possible, as has been illustrated for the pea (Lyndon, 1973), that the rate of displacement is relatively great compared to the length of the cell cycle so that a cell may always be in the process of speeding up its cell cycle and may not reach a steady state, so far as cell cycle length is concerned, until it is incorporated into a leaf primordium. In those cells which form the pith the cell cycle may no sooner get to minimum length than it begins to lengthen again as the cells mature and pass out of division. The only cells in a reasonably steady state of cell cycle length may be those in the primordium, those in the procambium (for a short time) and those at the extreme summit of the apex.

The observation that sometimes a carbon particle was not displaced from the summit of the apex over a period of several weeks (Soma and Ball, 1963) is not inconsistent with displacement of cells being the norm. For as Newman (1961) has pointed out, there must be at least three cells at the summit of the apex which are the descendants of previous apical cells and at least one of these must be the ancestor of subsequent apical cells. Newman has called these cells the “continuing meristematic residue”. Presumably the immobile carbon particles have been placed fortuitously over these particular cells.

Ball’s view (and Newman’s) seems to be that the cell which will be found at the centre of the dome cannot be predicted very far in advance, and may depend on the nutritional, hormonal and cellular environment. The likelihood of all cells eventually finding themselves in this position may not be the same, because in the steady state growth of the shoot the future roles of cells in different parts of the apical dome will be relatively fixed if the system is not perturbed.

In some pteridophytes there seems to be a specialized situation in which the cells at the summit of the apex may have lost their histogenic potentialities. The shoot apex of *Equisetum* resembles the root apex of other pteridophytes in having an apical cell which may divide but is polyploid (D’Amato and Avanzi, 1968; Avanzi and D’Amato, 1967). The structure of the apex suggests that when the apical cell has become polyploid it gives rise to adjacent polyploid cells by division but that the growth of the shoot and the initiation of leaves occurs as a result of the growth and division of the diploid cells less distal in the apex. This is at first sight comparable to a “méristème d’attente” except that it is not known whether the polyploid pteridophyte apex has any
histogenic contribution to make during subsequent development of the plant or whether this signals its end as an effective contributor to the growth of the shoot. The fern, *Dryopteris*, has been used for many experiments and observations on the functioning of the shoot apex. It would be of interest to know for this plant what contribution, if any, the apical cell and its immediate derivatives make to the regions of the apex where the leaves are initiated.

3. **Cell Division and the Shape of the Apical Dome**

The apical dome of many plants approximates to a hemisphere or a portion of a hemisphere. It can be demonstrated that this shape could be maintained at a growing point showing isotropic growth (equal in all directions) only if there is a gradient (a cosine gradient) of decreasing relative growth rate from a maximum at the summit of the dome to zero at the base (Green *et al.*, 1970). This is the opposite of what is characteristically found in shoot apices: a minimum growth rate at the summit increasing to a maximum growth rate on the flanks of the apical dome where the leaves are initiated. The growth of the apical dome therefore cannot be isotropic; there must be some anisotropy in its growth. One would expect that the longitudinal component of growth would increase with distance from the summit of the apex at the expense of the latitudinal component. These two components one would find to be equal if growth were isotropic.

Despite a claim to the contrary (Lyndon, 1970b), longitudinal growth does seem to predominate in the pea apex. Re-examination of my earlier data for orientation of mitotic spindles on the surface of the pea apical dome can be made from Fig. 3. It is difficult in view of the small number of divisions to know whether growth at the extreme summit is isotropic or not. Over most of the surface of the apical dome the longitudinal (radial) component of growth is about twice as great as the latitudinal component. The anisotropic growth on the surface of the pea shoot apical dome therefore appears to be consistent with that expected for a markedly hemispherical apex with a gradient of increasing rate of growth from the summit downwards. The predominance of the longitudinal component of growth was also seen in *Impatiens* apices which had had marks of carbon black placed on their summits. As the marks became displaced down the apical dome they often became elongated radially (Loiseau, 1962). In apices which are less markedly domed than the pea one might expect growth to be less anisotropic and in apices like the *Helianthus* apex, which are essentially planar, to be isotropic.

These considerations lead to the conclusion that the shape of the
8. The Shoot Apex

![Diagram of the shoot apex]

Fig. 3. Orientation of growth as indicated by the orientation of mitotic spindles in the surface cells of the shoot apex of *Pisum sativum*. (a) Minimal area stage (just after initiation of a leaf at P1). (b) Maximal area stage (just before initiation of a leaf at P2).

The diagrams represent the surface of the apical dome as seen from a point directly above the highest point of the dome. As seen from the tables which summarize the diagrams, the orientation of the mitotic spindles is predominantly longitudinal (radial) rather than latitudinal (tangential). (Data from Lyndon, 1970b.)

The shoot apex is probably governed not by variations in the growth rates of the cells in the apical dome but by the relative amounts of longitudinally orientated and latitudinally orientated growth, i.e. by variations in the directions of growth. Experimental observations which support this view are those which were made on *Chrysanthemum* apices treated with tri-iodobenzoic acid (Schwabe, 1971). The rate of growth, measured as the cell doubling time, did not change in the treated plants (compared with the untreated controls), although their apices became narrower and more elongated. Unless there had been a complete reversal in the growth rate gradient in the apex (and this seems unlikely) this change in shape could only have been achieved by a change in the
direction of growth with the longitudinal component being emphasized at the expense of the latitudinal component.

If the gradient of increasing rates of cell division and growth from the summit of the apex downwards is not involved in maintaining the shape of the apex, then its significance must be looked for in other aspects of apical structure and function, of which leaf initiation is the most likely.

C. Control of Division Rates

1. Control Points in the Cell Cycle

The information from the colchicine data in conjunction with measurements of mitotic index indicates that in shoot apical cells with different cycle lengths the absolute time spent in mitosis is constant, and it is interphase which varies (Lyndon, 1973). With more detailed information about the lengths of the various parts of interphase \( G_1, S, G_2 \) it is possible to see what stage in the cell cycle is blocked in slowly dividing cells. For instance, in the quiescent centre of the root the cells are held in the \( G_1 \) phase so that the bar to progress through the cell cycle is a block at the stage of DNA synthesis (see Chapter 7, this volume).

Despite the experimental difficulties involved in labelling shoot apices, Jacquemard (1970) succeeded in measuring the lengths of the component phases of the cell cycle in the cells of the flanks and the incipient pith of the \textit{Rudbeckia} apex, although complete measurements could not be made for the summit cells (central zone). \( G_2 \) and \( S \) were longer in the summit cells than in the cells on the flanks of the apex, but neither the length of the whole cycle nor \( G_1 \) could be obtained for the slowly dividing summit cells. However, these results showed that the cells of the shoot were probably different from those of the root in having both \( G_2 \) and \( S \) extended, whereas in the root the major extension was in \( G_1 \).

The only estimates of the lengths of all the phases of the cell cycle in each region of the shoot apex are those for the pea (Lyndon, 1973). The method used was that of Mak (1965). This involves labelling the cells with a short pulse of \(^3\text{H-thymidine} \) so that the cells in \( S \) become labelled. The apices are then sectioned and stained with Feulgen. Autoradiographs are made of the sections and the percentage of labelled nuclei (those in \( S \)) is scored. The DNA content of each unlabelled interphase nucleus is then measured by microdensitometry so that the proportions of \( 2\text{C} \) (\( G_1 \)) and \( 4\text{C} \) (\( G_2 \)) nuclei can be found. The proportion of cells in mitosis is given by the mitotic index. From these data the proportions of cells in each phase of the cell cycle (\( G_1, S, G_2 \))...
and M) can be calculated, and when a correction is made for the age gradient in a population of exponentially dividing cells (Nachtwey and Cameron, 1968) the relative lengths of time spent in each phase can be calculated. If the length of the whole mitotic cycle in each region is also known (e.g. from colchicine-metaphase data) then the absolute lengths of the phases in each part of the apex can be calculated. A simpler method for finding the proportions of cells in $G_1$ and $G_2$ can be used which requires only the measurement of average values for DNA per cell (Lyndon, 1973). Both methods gave similar values for the lengths of the phases of the cell cycle in the pea apex and the means of both sets of measurements are given in Table II. The cell cycle in the central zone was more than twice as long as in the rest of the apex. This longer cycle was not because of the extension of any one part of interphase; $G_1$ and $G_2$ were extended by about 2.5 times and 3.6 times respectively, and $S$ was also extended but only by about 1.5 times. Since the amount of DNA to be synthesized during $S$ was the same in all parts of the apex (from 2C to 4C) the longer period in $S$ means that the actual rate of DNA synthesis is slower in the more slowly dividing cells.

It remains to be seen whether the plants so far examined are typical. If they are, the results suggest that there is no one point in the cell cycle which is blocked in the cells at the summit of the shoot apex but that the whole cycle is slowed down, except for the actual process of division (M) itself. The lengthening of $G_1$ and $G_2$ to a greater extent than $S$ suggests that the main control points are at the transition from $G_1$ to $S$, and $G_2$ to M. Gibberellic acid, when it affects the cell cycle (probably shortening it) in the apical meristem, increases the proportion of cells in M and S as indicated by the increase in the mitotic index and labelling index respectively (Bernier et al., 1967a). These observations suggest that gibberellic acid may also act at the points of entry into S and M. These are the main points at which carbohydrate level also controls the rate of progress through the cell cycle in roots (Van't Hof
and Rost, 1972). The rate of cell division in cultured *Vicia* shoot apices is a function of the glucose concentration (Ball and Soma, 1965) which could presumably be controlling entry into S and M as in the roots.

What the relative concentrations are of substances such as gibberellic acid and sugars in the different regions of the shoot apex is unknown. However, the concentrations of macromolecules such as protein and nucleic acid are less in the slowly dividing cells at the summit of the pea apex than on the flanks of the apex where cell division is faster (Lyndon, 1970c). This is also shown by the ubiquitous occurrence of zonation patterns, visible after staining, in which the lightest staining, indicating the lowest concentration of protein and nucleic acid, is in the cells at the summit of the apex. It could possibly be that the concentration of small molecules is also less at the summit in the slowly dividing cells, but this is complete speculation.

2. Replication of Sub-cellular Components

Measurements of the amounts of RNA and protein per cell show that in the pea apex all cells have the same gross composition and so the rate of increase in RNA and protein is a function of the rate of cell division (Lyndon, 1970c). Whether this means that the rates of *synthesis* of these substances are also a function of the rates of cell division cannot be demonstrated until adequate experiments are done which take into account the possible complications of differential rates of uptake of precursor molecules and the sizes of the pools of endogenous metabolites (Lyndon, 1972b).

But the cells in different regions of the apex do not have the same ultrastructural composition (Gifford and Stewart, 1967) and this implies that the rate of formation or replication of subcellular components may not be correlated with the rate of cell division but may be controlled independently. A quantitative examination of the ultrastructure of the pea apex has shown that as cells are displaced down the apical dome and become incorporated into the leaf primordia the number of organelles per cell tends to increase, implying that the replication of organelles is going on faster than cell division (Lyndon and Robertson, 1975). In other cells, which eventually form the pith of the stem, the number of mitochondria per cell increases but the number of plastids per cell remains the same, suggesting that the rate of replication of at least mitochondria and plastids may be controlled by different mechanisms (see Leech, Chapter 4). The control of the rate of cell division is therefore only one facet of the problem of the control of replication at all levels of organization from the molecule to the cell.
3. Diurnal Rhythms

A plant growing under natural conditions is exposed to a diurnal fluctuation of light intensity and often of temperature as well. The plant might be expected to alter in carbohydrate and metabolic status, and it would not be surprising if the rate of growth and cell division at the shoot apex also varied diurnally.

There are a number of instances in which diurnal peaks of mitotic index have been found. In many cases (Bünning, 1952; Denne, 1966b; Karsten, 1915; Lance, 1952) the experiments have been done under normal conditions, i.e. alternations of light and dark periods and uncontrolled temperatures. The mitotic peaks are usually found during the dark period, but Denne (1966b, c) found the maxima to be in the light, in the afternoon. Other workers have found no evidence of a mitotic rhythm (Savelkoul, 1957; Popham, 1958; Jacobs and Morrow, 1961). Rotta (1949) and Karsten (1915) were able to demonstrate that the mitotic peak recurred after 24 h even when the plants were held in constant darkness at constant temperature. Rotta’s data showed the peak mitotic index to be about 90%. This can only mean that the cells were highly synchronized. This being so, the recurrence of the peak after 24 h in constant conditions implies that the cell cycle was 24 h in these plants. In Tradescantia (Denne, 1966c) the average cell cycle was 4 days, and this may be partly why the maximum values for mitotic index were only about 7%. Similarly, in Trifolium (Denne, 1966b) with a cell cycle of 64 h, the maximum mitotic index was only about 5% but was again in the light.

The occurrence of periodicity has been linked to the occurrence of the light/dark cycle, but it is puzzling why it is sometimes not found and why, when it is, the peak is in some plants in the dark and in others in the light. It may be of significance that in the plants in which maxima have been found only in the dark, the light has been provided by artificial illumination, of the order of a few hundred foot-candles, but that in the other instances, when the maxima have been smaller or occurring in the light, or non-existent, the plants have been grown under the much greater intensity of natural illumination. It is relevant here to consider the situation in unicellular algae, in which cell division is usually restricted to the dark period (Jones, 1970). In Euglena gracilis, which can be autotrophic or heterotrophic, the nocturnal periodicity of division can be removed, so that the cells will divide during the light as well, by the provision of a rich food supply (Leedale, 1959). If the periodicity which has been observed in the feebly lit shoot apices is a similar phenomenon, then it would be expected that strong
illumination (as with natural light) could allow sufficient photosynthesis and thus increased food supply to suppress the periodicity of division. If this is so, and the observations strongly suggest that it is, then a periodicity in cell division might occur if the plants are grown under artificial conditions and insufficiently illuminated, but under adequate conditions of illumination one might expect a periodicity to be absent.

The other usual variable in these experiments is temperature. Bünning (1952) found that periodicity of mitosis was absent in fluctuating temperature in *Vicia* roots. This does not necessarily imply that there was no effect on the rate of division, because Burholt and Van’t Hof (1971) showed that the mitotic index remained constant, at 7%, in *Helianthus* roots over the temperature range 10–35°C even though the cell cycle varied from 46 h at 10°C to 6 h at 35°C. Lack of change in the mitotic index does not therefore imply a lack of change in division rates if the cells remain asynchronous. On the other hand, if the cells are synchronous, then the cell cycle can remain constant even though there are peaks of mitotic index (which is what one would expect). If a mitotic maximum occurs for the first time, the occurrence of a peak implies that either cells have been speeded up in interphase, so that a greater than usual number of cells reach mitosis together or else that mitosis itself has been retarded so that cells tend to pile up in mitosis. The occurrence of a single mitotic peak gives no information about how the cell cycle has temporarily changed, but it does indicate that some degree of synchrony has been introduced into the system (see Chapter 3). Even if there are successive or diurnally recurring peaks of mitotic index these are not necessarily at intervals of one cell cycle unless each peak is large enough to account for the division of all the cells.

Although the evidence suggests that there are plants, grown under some conditions, which show a diurnal periodicity in cell division it is doubtful whether this is a universal feature of shoot apices. When it does occur it may be accompanied by diurnal fluctuations in growth rate at the apex, but this possibility has yet to be investigated.

III. CELL DIVISION AND LEAF INITIATION

A. Is an Increase in Division Rate Necessary?

In considering the growth of the apex as a whole (p. 287) it was demonstrated that once the young primordium is initiated its relative growth rate need be no greater than that of the apical dome, and could in fact be less, and yet the form of the apex, with the young leaves overtrop-
An increased rate of cell division may not, therefore, be necessary to maintain the growth of the primordium once it is initiated, but is it necessary for the initiation process? Since the primordium grows out in a direction different from that of the continued growth of the apical dome then this different direction of growth is somehow brought about during primordial initiation and first becomes obvious with the occurrence of periclinal divisions in an inner layer of the tunica, which otherwise shows only anticlinal division. But is this the primary event or is it a secondary event, as it would be if the change in the direction of growth were brought about by pressure of a growing mass of cells inside it in the corpus? To what extent is the inception of a primordium the result of a change in the polarity of growth and to what extent a change in the rate of growth?

The changes in the rate of cell division that are associated with leaf initiation can be followed by comparing the rates of division in those regions of the shoot apex which represent a developmental sequence leading to leaf formation. The position occupied by the cells which form the incipient primordium are, in the plastochron before the primordium becomes visible, designated I₁ according to the terminology of Snow and Snow (1931) and in the plastochrons before this, I₂, I₃, etc. Since these represent the positions of future leaves, which are predictable, the cells in these stages of the developmental sequence can be identified by their position alone. Plants so far examined in sufficient detail are Trifolium (Denne, 1966a), Datura (Corson, 1969) and Pisum (Lyndon, 1970a). In each case the rates of division in the I₂ and I₁ regions were similar, differing at the most by no more than about 30%, so that the increase of cell division rate leading to leaf initiation was not very marked. However, these represent measurements of regions of the apex of which the delimitation is necessarily arbitrary, and if there are variations in division rates within these regions then the overall values may be misleading.

The detailed maps of division rates in the pea apex (Lyndon, 1970a) showed that the I₂ and I₁ region as delimited each consist of distal regions of slow division in the apical dome and regions of much faster division in the more proximal region at the base of the apical dome (Fig. 1a). Hussey (1972) pointed out that in fact there was in the I₁ region of the pea a small part which had an apparently higher rate of division than anywhere else in the apex and he suggested that this was a growth centre responsible for a rapid growth which resulted in
the formation of the primordial bulge. A similar growth centre consisting of cells with a higher rate of division was also observed in the apex of the tomato (Hussey, 1971). It was suggested that the initial increase in division rate which led to the formation of the primordium in the pea was the increase in division rate from \( I_3 \) to \( I_2 \). Hussey (1972) is probably right in concluding that this increase in division rate in \( I_2 \) is an essential part of the functioning of the shoot apex, but it seems less certain that it is an obligatory part of the processes leading to formation of the primordial bulge itself, especially since the initial increase in division rate occurs (at the transition from \( I_3 \) to \( I_2 \)) two plastochrons, or approximately four cell cycles, before the primordial bulge is formed.

Would the existence of a greater rate of division at the base of the \( I_1 \) region necessarily lead to the formation of a bulge? This would seem to depend on the cellular structure of the apex. If the apex consists of a tunica of two or three layers of cells, overlying a corpus in which divisions are in all planes, then an increase in division rate in the corpus would result in a nodule of cells which could be visualized as exerting pressure on the tunica cells and so stimulating them into division (Fig. 4a). Alternatively, if the apical dome in the first half of the plastochron is all tunica, as claimed for the pea (Lyndon, 1970b), then an increase in division rate should still give anticlinal growth but with some outward buckling of the surface, which could be visualized as resulting in a tension on the inner layers and thus stimulating periclinal divisions.

![Fig. 4](image-url) Possible modes of growth of the apex during leaf initiation. (a) An increased rate of division in the shaded part of the corpus (C), giving growth of the shaded tissue, could result in pressure on the tunica in the region T so that it would be forced out as a bulge. On this model the divisions in C would be typical corpus ones, in all planes, throughout the plastochron. (b) An increased rate of anticlinal division in the shaded regions of the corpus, C, and tunica, T, could result in a tension on the cells at C which might result in outward growth to relieve the tension. The divisions in C would then contain a periclinal component, as a result of the outward growth, just before the leaf primordium began to be visible.
Fig. 5. Relative division rates, as shown by the distribution of colchicine-metaphases, in the epidermis of the median part of the pea apex at the point of leaf initiation. (a) Just before the leaf is formed. (b) At the initiation of the leaf primordium. (c) After one plastochron's growth of the leaf primordium.

The density of the dots is proportional to the rate of division. The greater rate of division in the basal part of the region (a and b) leads to faster growth of the abaxial surface of the leaf primordium in the early stages of its growth. The rates of division are less different on adaxial and abaxial surfaces once the primordium has become established (c).

in these inner layers (corpus). Note that the first possibility means that periclinal divisions are present in the corpus at all times, the second, that the formation of the bulge would be accompanied by tensions in the tissues at the onset of bulge formation (Fig. 4b). Neither of these explanations accords with the facts. The occurrence of a dome which is all tunica for part of the plastochron finds support in a number of observations (see below) and in several apices, including the pea, the tissues are not under tension (Hussey, 1973). The most plausible explanation is that the primary process in leaf initiation is the change in the plane of growth and that changes in the rate of growth are associated, but secondary, processes.

A characteristic feature of the early growth of the leaf primordium is that the growth of the abaxial surface is greater than that of the
adaxial surface so that the young leaf tends to grow upwards (Gifford, 1951; Girolami, 1954; Hussey, 1971). The increased division rate in the basal part of the I₁ region may be important in this upward growth of the primordium when it has been initiated, and Hussey (1972) gives measurements for the pea apex to support this contention. He shows that the increase in cell number in the longitudinal plane is because of a higher rate of cell accumulation (1.8 cells per cell per plastochron) on the abaxial side of the young primordium than on the adaxial side (1.1 cells per cell per plastochron). Since these are data of increases in cell number in only one plane (in the epidermis) they would accurately reflect the actual rates of division only if the orientation of growth is equally longitudinal and transverse on both the adaxial and abaxial sides of the primordium. If the proportions of longitudinal and transverse growth differed on the two surfaces of the primordium then these values would not represent the true division rates. Values for the actual division rates in the epidermis are available from measurements made by Lyndon (1970a) and from supplementary data, and are shown in Fig. 5. These confirm that the rate of division is indeed greater on the abaxial surface of the incipient primordium than on the adaxial surface. This difference can be regarded as a natural extension of the difference in division rates which is established as early as I₂ between the upper and lower parts of this region. It should, however, be noted that this process is not necessarily involved in the formation of the primordial bulge, but in the establishment of the shape of the bulge while it is being initiated. This may well be a process that is concerned with the establishment of the dorsi-ventrality of the leaf.

B. Planes of Division and Growth

Whether or not there is an increase in the division rate of the cells at the point of initiation of the new primordium at the time it emerges as a bump on the apex, there are changes in the directions of growth both on the surface and within the tissues of the apex which are associated with leaf initiation.

A detailed examination of the pea apex at intervals throughout a single plastochron showed that essentially no mitotic spindles which would give periclinal divisions could be found in the apical dome in the first half of the plastochron, whereas one-third of all mitotic figures would give periclinal divisions during the second part of the plastochron (Lyndon, 1970b, 1972a). This implies that for about 30 h out of the 46 h of the plastochron, i.e. for almost two-thirds of the time, there were no periclinal divisions in the whole of the summit of the apex and that
this was therefore all tunica, with only anticlinal divisions, so that the tunica at this time effectively extended inwards about six layers of cells from the surface. In the second part (one-third) of the plastochron, periclinal mitoses suddenly appeared and could almost always be found, so that the tunica became reduced to the typical single layer at the point of leaf initiation and to two layers at the extreme summit of the apex (see Fig. 3 in Lyndon, 1972a). In the pea the number of tunica layers at the summit (and not just where the leaf forms) therefore seems to vary according to the stage of the plastochron. Is this an observation of general validity? There is in fact quite a considerable amount of evidence that suggests that it might be. Gifford (1954) cites a number of examples (Gifford, 1950; Cross and Johnson, 1941; Schnabel, 1941; Reeve, 1942, 1948; Rouffa and Gunckel, 1951; Zimm- erman, 1928) in which a more stratified appearance of the apex has been noted at certain times of the plastochron, often apparently at the maximal area phase, just before initiation of the new primordium. This would tend to correspond to the second half of the plastochron in the pea in which the periclinal divisions are found. However, it must be remembered that the most stratified appearance will be only after antici- nal divisions have gone on for some time, i.e. about half-way through the plastochron and at the time when periclinal divisions begin. Other workers have also noted changes in the number of tunica layers with the plastochron stage. In *Cosmos* periclinal divisions were found only in the corpus cells of the flanks (peripheral region) and only during the minimal area stage of the plastochron (Molder and Owens, 1972). The maximum degree of stratification was seen during the maximal area phase of the plastochron, i.e. when periclinal divisions were not found. In *Acorus* the stratification was also greatest during the maximal area phase of the plastochron, just before a new leaf emerged, and extended into the corpus so that as many as seven layers of cells were apparent at this stage (Kaplan, 1970). Soma (1958) also found in *Euphorbia* that maximal stratification occurred "in the stage prior to the maximal area phase" with minimal stratification "just after the in- itiation of leaf primordia". Observations on other plants can be inter- preted as showing maximal stratification in the minimal area phase of the plastochron (Shushan and Johnson, 1955; Sterling, 1949). Although changes in stratification have not always been seen, there is quite a body of evidence which strongly suggests that the absence of periclinal divisions in the apical dome for about a third or a half of the plastochron could be a characteristic feature of shoot apices. If so the pea would be typical, and the change in plane of growth would be, as suggested (Lyndon, 1970b, 1972a), the primary event in leaf initiation.
The occurrence of periclinal divisions in the $I_1$ region during the 16 h before the leaf buttress forms in the pea has been regarded not as the imposition of a new direction of growth but rather the lifting of a constraint so that the cells can divide and grow in all directions. The occurrence and maintenance of a 1:1:1 ratio of divisions in the three planes into which the data were classified are most readily understandable as a random orientation (Lyndon, 1972a). The control of the planes of growth and division is therefore thought to be exerted in the apical dome, to allow only anticlinal divisions during the first part of the plastochron. Leaf initiation is visualized as a process which depends on the temporary lifting of a restraint so that random growth can occur.

C. Significance of the Gradient in Division Rates: Models of Apical Growth

One way in which the gradient in the rate of division down the apical dome may be of importance is in its providing a difference in growth rates between the upper and lower surfaces of the primordium, as discussed on p. 308. But this does not seem a sufficient explanation if the growth of the abaxial surface of the primordium results from the establishment of a locus of rapid division only in the $I_1$ region of the apex.

It would be difficult, if not impossible, to alter experimentally the rates of growth and division in different parts of the apex, or to reverse the gradient, to see what effects this might have on the growth of the apex and the initiation of leaves. However, it might prove possible to gain some insight into the functioning of the apex if it were possible to build a convincing model in which the effects of altering division rates in different parts of the apex could be tested. With this in view, a computer simulation of the growth of the pea apex has been attempted, using as its basis the data obtained from the growth of living apices (Lyndon, 1975). The rate of division was programmed to increase as a function of distance from the summit of the apex to the region (on the flanks of the apex) about ten cells from the summit and then to decrease to a constant value with further distance from the summit of the apex, thus simulating the situation in a growing apex (Lyndon, 1970a, 1973). The plane of division was programmed to be entirely anticlinal throughout the whole of the apical dome for 30 h and then random (and so including periclinal divisions) in all cells, other than the epidermis, as apparently happens in the growing apex during the plastochron immediately before and during the emergence of a new primordium as a bump (Lyndon, 1970b, 1972a). The result of this simulation was the formation of a bulge on the flanks of the apex which
after two plastochrons of growth was comparable in size and position to the primordium which develops in the living apex.

The results obtained by varying the input parameters in this model suggest that, if the rate of division and growth did not increase with distance from the summit of the apex, primordia would not be formed on the apex at all. The apex would simply grow on like a filament showing tip growth. The increasing rate of growth and division of cells displaced down the apical dome ensures that, when the plane of growth is not restricted, the apex below the summit can grow out into lateral protuberances, the leaf primordia.

D. Rates of Division and Growth in Relation to the Length of the Plasto-chron

So far we have considered the factors involved in the initiation of a leaf and the processes leading to the growth of the bulge which is the young primordium. Other factors which require consideration are those which determine the site of leaf initiation and the length of the plastochron, which is a measure of the frequency of leaf initiation. Richards (1951) showed that the phyllotaxis is the resultant of three things: the rate of growth at the apex (i.e. the radial relative growth rate); the shape of the apical dome (which modifies the rate of radial displacement of cells and the rate of their tangential separation); and the size and shape of a primordium on initiation in relation to the size of the apical dome. The plastochron, the interval between the initiation of one leaf and the next, is usually considered to be the resultant of these factors.

The shape of the apex is probably determined by the directions of growth, as reflected in the planes of cell division. To what extent the rates or planes of cell division are involved in determining the size of a primordium on initiation is not known. The rate of cell division is a function of the relative growth rate, which is the third determinant of the rate of leaf initiation. One might expect that the faster the growth rate the faster would leaves be initiated. This is what was found for the maize, in which the relative growth rate of the apex increased during vegetative development (Abbé et al., 1951). The rate of leaf initiation also increased so that the plastochron decreased from 4-7 days to 0-5 day. The growth rate increased sufficiently also to allow an increase in the size of the apical dome as well as a corresponding increase in the size of each primordium on initiation (Abbé and Phinney, 1951; Abbé et al., 1941).

The rate of growth of the apex and the rate of leaf initiation are not
always positively correlated. In both the lupin and the rye while the apex was still vegetative the relative growth rate of the apex decreased, but the plastochron remained unchanged. Without other compensatory changes the apical dome would have become smaller and smaller, a trend which could not have been kept up for very long. In fact the apical dome actually increased in size. This was possible because "the size of each primordium, and the number of cells in it, progressively decrease as the dome develops. The size of the dome therefore increases because at the differentiation of each primordium a progressively smaller proportion of the cells in the dome is given over to the new primordium and a correspondingly larger proportion remains to constitute the new dome" (Sunderland, 1961). It is curious that in each case these factors compensated for each other so precisely that the plastochron remained constant.

The spruce (Picea) is similar to the lupin and the rye in that the relative growth rate of the apex decreased during development and the apical dome increased in size, but in Picea the rate of leaf initiation did not remain constant but became much faster, so that the plastochron shortened (Gregory and Romberger, 1972). Presumably the size of the primordia on initiation became smaller; unfortunately no data about this were given.

Another case in which the rate of growth and the rate of leaf initiation were not correlated was in Chrysanthemum apices, some of which were treated with tri-iodobenzoic acid (Schwabe, 1971). In the treated plants the relative growth rate of the apex remained unchanged, but the plastochron became longer. This was because the shape of the apex changed so that primordia were initiated further down its flanks.

These examples show that the relative growth rate, and the rate of cell division, is only one of the components of the apical system that determine the rate of leaf initiation. The site of initiation of a new leaf is also a function of these components, none of which can properly be considered in the absence of the others. So far, experimental analyses of the growth of the apex taking all these factors into consideration simultaneously do not seem to have been attempted, although the theoretical basis for their understanding has been carefully worked out (Richards, 1948, 1951, 1956).

IV. CELL DIVISION AND FLOWERING

The formation of the flower or inflorescence is generally thought to be accompanied by an increase in the rate of cell division in the apex.
The evidence for this is of several types. First, the increase in mitotic index which is often seen at the onset of flower formation has been cited as indicating an increase in the rate of cell division and a similar increase in the labelling index has been put forward as supporting evidence (Nougarède, 1967). Neither of these observations necessarily shows that the rate of division has increased. They only show that S and M occupy a larger part of the cell cycle in reproductive than in vegetative apices. Although this is consistent with a shortened cycle it could equally well be consistent with a lengthened or unchanged cycle time. This evidence is therefore not conclusive.

The increase in mitotic index which occurs in a number of plants just after floral induction (Bernier, 1971) has been studied in detail in Sinapis (Bernier et al., 1967b). The increase in mitotic index which occurs 30 h after the beginning of the inductive long day is associated with a release of cells from $G_2$ (Kinet et al., 1967) and is followed by an accumulation of cells in $G_1$ (Jacqmard and Miksche, 1971), indicating that the cells have been synchronized by the floral stimulus. A further peak of mitotic index occurs 32 h later, when floral morphogenesis is beginning, and indicates that a further degree of synchrony has been imposed on the cells (Bernier et al., 1967b). These changes are accompanied by increases in the rate of cell division as shown by the rate of accumulation of colchicine metaphases (Bodson, 1975). The first increase of mitotic index does not always need to be followed by induction. If Sinapis plants are given a 12 h photoperiod, which is not sufficient to induce them, the first rise in mitotic index still occurs, showing that it can be separated from the flowering process (Bernier et al., 1970). Although data are not available, it seems probable that the rise in mitotic index in this case is indicative of a temporary increase in division rate.

The rates of division during and subsequent to induction were measured in Datura by the rate of accumulation of colchicine metaphases (Corson, 1969). The cell cycle decreased from 46 h to 26 h on induction, but, as in Sinapis, the differential between the lateral and summit regions of the apex was maintained so that the summit cells were still the slower dividing cells as they are in the vegetative apex. In Coleus, however, the rate of division of the summit cells increased so that in the flowering apex it was the same as in the cells on the flanks (Saint-Côme, 1971). The changes in the MCGT during and after transition to flowering in lupin and vernalized rye were measured by rates of increase of cell number (Sunderland, 1961). The MCGT of the shoot meristems and the developing leaf primordia fell during vegetative growth, increased again on transition to flowering.
and, at least in the lupin, fell again when this transition had been accomplished.

This evidence is consistent with the idea that the rate of division increases during induction and may increase even more when the flower is formed. All these plants considered so far produce inflorescences, and an integral part of the flowering process is the growth of the axis to support the many flowers. It could be argued that in these plants there is a growth of the shoot apex during flowering which is not concerned with the formation and morphogenesis of the flowers themselves but which is concerned with the formation of the structure which subsequently bears the flowers. An attempt was made to separate these two processes—growth of the axis (essentially a continuation of the vegetative mode of growth) and formation of the flower itself—by the use of a plant in which the first-formed flower was terminal so that the apex itself became transformed into a flower as a first and direct result of induction. This plant was *Silene coeli-rosa*, a long-day plant. The MCGT of the cells in the apical dome (the apex distal to the youngest primordia) was measured by counting the number of cells in it at daily intervals over the course of several plastochrons, a plastochron being about 3.7 days (Miller, 1975). The MCGT was calculated to be 20 h in vegetative (short-day) plants and in plants during the 7 days required for induction, and 9 h in plants in which flower morphogenesis was just beginning (2–4 days after the end of the inductive period). This result, that the rate of division remained unchanged during induction and increased only during the process of flower morphogenesis, was confirmed by measuring the length of the cell cycle by a double labelling technique, which gave values of 20 h for plants which were vegetative or were undergoing induction and 10 h for plants in which flower formation had begun (Miller and Lyndon, 1975). The conclusion to be drawn from these experiments is that although an increased rate of cell division appears to be a necessary part of flower formation and morphogenesis it does not necessarily accompany the inductive process.

The rates of cell division and the changes in the planes of growth which accompany flower development and the formation of the floral parts have not yet been measured or described quantitatively. Only when this has been done, and the rates of formation of the primordia have also been measured, will there be a basis for interpreting the growth and morphogenesis of the flower in a way which has now become possible for the vegetative shoot apex.
7. The Shoot Apical Meristem

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MORPHOLOGY AND ANATOMY

A. Morphology

The external morphology of the pea shoot apex is shown in Fig. 7.1. Below the rounded apical dome the youngest leaf primordium is seen as a swelling on the side of the apex. As the primordium grows it extends around to the sides of the apex so forming a V-shaped protuberance, the lateral parts subsequently developing into the stipules. About one plastochron after its first appearance the primordium develops two lobes or protuberances about halfway between the axil and the tip. These are the first leaflet primordia. Subsequent pairs of leaflet primordia develop in acropetal succession as the primordium lengthens. Sometimes one or more pairs of leaflet primordia develop into tendrils or fail to develop at all. The stipules of the pea are characteristic in developing from the lateral parts of the primordium which gives rise to the leaf, and not from separate primordia of their own. The development and vasculature of the stipules have been described by Mitra (1950) and Nougarède
Fig. 7.1. Scanning electron micrograph of the pea shoot apex (cv. 'Lincoln') with four leaf primordia. The larger leaves and stipules have been removed. The youngest primordium (on the left of the apical dome) is a bulge extending round to the sides of the apical dome. One plastochores later (the next youngest primordium, on the right of the apical dome), one of the leaflet primordia and a developing stipule are visible. Another plastochores later (larger primordium at left), the leaflet primordia and the stipules have enlarged. After a further plastochores, two shoots are deutogonous.
7. The Shoot Apical Meristem

and Rondet (1973a). Successive leaves are formed alternatively on opposite sides of the apex so that the divergence angle between successive primordia is 180°. The phyllotaxis is therefore distichous, the leaves being in two vertical ranks.

B. Anatomy

The pea apex, as seen in a longitudinal section passing through the young leaves, has an epidermis in which all cell divisions are anticlinal (Thomson and Miller 1962; Lyndon 1971). The tunica (which includes the epidermis) is usually two-layered (Mitra 1950; Thomson and Miller, 1962; Nougarède and Rondet 1973b), although up to four layers have been observed (Reeve 1948). The tunica is distinguished from the corpus by the fact that it consists of cells which appear, from the arrangement of their cell walls, to have divided only anticlinally. If there are cells in the outer layer of the corpus which sometimes divide only anticlinally, while at other times they also divide periclinally, then these would not have only anticlinal walls as the tunica cells have. When the orientation of mitotic spindles was used as the sole criterion the tunica was found to be about seven-layered for one-half of the plastochron and to be two-layered for the other half of the plastochron (Lyndon 1970b, 1972a). Variation in the number of tunica layers during the course of the plastochron has been noted in other plants (Gifford, 1954) and is of possible significance in leaf initiation (Lyndon 1976; see Section VI). Variation is greatest at the point where a new leaf primordium is to be formed, for here the tunica becomes reduced to a single layer (the epidermis) by the periclinal divisions which occur in the sub-epidermal cell layers.

The tunica–corpus arrangement of the pea shoot apex develops during late embryogeny (Reeve, 1948). The apex arises between the two cotyledons and at an early stage of its development shows two distinct outer cell layers. However, periclinal divisions are often observed in both layers during the early development of the apex, so there is no clear tunica at this stage. Even when the embryo apex has initiated the first primordium, occasional periclinal divisions may still be found in the epidermis. The apex does not show any appreciable change in the degree of organization until the tissues of the axis are well differentiated, and by the time the embryo is fully developed, two tunica layers have become sharply defined with sometimes a third, less discrete, layer also present in the mature embryo. On the flanks of the apex more than three tunica layers can sometimes be seen. The anatomy of the fully developed embryo apex differs very little from the apices of seedling and adult plants (Reeve, 1948); during development in embryogeny the apical dome increases slightly in size, and may continue to increase after germination and during vegetative growth of the plant.
The first sign of cell differentiation in the apex is a slight elongation of cells in the centre of the apex about level with the youngest primordium. These are the cells which are beginning to vacuolate and form the pith of the stem. In etiolated plants grown in complete darkness there are numerous starch grains in these cells which are not present in light-grown plants. Apart from this, the external and internal structures of the apex are exactly the same in plants grown in the light as those grown in the dark (Thomson and Miller, 1962). Procambium is first visible in the apex in relation to the development of the youngest leaf primordium, while the procambium in relation to the stipules develops later (Mitra, 1950).

The intensity of staining with pyronin or with general stains in longitudinal sections of the pea apex (Fig. 7.2) is greatest in the young leaf primordia and on the flanks of the apex, and is least in the enlarging cells of the pith and in the central zone (which consists of the cells at the extreme summit of the apical dome). The zonation pattern in the pea is not as marked as in most other plants (Nougarède and Rondet, 1973b). The lower intensity of staining in the pith is clearly because the cells are larger and more vacuolated than elsewhere in the apex. The lower staining intensity in the central zone is due to a slightly larger cell size than in the rest of the apical dome. The pattern of cell density, which is the inverse of cell size, is obtained by scoring the positions of individual cells in a median longitudinal section of the apex. By superimposing the drawings obtained from several apices, all at exactly the same stage of development, the mean cell density per unit area can be obtained for each position in a grid placed over the drawings (Lyndon, 1972a). From the values so obtained a diagram can be prepared in which the density of shading is directly proportional to the numbers of cells per unit area (Fig. 7.2B). The areas of densest shading are therefore the areas of smallest cell size and the areas in which the shading is lightest are those in which cells are largest. A comparison of the distribution of cell density with the distribution of staining intensity shows that they are very similar. The staining intensity is therefore a direct function of cell density and the zonation pattern seen in the pea is consistent with there being a constant amount of stainable material per cell throughout the apex. This has been confirmed by histochemical measurements (see Section III).

The central zone is also characterized by having larger nuclei than the cells in the surrounding tissues. Measurements of the sizes of the nuclei, in conjunction with microdensitometric measurements of the DNA value for each nucleus, showed that the 2C nuclei in the central zone were approximately twice the volume of 2C nuclei in the rest of the apex (Lyndon, 1973). The difference between 4C nuclei in the central zone and the rest of the apex was less marked.
Fig. 7.2. (A) A longitudinal median section of the shoot apex stained with pyronin. (B) The relative densities of cells. In (A) the flanks of the apex and the young leaf primordium are darkly stained and the central zone and incipient pith are lightly stained, corresponding to the distribution of RNA. This is similar to the pattern of cell density (B) showing that the different concentrations of RNA throughout the apex are a function of cell density and inversely proportional to cell size. This is consistent with a constant amount of RNA per cell throughout the apex. Bar = 30 \mu m
C. Plastochnomic Changes

As each new leaf primordium is formed, the shape of the apex changes. The formation of a primordium on the flanks of the apical dome reduces the apical dome to minimal area. The apical dome then grows to maximal area just before the initiation of the next primordium. The interval between the formation of successive leaves is a plastochron. Associated with the formation of leaves are changes in the structure of the apex, these changes being repeated at each plastochron. From the time of its first emergence as a bump on the flanks of the apex, the youngest primordium grows outwards about 60 μm before the next leaf is formed on the opposite flank of the apex. The development of the apex within a single plastochron has been followed by classifying apices according to the stage of development of the youngest primordium (Lyndon, 1968a). The base of the primordium was taken as the line joining the axil of the youngest primordium itself and the axil of the next primordium immediately below it. The length of the youngest primordium was taken as the length of the perpendicular from this base-line to the tip of the primordium. Since the primordium increased in length by 60 μm during the first plastochron of its existence, i.e. before the next primordium emerged, the plastochron could be divided into 10 distinct and morphologically recognizable stages, each represented by a 6 μm increment in the height of the youngest primordium.

A more sensitive method for measuring plastochron stage was devised by Hussey (1971a) and was used to follow the development of pea apices (Hussey, 1972). As soon as the youngest primordium is large enough to form an axil, a line joining the surface of the primordium to the surface of the apical dome no longer touches the surface of the apex in the region of the axil. The shortest distance from the axil to this line is the axillary distance. The value for the axillary distance at the time that the axil of the next leaf primordium becomes formed is the axillary distance increment during the course of a single plastochron. In this way it was possible to measure the developmental stage of an apex to within 1/100 of a plastochron.

When following changes in the morphology or physiology of a plant it is usually more useful to measure the time taken for the plant to reach a given developmental stage than to measure the average developmental stage reached by a certain time. By the latter method differences between developmental stages can be obscured because of the averaging of plants which are not exactly synchronous in their development. The former method, which is essentially a modification of the plastochron index (Erickson and Michelini, 1957), is to be preferred since plants at a given developmental stage can then be compared with plants at another clearly different and defined developmental stage.

The changes in structure of the pea shoot apex during the course of a
plastochron have been described by Nougarède and Rondet (1973b) and Lyndon (1968a, 1970b). The formation of a new leaf primordium is accompanied by the occurrence of periclinal divisions in the underlying tunica and corpus. These are first seen as a change in orientation of the mitotic spindles in the II region of the apex which occurs about half a plastochron before the new primordium emerges as a bump (see Section V). The incipient pith region is regenerated by cell division and growth at the base of the apical dome, the mitotic spindles being about 15° from the vertical so that the axis is inclined in successive plastochrons about 30° to the right or left of the previous orientation. Longitudinal mitotic spindles on the flanks of the apical dome are associated with the anticlinal divisions which result in the upward growth of the apical dome. In the incipient pith region there is a sudden increase in the proportion of plastids with starch which also occurs half a plastochron before the new primordium emerges and at exactly the same time as the change in orientation of the mitotic spindles in the II region (see Section II).

These changes in structure and shape of the apex are associated with changes in the overall dimensions. The height of the apical dome increases slowly just after a leaf has been initiated but increases much more rapidly during the second part of the plastochron, just before the initiation of the next leaf (Lyndon, 1968a). The increase in size of the apical dome was also measured by the increase in cell number during the plastochron. The increase in cell number was slow during the first part of the plastochron and rapid during the second part just before the initiation of the next leaf. Conversely, the increase in cell number in the incipient primordium and the associated axial tissue was more rapid during the first part of the plastochron, when the primordium had just been initiated, and was slower during the second part when the apical dome itself was growing more rapidly. The increase in cell number, and size, of the whole apex was nevertheless exponential, and the growth of the young primordium was also exponential (Lyndon, 1968a). The apparently discontinuous growth of the different regions of the apex is because the apex changes in shape as it grows.

The central zone at the summit of the apical dome appears to change in size during the plastochron, being at a maximum of about 100 cells in the middle of the plastochron and falling to a minimum at the times of emergence of a new leaf, i.e. at the beginning and end of a plastochron (Lyndon, 1968a). The rate of cell division also changes in the central zone, being lowest in the middle of the plastochron and reaching its highest value at the beginning and the end of the plastochron (Lyndon, 1970a).

* The II region is that part of the apical dome which will form the next primordium, and the I2, I3, etc. regions those which will form the next +1, next +2, etc. primordia.
Fig. 7.3. A. Plastids from the II region of the apical dome have poorly developed membranes and lack starch. Bar = 1 μm.
Fig. 7.3. B. Plastids from the incipient pith are larger, have better developed membrane systems and contain starch. One of the plastids (left) appears to be just completing division. Bar 1 μm
II. ULTRASTRUCTURE

A systematic survey of the ultrastructure of the pea apex (Lyndon and Robertson, 1975) showed that most of the differences between cells in different parts of the apex were quantitative rather than qualitative. Observations were made on longitudinal sections which were cut near the median line of apices at six defined stages within a single plastochron. The sections were mounted on formvar/carbon films so that the whole of each section could be seen and eleven predetermined positions could be photographed. On each of the resulting electron micrographs the relative volumes of the different classes of organelles were measured by quantitative stereological methods (Weibel, 1969). The total numbers of profiles of each recognizable organelle were also counted. From these primary data the relative volume occupied by each class of organelle, the numbers of organelles per cell, and the sizes of the organelles, were then calculated. The results were subjected to statistical analyses of variance.

Within the course of a single plastochron there were no changes in the volumes per cell of the different classes of organelles, or of the numbers of organelles per cell, or of the sizes of the organelles. The only change which could be linked to the plastochronic functioning of the apex and the regular initiation of leaves was an increase in the percentage of plastids with starch in the central, axial parts of the apex and in the developing leaf axils, but not in the apical dome and the youngest primordium. This increase occurred at plastochron stage 9-5, which is precisely the point in the plastochron at which the orientation of the mitotic spindles changes in the II region just prior to the emergence of a new leaf (Lyndon, 1970b). The cells in which the increase in starch was most marked (the developing leaf axils and the incipient pith) are cells which are not destined to play any part in the formation of the leaf itself. The starch was almost certainly synthesized from precursors already present in the apex and not as a result of photosynthesis, because the membrane systems of the plastids were poorly developed (see Fig. 7.3 A, B).

The quantitative differences in ultrastructure between different regions of the apex were related to cell differentiation (Figs 7.4 and 7.5). In the course of development of cells from the summit of the meristem to the incipient pith, the cells enlarged and the volume and numbers of most of the cytoplasmic components increased. The amount of endoplasmic reticulum and dictyosomal material per cell and the numbers of microbodies and vacuoles per cell increased. Plastids and mitochondria differed in that the number of plastids per cell remained constant though their size increased, whereas the number of mitochondria per cell increased although total mitochondrial volume did not increase and therefore the size of individual mitochondria decreased slightly.
This implies that as the cells enlarged, mitochondrial replication out-stripped cell replication whereas plastid replication kept in step with cell replication.

In the development of the leaf axils the number of plastids per cell remained the same from the axil of the eleventh (youngest) leaf to the axil of the ninth leaf, but the number of mitochondria per cell decreased. This was associated with a decrease in cell size in this axillary region during its development and again implies that the plastid replication has kept pace exactly with cell replication, whereas mitochondrial replication has not kept up with the rate of cell division.

In the development of cells which are displaced from the summit of the apex to the flanks and then incorporated into the developing leaf primordia, the numbers per cell of plastids, mitochondria, dictyosomes, microbodies and vacuoles all increased. This is the only cell differentiation sequence in which the number of plastids per cell increased and it implies that in this case plastid replication, like mitochondrial replication, is faster than cell replication. This probably represents the beginning of the increase in plastid number per cell which is usually associated with leaf development.

Since mitochondrial replication occurred faster than plastid replication in developing pith cells, slower in developing axillary cells, and at the same rate in developing primordium cells, the implication is that the replication of mitochondria and plastids are not under a common control and that neither are under the same control as cell replication.

In all three sequences of cell differentiation—the formation of the incipient pith, the development of the axillary position and the development of the young leaf cells—there was an increase in the number of vacuoles per cell. In the pea apex therefore, vacuolation accompanies cell differentiation, even, as in the case of the axillary development, when the cells are becoming smaller as a consequence of not regaining their former size after division. Although there are differences in the rate of division of cells within the apical meristem of about three- or four-fold (Lyndon, 1973) there were no ultrastructural differences that could be correlated with the different rates of cell division.

The zonation patterns (Fig. 7.2) which are seen in stained sections of apices (Lyndon, 1972a) were not reflected in the quantitative distribution of any of the recognizable organelles in this study. However, ribosomes were not examined, and in other apices (Nougarède, 1967) the greater density of stain on the flanks of the apex and in the incipient primordium has been shown to be associated with a higher density of ribosomes in these regions. The zonation pattern in the pea is therefore presumably a reflection of the different concentrations of ribosomes and possibly soluble proteins in the apex but is not a reflection of the general composition of the cells in terms of other organelles.

The mean numbers of organelles per cell in the central zone of the pea apex were 11 plastids, 58 mitochondria, 15 dictyosomes, 4 microbodies and
FIG. 7.4. Electron micrograph of cells in the H region of the apical dome (fixed in permanganate, stained with uranyl acetate and lead citrate). The plastids have very little internal structure; the endoplasmic reticulum tends to be sparse; and the vacuoles are small and infrequent. Bar = 2 μm.
Fig. 7.5. Electron micrograph of cells in the incipient pith region of the apex (fixed in permanganate, stained with uranyl acetate and lead citrate). The internal membranes of the plastids are developing and often have starch associated with them. Vacuoles (V) are present. Bar = 2 μm
1 vacuole. This is comparable to the 11 plastids, 60 mitochondria and 24 dictyosomes in each telophase daughter cell in the *Epilobium* shoot meristem (Anton-Lamprecht, 1967) and the 12 plastids per cell in the shoot apex of spinach (Cran and Possingham, 1972). Although there was on average 1 vacuole per cell in the central region of the pea apex these were only small vacuoles and the apical dome has the general appearance of being non-vacuolated. This single average vacuole in the central zone was about half the volume of the typical plastid.

### III. CELL GROWTH AND METABOLISM

The relative amounts of RNA, DNA and protein per cell in six regions of the apical meristem of the shoot were measured histochemically (Lyndon, 1970c) in sections stained with gallocyanin, Feulgen and dinitrofluorobenzene respectively. The absolute values for RNA and DNA per cell could be calculated because the 2C DNA amount was known to be 9.5 pg (Lyndon, 1967) and mitotic figures could therefore be used as an internal standard for the histochemical measurements. Independent chemical measurements indicated a protein:DNA ratio of approximately 6. The mean composition per cell in all regions of the shoot meristem was approximately 12 pg DNA, 9 pg RNA and 70 pg protein. The enlarging cells of the incipient pith were not measured but presumably would have greater amounts of RNA and protein per cell. The ratio of RNA:DNA is less than 1 in most of the apex and this is much lower than is usually found in plant cells, but is in fact comparable with the ratios of 1 or less recorded for the shoot apex of *Lolium* and for young wheat leaves (Rijven and Evans, 1967; Williams and Rijven, 1965). A low RNA:DNA ratio in the pea apex is also suggested by the RNA:DNA ratio in apical segments, each consisting of the apical dome plus the five youngest primordia and therefore containing a considerable number of enlarging and differentiating cells. Scans of the total nucleic acid extracted from these segments and subjected to electrophoresis on acrylamide gels shows that the RNA:DNA ratio was not more than about 2 (Fig. 7.6). The usual characteristic RNA components are present except for plastid RNA which would presumably occur only in small amounts, and is also easily degraded.

Since the gross composition of all the cells in the apex which were measured was the same it follows that all these cells have the same amounts of constituents to synthesize during the cell cycle. The rates of increase in the amounts of RNA and protein are therefore presumably a function of the rates of cell growth and division and will be less in the slowly dividing cells at the summit of the apex than in the more rapidly dividing cells. If the different rates of increase result from different rates of synthesis, and if the rate of incorporation of RNA and protein precursors is a measure of the rate of
synthesis, then the incorporation of \(^{1}\)H-uridine and \(^{3}\)H-leucine should be least at the summit of the apex and greatest on the flanks. However with both precursors the labelling was uniform throughout the pea meristem (Lyndon, 1972b). If the rates of incorporation of precursors were indicative of the rates of RNA and protein synthesis then this would mean that these rates were uniform throughout the apex. Were this so then the slower increase in the amounts of RNA and protein at the summit of the apex could result only from enhanced rates of RNA and protein breakdown.

A more probable and plausible explanation is that the rates of incorporation of precursor are not an indication of the rates of synthesis. This would only be so if the incorporation of the labelled precursor into the RNA or protein was itself the limiting step in the process. If some other step is limiting, such as the rate of uptake of precursor into the cells, or if there is an endogenous precursor pool already present in the cells, thus diluting the exogenous label to different extents in different parts of the apex, then the rate of incorporation will reflect these parameters rather than the rate of synthesis. Further experiments and kinetic data are necessary to determine whether or not these results can be explained, for example, by smaller pool sizes of precursors in the central zone rather than in the flank regions, or whether there is some other explanation. Rates of incorporation, in the absence of other data, obviously do not provide sufficient information from which to draw conclusions about the rates of synthesis of the compounds which become labelled.
The rate of DNA synthesis seems to be a function of the rate of cell division because the length of the S phase of the mitotic cycle is a function of the length of the whole cell cycle (Lyndon, 1973). In the cells on the flanks of the apex and in the incipient primordium the length of S was about 7 h, whereas in the more slowly dividing cells in the central zone it was about 11 h (Lyndon, 1972b, 1973; see also Table 7.1). Presumably the number of initiation sites for the replication of the DNA molecule is less in the central zone than in the rest of the apex, as it is in slowly dividing animal cells (Callan, 1972).

One aspect of the growth of the cell which can be measured visually is the growth of the nucleolus during the cell cycle. The average sizes of telophase and prophase nuclei in the pea shoot meristem are given in Fig. 7.7. Nuclei which are intermediate in size represent nuclei at some stage of interphase, progressively larger nuclei being progressively nearer prophase. Taking nuclear volume as an indicator of progress through the cell cycle (although the x axis may not necessarily be a linear time-scale) nucleolar volume per cell may be plotted against size of nucleus and so the increase in nucleolar volume per cell through the cell cycle from telophase to prophase may be inferred (Fig. 7.7). Nucleolar volume per cell increases from zero at telophase to 6.5 μm³ in early prophase, just before the nucleolus disappears at mitosis.

![Fig. 7.7. Growth of nucleoli during the cell cycle in the pea shoot meristem. Mean volumes of 10 telophase and 10 prophase nuclei are indicated. Each point is the mean of 4–15 values, measured from sections.](image-url)
This increase is slower than in the root meristem, in which the increase in nucleolar volume during the cell cycle is three times as great as in the shoot (Lyndon, 1968b). Since the cell cycle in the root meristem is probably shorter than in the shoot (Lyndon, 1973) this implies a rate of increase of nucleolar volume in the cell cycle in the shoot meristem of about one-sixth or less of that in the root.

The values in Fig. 7.7 refer to total nucleolar volume per cell. This is because there may be more than one nucleolus per cell and per nucleus. There are seven chromosomes in the haploid complement of *Pisum* and two of these have nucleolar organizers (Atakebowa, 1959). In the diploid cells of the shoot apical meristem one would therefore expect to find four nucleolar organizers per chromosome complement with a maximum number of four nucleoli per cell. In the pea shoot apical meristem four nucleoli are very rarely observed but three nucleoli can often be seen and most cells have one or two nucleoli. This suggests that fusion of the nucleoli occurs fairly quickly. Although different varieties of peas may have slightly different karyotypes, with differences in the position of the nucleolar organizing region, the total number of nucleolar organizers in the diploid cell is apparently always four (Sen and Tiwari, 1966).

IV. RATES OF CELL DIVISION

A. Mean Cell Generation Time

The overall rate of cell division for the meristem as a whole, the mean cell generation time (MCGT), can be calculated most readily from the rate of increase in cell number during a plastochron. Counts of the number of cells in the apical dome at different times during a single plastochron show that there is an increase from about 830 cells just after the formation of a leaf primordium to about 2600 just before the emergence of the next. Since the increase appears to be exponential (Lyndon, 1968a) the MCGT can be calculated as 28 h (Lyndon, 1970a) either graphically or from the formula:

\[
\text{MCGT} = \frac{t \log 2}{\log y - \log a}
\]

where \(t\) = the plastochron (h); \(a\) = number of cells in the apical dome at the beginning of a plastochron; \(y\) = number of cells in the apical dome at the end of a plastochron.

B. Division Rates Measured by C-metaphase Accumulation

The rate of cell division has also been measured by the method of accumulation of colchicine-metaphases (Lyndon, 1970a). However, it was observed that the maximum rate of accumulation of C-metaphases which could be
achieved was about 1.5% h⁻¹. This was less than the 2.5% h⁻¹ which was theoretically necessary with a MCGT of 28 h. Numerous attempts with different methods of application of colchicine and with different batches of colchicine all failed to increase the rate of accumulation of C-metaphases above 1.5% h⁻¹. Had this been the only piece of data available the MCGT would have been calculated as about 45 h. The colchicine was apparently inhibiting not only exit from metaphase but also progress through interphase or prophase, i.e. at some point before entry into metaphase. The use of lower concentrations of colchicine might have resulted in the calculation of shorter MCGTs but since such lower concentrations did not eliminate anaphases and telophases their efficacy was not certain and the values for the MCGT would have been of doubtful validity. It is clearly necessary to corroborate MCGTs obtained from accumulation of C-metaphases with independent data for the MCGT in untreated plants. Only in this way can true cell cycle times be obtained rather than lengthened times which are in fact experimental artefacts.

Relatively high (0.5%) concentrations of colchicine were necessary to inhibit division in the shoot apex of the pea, and although lower concentrations can be used for roots, the pea does seem to be relatively insensitive to colchicine. Higher concentrations than those which are applied to the root must be applied to the shoot apex because the latter seems relatively impermeable to substances placed on it. The entry of substances into the shoot apex is facilitated by wounding or excising tissues near the apex (Bernier and Bronchart, 1963; Lyndon, 1973).

Although colchicine inhibited processes other than the exit of cells from metaphase, and although after 8 h of treatment with the 0.5% colchicine solutions the accumulation of metaphases ceased in the tissue altogether, the distribution of C-metaphases did not change. This showed that the degree of inhibition of entry into metaphase was the same in all parts of the apex and that the accumulation of C-metaphases did not cease in some regions sooner than others. Had this occurred then the pattern of C-metaphases would have changed with time. Since it did not, then the pattern of C-metaphases was always indicative of the pattern of rates of cell division, and it was valid to use apices which had been treated with colchicine for any length of time to observe the distribution of metaphases. Knowing the absolute mean rate of cell division for all cells in the apex (the MCGT) it was then possible to convert the relative rates of cell division obtained from the C-metaphase data into absolute rates. The absolute rate of division and the length of the cell cycle corresponding to a given density of C-metaphases could then be calculated so that lines joining cells having the same rate of cell division and the same cell cycle length could be superimposed on the outline of sections of the apex (Lyndon, 1973).

By recording the positions of all the C-metaphases in all the serial sections through pea apices it was possible to reconstruct sections in any plane and so
examine the distribution of rates of cell division throughout the apex in three dimensions. The general structure of the apex in terms of cell division rates was most easily seen from median longitudinal sections (Lyndon, 1970a). At the beginning of a plastochron when the apical dome is at minimal area the whole of the dome is occupied by cells dividing relatively slowly. During the course of the plastochron a band of cells showing a more rapid rate of division develops at the base of the apical dome between the axil of the youngest leaf primordium which is just emerging and the axil of the next oldest leaf primordium. Reconstructions of sections in other planes show that this band of rapidly dividing cells is, in fact, a plate of cells. At the end of the plastochron the apex consists of a region of slowly-dividing cells which occupies most of the apical dome. This region is separated from that of the youngest primordium and the axial tissue which it subtends (and which has an intermediate rate of division) by a region of much more rapid divisions at the base of the apical dome. There does not seem to be any clear distinction in terms of rates of division between the central zone at the summit of the apex and the other tissues of the apical dome which surround it. The region of slow division rate in the apical dome is much larger than the central zone. Since the cytological characteristics of the central zone are not a function of a lower rate of division compared with the adjacent cells of the apical dome this suggests that the distinctiveness of the central zone has a physiological basis not related to division rate. When sections were reconstructed in other planes it was seen that the plate of rapidly dividing cells at the base of the apical dome was continuous with regions of rapid division lying in the positions of the incipient procambium at the stipular sides of the apex. At this stage of development this procambium is difficult to pick out by microscopical examination of anatomical preparations. It is notable that the procambium in the region of the young primordium, where the procambium is most distinct, does not have a higher rate of division than the cells around about it (Lyndon, 1970a). This may indicate that the high rate of division which is associated with the incipient procambial strands occurs only at the very earliest stage of procambium formation, and before the strands are anatomically distinct, the rate of division may diminish to that of the cells around them. The procambium in the region of the young primordium is seen to have a higher rate of division only at the stage before the primordium itself is formed, i.e. in the late II phase just after the orientations of the spindles have changed and before the primordium has begun to grow out as a bulge.

The recording of the C-metaphases in all the sections of the apical dome allowed reconstruction of the distribution of divisions over the surface of the apical dome. At the summit of the apex, in the region of the central zone and the tissues immediately adjacent to it, the rate of division was lower than in the rest of the apex, but divisions were present over the whole surface of the apex (Lyndon, 1970a).
The rate of division in the epidermis was in general about two-thirds of that of the underlying tissues (Lyndon, 1971). This is what one would anticipate on geometrical grounds because the surface of a solid body increases as the square of the linear dimensions, whereas the volume increases as the cube (see also p. 207).

C. Cell Cycle

The cells in different parts of the pea apex have different rates of division and it is clearly of interest to know whether certain parts of the cell cycle are extended or reduced at the expense of others. The only measurement of the lengths of the phases of the cell cycle in the pea (Lyndon 1973) have been done using the method of Mak (1965). The cells are labelled with $^3$H-thymidine and the nuclei stained with Feulgen. The nuclei of the apex which are not labelled are therefore in parts of interphase other than S. From microdensitometric measurements of the DNA content of unlabelled nuclei the relative numbers of 2C and 4C nuclei can be obtained and from these values the relative lengths of the $G_1$ and $G_2$ phases of the cell cycle can be calculated. The relative length of the S phase is obtained from the proportion of labelled nuclei. The length of mitosis is obtained from the mitotic index. If the absolute length of the cell cycle is known then the absolute lengths of the different phases can easily be calculated. This technique was used by Lyndon (1973) to obtain the lengths of the phases of the cell cycle in the different regions of the pea apex. Very similar values were obtained by another method which depended on the measurement of average amounts of DNA in cells in different regions of the apex. Previous measurements of the DNA values (Lyndon, 1970c) had given mean C values for the DNA per cell in different regions of the apex. These values depend upon the relative numbers of nuclei in $G_1$, $G_2$, S and M and since the characteristic C amounts of DNA associated with each of these phases can be assumed it is possible to work out a formula from which the proportion of nuclei in all the four phases can be found if the proportions of nuclei in S and M are known (Lyndon, 1973). The values for the lengths of the phases of the cell cycle obtained by both methods agreed and the means are shown in Table 7.1. In the central zone, where the mitotic

<table>
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<th>Region</th>
<th>Whole Cycle</th>
<th>$G_1$</th>
<th>S</th>
<th>$G_2$</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Zone</td>
<td>69</td>
<td>37</td>
<td>13</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Flanks of the</td>
<td>30</td>
<td>14</td>
<td>8</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Apical Dome</td>
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<td>15</td>
<td>8</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Leaf Primordium</td>
<td>29</td>
<td>15</td>
<td>10</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
7. The Shoot Apical Meristem

The shoot apical meristem is characterized by a cycle that is longer than in the rest of the apex. The lengths of S, G₂, and G₁ are all longer than elsewhere in the apex, with S being extended by about 50% and G₁ and G₂ by 150%. The length of M (mitosis) remains constant, irrespective of cycle length. The different rates of division in the pea apex therefore seem to be the result of an extension of all the phases of interphase.

Since the length of S is extended according to the length of the cell cycle, it follows that the proportion of cells labelled by ³H-thymidine, i.e., the proportion of cells in S, will remain more or less constant irrespective of the rate of cell division. This is in fact what was found for Lincoln peas (Lyndon, 1972a), for apart from the incipient pith all regions of the apex showed a similar labelling index. The lesser extension of S, compared with G₁ and G₂, in the slowly dividing summit cells was shown more clearly by the lower labelling index for the summit than for the flanks of the apex in the peas used by Nougarède and Rondet (1973b).

V. PLANES OF CELL DIVISION AND GROWTH

The direction of growth and the plane of cell division are related in tissues in which the cells remain isodiametric, since the isodiametric shape can only be maintained if the axis of growth of the cell is the same as the axis of the mitotic spindle. If the axis of the mitotic spindle is at right angles to the direction of growth then the cells change in shape as they do in the procambium. The axis of the mitotic spindle in the procambium can be across the cell whereas the cell may grow in length and so become longer and thinner. Where the cell shape is isodiametric and remains so then the direction of growth can be inferred from the orientation of the mitotic spindles. The planes of growth throughout the pea apex have been measured and recorded by noting the orientation of all anaphase and telophase mitotic spindles (Lyndon, 1970b).

The distribution of spindles on the surface of the apical dome (i.e., in the epidermis) is predominantly radial, i.e., longitudinal. This, together with the gradient in the rate of growth and division from a minimum at the summit of the apex to a maximum on the flanks, results in the hemispherical shape of the apical dome in the pea (Lyndon, 1976).

In the sub-epidermal cells (i.e., all cells other than the epidermis) the orientation of growth is somewhat more complex and changes during the course of the plastochron. Median longitudinal sections of the apex show that the growth on the flanks of the apex where the primordium is about to appear is predominantly longitudinal and mitotic spindles are in the plane of the section (Lyndon, 1970b; Nougarède and Rondet, 1973b). This can be envisaged as giving rise to upward growth of the apex as a whole. In the central part of the apical dome the spindles (and hence the direction of growth) are
perpendicular to the plane of the sections and are transverse, i.e. the spindles are aligned across the apex (Lyndon, 1970b). This is thought to be associated with the growth of the stipules which are formed at the sides of the pea apex. The proportions of spindles in the longitudinal and transverse orientations were essentially the same throughout the plastochron except in the region of the apical dome immediately above and adjacent to the emerging primordium. Here the mitotic spindles and the plane of growth are essentially transverse in the first part of the plastochron when the young leaf primordium which is immediately adjacent is becoming established, whereas in the second part of the plastochron the orientation is predominantly longitudinal during the upward growth of the apical dome once the axil of the young leaf primordium has become established (Lyndon, 1970b). This change in the orientation of many of the spindles, and in the direction of growth, in this part of the apical dome (the I2a region) is restricted to the sub-epidermal cells. Such a change is not found in the epidermal cells and is consistent with the hypothesis that the changes in the directions of growth originate in the interior of the apex rather than at the surface.

The orientation of the spindles and the plane of growth can also be classified according to whether the subsequent cell divisions are periclinal or anticlinal. Except for the region immediately above the site of the incipient primordium the growth of the apical dome during the first part of the plastochron was almost exclusively the result of anticlinal cell divisions associated with growth parallel to the surface of the apex (Lyndon, 1970b). Only in the second part of the plastochron were periclinal divisions observed in that part of the apical dome where the next primordium was to appear (the I1 region). These divisions would be associated with growth perpendicular to the surface, resulting in the subsequent bulging which is seen as the formation of a new primordium. In the second part of the plastochron the number of mitotic spindles resulting in periclinal divisions increased considerably in the I1 region (excluding the epidermis in which all divisions were anticlinal) so that the proportion of spindles orientated in the three planes periclinal, anticlinal (plane of section) and anticlinal (perpendicular to section), were in the ratio 1:1:1 (Lyndon, 1972a). The periclinal divisions were found throughout both the corpus and the second tunica layer in the I1 region. The equal distribution of divisions between the three planes of growth in the sub-epidermal cells suggested that the orientation of the spindle (and hence the direction of growth) at this time during the plastochron was random. This suggests that there is a constraint upon the planes of division in the first part of the plastochron, when growth results in anticlinal divisions, and that this constraint is lifted and that the cells can divide in any direction during the second part of the plastochron in the I1 part of the apical dome where the leaf is about to be formed. This has been interpreted as the primary event of leaf initiation and primordium formation (Lyndon, 1976). In other words, leaf formation
appears to be associated with a loss of polarity in the apex rather than the imposition of a new polarity. The imposition of the polarity is in the maintenance of the anticlinal divisions and the planes of growth which allow the growth of the apical dome but not the formation of leaf primordia.

VI. MECHANISM OF LEAF INITIATION

The formation of a new leaf primordium at the shoot apex results from the outward growth of part of the apex to form a bulge. This change in shape could be accomplished by changes in the direction of growth, or by localized changes in the rates of growth with constraints on the directions in which the mass of dividing cells could grow, or it could result from a combination of both these factors.

Changes in the rates of growth in an apex with entirely anticlinal divisions would not result in a change in form of the apex and the formation of a new leaf. However, in an apex in which only the outer layers of cells are stratified and the inner cells divide and grow in all directions, i.e. there is a tunica and a corpus, then change in shape of the apex could occur if there were a change in the rate of division localized to one part of the apex. In this case one could expect to find the initiation of the leaf marked first of all by an increase in the rate of division localized to a particular part of the flank of the apex. If a change in the direction of growth were the main factor then the initiation of the primordium would be first marked by a change in orientation of growth and seen as a change in the orientation of mitotic spindles. Such a change in the orientation of mitotic spindles has been found in the pea shoot apex and occurs in the II region, where the new leaf primordium is about to be formed, about 16 h before it first emerges as a visible hump on the surface. The overall rate of division and growth in the II region of the apex was very much the same as in the I2 region in which a leaf was not to be immediately formed (Lyndon, 1970a). On the basis of this information Lyndon (1970a) argued that the formation of the leaf primordium depended primarily on changes in the directions of growth rather than changes in the rates of growth. However, Hussey (1972) pointed out that the region of fastest growth in the apex appeared to be the base of the II region and that Lyndon's data were inadequate in that they did not take into account the different rates of division in different parts of the II and I2 regions. Hussey (1971b) showed that in the tomato apex the formation of the leaf primordium was preceded by a localized increase in the rate of division of the cells in the corpus of the II region. He pointed out that in the pea apex there was a similar region at the base of the II region where divisions were faster than in any other part in the apex (Hussey, 1972). By placing marks of carbon on the apical dome of the pea and tracing their subsequent position after growth through a plastochron,
Hussey (1972) was able to show that it was the basal or abaxial part of the developing primordium which grew fastest in an upwards direction and he suggested that this followed from the establishment of the higher rate of division at the base of the II region. He concluded that the primordium was formed primarily because of this localized rapid growth at the base of the II region. Lyndon (1976) subsequently obtained data which supported Hussey's contention. The distribution of C-metaphases in the epidermis of the primordium indicated a greater division rate in the abaxial part of the primordium (Lyndon, 1976). Hussey (1972) also pointed out that the higher rate of division at the base of the II region was a continuation of a higher rate of division at the base of the I2 region. Hussey therefore concluded that the primary event in the formation of a leaf primordium was the increase in the rate of division which occurred on transition, or displacement, of a cell from the I3 to the I2 region. A cell being displaced down the apical dome into the leaf-forming region would therefore have its rate of division increased as it entered the base of the I2 region approximately two plastochrons before it would contribute to the initiation and formation of the bulge of the young leaf primordium.

The facts of the rates of division and planes of division within the pea apex are not in dispute. What has been contested is the interpretation placed upon these as to what is the primary event in leaf initiation. Lyndon (1976) argued that as the increase in the rate of division from I3 to I2 took place approximately four cell cycles before the leaf primordium was formed, and that since the I2 region itself did not grow out to form a leaf primordium, then this increase in the division rate was not an event associated with the formation of the bulge of the new primordium itself. He argued that the high rates of division at the sides of the apex and in the II region seemed to be more connected with the differentiation of the procambial strands, and that the plate of rapidly dividing cells was concerned with the elevation of the apical dome during the second part of the plastochron. The increased rate of division at the base of the II region was interpreted by Lyndon as resulting in the upward growth of the abaxial surface of the leaf primordium, as also claimed by Hussey, but Lyndon pointed out that this was a factor affecting the shape of the primordium rather than the emergence of the primordium itself. It seems clear that the immediate event resulting in the formation of the bulge, which is the new primordium, is the change in orientation of growth about 16 h before the primordium appears. Since each primordium (after the first) is part of an integrated system in which it has been preceded by other primordia and will be followed by further primordia it follows that the formation of a primordium results from the organized growth of the apex, and since the changes in rates of division are part of this organization it is difficult to point to any one event in this continuum of events which is likely to be the starting point for the formation of a new leaf primordium. Perhaps the question could
be resolved or at least clarified to a further extent by the examination of leafless mutants of peas or other plants.

The changes in the orientation of growth were in the first instance followed by comparing the rates of division with the rates of cell accumulation in arbitrary regions of the apex (Lyndon 1970a). This analysis was extended to the epidermis alone which could then be compared with the sub-epidermal regions of the apex (Lyndon, 1971). The bulging of tissues in one part of the apex was recorded as a displacement of cells from one arbitrary region to another. When the analysis was done for the epidermis it was found that the degree of displacement of cells from one region to another was less than it was for the underlying cells. This was interpreted as showing that the changes in the orientation of growth were predominantly internal phenomena and that the morphogenetic changes occurring in the apex and associated with leaf initiation were therefore events which were initiated deep within the apex rather than in the surface layers. The epidermis could be interpreted as behaving like a skin, growing locally in response to the bulging of the tissues within it.

Mechanical tensions do not seem to be involved in leaf initiation in the pea for all parts of the apex appear to be under slight compression, as shown by the lack of gaping (Gulline and Walker, 1957) or the closing up of incisions made in the apex (Hussey, 1973). Compression in the tissues of the young leaf primordium is consistent with the observation that here the rate of cell division is as high in the epidermal cells as in the underlying cells (Lyndon, 1971).

VII. RATE OF LEAF INITIATION

At the temperature at which peas are normally grown (15°-25°C) they appear to initiate leaves at the rate of about one leaf every 2 days. In *Pisum sativum* cv. ‘Lincoln’ no leaves were initiated during the first 3 days after sowing (Lyndon, 1968a). This is when the seed is imbibing water and the radicle is beginning to grow. Thereafter leaves were initiated quite rapidly from days 4 to 8 and the length of the plastochron during this stage of development was only about 23 h. Then the rate of leaf initiation settled down to a steady rate of about one leaf every second day, i.e. a plastochron of about 2 days. In *P. sativum* cv. ‘Alaska’ the rate of leaf initiation from days 2 to 16 after sowing seemed to be fairly constant and perhaps decreased with time, the plastochron again being just over 2 days (Thomson and Miller, 1961). The Alaska peas had six leaf primordia in the seed, and the Lincoln peas had seven primordia, the seventh primordium being very small.

Lincoln peas show strict apical dominance and flowering does not occur until about 19 leaves have been formed (Lyndon, 1968a). However in early
varieties, such as 'Feltham First', flower buds may be present as soon as the seed germinates.

The length of the plastochron as determined by the rate of leaf initiation may not be the same as the length of the plastochron measured by the rate at which unfolded leaves are formed. Measurements on Lincoln peas (Fig. 7.8) show that the rate of formation of unfolded leaves is slower than the rate of leaf initiation so that the number of young leaf primordia on the shoot apex increases as it develops. The plastochron measured in these two different ways is therefore different.

![Diagram](Fig. 7.8. The total number of leaves plus primordia (A) increases faster than the number of expanded leaves (B) so that the number of primordia plus folded leaves at the apex (the vertical difference between lines A and B) increases as the plant ages. Each value is a mean from 10 plants. The two small epicotyledonian leaves are included in both cases.)

There is some effect of light on the rate of initiation of primordia in Alaska peas (Thompson and Miller, 1961). In the light the length of the plastochron was about 2.4 days compared with 2.9 days for plants kept in complete darkness. This represented growth over the first 16 days and during the formation of five or six new leaf primordia. In *P. sativum* cvs. 'Greenfeast' and 'Telephone' the rate of leaf initiation was the same, about one leaf every 2 days, in light or in darkness (Low, 1971). However, in darkness leaf initiation
stopped abruptly when the 12th leaf was formed. When light-grown seedlings were transferred into darkness, if leaf 12 had not yet been initiated at the time of transfer then leaf 11 or leaf 12 would be the last to be initiated in the dark. If leaf 12 had already been initiated before the transfer, then leaf initiation either stopped immediately or after the initiation of one more leaf. Apices which had stopped leaf initiation in the dark resumed initiation 2–3 days after being transferred back into the light, irrespective of whether this was done immediately or after the plants had been up to 10 days in the dark.

VIII. CULTURE OF EXCISED APICES

Shoot apices consisting of the meristem and the youngest leaf primordium together with 3 mm of stem have been successfully cultured for approximately one plastochron (Hussey, 1972). The apices were obtained from seedlings grown in sterile conditions for 7 days after sowing. The 3 mm piece of stem was embedded in agar and particular care was taken not to touch or damage the apical dome. The nutrient medium consisted of the Murashige and Skoog salt mixture and 2% sucrose together with 100 mg l\(^{-1}\) myo-inositol and 0.5 mg l\(^{-1}\) thiamine.HCl in 1% agar. The cultured apices, maintained at 25°C in a light intensity of 10,000 lx, grew for one plastochron, which was about 4 days. Since the plastochron in an intact seedling is about 2 days this means that before their growth eventually stopped these explanted apices were growing at only about half the rate of intact apices. Despite this slower growth rate their growth pattern was apparently the same as that of intact apices as far as could be judged from their anatomy.

Apical domes, with no primordia attached, have been cultured on agar with varying concentrations of benzyladenine (BA) and naphthalene acetic acid (NAA), both singly and together (Kartha et al., 1974). The apical cells formed a callus and if BA was present (with or without NAA) shoots were initiated but not roots. Only when 10\(^{-6}\) M NAA was supplied, in the absence of BA, were roots as well as shoots formed. With lower concentrations of NAA (10\(^{-7}\) and 10\(^{-8}\) M) no roots were formed and only shoots were initiated. Calluses which initiated shoots could also be obtained from macerates of apical meristems (Gamborg et al., 1974).

IX. GRAFTING OF THE APEX

Successful grafting of apical meristems back on to the parent stock can be achieved when special precautions are taken to prevent the apices drying out (Gulline and Walker, 1957). Young seedlings were grown until they were
about 7 days old and their roots were about 3.8 cm (1.5 in) long. The seedlings were then decapitated above the first node. After pruning away the bracts, and axillary buds which had more than one primordium, new axillary apices were formed and were readily accessible for experimentation. Using pieces of thin razor blade as knives, apical segments 200 μm or less were cut from the summit of the apex. The apical segments were then replaced on the stock from which they had been cut. If there were primordia present then the apical segment was rotated through approximately 180° to ensure the discontinuity of the original conducting paths. In the cases where successful grafting occurred this sometimes began as little as 1 or 2 days after cutting, and the grafts developed into small buds within a week. The smallest piece of apex which was successfully grafted was 50 μm in height. This presumably represented only the apical dome itself. The smallest apex which regenerated a bud was 150 μm high and consisted of about 500 cells.

The critical procedure for successful grafting was the performance of the cutting and grafting in a cabinet in which the atmosphere could be maintained saturated with a mist. The slightest drying out resulted in the graft being unsuccessful. Gulline and Walker concluded that the limitations to the size of the graft were imposed by the technical difficulty of cutting smaller pieces and transferring them back to the parent stock, and in doing so avoiding damage to the apex, for even touching the surface of the apex without causing any visible damage could result in its death. Unsuccessful grafts were usually caused by the cut surfaces not being in sufficiently close contact.

REFERENCES


Summary. Differentiation is considered at three levels: (i) differentiation of cell types in the meristems, (ii) cell maturation and enlargement, and (iii) organ initiation. The possible control mechanisms are suggested as being respectively, (i) spatial control, (ii) temporal control, and (iii) control of polarity. Comparison of differentiation in root and shoot meristems leads to the suggestion that root meristems are dependent on a supply of morphogens from elsewhere in the plant whereas in the shoot apex localised habituation may occur to give sites of morphogen production.

INTRODUCTION

Differentiation in the apical meristems of plants may be considered at three levels:

1. Differentiation of cell and tissue types consisting of the development of different cell types from a common set of initial cells, to give a patterned formation of tissues.

2. The development of cells as they are displaced from the meristem by growth of the cells distal to them. As they are displaced (or more properly, as the apex grows away from them) they enlarge and mature to their fully differentiated state, a process which may termed cell maturation (i).

3. The initiation of organs - lateral roots in the root, or leaves in the shoot - involving the imposition of new directions of growth and implied changes in polarity, and resulting in changes in the shape and form of the apex in the shoot or of the root system in the root.

These three levels of differentiation may be conveniently considered separately, especially since the controls may be different in each case. In the definition of tissue patterns in the meristem and the determination and differentiation of cell types the controls seem to be spatial controls, depending on the positions of cells within a morphogenetic field and depending in some way on cell division. The maturation of cells as they are displaced along the axis, basipetally in the shoot and in the body of the root, and acropetally in the root cap, on the other hand seems to depend on a temporal control, the cells following a developmental sequence in time irrespective of their position, and cell division perhaps occurring and being superimposed on this process rather than being an obligatory part of it. The differentiation of organs and the maintenance of shape of the meristems themselves may be principally a result of changes in, or the maintenance of, polarity and the main role of cell division may be in the differentiation of different cell types in the new organ.
Determination may be defined as the process by which a cell becomes committed to a particular pathway of differentiation (1). The determination of different types of cells occurs in or just proximal to the promeristem in the shoot and root apices. In the shoot apex the investigation of the differentiation of cells and tissues is complicated by the fact that the leaves which are also formed at the apex can themselves induce the formation of procambium and vascular tissues (2,3), at about the same level in the apical meristem as the procambium that is not associated with the leaves may also become differentiated (4) as, for example, in a pea apex on the sides of the stem on which leaves are not initiated (5). To talk of the differentiation of the procambium may perhaps be misleading, for it consists of the cells which remain meristematic when the adjacent cells differentiate into parenchyma (Li). This does, however, draw attention to the fact that differentiation of cells necessarily consists not of the formation of a single cell type but the divergent and simultaneous development of two or more cell types. Differentiation of cells is more easily studied in the root, because no lateral organs are formed at the meristem, and the promeristem in which differentiation occurs is smaller and more accessible than in the shoot.

The origin of different types of cells and tissues at the root tip poses several questions:

1. What are the differences between cell types? Where in the meristem do these differences arise?

2. What is the role of cell division?

3. What is the nature of the positional information which results in patterned initiation of cells and tissues?

We recognise different cell types by their anatomical and cytological characteristics, and since the most obvious differences between cells are in their shape, their wall structure and in the presence or absence of cell contents it is mainly these aspects of cellular structure that we think of as constituting differentiation. The known differences between, for example, xylem vessels and sieve tubes lie in the different degrees to which lysis of the cell contents and the end walls has occurred and the different ways in which the cell wall has developed. Apart from the differences in the chemistry of the walls and changes in activity of enzymes concerned with wall metabolism (6) virtually nothing is known about the biochemistry or ultrastructural changes peculiar to different cell types in the early stages of cell differentiation.

In the root meristem the origin of differences between cells can be traced back into the quiescent centre itself (7,8) and of course the recognition of histogens (Li) depends on cell lineages being traceable back to the epidermis/root cap junction. In maize roots the metaxylem vessels can be distinguished in sections within 5 or 6 cells (50 or 60 μm) of the root cap junction, and stele, endodermis, cortex and epidermis may each be distinguished within 50 μm of the root cap junction (9). Since these are differences which have become visible we may infer that differentiation of cells in the root occurs within the promeristem itself. In the shoot, with a more extensive meristem than the root, differentiation probably occurs further from the tip of the meristem. Vascular tissues are not usually distinguishable in the apical dome distal to the youngest primordium. In both root and shoot the epidermis is a tissue which is distinct in many plants from the very tip of the meristem.
Cell division is a characteristic of meristems and may be regarded as a process superimposed on the cell growth and enlargement which is also characteristic of meristems (10). What is the role of cell division in cell determination? Does it have a role other than the partitioning of the protoplast? Clearly without cell division we would not recognise cells. But differentiation in plants can occur within a single protoplast, as it does in coenocytes such as Acetabularia and Caulerpa. However, such coenocytes are very much larger than the cells of higher plants and it may be that for differentiation to occur over distances of microns rather than millimetres the plant protoplast must be partitioned to allow the differences to exist in such close proximity. That cell division is not essential to allow differentiation may be suggested by the observation that xylem wound vessel members can be induced to differentiate in lettuce pith in the absence of cell division (11). In meristems, however, differentiation always seems to occur in conjunction with cell division. In many, perhaps all, cases of cell differentiation in meristems, the initial process depends on the formation of two dissimilar daughter cells from a parent cell. Until a parent cell has given rise to daughters then of course differentiation of the products cannot occur. In many cases the products of the division differentiate along distinctive lines, for instance the sieve tube and the companion cell, the epidermis and the root cap initials, the endodermis and cortical cells, the pericycle and stelar cells. There seems a possibility that the original differentiation of daughter cells along different pathways might be reinforced and the differences accentuated by each cell imposing a new environment on its neighbour derived from the same parent cell and so setting up a feedback system in which the differentiation of one daughter cell is complementary, in a biochemical sense, to its sister cell. This is a possibility which has yet to be examined experimentally. Such differences between daughter cells could perhaps be observed at the ultrastructural level when unequal divisions occur, as in the formation of stomata (12) or trichoblasts (13). Even a careful and quantitative ultrastructural investigation of the differences between cell types at the time of determination might reveal early ultrastructural differences between adjacent cells at the time of determination.

Is cell division necessary for continued growth and differentiation of the cells after determination? Some answers to this question may be obtained from experiments in which cell division is inhibited but growth is not. This can be achieved by several treatments, including radiation, colchicine, or hydroxyrea, all of which may allow growth to continue in the absence of cell division at least for a few days (14, 15, 16). The implication is that cell growth and cell division are processes which are not necessarily linked (10). In the differentiation of xylem vessels, cell division ceases but growth continues so that the cells become endopolyploid (17). The possibility of hormonal control of cell division in relation to expansion is suggested by the experiments which show that division can be re-started in diploid and polyploid cells by the application of IAA or kinetin to segments from that region at the root where cell division and cell expansion had ceased (18). Barlow (19) has proposed a model to account for the distribution of cell division and endopolyploidy along the root which depends on gradients along the root of two interacting substances which he suggests are auxin and cytokinin.

The most precise study of the positions in which cells are determined and begin differentiation in the root meristem has been made by Feldman (7). He traced back the files of metaxylem cells in the maize root to their origin at a longitudinal division of the mother cell. In all cases this was between 40 and 155 μm from the epidermis/root cap junction. However, all files did not originate at the same level. At least four
files appeared to originate more or less simultaneously at 40 to 80 \( \mu \)m from the root cap junction and proximal to this further files up to a total of eight were differentiated. New files originated when the radial enlargement of the root which occurs at the level of the promeristem was sufficient to permit it (Table 1). Two points may be noted: determination occurred not only at a given distance from the root tip but over about 50 \( \mu \)m longitudinally, and new files arose only when the lateral distance between existing files was more than 50 \( \mu \)m. These observations indicate that the origin of the later files was only in cells more than about 25 \( \mu \)m from existing files. At their origin the initial four files were also more than 25 \( \mu \)m from each other. These observations lead to the hypothesis that the origin of new files of xylem vessels in the root occurs in the meristem only when and because there is a locus sufficiently far removed from other strands. This has obvious similarities to the field theory of the initiation of leaf primordia where it is assumed that a young primordium inhibits the initiation of new primordia and that new primordia are initiated at minima in an inhibitory field (20). Perhaps similar processes are operative in the differentiation of cells and in the initiation of organs.

If the initiation of new files is dependent on the position and spacing of existing files, what determines the initiation of the original files? Why do we not see, in the maize root, first one, then two, and more files originating in basipetal sequence as the meristem widens with distance back from the root cap junction? There seem to be two problems: 1) the specification of the distance behind the root cap junction that any metaxylem files at all will differentiate, and 2) the regulation of the diameter of the stele, which seems to determine whether further files may be initiated. The importance of the diameter of the organ is suggested by the correlation between the diameter of the root, and particularly the diameter of the procambium, with the number of xylem poles in pea root tips cultured in different auxin concentrations (21). A similar correlation may also be found in mature organs in which the number of vascular strands is very often a function of the diameter of the axis (22).

All cells in the promeristem would seem to be potentially any kind of cell; this follows from the ability of the meristem to reorganize itself when mutilated (23, 24, 25). When a cell is sufficiently distant from the root cap junction it has the potentiality to differentiate into metaxylem. This differentiation could perhaps be inhibited near the root cap junction because some inhibitory substance is in highest concentration here. Such a substance might possibly be abscisic acid which is apparently produced by the root cap (26). If this were so one might expect the level of metaxylem differentiation to be different in roots in which the cap produces abscisic acid (roots exposed to light) and in roots in which it does not (roots not exposed to light) (26).

The number of files initiated depends on the size of the quiescent centre, probably more specifically its diameter (27) (Table 2). What might be the mechanism whereby a ring of xylem differentiates? With callus, supplying auxin from a point on the surface induced the differentiation of a ring of vascular tissue within the callus (28). By analogy it does not seem appropriate to postulate gradients from inside to outside of the root but rather a supply of auxin and sucrose down the centre of the axis i.e. in the stele. Auxin concentration is higher in the stele than in the cortex of the root (29) and if there is a supply of auxin from the tip of the stele then this could provide a supply of morphogen to the promeristem of the root.
### TABLE 1

**ORIGIN OF METAXYLEM FILES**

<table>
<thead>
<tr>
<th>Metaxylem file number</th>
<th>Distance (μm) proximal to the root cap junction at which file originates</th>
<th>Mean distance (μm) between existing files between which file originates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>$63 \pm 2.5$ (15)</td>
<td>$42.5^*$ (2)</td>
</tr>
<tr>
<td>5</td>
<td>$78 \pm 2.9$ (15)</td>
<td>$52.5$ (2)</td>
</tr>
<tr>
<td>6</td>
<td>$84 \pm 4.9$ (9)</td>
<td>$59.5$ (2)</td>
</tr>
<tr>
<td>7</td>
<td>$93 \pm 5.9$ (7)</td>
<td>$70.0$ (1)</td>
</tr>
<tr>
<td>8</td>
<td>$110 \pm 11.1$ (6)</td>
<td>-</td>
</tr>
</tbody>
</table>

* The distance between the first 4 files is the average of the 4 distances between each of these files at their level of origin.

Numbers in parentheses are numbers of roots examined.

(From Feldman, 1977). (7)

### TABLE 2

**NUMBER OF METAXYLEM FILES AND SIZE OF QUIESCENT CENTRE IN EXCISED MAIZE ROOTS GROWING IN SUCROSE SOLUTIONS**

<table>
<thead>
<tr>
<th>% Sucrose</th>
<th>Average number of metaxylem files at apex</th>
<th>Average height of quiescent centre (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>6.5</td>
<td>55</td>
</tr>
<tr>
<td>0.10</td>
<td>5.7</td>
<td>52</td>
</tr>
<tr>
<td>0.25</td>
<td>2.2</td>
<td>43</td>
</tr>
<tr>
<td>0.50</td>
<td>5.0</td>
<td>60</td>
</tr>
</tbody>
</table>

(From Feldman and Torrey, 1975). (27)
Potentially all cells at a particular radius from the morphogen source would then be able to differentiate into metaxylem. This might be expected to give a continuous ring of tissue or differentiating cells. Where only some of the cells do this, whether it is in the differentiation of metaxylem or phloem in the root, or in the differentiation of nodules of vascular tissue in a callus (30, 28), the problem is why cells in between the differentiating loci do not also differentiate in the same way. A possible hypothesis is as follows.

Distinct loci of differentiating cells would arise if:

1. A specific concentration of a morphogen initiates a given cell type.
2. The initiation process is not exactly synchronous at all potential sites.
3. The differentiating cells inhibit the initiation of other cells of the same type in their immediate neighbourhood.
4. The earliest stages in the initiation process are reversible, i.e. there is a finite time for determination to occur.

A possible corollary is that if the morphogen concentrations allow more than one cell type to be initiated at the same radius the second cell type might be expected to be biochemically complementary to the first in its differentiation.

As a basis for the inhibition of differentiation of other cells in the immediate neighbourhood of a small group of already differentiating cells Bühning (31) has suggested that the environment of the differentiating nodule of cells might become impoverished, not of nutrients in general but of specific substances, namely the morphogens themselves. Sachs (32) suggests the process is one of "differentiation-dependent pattern formation" in which differentiation, which is dependent on the distribution of morphogens, at the same time also alters the distribution and concentrations of the morphogens.

If these suppositions are correct we might anticipate that one tissue type (or cell type) would differentiate before another but associated tissue. There is indeed evidence that in vascular strand regeneration phloem is initiated before xylem (33) and that in callus xylem is initiated before phloem (6). On the hypothesis just outlined the initiation of xylem and phloem in the root could occur in the same pattern irrespective of which might be initiated first.

Has auxin then two effects? One would be to initiate vascular tissue directly when at a specific concentration; the other would be to modify the diameter of the system in which initiation was occurring (34). The tentative conclusion is that vascular tissues in the root tip may require auxin and sugar for their initiation, that this occurs in the promeristem, and that new strands may arise when existing strands become separated by the radial growth of the axis. We may note the possible similarity with the positioning of leaf primordia at the shoot apex. We may also note that the control of the diameter of the axis is not understood. It seems reasonable to postulate that in an organ such as a turgid root the cylindrical form may depend on the properties of the skin. If this were so then the effects of auxin in increasing root diameter might act through effects on the epidermal cell walls - as in stems (35) - allowing radial growth of the axis and so creating new sites which can be induced to initiate vascular tissue as long as the appro-
priate concentrations of morphogens also exist within the initiating region and the cells are still competent to respond.

CELL ENLARGEMENT AND MATURATION

The course of cell development is very conveniently studied in the root, because if we assume that the root is growing in a steady state then the spatial sequence of cells backwards along the root represents a sequence in time. The development and maturation of a cell as it is displaced from the meristem may therefore be followed by analysing successive segments cut from a single root. When combined with a kinetic analysis of the growth of the root and the rates of displacement of cells at different distances behind the meristem (36) it can provide a very clear picture of cell development. As cells mature in the root they enlarge, and synthesise cell wall and proteins and other cellular constituents (36, 37, 38, 39). Also the activities of enzymes change as the cells develop and the activities of different enzymes change relative to one another (40, 41, 42).

The cells still enlarge and changes in enzyme activities occur if the root is cut into segments which are then cultured in isolation from each other (43). Clearly the presence of neither the meristem nor the older parts of the root is necessary for increase in cell length and volume. These developmental changes cannot depend for their continuation and completion on positional effects, which are completely absent in isolated segments of root. The developmental sequence seems to continue as a function of time. Furthermore, it does not depend on the net increase in the amount of protein (which does not increase) or nucleic acid (which decreases) in the root segments (44). If development is mainly a function of time then the time course of development of enzyme activity and cell elongation should be similar in intact roots and in the isolated segments. Unfortunately, kinetic analyses of the growth of intact roots and measurements of isolated segments have not been done on the same species. Heyes and Vaughan (45), using pea segments, got durations of growth closely comparable with those in the intact root and suggest that the differences between their work and that of others (46) was because they had grown the segments in rotating liquid culture, providing adequate aeration, rather than on sintered discs. This latter method was the one used by Robinson and Brown (40) and may be why the duration of development of their Vicia segments was apparently longer than for the comparable segments in the intact root. That the duration of development is dependent on respiration rate is supported by the observation that if bean segments were grown in 10% oxygen, expansion of the segments took longer and was slower than in air (46). The duration of extension in lupin roots was also extended in low oxygen concentrations (47) and suggests a dependence on oxidative phosphorylation for the processes of cell maturation. But when respiration was stimulated by 20% in the presence of 2-thiouracil, without affecting oxidative phosphorylation, neither the duration of expansion nor the course of enzyme development was altered (44, 48).

When, in a particular experiment, the effect of growth stimulators and inhibitors was tested on cell elongation, the development of enzyme activity, and xylem and phloem differentiation, it was the rate rather than the duration of development that was affected (44, 48). The effect of temperature was also on the rate of expansion but not its duration (49). The stimulation of cell expansion by treatment of pea root segments with ribonuclease again affected only the rate and not the duration of elongation (50).
### Table 3

**RATIO OF INVERTASE:PHOSPHATASE IN ENLARGING CELLS OF Vicia Faba ROOTS**

<table>
<thead>
<tr>
<th>Intact roots</th>
<th>Isolated 3-4 mm segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm from apex</td>
<td>Ratio</td>
</tr>
<tr>
<td>3-4</td>
<td>5.6</td>
</tr>
<tr>
<td>4-5</td>
<td>8.4</td>
</tr>
<tr>
<td>5-6</td>
<td>9.6</td>
</tr>
<tr>
<td>6-7</td>
<td>9.1</td>
</tr>
<tr>
<td>7-8</td>
<td>8.3</td>
</tr>
<tr>
<td>8-9</td>
<td>7.0</td>
</tr>
<tr>
<td>9-10</td>
<td>7.1</td>
</tr>
<tr>
<td>10-11</td>
<td>4.6</td>
</tr>
<tr>
<td>11-12</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Note that the times in hours for the isolated segments do not correspond with the times for cells in an intact root to reach the positions shown.

(From Robinson and Brown, 1952, 1954). (140,143)

### Table 4

**ORIENTATION OF CELL DIVISIONS IN THE NON-EPIDERMAL CELLS IN THE I1 REGION OF THE PEA SHOOT APICAL DOME DURING A PLASTOCHRON OF 46 HOURS**

<table>
<thead>
<tr>
<th>Orientation of cell division</th>
<th>Percentage of mitoses 0-30h</th>
<th>30-46h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticlinal in vertical plane</td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td>Anticlinal in horizontal plane</td>
<td>34</td>
<td>29</td>
</tr>
<tr>
<td>Periclinal</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>19</td>
<td>12</td>
</tr>
</tbody>
</table>
When isolated segments are compared with intact roots it can be shown that the changes in the relative activities of the enzymes invertase and phosphatase—an increase in this ratio and then a decrease—which was found in intact roots as the cells were displaced back along the root could also be found in isolated segments as a function of time, although the changes were not so obvious in the isolated segments (Table 3).

A temporal control for the development of root cells is also suggested by the observations that when growth of roots is retarded or inhibited the differentiation of vascular tissues takes place nearer the tip than in rapidly growing roots. In roots of incense cedar the distance from the root tip that differentiation occurred was positively correlated with the rate of growth of the root (51). Similarly, in the growth of isolated root tips in culture the size of the meristem decreased when cell division was slow, apparently because the time between cell formation and vacuolation and enlargement was independent of the rate of division and the position of the cells relative to the root tip (52).

The development and maturation of cells as they pass back along the root from the meristem and enlarge therefore seems to be time-dependent and not position dependent. The duration of development of excised segments may be about the same as in the intact root or it may take much longer if oxidative phosphorylation is limiting, but when aeration is adequate experimental treatments seem to alter the rate of cell development rather than its duration.

In the root cap there is also evidence for a time-dependent differentiation. The cells of the root cap of maize become endopolyploid when they have become displaced to about the 8th cell tier from the cap initials, i.e. about 3 way down the cap and about 3 days after their formation by growth and division of the cap initials (53). When roots are treated with colchicine, which prevents cell division and hence also prevents cell displacement, the cells of the root cap initials become polyploid (54) and in 3 days they have undergone about 3 rounds of DNA replication in the presence of the colchicine, about the same number of rounds of replication that they would have undergone in the untreated root. The synthesis of DNA in the root cap therefore continues irrespective of the position of the cells in the cap and seems to be a function of time from the formation of the cells from the root cap initials. The root cap also becomes smaller because of the continued sloughing of cells from it (54); the loss of cells would therefore also seem to be a function of time rather than position, although it could be position on the outside of the cap that determines that the cells are sloughed off. It is apparently not their position with regard to the cap initials or the centre of the cap. Also cells with the 16C amount of DNA are characteristically located at the outer surface of the cap in untreated roots (53) but disappear in the colchicine-treated roots as they become sloughed off and are replaced by cells with lower ploidy levels (54). The attainment of a 16C ploidy level is therefore not due to their superficial position, but apparently a function of the time since the cells would normally have been displaced from the cap initials.

Another instance of the possible temporal control of cell development in the root is the initiation of lateral roots. This characteristically occurs some distance back from the root tip. When the meristems of bean roots were cut off the initiation of lateral roots on the stumps continued and followed the same time course as in the intact roots (55). The cut surface excepted, the number of roots and the time of their appearance was the same as in the intact roots, suggesting that the development of the pericycle to give initiation of lateral roots may be a function of time rather than of distance from the meristem.
Differences in the elongation of isolated stem segments given different experimental treatments also seem to be the result of the rate rather than the duration of growth (56, 67). Even in intact plants the differences in internode length occurring naturally (58) or induced experimentally (59) depended on the rate rather than the duration of growth.

These various pieces of evidence all suggest that development of cells which have passed out of the meristem may be a function of time. The temporal control could be of several kinds. Robinson and Brown (61) suggested that a series of protein states (P₁, P₂ etc.), would result in a corresponding series of catalytic states (B₁, B₂ etc.) each state resulting in its replacement by a subsequent state via effects on the control of the transcription of the genes (61).

However, it is not certain that the changes in relative activities of enzymes occur within a single cell during its development. The elongating zone of the root consists of several tissues and about ten or more different cell types which can be seen, from sections, to differentiate in different ways. The changing enzyme ratios may reflect the changing proportions of different cell types as a segment of the root matures, with different rates and degrees of lysis (as in xylem and phloem) or activity of enzyme systems, as exemplified by the characteristic accumulation of starch in the cortex. Different enzymes may develop at different rates. For instance, the changing ratio between the pentose phosphate and glycolytic pathways of respiration is the result of a greater increase of pentose phosphate pathway enzymes than of glycolytic enzymes (85), with most of the changes ascribable to changes in the stele rather than the cortex (86).

This suggests an alternative possibility for cell maturation: that the changing enzyme activities as the root cells mature is because of (a) the development of qualitatively different protein complements in different cell types, or (b) the presence of the same basic protein complement in all cell types but with specific proteins increasing at different rates in the different cell types.

Thus the specification of the protein complement of a cell may be fixed once it is determined in the meristem and the development of the cell would then depend on the rate at which the different proteins are accumulated and the duration of this process. However, the increase of one enzyme, invertase, in pea cells as they mature seems to be a consequence of expansion rather than its cause. When cell expansion in root segments was inhibited by indole acetic acid or mannitol the increase in invertase which occurred in growing segments was also inhibited (42). Mannitol presumably would be preventing expansion only by reducing cell turgor. Whether there are other enzymes which, like invertase, may be bound to the cell wall and which also increase in activity or amount as a function of cell expansion, is not known.

If cell maturation is time-dependent then the duration of cell growth must in some way be specified, perhaps by the rate of accumulation of an inhibitor, or the progressive degradation of a key enzyme, or the utilisation of a key substrate. Another possibility is that cell to cell communication via the plasmodesmata is essential for continued growth. Since the number of plasmodesmata in cell walls seems to remain constant after their initial formation during cytokinesis (60, 61) then the number per unit area of cell wall decreases, perhaps to below a critical value necessary to maintain adequate cell-cell communication (62). At the moment we do not know why cell expansion and maturation follows the course it does whether in an intact root or in an isolated segment. It is perhaps symptomatic of the way the problem of cell maturation has been
approached, that in apparently all investigations on intact roots cell development has been followed as a function of the position of the cells, and the time course of cell development seems not to have been studied at all.

INITIATION OF ORGANS

Lateral root initiation

This occurs in the pericycle or endodermis (63). The first sign of lateral root initiation in the maize is an increase in cytoplasmic basiphilia and a thinning and delignification of the cell walls in pericycle cells opposite the xylem poles. These changes occur before much apparent cell extension occurs (64). In other plants the pericyclic cells can be seen to expand radially before division occurs, as in Pontedaria (65).

There are several questions that spring to mind:

1. What triggers lateral root initiation in the pericycle?
2. What determines the polarity of growth, which is at right angles to the parent root?
3. What determines the radial and longitudinal spacing?

If lateral initiation is a function of time since the cells were in the meristem then perhaps a specific trigger is not necessary. However, not all pericyclic cells form lateral roots. Typically it is only those opposite the xylem poles which do so, suggesting that something from the xylem may be necessary for triggering. Since lateral initiation is stimulated by auxin in pericyclic cells which would not normally form roots (66) this might suggest auxin as being the normal substance which travels in the xylem and triggers lateral initiation. The time function might simply be a time required for the cells to become sensitised to substances available from the xylem. Alternatively, it may be that only when the xylem has become sufficiently differentiated can it transport or leak sufficient auxin to allow lateral initiation. One can envisage as a mechanism the following sequence of events:

1. Time-dependent differentiation of xylem.
2. Auxin transported by differentiated xylem or produced during its differentiation (67) diffuses out to the adjacent pericyclic cells.
3. Auxin-stimulated growth and outward expansion (as the only possible direction of expansion because of the constraints of the tissues).
4. Cell division and orientation of the cell plate in the plane of least stress (68) so that periclinal divisions occur.
5. Longitudinal spacing determined by each lateral initial acting as a sink for auxin and so preventing initiation of other laterals in its immediate vicinity.

That it is auxin that is important is suggested by the observation that only auxin is necessary as an addition to the medium for the stimulation of root initiation (66). The auxin may perhaps itself result in the formation of a sink for metabolites.

One of the difficulties with any theory of lateral root initiation is to account for the fact that the roots are initiated only opposite the xylem poles, even when root initiation is stimulated by exogenous auxin. This implies that auxin is not the only factor necessary and that either some other substance is produced by the xylem or else only cells opposite
the xylem are competent to respond. This could be because final differ-
etentiation of the pericycle cells opposite the xylem poles is delayed; 
this seems possible because it is at these positions that cell division 
in the pericycle persists longest (69). Factors other than auxin are also 
required for lateral root initiation (21) and it could be that one factor 
is required to delay differentiation (or rather maturation) in the peri-
cycle and another, auxin, to allow the recrudescence of growth. The 
initiation of laterals in roots in which cell division was prevented by 
coleopticine (15, 70) shows that cell division is not necessary for root 
initiation or for the re-orientation of polarity in the lateral root 
initial.

Initiation of leaves in the shoot

Is cell division necessary? The protrusion of a leaf primordium and the 
changed polarity of growth which this entails can occur in the absence of 
cell division in irradiated wheat seedlings (71). However, it seems poss-
able that the primordium was determined at the time of treatment for the 
experiments of Snow and Snow (72) showed that the site of a leaf primordium 
became fixed half a plastochron before the primordium became visible,* 
which is also the time that the polarity of growth in the I1 position of 
the apical dome changes (73). The primordium which appeared in the 
irradiated wheat seedling may therefore already have been determined at 
the time of irradiation and the commitment to a change in polarity may 
already have taken place. If so then this experiment would demonstrate 
that the initial outgrowth of the primordium does not require cell 
division but it may not tell us whether cell division is required for the 
initial determination of the primordium.

Mechanism of leaf initiation. Measurements have shown that in the 
initiation of pea leaves the first detectable event is a change in pol-
arity in the I1 region of the apex as shown by the appearance of periclinal 
cell divisions half way through the plastochron,** i.e. about 16 hours 
before the primordium appears as a visible bump (73). When the epidermis, 
in which all divisions were anticlinal, was not included, all divisions 
were anticlinal in the first half of the plastochron but in the second 
half of the plastochron were distributed between the two anticlinal planes 
and the periclinal plane in the ratio 1:1:1, i.e. indistinguishable from 
random orientation (74) (Table 4). The interpretation of the growth of 
the apical dome was therefore that in the first half of the plastochron 
there was a restraint of some sort on the apex which resulted in only 
anticlinal divisions and growth parallel to the surface, and that half 
way through the plastochron this restraint was lifted so that growth and 
divisions could occur equally in all planes. The initiation of a leaf 
primordium is therefore viewed as the result of a lifting of a restraint 
on the growth of the apex rather than the imposition of a new direction of 
growth.

It is assumed that the change in the plane of cell division from anti-
clinal to random is not the cause of the change in polarity but the result, 
in perhaps the way proposed by Lintilhac (68). His hypothesis is that the 
cell plate forms across the cell in the plane in which there is least 
shear. This would be normal to the direction of stress imposed by cell

* The next (Invisible) primordium will appear at the I1 position, the 
next after that at I2, etc.
** The interval between the initiation of primordia.
compression or tension or elongation (i.e. normal to the axis of elongation). If the direction of principal stress should change because of a change in the principal axis of expansion of the cell, i.e. a change of polarity, then the plane of the cell plate would also change accordingly.

According to this theory the randomisation of the plane of division would be a consequence of the relief or equalisation of stresses in the $I_1$ region. Is there evidence of stress in the $I_1$ region in the first half of the plastochron? When cuts were made in the apical dome or young primordia tensions were found in tomato and Euphorbia apices but not in any parts of pea apices (75). Except for compression at the developing leaf axils, there were no differences in the effects of cuts made at different times throughout the plastochron (75, 76) and so no evidence for changes in stress associated with leaf initiation, but these experiments probably do not rule them out.

The problem remains of explaining how changes in polarity may be brought about, especially since the rate of growth in the pea apex did not change appreciably at the site of leaf initiation (5). Changes in polarity of growth can presumably occur only in conjunction with or because of changes in the properties of the cell walls and particularly the walls of the limiting layer of cells, the epidermis. The epidermis in the pea is typical of dicotyledons in having only anticlinal divisions even when all the underlying tissues contain periclinal divisions (73). The epidermis therefore remains distinct. The outer wall of the epidermis, as well as being cutinised, is also much the thickest cell wall in the apex. Is this the restraining layer which allows only anticlinal divisions in the apical dome for half of each plastochron?

In the pea, measurements showed that the growth of the epidermis was more localised than in the underlying cells (77). The interpretation was that the epidermis was behaving like a skin, growing in response to the growth of the tissues beneath it. However, an equally possible interpretation is that the localised growth of the epidermis allowed the growth of the tissues beneath it. That this is the correct interpretation is suggested by the experiments showing that in the protrusion of a leaf primordium in irradiated wheat in the absence of cell division only the epidermis was involved initially and not the underlying cells (71). The simplest hypothesis is to suppose that this is true for other plants as well.

The substance which par excellence alters wall properties and promotes wall loosening is auxin (78), and its effects seem to be mainly on the walls of the epidermis (35, 79). Auxin, and auxin antagonists, are also the principal effective substances in modifying the initiation of leaves at the shoot apex (80, 81, 82). Auxins also seem to be produced by very young leaf primordia (2). These observations are consistent with the hypothesis that in leaf initiation a local increase in auxin concentration causes wall loosening, and that the main wall affected is the outer wall of the epidermis. This would allow a lifting of the restraint on the underlying cells and result in non-polarised growth in these cells.

Thornley (20) has shown that the initiation of leaves is explicable in terms of changes in the concentration of a morphogen produced by existing primordia which inhibits the initiation of new primordia except at minima in the morphogen field. The nature of this supposed morphogen is unknown but it could possibly be auxin. A major problem is the necessity to postulate that a substance produced by primordia inhibits the initiation of new primordia but that its synthesis is induced in regions where it is at a critically low concentration. If the substance is auxin the postulate
would have to be that auxin is produced by primordia, that it inhibits the initiation of new primordia, and that synthesis of auxin is induced at low auxin concentrations. Experiments which suggest that this is a real possibility have been done with tobacco callus (83) in which auxin non-requirement (i.e. competence to synthesise auxin, or habituation) was induced by culturing on low concentrations of auxin, and auxin requirement was induced by relatively high concentrations of auxin. These experiments are consistent with the auxin-synthesising system being induced by low concentrations of auxin and being inhibited by high concentrations, and recall Thornley’s (20) postulates that leaves are initiated at minimal morphogen concentrations and that the degradation of morphogen is concentration-dependent.

Based on these considerations we can devise a scheme to attempt to explain leaf initiation.

- Auxin concentration falls in regions between primordia as apex grows and primordia grow apart
- Localised induction of auxin synthesis
- Local concentration of auxin
- Wall-loosening of epidermis and release of stress
- Growth and bulging of I1 region to give primordium initiation
- Continued growth of apex, moving primordia apart.

Evidence is lacking on whether auxin levels in the shoot apex change during a plastochron. However the effects of applied 2,4-D were different at different stages of the plastochron (81), and it was easier to induce extra leaves at some times than others.

The most difficult thing to understand is how the restraint on the shoot apex between the initiation of primordia results in only anticlinal divisions, and how in the dicotyledons, the epidermis in the apex retains its distinctness as a tissue in which periclinal divisions almost never occur.

**A COMPARISON OF THE INITIATION OF LATERAL ROOTS, LEAVES AND VASCULAR TISSUES**

First, auxins have the ability to stimulate the initiation of roots, leaves and vascular tissues. Probably for lateral roots a supply of auxin from the xylem is necessary, whereas in the shoot leaf primordia are autonomous for auxin production and the suggestion is that they become induced to be so by low concentrations of auxin. Here is a possible difference between root and shoot: we can postulate that the root is auxin-requiring and auxin synthesis in it cannot be induced whereas auxin synthesis can be induced in the shoot by low auxin levels.

Another point of comparison between leaf primordia, lateral root initials and nodules of differentiating vascular cells is that each seems to inhibit the initiation of similar loci in its immediate vicinity, even where conditions would otherwise seem favourable. This is most readily
explained by idea that differentiating loci alter the morphogen concentration in their vicinity (31) and lead to the concept of "differentiation-dependent pattern formation" (32). But whereas in the initiation of vascular tissues and lateral roots the differentiating loci seem to be locally depleting the tissue of morphogen and so preventing the initiation of new loci, in the shoot apex the differentiating loci (the primordia) are producing the morphogen, which leads to the same result, inhibition of initiation of new loci. The role of cells in organ initiation may perhaps be that of allowing interactions between neighboring loci during differentiation over shorter distances and therefore also shorter times than would otherwise be possible in a coenocytic plant.

IN CONCLUSION

In this contribution the emphasis on hypotheses has been deliberate, for only by trying to integrate the facts by hypotheses which are experimentally testable will we increase our understanding of differentiation and of the underlying principles which we might hope to find common to the processes of differentiation at all levels of organisation within the plant.

References

Changes in Polarity of Growth During Leaf Initiation in the Pea, *Pisum sativum* L.

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ABSTRACT

In the apical dome of the pea shoot apex the axis of growth of the epidermal cells becomes predominantly longitudinal in the I_1_ region one plastochron before a leaf is initiated, and this orientation persists into the young primordium. In contrast, in the underlying, non-epidermal cells the growth axis in the I_1_ region becomes randomized half a plastochron before leaf initiation, but in the young primordium it becomes the same as in the epidermis. The initiation of a leaf primordium therefore takes place without any major change in the orientation of growth axes in the epidermis. A controlling rôle for the epidermis is therefore suggested. No marked reorientation of the growth axis occurs on the sides of the newly initiated primordium. The shape of the young primordium can be related to the differential rates of growth and division within it rather than to changes in growth orientation.

Key words: *Pisum sativum*, pea, shoot apex, meristem, growth, epidermis, polarity.

INTRODUCTION

If a new lateral axis, a branch or a leaf, arises on the flanks of a cylindrical parent axis in which the predominant direction of growth is longitudinal, and if the new axis itself becomes radially symmetrical, then there will be a change of growth orientation through 90° on the sides of the new organ (Fig. 1). As Green (1980) has pointed out, the initiation of a new stem or leaf axis has almost always been studied from sections in a longitudinal plane which is common to the parent and lateral axes, whereas the major changes in reorientation of growth would be expected to be normal to this plane (on the sides of the new axis) and so are usually ignored. Relevant data for leaf initiation were potentially available in a study of the changes in the planes and directions of growth during leaf initiation in the pea but these data were not analysed to see what happened on the sides of the emerging primordium (Lyndon, 1970b, 1972).

Green (1980) quotes unpublished observations which show that during the initiation of leaves on a decussate stem apex ‘the whole dome expanded, first in one direction, then at 90° to this direction, then back to the original direction, etc.’ and he proposes a model which reconciles these changes in directions of growth with the initiation of leaves which are assumed to be (at least at initiation) radially symmetrical axes. If, during leaf initiation in the pea, there are changes of 90° in the orientation of growth on the sides of an emerging primordium like those postulated in Green’s (1980) model or like those observed during initiation of a new stem axis (Green and Brooks, 1978), then it should be possible to detect them.

Changes in the plane or axis of growth can be followed in several ways. The predominant orientation of the cellulose microfibrils in the cell wall is often, but not always (Green and Brooks, 1978), normal to the axis of growth of the cell and can be determined for the surface layers of a growing region by stripping them off and examining them in plane polarized light (Green, 1964, 1980). Alternatively the plane of the latest
Fig 1. When a new axis is initiated on the flank of a radially symmetrical parent axis (a) the longitudinal polarity of the flanks of the parent axis will become altered in the new axis as it, too, becomes radially symmetrical (b). The major change in the direction of growth is on the sides of the new axis where it becomes re-orientated through 90°. Double-headed arrows indicate predominant growth axes. (After Green and Brooks, 1978).

cell divisions in surface cells can be seen with the use of a scanning electron microscope, but this method does not yet seem to have been used, perhaps because it depends on excellent preservation of the surface structure to allow young cell walls to be distinguished from older ones. These two methods are restricted to observations of the directions of growth in surface layers or in the epidermis. A third method is that of recording the orientation of mitotic spindles as seen in sections, the axis of growth of the cell being parallel to that of the spindle if the average cell shape remains unchanged (Lyndon, 1970b).

Detailed data showing the location and orientation of mitotic spindles in the pea apex during a plastochron (from the initiation of one leaf to the next) have been obtained previously and some of these data have been published (Lyndon, 1970b). It is now appropriate to re-examine the original data to see how the orientation of growth changes at the sides of the incipient pea leaf primordium during its initiation and early growth. It will be shown how this leads to a reappraisal of the rôle of the apical dome and of the epidermis in establishing the polarity and dorsiventrality of the young leaf primordium.

MATERIALS AND METHODS

Plants of *Pisum sativum* (cv. Lincoln) were grown and sampled, and apices were sectioned longitudinally and stained by the methods already given (Lyndon, 1968). The positions and orientations of mitotic figures were recorded, and the rates of cell division were measured and calculated as described previously (Lyndon, 1970a, b). The apices from which the sections were cut were classified according to the stage of the plastochron they represented, plastochron stages 9·0 and 10·0 marking the beginnings of the emergence of the 9th and 10th true leaf primordia respectively (Lyndon, 1968). The plastochron was divided into stages differing by 6 μm increments in the length of the 9th primordium. The time taken for growth from plastochron stages 9·0 to 9·5 was about 30 h and from plastochron stages 9·5 to 10·0 about 16 h (Lyndon, 1968). These are referred to as the first and second parts of the plastochron and correspond to the periods when periclinal divisions in the apical dome were respectively absent and present (Lyndon, 1970b). When referring to growth in the apical dome the terms longitudinal and latitudinal are used in the same way as they are for describing orientation on the
surface of a sphere, the position corresponding to the pole being the summit of the apical dome midway between the axils of the youngest and the next older primordium. The I₁ and I₂ regions are where the next and next but one primordia will arise respectively.

RESULTS

Changes in the directions of growth in the epidermis

The orientation of mitotic spindles in the epidermis is shown in Fig. 2. There were no obvious differences between consecutive plastochron stages and so the data for the first part of the plastochron are consolidated in Fig. 2(a) and for the second part in Fig. 2(b). Assuming that the events in the apex are the same in the 8th and 10th plastochrons as in the 9th plastochron (recorded here) it is possible to consider the progression from I₂ to I₁ to primordium as a temporal sequence.

Fig. 2. Orientation of the axes of mitotic spindles in the epidermis of the pea apex, as seen from the side, during the first part (a) and second part (b) of the plastochron. The new primordium (P) has just been initiated in (a) and has grown larger in (b). The next primordium will be initiated later in the I₁ region. The orientation of mitotic spindles shown as dots is normal to the plane of the sections. Data from both sides of the apex are shown superimposed as they would appear to be in a transparent model viewed from the side. Where divisions appear to be more frequent along the outline of the apex this is because several of the most median sections are seen superimposed. The central zone is that part of the I₁ and I₂ regions at the summit of the apex enclosed by the dotted line.

In the I₂ half of the apical dome the longitudinal and latitudinal components of growth are about equal [Fig. 2(a), (b) and Table 1]. On initiation of a primordium on the opposite side of the apex this region becomes I₁ in which the orientation of growth becomes predominantly longitudinal in the first part of the plastochron [Fig. 2(a) and Table 1], but in the second part of the plastochron there is an increasing latitudinal component,
Table 1. Orientation of mitotic spindles in the epidermis in the I₁ and I₂ halves of the apical dome and in the leaf primordium (P) during (1) the first part and (2) the second part of the plastochron. Data from Fig. 2

<table>
<thead>
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<th>Latitudinal</th>
<th>Intermediate</th>
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<td>0</td>
<td>1.8</td>
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</tbody>
</table>

both on the outer surface of I₁ where there are more spindles normal to the sections and also on the sides of the apex [Fig. 2(b) and Table 1]. The longitudinal component, however, continues to predominate.

When the primordium begins to emerge, the latitudinal component of growth evident in I₁ in the second part of the plastochron [Fig. 2(b)] seems to persist at the tip of the primordium. Because the primordium consists of only a few layers of cells at this stage [Fig. 2(a)] this results in a ratio of longitudinal to latitudinal growth of only 1:2 (Table 1). On the sides of the developing primordium relatively few spindles were recorded (Fig. 2) and so no obvious change in the growth axis can be seen although there is a hint that an axis of growth orientated towards the tip of the primordium may be becoming established near the tip, as would be expected on Green's (1980) model for leaf initiation. There is, however, a noticeable persistence, on the upper and lower surfaces of the primordium, of the predominantly longitudinal orientation of the growth axis (now directed towards the tip of the primordium) which originated when the cells were part of the I₁ region. On the sides of the apex, proximal to the young primordium, where the stipules are initiated the growth axis at first [Fig. 2(a)] remains predominantly longitudinal (vertical) as in I₁ but later [Fig. 2(b)] becomes less marked and not clearly orientated in any preferred direction.

Changes in the directions of growth in the underlying (non-epidermal) cells

The persistence of a predominantly longitudinal polarity of growth in the epidermis of the I₁ region, at the site of leaf initiation, contrasts with the randomization of the planes of growth which occurs in the underlying (non-epidermal) cells, beginning at plastochron stage 9-5 (Lyndon, 1970b, 1972). This raises the question of whether the random orientation of the plane of growth in these sub-epidermal cells persists into the primordium even though growth in the epidermis is fairly strongly orientated.

Analysis of the plane of growth in the primordium with respect to the surface becomes more difficult as the shape of the apex changes and the surface/volume ratio of the primordium increases relative to that in I₁. The least unsatisfactory way of being able to compare growth axes in the epidermis and the underlying cells in the young primordium, and to allow approximate comparison with the orientations which were measured with respect to the surface of the apex in I₁ (Lyndon, 1972) seems to be to classify the mitotic spindles as being in one of three orientations. These are: (1) directed towards the tip of the primordium, and (2) and (3) not directed towards the tip of the primordium, (2) being normal to orientation (1) and in the same plane as the sections, and (3) being normal to the plane of the sections.
The orientations of the directions of growth, classified in this way, in the epidermis and underlying cells of I₁ and the young primordium are shown in Table 2. In the epidermis of I₁ and the primordium about half the spindles are orientated towards the existing, or (in I₁) the putative future, primordium tip. The sub-epidermal cells in I₁ differ in having approximately one-third of the spindles in each of the three orientations defined here. These orientations are slightly different from those used for the analysis previously made (Lyndon, 1972) but represent the same sub-epidermal cells in which the plane of growth was judged to be random during the second part of the plastochron, just before emergence of the leaf primordium. In the primordium, however, the orientation of the directions of growth in the sub-epidermal cells becomes much more similar to that in the epidermis, with over half the spindles being orientated towards the tip of the primordium. The orientation of the spindles in the sub-epidermal cells in I₁, which corresponds to a random orientation of growth, is therefore lost in the developing primordium. In the epidermis the orientation of the growth axis therefore does not change as the cells in I₁ become part of the emerging primordium, whereas in the underlying cells the orientation becomes random in I₁ and then in the primordium orientation is restored and comes to resemble that which has persisted throughout in the epidermis.

**Rates of cell division in the primordium**

The persistence of a predominantly longitudinal axis of growth in the young primordium accounts for the longitudinal files of cells seen on the surfaces of young leaves and primordia under the dissecting microscope or by scanning electron microscopy (Fig. 3). However, the primordium in the pea becomes quite pointed just over a plastochron after its initiation, and since the files of cells remain remarkably parallel and do not conform to the shape of the primordium [Fig. 3(b)] this implies that the shape may be determined by differential rates of growth, the growth rate being fastest in the mid-line of the primordium. This is confirmed by measurements which show that the rate of cell division in the median 30 μm of the primordium is 24 per cent faster than in the lateral regions for both epidermal and non-epidermal cells, but only during the first part of the plastochron after initiation (Table 3), and not after this [Primordium (2), Table 3] or immediately before [I₁ (2), Table 3].

The apparent presence of a region of faster cell division rate near the tip of the
primordium (Lyndon, 1973) suggests that there is tip growth at this stage (Esau, 1965). This should be apparent from diagrams showing the distribution of colchicine-metaphases over the surface of the primordium as seen in face view [Fig. 4(a), (c)] Surprisingly, the epidermis at the very tip of the primordium is relatively free of cell divisions. The maximum division rate seems to be just proximal to the extreme tip, overlying the region of sub-epidermal cells with a faster division rate (Lyndon, 1973). As the primordium develops, in the second part of the plastochron after its initiation, there seem to be four main regions of maximum division rate in the epidermis [Fig. 4(d)]: the regions on the sides of the upper surface of the primordium where the leaflets will be initiated, and the regions on the sides of the apex which give rise to the stipules.
TABLE 3. Rates of cell division (percentage cells per hour) in the epidermal (E) and subepidermal (S) cells of the median 30 μm and the lateral (remaining) regions of the apex in (1) the first part and (2) the second part of the plastochron. The central zone at the summit of the apex is not included in these values. (Data for primordium from Lyndon, 1971)

<table>
<thead>
<tr>
<th>Rate of cell division</th>
<th>Median 30 μm</th>
<th>Lateral regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>S</td>
</tr>
<tr>
<td>I₂ (1)</td>
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</tr>
<tr>
<td>(2)</td>
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<td>Primordium (1)</td>
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</tr>
<tr>
<td>(2)</td>
<td>1.4</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Fig. 4. Rates of division in the epidermis of the apex as shown by the distribution of colchicine-metaphases in the median 80 μm of the primordium as seen in face view [(a), (c)] and on the sides of the apex [(b), (d)] in the first part [(a), (b)] and second part [(c), (d)] of the plastochron. Each dot represents a colchicine-metaphase.
DISCUSSION

The data presented here show that the leaf primordium in the pea is probably not at any time a cylindrical axis like the young leaf of *Graptopetalum* (Green and Brooks, 1978). Instead it tends to retain the original longitudinal polarity of the flanks of the apical dome. The initiation of a leaf primordium in the pea results not so much from the establishment of a new axis but rather from an outgrowth forming on the flanks of the apex without a major change of polarity in the epidermal cells.

The growth of a decussate apex having an apical dome with radial symmetry like the pea's would not require a 90° change of growth axis on the initiation of each successive leaf pair as postulated by Green (1980). Instead, 90° changes in growth axis would be understandable as a consequence of the longitudinal (or radial) polarity on the flanks of the apical dome. Outgrowths at 90° from each other would necessarily result in directions of expansion also at 90° from each other. If the resulting primordia are dorsiventral rather than centric, then although there may be a change in the orientation of growth on the sides of the primordia just after initiation this may be difficult to detect because the sides of a young leaf would consist of relatively few cells.

On the other hand, in a shoot apical meristem such as that of *Graptopetalum*, which at least in the early stages of its establishment does not seem to be radially symmetrical (Hardham, Green and Lang, 1980), switches in growth orientation in successive plastochrons, as proposed by Green (1980), would be unavoidable.

Since in the pea there is continuity of the same orientation in the epidermis from I, into the upper and lower surfaces of the primordium and since this same orientation is subsequently adopted by the non-epidermal cells, which lose all orientation in I, (Lyndon, 1972), this strongly suggests that it is the epidermis which is responsible for maintaining polarity and for conferring dorsiventral structure on the growing leaf.

The observation that cell divisions in the epidermis are more localized than in the underlying cells has led to the conclusion that the epidermis behaves like a skin responding, by localized growth, to the bulging of the tissues beneath it (Lyndon, 1971). The contrary hypothesis, which agrees equally well with these earlier observations and better with the present data, is that the change in shape of the apex and the bulging of the underlying tissues is controlled by localized changes in growth and cell division rate in the epidermis, which also confers its own polarity on the emerging primordium. This interpretation is consistent with the observation that there is no increase in the division rate of the epidermal cells in I, (when the shape of the apical dome in the I, region is not changing) but there is an increase when the apex changes shape as the primordium begins to emerge. This is also when the rate of cell division is highest in the underlying cells (Table 3). On this view the longitudinal orientation of growth in the epidermis of I, would be an enabling condition for subsequent formation of a dorsiventral protuberance (i.e. showing longitudinal polarity) and the increase in division rate in the epidermis at the moment of primordium emergence would be the event facilitating primordium initiation.

If control of leaf initiation resides in the epidermis then this raises three questions: (1) is it the properties of the epidermis which confer shape and form on the shoot apex and control the initiation and shape of primordia? (2) how is the predominantly longitudinal polarity in the apical dome established and maintained? and (3) is the degree of dorsiventrality of a leaf a consequence of the structure of the apical dome? Clear evidence for epidermal control of the initiation of stamen and carpel primordia is provided by the *Camellia* chimera and its parents in which the occurrence or otherwise of androecium and gynoecium depended only on the nature of the epidermis (Stewart, Meyer and Dermen, 1972). Answers to the remaining questions depend on a deeper understanding of the structure and physiology of the apical dome than we have at the moment.
Some events apparently linked to primordium initiation are, however, left unexplained. Although the underlying tissues conform to the polarity of the epidermis in the primordium (Table 2) this is not so in the \( I_1 \) region for the 16 h just before primordium emergence. During this period the planes of division in \( I_1 \) are random in the underlying cells although the epidermis retains its strongly longitudinal polarity (Table 2 and Lyndon, 1972). At first sight it seems unlikely that the randomization of the axis of growth in the sub-epidermal cells of the \( I_1 \) region is owing to a local release of physical constraint by the epidermis because the shape of the \( I_1 \) region does not change for a further 16 h and the surface of the pea apical dome is not under tension (Hussey, 1973). However, the expansion of the apical dome which occurs in the second part of the plastochron (Lyndon, 1968) could allow growth in a direction normal to the surface, resulting in periclinal divisions, mainly in the \( I_1 \) region (Lyndon, 1972). What this does not explain is why periclinal divisions are not equally frequent throughout the apical dome. Since the localization of the randomization of the growth axis to the \( I_1 \) region does not seem to be explicable as a result of local changes in shape, and therefore of local changes in physical restraint, one may perhaps tentatively conclude that it results from localized chemical changes, of a nature so far unspecified.

The hypothesis that primordium initiation is under epidermal control also leaves unexplained the plastochronic events which are not obviously linked to events in the epidermis. Snow and Snow (1933) showed that in lupins the position of a primordium became determined in \( I_1 \) about half a plastochron before its emergence, which also corresponds with the time when four events occur simultaneously in the pea apex. These events are (1) the randomization of the axis of growth in the non-epidermal cells of \( I_1 \) (Lyndon, 1970b), (2) the formation of a distinct axil between the previously initiated primordium and the apical dome, i.e. an axillary distance (Hussey, 1971) becomes measurable, (3) a marked increase of starch in those regions of the apex near this newly formed leaf axil and a sudden decrease of starch in the \( I_1 \) region (Lyndon and Robertson, 1976), and (4) a transient increase in cell division rate in the central zone, at the summit of the apex (Lyndon, 1970a). Significantly it is at this stage of the plastochron that periclinal divisions, which are characteristic of leaf initiation (Esau, 1965), first appear in the \( I_1 \) region and therefore anticipate the emergence of the primordium by half a plastochron. Although it might be plausible to suggest that the increase of starch, and the increase of division rate in the central zone, could result from a sudden change in concentration of a metabolite or a growth substance in the apex, it is not obvious what substance might be expected at the same time to bring about a localized loss of polarity of division as well as the determination of the leaf axil.

There are, therefore, two sets of events involved in leaf initiation: (1) those occurring half a plastochron before primordium emergence and concerned with determination of the leaf axil of the youngest primordium and determination of the site of initiation of the next primordium, and (2) those events occurring in, and perhaps controlled by, the epidermis at the moment of emergence of a primordium. Clearly experiments are needed to investigate further the presumed biochemical changes in the apex half way through the plastochron and also the possible effects of alterations of the structure of the apical dome on leaf initiation and form.

ACKNOWLEDGEMENT

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LITERATURE CITED

Lyndon—Leaf Initiation in the Pea


The mechanism of leaf initiation

R. F. LYNDON

Introduction

The whole of the leafy shoot originates from the activity of the meristem at the shoot apex, a minute part of the plant usually about 100–300 μm in diameter and usually consisting of no more than a few hundred cells. The leaves are formed below the extreme tip of the apex, on the flanks of the apical dome which is that region of the shoot meristem distal to the youngest primordium. The apical dome is free of primordia until a terminal flower is formed. A major change in the functioning of the shoot apex is when it makes the transition from vegetative to reproductive growth. Here we will be concerned only with vegetative growth and in particular with the initiation of leaves. Various aspects of leaf initiation and the structure of the shoot apex are reviewed by Cutter (1965), Gifford & Corson (1971), Lyndon (1973, 1976, 1977), Halperin (1978) and Green (1980).

Problems of leaf initiation

The process of leaf initiation poses two questions: (1) how is a leaf primordium formed and (2) where is it formed? The latter is really the question of phyllotaxis and is considered in Chapter 3. Here we will confine ourselves to the question of how a primordium is formed, although the question of where it is formed inevitably arises since the position is a function of the size of the primordium and the area of the apex over which the events of primordial initiation occur.

The problems of leaf initiation are:
(1) What is the mechanism of leaf primordium initiation?
How is apical structure related to leaf initiation?
(3) How is apical structure maintained?
The structure of the apex is considered first, then how leaves are initiated, and lastly how structure and functioning may be linked.

The structure of the shoot apex: the basis for leaf initiation

The cellular pattern

The apical dome in many plants is radially symmetrical, as best seen in those plants, such as conifers or the lupin, in which leaves are initiated in a spiral or helix. Its structure is less symmetrical where the apical dome is small relative to the primordia as, for example, in Clethra (Hara, 1971) or in plants with decussate or distichous phyllotaxis. In pteridophytes with a large apical cell which cuts off cells on three faces, radial symmetry may be replaced by trilateral symmetry. But even where the sectors of cells so generated are clearly distinguishable there is no correlation between the geometry of the cell sectors in the apex and the positions of leaf initiation (Golub & Wetmore, 1948; Hébant-Mauri, 1975).

A pattern seen in many angiosperm apices is the tunica-corporus arrangement of the cells in which the outer layers of cells (the tunica) show only anticlinal divisions (Clowes, 1961; Esau, 1965). It is most marked in dicotyledons, and in Acorus, for example, up to seven tunica layers may be discerned (Kaplan, 1970). However, in grasses the tunica typically consists only of the epidermis (Esau, 1965) and in gymnosperms there often does not appear to be a distinct tunica; instead, the epidermis often shows periclinal divisions and so contributes cells to the inner layers. The number of tunica layers can vary from plant to plant of the same species and also varies according to the stage of the plastochron (Clowes, 1961; Lyndon, 1976; Agarwal & Puri, 1977). In the grasses Saccharum and Erianthus it varies according to the age and the size of the apex, periclinal divisions occurring frequently in the epidermis of the apical dome of young plants and those with small apices (Thielke, 1965). In Silene plants subjected to severe nutrient deficiency, the apices are small, the number of tunica layers is reduced, and periclinal divisions may be seen in the epidermis but the apices function apparently normally (R. F. Lyndon, unpublished). Periclinal divisions in the epidermis of the apical dome have been recorded as occurring rarely in a number of dicotyledons and monocotyledons (Popham, 1963;
I. The mechanism of leaf initiation

Stewart & Dermen, 1970) but may well be less rare than is commonly supposed.

Because of the limited applicability of the tunica-corpus concept apices have been classified according to more complex schemes (Popham, 1951; Newman, 1965; Cutter, 1971). Since all apices, with or without apical cells and with or without tunicas, seem to function similarly in producing leaves on the flanks of the apex but not on the apical dome itself, the tunica-corpus arrangement and the cellular pattern in the apical dome cannot be crucial to leaf initiation. This emphasizes that the differences are likely to be at the molecular level and are not dependent on the cellular arrangements we happen to see; moreover, there are no apparent ultrastructural differences between tunica and corpus cells (Mauseth, 1981a). Models indicate that the visible cellular configurations in the apex could result from the physical constraints and pressures resulting from the shape of the apex and its derivatives (Hejnowicz, 1955; Niklas & Mauseth, 1980). The important point then becomes how this shape is maintained.

**Cytochemical zonation**

A common, perhaps universal, feature of active vegetative shoot meristems is the occurrence of a zonation, revealed histochemically, of less dense staining at the primordia-free summit of the apex (central zone) and deeper staining on the flanks (peripheral zone) where the leaves are initiated. These regions correspond to the ‘meristème d’attente’ and ‘anneau initial’ respectively of the French school of anatomists (Buvat, 1952; Nougarede, 1967). This zonation reflects the greater concentrations of nucleic acids, especially RNA, and proteins, seen at the ultrastructural level as a greater concentration of ribosomes, in the cells on the flanks than in those of the central zone (Gifford & Stewart, 1967; Nougarede, 1967; Cecich, 1977). Other differences in ultrastructure have been looked for but have not been found (Lyndon & Robertson, 1976; Mauseth, 1981a). Non-ribosomal proteins may often be in greater concentration on the flanks of the apex than in the central zone (Jacqmard, 1978; Fosket & Miksche, 1966; Riding & Gifford, 1973) but this is not always so (Evans & Berg, 1972; Mia & Pathak, 1968; Vanden Born, 1963; Thielke, 1965).

The central zone can often be distinguished because its nuclei stain for DNA more diffusely than the nuclei of the peripheral zone. This is not because the DNA content per nucleus is any less but because the nuclei
are larger. In pea, for instance, nuclei with the 2C content of DNA are two to three times the volume of 2C nuclei in the peripheral zone (Lyndon, 1973; Toupiol, 1976). In contrast, in the cactus *Echinocereus*, the nuclei in the central zone are smaller than in the peripheral zone but the central zone cells are more vacuolate (Mauseth, 1981a) and this apparently leads to the lower concentration of proteins and nucleic acids.

The cytochemical zonation pattern is independent of the tunica-corpus arrangement or cell pattern since the differential staining of central and peripheral regions occurs not only in angiosperms with diverse tunica-corpus arrangements, but also in gymnosperms with mantle-core patterns and pteridophytes with single apical cells. In different species, the cell size can be larger or smaller in the central zone than on the flanks of the apex (Lyndon, 1972a), so cell size, too, seems to be independent of the cytochemical zonation. The universality of the cytochemical zonation in vegetative apices suggests that it may be essential for apical functioning and leaf initiation.

**Ultrastructure**

Although ultrastructural differences have been looked for, none (except for ribosome concentration) have been found which are correlated with the various cyto-histological patterns. The ultrastructural differences within the apex are related to the differentiation of the cells (Lyndon & Robertson, 1976; Mauseth, 1981b). As cells differentiate into pith in the pith-rib meristem the volume per cell of all organelles except the nucleus increases (Table 1.1) and the cells become much more vacuolate; the number of mitochondria per cell increases but plastid number remains the same as in the apical dome. The developing leaf axils consist of smaller cells with more densely-packed organelles, fewer mitochondria and less endoplasmic reticulum. In the young leaf primordium, although the volume of organelles is very much the same as in the rest of the apex, there are greater numbers of plastids, mitochondria and dictyosomes per cell. The initiation of the primordium is marked by plastid replication becoming faster than cell replication and this could be regarded as one of the first signs of leaf differentiation (Lyndon & Robertson, 1976). There is a similar major development of chloroplasts in the cortical chlorenchyma of the cactus *Echinocereus*, which does not have leaves (Mauseth, 1981b).

In other respects the ultrastructure of the shoot meristem appears to
1. The mechanism of leaf initiation

be unremarkable. No changes in ultrastructure during a plastochron have been detected (Lyndon & Robertson, 1976; Mauseth, 1981a) although there is an abrupt increase in the number of plastids containing starch half a plastochron before leaf initiation (Lyndon & Robertson, 1976). However, this is a metabolic rather than an ultrastructural change and serves to emphasize that the controls for leaf initiation are almost certainly at the molecular level and that the precise cellular or ultrastructural organization of the apex is irrelevant to the zonation.

**Gradient of growth rate**

It has been inferred from the cytochemical zonation and from mitotic indices that there is a gradient of growth and cell division rates from a minimum at the summit to a maximum on the flanks of the apex where the leaves are initiated (Nougarede, 1967). In plants in which direct experimental measurements have been made there is a two- to three-fold difference in division rates between the summit and the flanks (Lyndon, 1973) but in some plants (e.g. *Helianthus* and *Nicotiana*), the difference may be much greater and the central zone relatively less active (Steeves, Hicks, Naylor & Rennie, 1969; Sussex & Rosenthal, 1973). How this growth gradient is maintained is not known. It means that changes in form will tend to take place on the flanks of the apex because for most of the plastochron this is where the growth rate is fastest.

<table>
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<th>Region</th>
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<th>Number per cell</th>
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<tr>
<td></td>
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<td>Pla</td>
</tr>
<tr>
<td>Central zone</td>
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<td>23</td>
</tr>
<tr>
<td>Peripheral zone (I₁)</td>
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<tr>
<td>Leaf primordium</td>
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<td>25</td>
</tr>
<tr>
<td>Incipient pith</td>
<td>31</td>
<td>47</td>
</tr>
<tr>
<td>Axillary cells of P₂</td>
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<td>20</td>
</tr>
</tbody>
</table>

P₂, the second youngest primordium.

After Lyndon & Robertson (1976).
**Polarity in the apical dome**

A consequence of the gradient in growth rate, with a minimum at the summit, is that if the apex is domed, as it is in most plants, then the growth of the apical surface cannot be isotropic since this would require maximum growth rate at the summit and minimum on the flanks (Green, Erickson & Richmond, 1970). In fact growth is polarized predominantly longitudinally, or radially in a flatter apex, and is most obvious in the peripheral surface cells (Hara, 1962, 1971, 1975, 1980; Lyndon, 1976). Such polarity is not only compatible with the maintenance of domed apex but also with the extreme polarization of growth and the rapid longitudinal extension of the axis below the apical dome. Although such a longitudinal, or radial, polarization would not in theory be obligatory for a flat disc-like apex, in the flat apices of *Clethra* and *Ginkgo* radial files of cells are nevertheless a distinctive feature of the apical surface (Hara, 1971, 1980). In the newly regenerated *Graptopetalum* apex there appear to be two axes of longitudinal polarization normal to each other, corresponding to the axes on which the decussate pairs of leaves are initiated (Green & Lang, 1981).

**The significant structural features**

Three features seem to be universal to vegetative shoot apices: the cytochemical zonation of central and peripheral zones which reflects differences in the concentration of proteins and nucleic acids; the gradient of cell division and growth rates which are least at the summit and greatest on the flanks of the apex; and the radial or longitudinal polarity of growth in the apical dome. All three features suggest a lower rate of growth and metabolism at the summit of the apex than on the flanks. Labelling patterns, such as those after supplying radioactive precursors of RNA, show little difference between different parts of the apex (West & Gunckel, 1968; Lyndon, 1972b) but these data alone are not reliable indications of relative metabolic rates.

The patterns of cytochemical staining and of growth rates are similar but not necessarily coincident. In pea, for example, the central zone is smaller than the region of lowest division rate (Lyndon, 1970a, 1972a). They may, however, be functionally correlated since an increase in growth rate in the central zone at the moment of leaf initiation is accompanied by a reduction in size of the central zone (Lyndon, 1968, 1970a). Also, in *Cosmos* an increase in the mitotic index in the tunica of
The mechanism of leaf initiation

1. The mechanism of leaf initiation

The events of leaf initiation

In angiosperms the first visible sign of leaf initiation is the occurrence of periclinal divisions in the tunica, which in grasses is the epidermis. In some species of conifers these periclinal divisions may be seen in the epidermis as well as in the underlying cells (Fahn, 1967). It is frequently assumed that for a leaf primordium to be formed there must arise a growth centre where growth is faster than elsewhere in the apex.
We shall see that both types of change – in the direction of growth and in the rate of growth – are involved in leaf initiation.

**Changes in the rates of growth and cell division**

Although a faster growth rate at the site of primordium initiation might be expected, when direct measurements of the rates of cell division in the different parts of the apex of clover and pea were made only a relatively small difference was found between the site at which a leaf was about to be initiated (I₁) and the opposite flank of the apex (I₂) (Denne, 1966; Lyndon, 1970a). Even in the young primordium the rate of cell division and growth did not appear to be very different from that of most of the cells of the apical dome (Lyndon, 1970a). The only direct measurements of the changes in the rate of cell division throughout the plastochron and during primordium initiation are those for the pea, on which this account will be based (Lyndon, 1970a, b, 1971, 1982).

The rate of cell division increased in the I₂ region at the end of a plastochron (Table 1.2), was maintained at this higher rate as the cells were displaced into the I₁ region in the next plastochron and then increased at initiation of the primordium (Lyndon, 1970a). Two-thirds of a plastochron after its initiation, when its axil was formed, the division rate of the primordium fell again to the lower value originally found in I₂.

The values for rates of cell division were supplemented by maps showing the distribution of colchicine metaphases throughout the apex (Fig. 1.1). On the basis of such maps, Hussey (1972) pointed out that, in both pea and tomato, there was indeed a growth centre of cells with a higher rate of division in the I₁ region, and localized at the site of

<table>
<thead>
<tr>
<th>Table 1.2. Rates of cell division in cells as they pass from I₂ to I₁ and into the primordium; (1) = first 30 h and (2) = subsequent 16 h of each plastochron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
</tr>
<tr>
<td>Rate of cell division (% cells per h)</td>
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After Lyndon (1970a).
initiation of the future primordium. Although this growth centre was first apparent at the base of the $I_2$ region and persisted into the base of the $I_1$ region, in the incipient primordium itself there was no longer a region of high division rate and the procambium had differentiated (Lyndon, 1970a). In pea, other regions of high division rate which were present at the sides of the apex, where leaves were never initiated, were the forerunners of the procambium, well before it became visibly

Fig. 1.1. Rates of cell division as indicated by the distribution of colchicine metaphases in (a) a $30\mu m$ thick longitudinal median section and (b) a $40\mu m$ thick transverse section at the base of the apical dome. Both diagrams represent the pea apex towards the end of a plastochron. The youngest primordium is at the left in both cases; the next primordium is about to be initiated in the $I_1$ region, on the right.
differentiated. It can therefore be argued that the regions of high division rate ('growth centres') are regions in which procambium differentiation is beginning, one and a half plastochrons before leaf initiation, and may not be directly concerned with leaf initiation (cf. Chapter 2).

Changes in the orientation of growth and cell division

An event which seems to be more directly associated with the emergence of a primordium (i.e. the formation of a bulge on the apical flank) is the change in orientation of growth and the occurrence of periclinal divisions in the I1 region of the apical dome half a plastochron before primordium emergence (Fig. 1.2). In the I1 region in the first part of the plastochron only one mitotic figure out of 65 (from 30 apical domes) was a periclinal division whereas in the second part of the plastochron periclinal divisions were seen consistently (22 out of 111 mitotic figures from 36 apical domes). The increase in the proportion of periclinal divisions was quite abrupt at 16 hours before leaf initiation (Lyndon, 1970b). Divisions in the epidermis were always anticlinal but,

Fig. 1.2. Proportions of anticlinal and periclinal cell divisions in the non-epidermal cells of the pea apical dome (a) during the 30 h just after initiation of the leaf primordium shown to the left and (b) during the subsequent 16 h, just before initiation of the next primordium at the I1 region, at the right. Stippled, anticlinal divisions with new cell walls normal to the section; white, anticlinal divisions with new cell walls in the plane of the section; black, periclinal divisions. (Data from Lyndon, 1972a.)
when orientations of mitotic figures in other tissues were examined, it was found that the proportions of divisions in the three planes of space into which the data were analysed were equal. There was therefore no preferred orientation, a state which persisted until primordium emergence (Lyndon, 1972a). The occurrence of periclinal divisions is therefore not because this orientation is imposed on the cells but because the restriction on periclinal divisions is lifted. Moreover, the periclinal divisions were not restricted to the tunica but seemed to be more frequent in the corpus soon after they first appeared. This has been noted for other species (Tepfer, 1960; Rickson, 1969; Lyndon, 1972a; Sistrunk & Tucker, 1974). Since this change in growth orientation occurred without a change in the growth rate of the I₁ region as a whole, it was concluded that the primary event in primordium initiation, which took place half a plastochron before primordium emergence, was a change in the plane of growth rather than a change in the rate of growth.

Rates of growth and cell division in the median part of the incipient primordium

Closer examination shows the situation to be more complicated. When the division rates in the median and lateral parts of the incipient primordium and of the I₁ and I₂ regions were examined separately, it was found that the rate of division increased by about 30% in the midline of the primordium at initiation (Lyndon, 1982) but half a plastochron later the rate had fallen and become lower than in most other parts of the apex (Table 1.3). This more pronounced increase in division rate in the midline of the new primordium occurred 16 hours after Lyndon (1982).

<table>
<thead>
<tr>
<th></th>
<th>I₂ (1)</th>
<th>I₁ (1)</th>
<th>Primordium (1)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermis</td>
<td>0.3</td>
<td>1.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Underlying cells</td>
<td>1.0</td>
<td>2.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Median 30μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermis</td>
<td>1.1</td>
<td>2.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Underlying cells</td>
<td>3.0</td>
<td>3.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Lateral regions</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Table 1.3. Rates of cell division (% cells per h) in the median and lateral regions of the apex in the first 30 h (1) and the subsequent 16 h (2) of the plastochron.

After Lyndon (1982).
after the change in orientation of growth so that the two changes were not simultaneous. Despite the change in plane of growth in the underlying cells, the apex as a whole does not change shape substantially until the moment of primordium appearance.

**Growth of the epidermis and the non-epidermal cells**

Comparison of the rates of growth with the changes in shape of the apex suggested that during the first part of the plastochron there was a restraint such that growth within the apical dome led to a bulging into the tissues proximal to it (Lyndon, 1970a). A good candidate for a restraining layer would be the outer layers of the apex, perhaps the epidermis. Although it was concluded that the epidermis was acting like a skin, responding to the growth of the tissues within it (Lyndon, 1971), this view was influenced by the apparent deep-seated nature of the morphogenetic events, i.e. the occurrence of periclinal divisions which appeared to originate deep in the corpus and did not extend to the epidermis. If instead we regard the localized growth of the epidermis as being essential for primordium initiation, then the increase in growth rate in the epidermis and the underlying cells in the midline of the primordium at initiation becomes crucial, and we can tentatively assign a controlling role to the processes which allow this increased growth rate.

The orientations of growth of the epidermis and the underlying cells differ (Table 1.4). During the second part of the plastochron the subepidermal cells in $I_1$ show random orientation of planes of division.

<table>
<thead>
<tr>
<th></th>
<th>$I_1$</th>
<th>Primordium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>Epidermis</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Underlying cells</td>
<td>38</td>
<td>62</td>
</tr>
</tbody>
</table>

After Lyndon (1982).
1. The mechanism of leaf initiation

In the epidermis, however, the longitudinal orientation of growth which becomes established at the transition from $I_2$ to $I_1$ is largely maintained throughout $I_1$ and into the primordium and this polarity is adopted by the underlying cells in the developing primordium (Lyndon, 1982). This suggests a controlling role for the epidermis in the establishment of polarity in the young primordium. If the epidermis is the restraining layer preventing outgrowth of the apex then the immediate event resulting in primordium initiation would be an increase in growth rate of the epidermis.

Two independent observations are relevant and striking. The first is that in irradiated wheat seedlings the first stages of primordium initiation occurred in the absence of cell division and consisted of the swelling of the epidermal cells to form a bulge (Foard, 1971). The second is the overriding control of primordium initiation shown by the epidermis in a Camellia chimera (Stewart, Meyer & Dermen, 1972). The parents were C. sasanqua which shows normal flower development, and C. japonica, in which the flowers have sepals and petals but no stamens or carpels. In the chimera with a sasanqua epidermis and a japonica interior, stamens and carpels were formed. Whether or not stamens and carpels were initiated therefore depended solely on the genetic constitution of the epidermis suggesting that the control of primordium initiation is exercised by the epidermis.

The possible importance of epidermal control is also suggested by the observations of the reorientation of microtubules and cellulose microfibrils in the epidermal cells of the succulent Graptopetalum before primordium initiation (Hardham, Green & Lang, 1980) and before any cellular rearrangement or reorientation of growth direction can be seen in the underlying cells (Green & Lang, 1981). However, it is not known whether reorganization of the microtubules and cellulose microfibrils had also taken place in the underlying cells, or whether the orientation of their mitotic spindles had changed, and so it is not clear whether the reorientation of growth direction in Graptopetalum is confined to the epidermis. In pea, the reorientation of growth planes certainly occurs in the underlying cells before there is any evidence of the primordium as a bump (Lyndon, 1970b, 1972a), and in fact it is the epidermal cells which are conservative in their polarity (Lyndon, 1982). Pea and Graptopetalum differ, however, in that in the former the apex was already established and had been functioning for about four plastochrons after germination whereas in Graptopetalum the regenerating apex was being organized at the same time as it was initiating its first
leaves. It is therefore not entirely clear whether the epidermal reorganization seen in *Graptopetalum* was concerned primarily with leaf initiation or whether it might be more to do with the reorganization of the apex itself.

The reorientation of growth axes on the sides of the primordium, stressed by Green (1980), may be more obvious in *Graptopetalum* in which the leaves are more-or-less centric, than in pea in which the primordium, which is essentially a crescent, gives rise to a laminar leaf (Lyndon, 1982).

**The cellular basis for leaf initiation: changes during the plastochron**

We may distinguish two sets of events during a plastochron: those which occur before primordium emergence and which are therefore perhaps requirements for initiation but are not directly concerned with the actual change in shape of the apex, and those which are the immediate cause of the change in shape of the apex to give the bulge which is the primordium. There may be a third set of events, which so far have not been identified and which cause determination (i.e. fix the fate) of the primordium and its parts; these will be considered later.

*The first set of events: before leaf initiation*

Those events occurring half a plastochron before primordium initiation in the pea are:

(i) The randomization of the growth axis in the subepidermal cells of $I_1$.

(ii) A decrease of starch in $I_1$ and an increase on the opposite side of the apex.

(iii) Increase in division rate in $I_2$ and decrease in division rate in the youngest existing primordium.

(iv) A transient increase in division rate in the central zone.

(v) The formation of a distinct axil in the youngest existing primordium.

Since the randomization of the division planes in $I_1$ takes place 16 hours before any visible change in the shape of the apical dome at this point (Lyndon, 1970a) it presumably cannot be a response to removal of physical restraint. If it were, then it is not clear why other parts of the apical dome should not also show the same randomization of division
planes, which they do not (Lyndon, 1982). This points to the randomization being the result of localized chemical changes in the cellular environment of I\textsubscript{1}, which may also be responsible for causing the changes in starch accumulation in the plastids (Lyndon & Robertson, 1976) and the transient increase in division rate in the central zone (Lyndon, 1970a).

The axil of the youngest primordium also forms at this time, so that an axillary distance (Hussey, 1971) becomes measurable. It is an event which determines the diameter of the apical dome by fixing the position of the axillary cells at a more-or-less set distance from the opposite axil. If this did not occur the apical dome and primordium would continue growing as a single unit with the axillary cells being carried upwards passively by the growth of the tissues beneath them.

Whether the increase in division rate in I\textsubscript{2} and the decrease in the primordium at this time are also linked events is less certain.

The possibility then is that 30 hours after the initiation of the last primordium and 16 hours before the initiation of the next there is a change in concentration of a chemical substance at the apex which has these diverse effects. The different concentrations of starch on the opposite sides of the apex are consistent with these arising from the same stimulus as causes randomization of the plane of division in I\textsubscript{1} but not in I\textsubscript{2}. Since the position of the next leaf is governed by the positions of the existing leaves (Richards, 1951) the different effects of the stimulus on the opposite sides of the apex may well result from some influence from the existing leaf primordia.

\textit{The second set of events: at leaf initiation}

The second set of events is those occurring at primordium emergence. In pea these are:

(i) Increase in division rate in cells (epidermal and subepidermal) in the midline of the incipient primordium.

(ii) Beginning of outward growth of the primordial bulge.

(iii) Transient increase in division rate and reduction in size of the central zone.

All can be accounted for by an increase in the rate of growth and cell division, the significance of which is easier to comprehend for the emerging primordium than it is for the central zone. In order for the primordium to bulge out there must be a more rapid localized growth of the epidermis since this grows only by increase in area.
It is pertinent here to consider what might be meant in physiological terms by ‘the size of a primordium at initiation’. This may be regarded as the area of the apical surface and the associated cells beneath which contribute to form the primordial bulge. This area, relative to the apical area, is the principal determinant of phyllotaxis (Richards, 1951). The size of the incipient primordium could be described by two parameters: (1) the size of the region of the apical dome in which randomization of cell division planes occurs, this being probably larger than the primordial size (Lyndon, 1972a); and (2) the area over which the properties of the surface change so that a bulge is formed. This will be determined by the area of influence of whatever increases division rate in the midline of the incipient primordium and delimits the area of the epidermis that will grow faster to accommodate the bulge.

If a substance such as auxin, which could cause wall loosening in the epidermis and so allow the tissues to bulge, were produced in the inner tissues it would have to diffuse outwards to reach sufficient concentration at the epidermis at the time of primordium initiation for this event to occur. Is the randomization of division planes in I₁, 16 hours before this, the moment at which the localized activation of an enzyme system for synthesis of auxin, or some other morphogen, begins? If so, this might account for the apparent spread of periclinal divisions to the tunica from the inner tissues. But such divisions are found in the tunica at least 10 hours before primordium initiation and it would be necessary to make the further postulate that the threshold concentration of morphogen for changing the plane of division would have to be quite low and much higher for increased wall growth in the epidermis.

Such a hypothetical scheme would link the events occurring half a plastochron before leaf initiation and those occurring at leaf initiation, but raises the questions of whether a single substance could possibly be responsible for all events and whether a delay of 16 hours between events in the underlying cells and the epidermis could plausibly be accounted for by the need for different threshold concentrations.

The temporary reduction in size of the central zone (presumably by a change in the degree of hydration of the cells) and the simultaneous and also temporary disappearance of the gradient of growth rate in the apex suggests that whatever conditions maintain the cytological features of the central zone also maintain its low growth rate and that these conditions are changed transiently when a leaf is initiated. But whether the events in the incipient leaf control those in the central zone, or vice versa, is a matter for speculation.
I. The mechanism of leaf initiation

Structure of the apical dome in relation to leaf structure

In an established apex, such as that of the pea, the dome has an essentially radial structure with predominantly longitudinal polarity which corresponds to the requirements for a domed apex with minimum growth rate at the summit (Green, Erickson & Richmond, 1970). This polarity then may determine the polarity of growth in the leaves through the influence of the epidermis (Lyndon, 1982). However, an established radial structure of this kind is not essential since in *Graptopetalum* the polarity may have to change at the initiation of successive leaf pairs (Hardham, Green & Lang, 1980). If this is so, there are two possibilities: one is that this lack of radial symmetry may be related to the decussate phyllotaxis. The other is that the reorganization of polarity is specifically linked to and imposed by the processes of leaf initiation. The leaves of *Graptopetalum* are centric or nearly so, whereas pea leaves are strongly dorsiventral and it is possible that the degree of dorsiventrality of a leaf may therefore be a function of the degree of polarity already existing in the apical dome at the site of leaf initiation.

It is not clear exactly what are the properties of the apex which make a leaf dorsiventral. Experiments on potato apices showed that when a potential leaf site (I1) was isolated from the summit of the apical dome by cuts the resulting organ which developed was centric (either a leaf or a bud) instead of being dorsiventral (Sussex, 1951). This suggested that some influence, presumably chemical, from the apical summit was essential to cause dorsiventrality in the developing primordium. However, these results could not be repeated with a different cultivar of potato, although they could be repeated in *Epilobium* (Snow & Snow, 1959). Such experiments, and those in which the apical summit was destroyed by a prick but the meristems continued to produce dorsiventral leaves (Wardlaw, 1950; Sussex, 1954), suggests that induction from the summit is not needed to make the leaves dorsiventral. Snow & Snow (1959) suggested that ‘possibly in these species the tissues of the apical cone may become longitudinally polarized in their microstructure as they pass down from the summit of the apex to the leaf-forming zone so that the leaves arising from such polarized tissues are inherently dorsiventral from the start’. In view of what we now know this seems a very pertinent comment. A repetition of the experiments on potato apices in conjunction with a detailed examination of the surface structure could perhaps lead to an understanding of why contradictory results were obtained.
The dorsiventrality of the leaf may also, at least in part, owe its origin to the difference in growth rate between the adaxial and abaxial surfaces of the leaf which can be traced back to the difference in growth rates between the upper and lower parts of the I₁ region (Lyndon, 1970a, 1976; Hussey, 1972). The abaxial surface of the leaf characteristically grows quicker, resulting in the upwards growth of the young primordium and the resulting enshrouding of the apex by the growing leaves. These various considerations show the need to investigate in more detail the relationship between the structure of the apex and the form of the leaves which are initiated on it.

**Determination of the leaf**

There are probably several steps in the determination of a leaf of which it is possible to identify the following:

(i) The determination of a site on the apical surface as a primordium. This would mark the fixing of the position of a leaf.

(ii) The determination of the nature of this primordium as a bud, leaf or other organ (e.g. as a centric organ, or as a spine).

(iii) Determination of the parts of the primordium as axis, lamina, leaflets or stipules.

Surgical experiments on apices have shown that the position of the next primordium may be altered until half a plastochron before its emergence. Incisions made in the apex during the half plastochron before initiation resulted in the formation of bracts rather than leaves (Snow & Snow, 1933). These experiments therefore suggest that the position and the nature of the primordium both become determined at the same time, about half a plastochron before emergence. In ferns, however, the primordium is not committed to being a leaf until about three plastochrons after its initiation, until which time it could transform into either a bud or a leaf (Cutter, 1954). When the I₁ regions of potato apices were isolated by cuts they developed into leaves showing that this region was already committed as a leaf (Sussex, 1951). The upper and lower surfaces of the pea leaf may also be determined as such at the time of initiation. This is suggested by pea mutants for waxiness and leaf shape which show that the lower surface of the leaf corresponds in its expression of certain genes to the whole of the normal stipule but that the gene expression on the upper surface of the leaf may differ from that on the lower (Marx, 1977). The determination process (whatever it is) continues until one plastochron after initiation. By this time the excision
of parts of the leaf does not allow regeneration and so the form of the leaf has become fixed (Sachs, 1969).

The process of determination for a leaf may therefore extend over one and a half plastochrons, or longer in the ferns. Processes of determination continue in the leaf with the differentiation of the various tissues in the mature leaf. The maturation of the leaf progresses from the tip downwards (see Chapter 6). This process may start at the moment of leaf initiation when the epidermal cells at the tip of the incipient primordium begin to divide more slowly than the other cells (Lyndon, 1982), and prompts the interesting speculation that perhaps the epidermis is the site where leaf maturation begins.

In conclusion

It is easy to forget that the leaf is not a single but a dual structure – a leaf with a bud in its axil. The visible formation of the axil half a plastochron after leaf initiation may well be preceded by the determination of these cells as axillary long before this. The axil cannot be explained simply as a group of cells which grow more slowly. Apart from having the potential to grow into a new shoot with its own apical meristem, their significance for leaf initiation is that they remain in a particular spatial relationship to the opposite side of the apical dome. It may well be more pertinent to concentrate on what happens in the axillary cells and on the flanks of the primordium in order to understand the mechanisms controlling the changing shape of the apical system which we call leaf initiation.

References


1. The mechanism of leaf initiation


The Relationship Between the Distribution of Periclinal Cell Divisions in the Shoot Apex and Leaf Initiation

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ABSTRACT

Periclinal cell divisions in vegetative shoot apices of Pisum and Silene were recorded from serial thin sections by mapping all the periclinal cell walls formed less than one cell cycle previously. The distribution of periclinal divisions in the apical domes corresponded to the distributions subsequently occurring in the apices when the young leaf primordia were forming. In Pisum, periclinal divisions were almost entirely absent from the I region of the apical dome for half a plastochron just after the formation of a leaf primordium and appeared, simultaneously over the whole of the next potential leaf site, about half a plastochron before the primordium formed. In Silene periclinal divisions seemed to always present in the apical dome at the potential leaf sites and also round the sides of the dome where the ensheathing leaf bases were to form. Periclinal divisions therefore anticipated the formation of leaf primordia by occurring, in Pisum about one cell cycle and in Silene two or more cell cycles, before the change in the direction of growth or deformation of the surface associated with primordial initiation.

Key words: Pisum, Silene, planes of cell division, orientation of cell walls, leaf primordia, shoot apical meristem, plastochron.

INTRODUCTION

The first visible event in leaf initiation is the occurrence of periclinal cell divisions in the tunica layers at the site of the future primordium (Esau, 1965), but some also occur in the corpus (Lyndon, 1972). In the apical dome of the Pisum shoot meristem, periclinal divisions appeared in the I region (the site of the next primordium) about half a plastochron before the next primordium appeared. They remained at a frequency of approximately one third of all divisions (Lyndon, 1970b). The simplest interpretation was that the plane of division, which was entirely anticlinal in the apical dome in the first half of the plastochron, became random during the second half (Lyndon, 1972). This change was not accompanied by a change in the rate of division in the I region of the apical dome (Lyndon, 1970a). Only when the apex began to change shape as the new primordium was initiated did the rate of division increase in the epidermis (Lyndon, 1982). The events associated with leaf initiation in the Pisum shoot apex can therefore be placed into two groups: (1) those occurring in the internal cells about half a plastochron before the leaf primordium appears, when periclinal divisions are first seen, and not accompanied by changes in the form of the apex, and (2) an increase of the rate of cell division in the epidermis associated with the bulging of the apex and the change in the shape at the time of primordium emergence (Lyndon, 1983).

Since the change in orientation of cell division to predominantly periclinal in the Pisum shoot apical dome was not immediately associated with the bulging primordium, and the periclinal divisions were not confined to the I region (Lyndon, 1972), these changes could be interpreted as being necessary to enable the subsequent primordium to develop without themselves being causal. The determining events could be those occurring in the
epidermis at the time of primordium appearance: the increase in the rate of cell division and the imposition of the epidermal polarity on the underlying cells in the new primordium (Lyndon, 1982). Changes in the orientation of cell divisions and microtubules in epidermal cells are interpreted as being causal to the formation of a new shoot axis in *Graptopetalum* (Green, 1980; Hardham, Green and Lang, 1980). However, the changes in the epidermis could also be interpreted as being simply a response to the growth of the tissues beneath them (Lyndon, 1971). In this case the primary causal events in leaf initiation would be the occurrence of periclinal cell divisions in the internal cells almost half a plastochron before primordium appearance.

In order to distinguish between these different interpretations of leaf initiation, more information is required. Two possible situations exist. On one hand, a distribution of periclinal divisions generally throughout the apical dome with changes in the division pattern in the epidermis predicting the form of the primordium would point to the epidermis having the determining role with the underlying cells merely allowing rather than causing primordium formation. On the other hand, if it could be shown that the distribution of periclinal divisions anticipated the shape and position of the new primordium this might point to the early events in the internal cells being those determining the new primordium. This would be in agreement with the idea that the leaf primordium is determined about half a plastochron before it becomes visible, i.e. about half-way through I (Snow and Snow, 1933).

The purpose of this work is to examine the distribution of new periclinal divisions at different stages throughout the plastochron and to relate them to the position and shape of the primordium subsequently formed. In previous work, the position of periclinal divisions was recognized from the orientation of mitotic figures. These were sufficiently infrequent in the shoot apex that it was difficult to get the required data (Lyndon, 1972). Now, use has been made of both light and electron microscopy to examine the orientation of new cell walls rather than relying on the orientation of mitotic figures. The shoot apical meristems of two species, *Pisum* (with distichous leaves) and *Silene* (with opposite decussate leaves) were examined to determine whether the distribution of periclinal divisions corresponds with the area where a new primordium will form or whether they are more generally distributed throughout the apex, and whether they appear first at a particular locus and subsequently spread.

**MATERIALS AND METHODS**

*Pisum sativum* were grown in sand at 22 °C in cycles of 12 h light (100 \( \mu \text{M} \text{s}^{-1} \text{m}^{-2} \) provided by fluorescent tubes + incandescent lamps)/12 h darkness. Plants were taken for examination 11 d after sowing, when two to three leaves had unfolded.

*Silene coeli-rosa* (L.) Godron plants were grown at 20 °C in non-inductive short-day cycles of 8 h light (360 \( \mu \text{M} \text{s}^{-1} \text{m}^{-2} \) provided by fluorescent tubes + incandescent lamps)/16 h darkness (Miller and Lyndon, 1976). Plants were sampled 28 d after sowing. All apices were vegetative.

**Electron microscopy**

Apices were dissected out and immediately dropped into fixative containing 2 per cent paraformaldehyde, and 2-5 per cent glutaraldehyde in 0·05 M phosphate buffer at pH 7·0 for 4 h. The tissue was then washed in buffer for 1 h before post-fixation in 1 per cent aqueous osmium tetroxide for 2 h. Apices were further rinsed in distilled water for 1 h, then dehydrated through a graded ethanol series and finally embedded in araldite resin. Longitudinal sections, in the plane of the primordia, and transverse sections were cut for both light and electron microscopy. Serial sections were sampled at 10 \( \mu \text{m} \) intervals.
TABLE 1. Thickness of walls seen in cross section bounding cells in prophase and metaphase/anaphase and separating cells in telophase in the shoot apex of Pisum

<table>
<thead>
<tr>
<th></th>
<th>Mean cell wall thickness (µm) (± s.e.)</th>
<th>Number of walls measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophase</td>
<td>0.169 ± 0.062</td>
<td>33</td>
</tr>
<tr>
<td>Metaphase/anaphase</td>
<td>0.157 ± 0.048</td>
<td>104</td>
</tr>
<tr>
<td>Telophase</td>
<td>0.088 ± 0.019</td>
<td>14</td>
</tr>
</tbody>
</table>

through each apex. Where possible, serial thick sections (1 µm) were stained with 0.05 per cent toluidine blue and examined under a Vickers M41 photomicroscope. Thin sections were collected onto copper grids and stained with uranyl acetate followed by lead citrate and examined under a JEOL 100S transmission electron microscope.

**Measurement of cell wall thickness**

Measurements of cell wall thicknesses in thin sections of Pisum were made on cells in interphase and in mitosis. The walls surrounding interphase, prophase and metaphase/anaphase cells were found to be similar in thickness but newly formed walls in telophase cells were significantly thinner (Table 1). A cut-off point of 0.1 µm, below which all telophase cell wall measurements fell, was chosen to distinguish recently formed walls. Eighty-seven per cent of periclinal walls in the pea apices were found to be below this value. Subsequently, the recording of periclinal walls was carried out on photographs of thick (1 µm) sections. The most recently formed walls (i.e. those formed less than one cell cycle ago) could be distinguished as being thinner than the walls they abutted. In Silene as well as Pisum the criteria therefore used to distinguish recently formed periclinal walls were: (1) that the cell walls were apparently formed in the last cell cycle and were approximately as thin as a wall separating telophase nuclei; and (2) that they had no other walls abutting onto them and were lying parallel to the nearest epidermal surface in such a manner that they appeared to more or less bisect the cells in a plane parallel to that of the layer in which they were found and did not appear to be a corner of an adjacent cell.

**Criteria for determining cell wall orientation**

Periclinal divisions can be readily recognized in longitudinal median sections. However at the sides and towards the top of an apical dome periclinal and anticlinal divisions can be confused. To overcome the ambiguity the lateral extent of periclinal divisions was determined from transverse sections and compared with the results obtained from longitudinal sections. Many more periclinal walls appeared in the transverse than in the corresponding longitudinal sections. This was unexpected since reconstructions from both transverse and longitudinal sections would be expected to produce similar results. Closer examination showed that the increased number of periclinal walls in transverse sections was due to ambiguity in the recording of anticlinal and periclinal walls. This was resolved by tracing sections onto transparencies then stacking consecutive 1 µm sections at each sampling level through an apex so that the unambiguous orientation of walls relative to the nearest apical surface could be found and anticlinal and periclinal walls could be clearly distinguished.
Definition and classification of plastochron stage

In order to follow changes through a plastochron it was necessary to define when one (Pisum) or a pair (Silene) of primordia were initiated and to divide the plastochron into stages. For Pisum the leaf primordium was regarded as forming when the apex began to bulge. The protuberance of the primordium increased from 0 μm (plastochron stage 0-0) to 60 μm (plastochron stage 1-0) when the next primordium was formed. The plastochron could therefore be divided into 10 parts each measured as a 6 μm increment in the protrusion of the primordium beyond the line drawn from its axil to the axil of the leaf immediately below and therefore two plastochrons older (Lyndon, 1968). In the present work it was convenient to group plastochron stages 0-1, 0-2 and 0-3 together, as the beginning of the plastochron; stages 0-4, 0-5 and 0-6 as the mid-plastochron when changes occur in the I₁ region in the pea apex (Lyndon, 1983) and plastochron stages 0-7, 0-8 and 0-9 as the late plastochron. Here the stages are designated 0-1 to 1-0 where the units 0 and 1 represent successive leaves irrespective of their serial number.

In Silene a similar, and comparable system was worked out. A pair of opposite leaves is formed each plastochron, at 90° from the preceding pair. The rate of leaf initiation was given by the mean increase in the number of leaf pairs per day. This was linear with time \((r = 0.87, b = 0.243, n = 47)\), the regression coefficient \((b)\) implying an increase of 0.243 leaf pairs per day, equivalent to a plastochron of 4.1 d, which is very similar to the value of 3.9 d previously obtained (Lyndon, 1977). The plastochron was therefore taken to be 4 d. Measurements on median sections of apices showed that the height (μm) of the summit of the apex above the axil of the youngest, 2nd youngest, or 3rd youngest pair of primordia increased linearly with time in days. The respective correlation and regression coefficients were: \(r = 0.87, b = 13.3; r₂ = 0.86, b₂ = 14.3; r₃ = 0.86, b₃ = 16.9; n = 47\) in each case. The regression coefficients imply a mean increase in apex height of 15 μm per day and therefore 60 μm per plastochron. The best measure of plastochron stage was the total lateral extent of the primordia, measured as the maximum width across a pair of primordia seen in a longitudinal section in the plane of the primordial insertions, minus the width of the apical dome at the axil of these primordia. The lateral extent of the primordia, \(P\), was correlated with the height of the apex above the axil of \(P₋1\) \((r = 0.88, b = 0.97; n = 40)\) such that, to a close approximation, the lateral extent of the primordia, \(P\), increased by 60 μm, from 25 to 85 μm in the plastochron after initiation, while the apex height above axil \(P₋1\) increased from 65 to 125 μm. The value of 25 μm for the lateral extent of the primordia when first visible is because they arise on the sides of a domed apex and 25 μm therefore represents the difference in width of the apical dome at the levels of the putative primordial tips and their putative axils just before they become discernible. Silene apices were therefore classified according to the lateral extent of the primordia, or where this was not possible because of the plane of the sectioning, the height of the apex above the axil of the youngest or 2nd youngest pair of primordia. It was fortuitous that the plastochron of Silene could be divided into 10 stages each representing a 6 μm increment in the lateral extent of the primordia (as in the pea), or also by 6 μm increments in the height of the apex. Again, as in the pea, plastochron stages 0-1, 0-2 and 0-3 were grouped as the early plastochron, stages 0-4, 0-5 and 0-6 (when the leaf axils began to form, as in the pea at this stage) as the mid-plastochron, and stages 0-7, 0-8 and 0-9 as the late plastochron.

RESULTS

Pisum

Examination of longitudinal sections of apices showed there are very few p-divisions (periclinal divisions) in the \(I₁\) region during the early plastochron (stages 0-1-0-3) (Fig. 1). P-divisions begin to appear in mid-plastochron (stages 0-4-0-6) and increase in
Fig. 1. Distribution of periclinal divisions as seen in longitudinal median sections of Pisum apices. Periclinal divisions are scarce or absent in I, in early plastochron (stages 0-1-0-3) then appear from mid-plastochron (stages 0-4-0-6) onwards, mainly at the site of the next primordium. In the developing primordium (late plastochron, stages 0-7-0-9) the periclinal divisions tend to become concentrated in the centre of the primordium as it begins to protrude (cf. Fig. 2). Data from the three most median sections of each apex. Each diagram represents four to eight apices.

Table 2. Distribution of periclinal divisions between tunica layers in Pisum

<table>
<thead>
<tr>
<th>Plastochron stage</th>
<th>Number of periclinal divisions</th>
<th>I, cell layer</th>
<th>Primordium cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Early: 0-1-0-3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mid: 0-4-0-6</td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Late: 0-7-0-9</td>
<td>32</td>
<td>32</td>
<td>24</td>
</tr>
</tbody>
</table>

Tunica layers are numbered from the outside inwards; the epidermis (layer 1) is not recorded as periclinal divisions are almost entirely absent from it. The subepidermal layer is 2, the next layer 3, internal to that (inner cells) the layers are not readily distinguished. Each line of values represents data from five to eight apices, and the seven most median 10 μm-longitudinal sections from each apex.

Frequency in late plastochron. P-divisions can be assigned to either the first or second subepidermal tunica layer or to the underlying cells. P-divisions appear in mid-plastochron evenly throughout the I, region in almost equal proportions in the three layers distinguished (Table 2). However once the primordium has emerged (P1, early) the relative frequency of p-division in 3 layer decreases.

The p-divisions in the longitudinal sections can be remapped to show more clearly their vertical distribution. In the central part of the young primordium (P1) during early and mid-plastochron (stages 0-1-0-3 and 0-4-0-6) (Fig. 2) p-divisions extend from just above the axil of the leaf vertically below P1 to the approximate position at which the axil of P1 will form (Figs 1 and 2). The vertical distribution of p-divisions in I, in mid and late plastochron (stages 0-4-0-6 and 0-7-0-9) is very similar to that in P1, up to mid-plastochron (Fig. 2). The vertical distribution of p-divisions therefore does not appreciably change as I, becomes P1. In the late plastochron the p-divisions become more concentrated towards the central part of P1 and this is related to the increasing protrusion of this young primordium (Fig. 1.)

The lateral extent of p-divisions is found by remapping the divisions in the longitudinal sections to show their distribution as though seen by looking down onto an apex (Fig. 3). The top 30 μm of the apical dome, in which p-divisions tend to be parallel to the plane
of the section, have been omitted. As previously noted, very few p-divisions are seen in \( I_1 \) during the early plastochron (Fig. 3A). By mid-plastochron (stages 0-4-0-6) and late plastochron (stages 0-7-0-9) more p-divisions appear in the \( I_1 \) region and assume a lateral spread similar to that in the \( P_1 \) region. Since in longitudinal sections, p-divisions at the sides of the apex tend not to be recognized, there is an apparent lack of p-divisions at the sides of the apex. However the full extent of the spread of p-divisions is seen in the transverse sections at mid-plastochron shown for comparison (Fig. 3B). In mid to late plastochron in \( I_1 \), the lateral distribution is very similar to that in the primordium, and covers an arc of about half the circumference of the apex.

The vertical and lateral extents of the p-divisions in the \( I_1 \) region during the second half of the plastochron therefore more or less correspond to the shape and extent of the subsequent primordium so that the distribution of p-divisions in \( I_1 \) anticipates the distribution when the primordium becomes visible and is growing out. However once the primordium emerges there is a shift in the location of divisions within the tunica layers and towards the more central parts of the primordium.

**Silene**

The distribution of p-divisions as seen in longitudinal sections in the plane of the youngest pair of primordia in the early plastochron is shown in Fig. 4A. Most of the p-divisions are associated with the bulges which are the young primordia but there are also p-divisions in the apical dome. Longitudinal sections in the plane at 90° to the young primordia in late plastochron (Fig. 4B) show that most p-divisions are on the flanks of the apex at the base of the \( I_1 \) region, anticipating the emergence of the next pair of primordia, and also lower on the sides of the apex where the ensheathing bases of the existing primordia are growing. Transverse sections of the lower half of the apical dome in the late plastochron, corresponding to the region indicated in Fig. 4A, show that p-divisions may be more frequent at the sides of the apical dome than at the \( I_1 \) sites where the next pair of primordia will form (Fig. 5B). In the early plastochron p-divisions are
Fig. 3. Lateral extent of periclinal divisions as seen in transverse sections of Pisum apices, (A) reconstructed from data of longitudinal sections and (B) obtained directly from transverse sections. The top 30 μm of the apical dome, in which periclinal cell walls tend to be parallel to the plane of the paper, are omitted. The extent of periclinal divisions in I₁ at mid-plastochron (stages 0·4 to 0·6) is similar to that in the young primordium (P₁, stages 0·1 to 0·3) to which it gives rise. Each diagram represents three to six apices.

also present in the I₁ region (Fig. 5A) so that there is apparently no time when the I₁ and I₂ regions in Silene are free of p-divisions, as in the case in the pea. The apparently higher frequency of p-divisions in the late plastochron at the sides of the apical dome which will give rise only to the ensheathing bases of the next leaf pair (Fig. 5B) is not dissimilar from the distribution of p-divisions at the level of young primordia in the early plastochron (Fig. 5C) where there is a surprisingly high frequency of p-divisions at the sides of the apex. The distribution of p-divisions in I₁ therefore seems to anticipate the distribution found when the young primordia are forming.
Fig. 4. Distribution of periclinal divisions in longitudinal sections of Silene apices. (A), Sections at 90° to the plane of the youngest pair of primordia, in late plastochron (stages 0-7-0-9). The ensheathing bases (EB) of these primordia, which would be in front of and behind the page, are seen as the slight bulges at the sides of the base of the apex. (B), Section in the plane of the youngest pair of primordia (P₁), in early plastochron (stages 0-1-0-3). The apical dome in (A) in late plastochron becomes the apex as in (B) in the next plastochron, as indicated by the arrow. The diagrams represent the three most median sections of (A), 10 and (B), six apices.

Fig. 5. Distribution of periclinal divisions in transverse sections of the lower half of the apical dome of Silene in (A), early plastochron (stages 0-1-0-3) and (B), late plastochron (stages 0-7-0-9), and (C) of the apex at the level of the young primordia in early plastochron (stages 0-1-0-3). The distribution of periclinal divisions in the apical dome in late plastochron, (B), anticipates the distribution early in the next plastochron, (C), when the primordia (P₁) have formed at what were the I₁ sites. (A) and (B) represent the basal 20 μm of the apical dome, of four to six apices, in the positions indicated by the vertical bar at the left of Fig. 4A; (C) represents a 20 μm slice at the position indicated by the bar at the right of Fig. 4B.

DISCUSSION

The formation of leaf primordia in Pisum and Silene is preceded by the occurrence of p-divisions not only in the tunica but also (and perhaps mainly) in the corpus. The distribution of p-divisions within the I₁ region of the apical dome corresponds to the distribution of p-divisions in the young primordia which are formed from the I₁ region and also corresponds in extent to the whole of the region which forms the new primordium. In Silene this includes the sides of the apex where the bases of the opposing primordia meet to form a sheath round the apex.

In Pisum p-divisions are present at least half a plastochron before the apex begins to bulge to form the primordium. They do not originate from a locus and spread, as might be expected if they resulted from some substance diffusing from a particular locus but appear simultaneously over the whole of the region of the putative primordium. In Silene, p-divisions are always present probably because the apical dome is much smaller. Being a segment of a hemisphere, any growth occurring between the initiation of successive primordia involves a greater proportion of the cells than in Pisum.
In Pisum p-divisions appear about 16 h before the primordium begins to form, which is similar to the length of the cell cycle of 15 h in the lower part of the I region (Lyndon, 1973). The change in division orientation is therefore expressed as a new direction of growth after the cells have grown for one cell cycle. In Silene, however, the plastochron is about 4 d but the cell cycle is 20 h or less in vegetative plants (Miller and Lyndon, 1975, 1976; Ormrod and Francis, 1985), so that there are several cell cycles in the apical dome before a change in shape occurs. The implication is that p-divisions do not lead to an immediate change in growth direction although they may be necessary for it to occur later. A further implication is that there may be two controls on primordium formation: (1) the control of the plane division in the apex and (2) the control of the change in the direction of growth, which occurs only in the region where there have been previous p-divisions. This is in accord with the observation of Selker and Green (1984) that a new cellular polarity and a new direction of growth, as shown by the reorientation of microtubules and wall microfibrils, is seen only in cells which have previously divided periclinally. In Silene several successive p-divisions may be observed at the same position within a cell layer, a consequence of there being two or more cell cycles between the first occurrence of p-divisions in the I regions and the formation of the primordia.

Since p-divisions occur in the I region before the apex begins to bulge to form the primordia the question arises of why the orientation of divisions in the apical dome changes or why p-divisions occur which are not immediately involved in an outward growth of the apical surface. In Silene the growth and cell division rate is apparently relatively uniform throughout the apical dome (Miller and Lyndon, 1976) and therefore p-divisions may be expected to contribute to the general outwards and upwards expansion of the apical dome, with an increased frequency of p-divisions associated with the localised outward growth at the level of the incipient primordia. In Graptopetalum, which also has opposite, decussate leaves, the occurrence of p-divisions in the underlying cells was concurrent with the changes at the surface associated with the bulging to form primordia (Selker and Green, 1984). However, in Silene p-divisions are present in the I region for several cell cycles before the formation of primordia. Pisum presents a somewhat different picture in that p-divisions suddenly become frequent in I, half a plastochron before the leaf is formed there. This might be because in Pisum the summit regions of the apical dome grow much more slowly than the flanks (Lyndon, 1970a, 1973) and upward growth of the dome seems more periodic (Lyndon, 1968). The occurrence of p-divisions in Pisum as a result of a release of physical restraint seems unlikely. The opposite side of the apex is already bulging, as the primordium protrudes in mid-plastochron, and there is no change in shape of the apex at I. The tentative conclusion is that the occurrence of p-divisions is the result of a change in the concentration of some substance within the apex which also causes starch synthesis in most of the apex (Lyndon and Robertson, 1976). The present findings also suggest that if a morphogen causes p-divisions (or there is the lack of one which inhibits their occurrence beforehand) then its distribution would be expected to correspond to the subsequent region over which the primordia form. Our conclusion is that whatever causes the occurrence of p-divisions also determines the maximum extent of the future primordia on the apical surface.

The importance of the epidermis has been stressed in the initiation of a new axis of growth and the initiation of leaf primordia (Green, 1980, 1985). Were the properties of the epidermis and the changes in division planes there to dictate the form of the plant then the changes in division planes in the underlying cells may be regarded (1) merely as accompaniments or consequences of the epidermal changes, if they occur simultaneously or later than in the epidermis, or (2) as providing an ‘enabling’ condition if they occur before. Clearly, in Pisum and Silene p-divisions occur in the regions of the apex where primordia are to be formed before the change of shape to produce the primordia. If it is the changes in the epidermis which are of prime importance and causative (Green,
1980), then they would be expected to occur at least as early as the changes in the underlying cells. The pattern of cell division in the epidermis in *Pisum* and *Silene* is now being examined to see whether changes in it precede or follow the events in the underlying cells, and how events in the epidermis and underlying cells are related during leaf initiation.

ACKNOWLEDGEMENT

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LITERATURE CITED


CONTROL OF SHOOT APICAL DEVELOPMENT VIA CELL DIVISION

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Summary

Cell division in plants not only partitions the protoplast but also provides the architectural framework for plant form. The shape of the shoot apical meristem is produced and maintained by gradients in the rate and plane of cell division, from the summit to the base of the apical dome, which also determine the region in which primordia can be formed. In *Pisum* leaf initiation is mainly the result of changes in the frequency of periclinal divisions at the leaf site whereas in *Silene* an increase in the rate of cell division seems more important since periclinal divisions are always present. Periclinal divisions may be permissive of primordium initiation rather than causal and may define the maximum area over which primordia can form. The occurrence in *Pisum* and *Silene* of periclinal divisions which do not seem to be related to concurrent outward growth suggests that the plane of division and the direction of growth may be controlled separately and in different ways. The control of outward growth during primordium initiation may lie in the epidermis, which necessarily grows faster at the leaf site. The initial orientation of epidermal growth at the primordial site, inferred as being normal to the plane of cell division, is predominantly longitudinal in *Pisum* but transverse in *Silene*. Longitudinal growth becomes dominant later in leaf development in *Silene*, as in *Pisum*. Several lines of evidence suggest a crucial role for the epidermis in the initial stages of primordium formation although the initial orientation of division planes in it may be concerned more with the shape of the young leaf than with initiation itself. In flower initiation primordial size becomes reduced, and in *Silene* there are alternations of higher and lower rates of cell division in successively initiated primordial types. A fuller understanding of the role of cell division in apical growth depends on better knowledge of the functional relationships between the plane of cell division, the orientation of microtubules and wall microfibrils, and the effect that division in one cell has on its neighbours.
Introduction

Cell division and organogenesis are the dominant characteristics of the shoot apical meristem, but it is far from clear to what extent the division of the meristem into cells is basic to organogenesis. Division into cells may permit more efficient expression of genes at a local level than would be the case in large cells where diffusion rates could perhaps become limiting. This compartmentation of the protoplast may be viewed as a way of erecting, between cells, potential barriers in the form of plasmalemmas through which the passage of molecules from cell to cell may be controlled via plasmodesmata (Goodwin & Lyndon, 1983), thus allowing different pathways of development to be followed in parts of the organism only micrometres apart.

In plants, a further consequence of cell division is the formation of new cell walls as part of the architecture of the organism. Since plant cells do not move with respect to each other the pattern and orientation of cell walls, and the way they subsequently develop, determines the form of the mature plant. The plane of cell division is therefore important in plants because it determines the structure of the framework within which the direction of growth of the cells may be controlled by the orientation of the cellulose microfibrils in the wall (Green, 1980; Taiz, 1984). We would therefore expect that in plants the extent and orientation of cell divisions may alter when the pattern of growth changes.

Growth of a plant can occur in two different modes, iterative and sequential. We may broadly equate iterative with indeterminate growth and sequential with determinate growth. A dominant feature of the plant (as opposed to the animal) is that growth continues throughout its life by the production of repeating units, usually fairly simple and each consisting of node, internode, leaf and axillary bud. An implication of such iterative growth is that the growth pattern changes during formation of the unit and then reverts to its original state to produce the next unit, implying some sort of feedback mechanism. Since the generation of the unit involves cell division we may ask whether there is a cyclic aspect to the rates or planes of cell division during iterative growth. If so, then an understanding of the control of cell division should throw light on the control of the generation of form, or morphogenesis.

Sequential growth is particularly characteristic of organs of limited or determinate growth, occurring for instance, in the development of a leaf. The simple leaf is formed by differentiation of the cells in sequence (Dale, 1982), although there is iteration at the meristematic level to generate the leaf blade for example. Sequential growth is also seen at the terminal
meristem when the flower or inflorescence is formed. The growth of a flower is a sequence of different organs although there is iteration within each organ type, as in the repeated initiation of stamens in helical sequence in a Ranalean flower. The particular involvement of cell division in sequential growth is that it leads to the formation of groups of cells which differentiate to form organs and tissues.

**Cell division in shoot apical growth and initiation of primordia**

*Rates and planes of cell division in the shoot apical dome*

In meristems in which mean cell volume stays approximately constant the rate of cell division and the rate of cell growth parallel each other. Since the cells can be recognized as discrete units it is not only convenient to measure rates of cell division in order to measure growth rates but extremely useful and informative to be able to measure differences in rates of cell division and hence differences in growth rates in different parts of the meristem. The rate of division is typically least at the summit of the shoot apex, increasing down the flanks (Lyndon, 1973; 1983), and is maximal where the primordia are formed (Fig. 1). Although the rate of cell division may be greatest in the region of primordial initiation the growth rate may be as great or greater in the stem internodes below the apex mainly because of cell enlargement with little cell division.

The plane of cell division is not random throughout the apical meristem of the higher plant. The existence of a tunica of one or more cell layers

![Fig. 1. Distribution of cell division in median longitudinal sections of vegetative shoot apices of (A) Pisum sativum and (B) Silene coeli-rosa. The points represent colchicine-metaphases, the density of which is proportional to the rate of cell division. The youngest leaf primordia (P) are initiated singly in Pisum and in pairs in Silene. The next primordium will be formed at I₁ in Pisum and at the base of the apical dome but at 90° to the existing pair in Silene. Bar = 50 μm. (From Lyndon, 1970a and Miller & Lyndon, 1976.)](image-url)
implies that within it the plane of cell division is predominantly normal to the surface of the apex. When periclinal divisions do occur in the tunica they are obvious because of the layered structure of the apex. When a leaf primordium is formed the layered structure is lost by the occurrence of many periclinal divisions but can still be inferred from the pattern of the older cell walls (Fig. 2).

The occurrence of anticlinal and periclinal cell divisions is useful in distinguishing regions of the apex or cell layers in which the potential direction of growth is, respectively, in the plane of, or normal to, the apical surface. However, the planes of anticlinal divisions can be various. The surface of the shoot apex typically approximates to the form of a hemisphere. Such a domed structure could in theory be maintained by surface growth that is either isotropic, or anisotropic with a strong longitudinal component or anisotropic with a strong transverse component (Green & King, 1966). However, only anisotropic growth with a strong longitudinal component is consistent with a minimum growth rate at the summit of the dome and a maximum on the flanks (Fig. 3) and this corresponds to what is found in the shoot apical dome (Lyndon, 1983). The gradient of basipetally increasing growth rate will tend to be more marked
Fig. 3. The elemental rate of meridional extension \[\frac{dm}{dt} \cdot \frac{1}{m}\] on the surface of a hemisphere as a function of distance down the meridian (m) from the summit of the hemisphere (m = 0) to the base (m = \(\pi/2\)). (A) Isotropic extension – the growth rate is maximum at the summit falling to zero at the base. (B) Anisotropic extension, rate of transverse twice that of longitudinal extension – growth rate is maximum at the summit falling to zero at the base. (C) Anisotropic extension, rate of longitudinal twice that of transverse extension – growth rate is minimal at summit, maximal on flanks falling to zero at the base. Dashed line – with an increasing longitudinal component, so that transverse extension is zero at the base of the hemisphere, the growth rate can be maintained and persist as longitudinal extension below the apical dome. (After Green & King, 1966.)

in the inner parts of the apex than on its surface because in a domed apex the growth rate of the inner, axial cells near the summit will be much less than the surface cells at the same level (Hejnowicz & Nakielski, 1979). With increasing distance from the summit the difference in growth rates between axial and surface cells will diminish until at the base of the dome the rates will be equal. Since the degree of anisotropy in surface expansion and the form of the growth gradient from the summit to the base of the apical dome are linked, we would expect to find that changes in the shape of the apical dome are accompanied by changes in the elemental growth rate of the surface. Conversely, when growth rates change to different extents in different regions of the apex the directions and planes of cell division may also change.
During growth of the shoot apex, cell size and shape remain roughly constant, so the principal direction of growth will be normal to the principal plane of cell division. The significance of the observed gradients in cell division rate and the distribution of anticlinal and periclinal divisions within the apex can be appreciated by considering the relative importance of changes in growth rates and growth directions during apical growth and the formation of primordia. We may ask to what extent the formation of primordia depends on the existence of a gradient in the rate of cell division from a minimum at the summit of the apex to a maximum on the flanks. If growth is isotropic, then the apex would bulge on its flanks wherever the growth rate (cell division rate) of the underlying tissues is greatest. If the gradient of growth rate were reversed, to be greatest at the summit, it would be the tip which would bulge, giving tip growth.

A gradient in the frequency of periclinal division from summit to flanks would cause the apex to bulge where the frequency of periclinal division was greatest (when growth rate is uniform throughout). If the frequency of periclinal division were greatest at the summit of the apex, tip growth would again result. Clearly, if the maximum in the growth rate gradient coincides with the maximum frequency of periclinal divisions then each would reinforce the other and a bulge would form at the site of the maxima. This corresponds to what occurs in the living plant in which the maximum cell division rate is on the flanks of the apex and periclinal divisions occur mainly on the flanks of the apex at the sites of leaf initiation (Esau, 1965). A primordium might be able to form as a result of either a maximum in the rate of cell division or an increased frequency of periclinal divisions. However just as some growth is essential for a change in form so are some periclinal divisions; without them all divisions would be anticlinal and whatever the rate gradient, growth would simply contribute to the longitudinal elongation of the apex. Periclinal divisions, even if only as part of a random orientation of the plane of cell divisions, are therefore essential for primordium formation if cell size and shape are to remain roughly constant.

The implications are that formation of primordia could result from either a local increase in growth rate (as long as there are periclinal divisions), as seems to be the case in *Silene*, or a local increase in the frequency of periclinal divisions, as occurs in *Pisum*. If there are two such potentially independent systems reinforcing each other then this might help to explain why apical functioning is so stable in a very wide range of environments. It would require an unusual set of environmental conditions to upset both gradients if each were dependent on different cellular and metabolic bases.
The control of the division of individual cells within the meristem is not
understood. For instance, if a cell elongates and divides, the adjacent cells
must necessarily elongate, but we do not know whether they necessarily
divide nor if a cell does divide, whether this affects the probability of
division in adjacent cells. The rate and plane of cell division may be altered
by mechanical deformation (Yeoman & Brown, 1971; Lintilhac & Vesecky,
1976), but the physiological mechanisms are unknown. If growth and cell
division are stimulated at a locus within a tissue, mechanical stress or other
effects could perhaps be transmitted to neighbouring cells, so causing them
to grow or divide or both. The existence of two populations of cells, slow
and fast cycling, in the Sinapis apex (Gonthier, Jacqmard & Bernier, 1985)
suggests that the shoot meristem may be heterogeneous with respect to
cellular growth rates, and this may be possible because of its shape in a way
that is not possible in the root, which grows essentially in a single dimension
(Green, 1976).

**Leaf initiation in Pisum and Silene**

In the Pisum shoot apex periclinal divisions are absent from the summit
of the apical dome and also from the side of the apical dome at which the
next primordium is about to arise until about 16 h or half a plastochron
before the primordium begins to be seen as a bulge, when periclinal
divisions suddenly appear in this I_1 region (Fig. 4). Since the rate of cell
division does not seem to change appreciably in the apical dome at the site
of a potential primordium for almost two plastochrons before it is formed
(Lyndon, 1970a) the initiation of a primordium in the pea seems to occur
mainly as a result of a change in the plane of division at the primordium site
(Lyndon, 1983). The position of the primordium in relation to the summit

Fig. 4. Distribution of periclinal divisions in median LS Pisum apices. (A) When a leaf
primordium, P, has just formed there are no periclinal divisions at the site, I_1, of the next leaf.
(B) Later in the plastochron, periclinal divisions appear in I_1 before the next primordium is
formed there. Bar = 50 μm. (From Cunninghame & Lyndon, 1986.)
of the apex will, however, still be dependent on the rate of growth and cell division being maximal on the flanks of the apical dome.

In *Silene*, on the other hand, periclinal divisions are present in the apex and the apical dome at all times and there is no abrupt change in their distribution during the plastochron as there is in *Pisum* (Cunninghame & Lyndon, 1986). The distribution of periclinal divisions is not obviously directly related to the occurrence of the bulges which form the paired primordia in *Silene*. Periclinal divisions are present in much of the apical dome (Fig. 5) and in the plastochron before a pair of primordia are formed the distribution of periclinal divisions which is then established does not change appreciably but persists until the primordia are at least a plastochron old (Fig. 5). The position and formation of primordia in *Silene* seem to depend on an increase in the rate of growth and cell division at the primordial sites, which is seen as a high rate of cell division in the young primordia (Fig. 1). The primordia are able to grow out because the occurrence of periclinal divisions allows this. In the apical domes of *Silene* the periclinal divisions may be associated with a less anisotropic and more uniform expansion of the apical dome, since cell division rates seem to be similar throughout the apical dome (Fig. 1).

The position at which primordia arise on the flanks of the apical dome may therefore be fixed in both *Pisum* and *Silene* by a gradient of increasing rate of growth and cell division from the summit to the flanks of the apical dome. However the actual formation of the primordia themselves may depend mainly on a localized increase in the frequency of periclinal divisions in *Pisum* but a localized increase in the rate of growth and cell division in *Silene*. In both, the distribution of periclinal divisions in the apical dome just before primordium formation foreshadows the distribution when the young primordia are forming (Figs 6, 7) (Cunninghame &

Fig. 5. Distribution of periclinal divisions in median LS *Silene* apices. Periclinal divisions are present in the apical dome and at the potential leaf sites (A) before the leaf primordia are formed, at the level of the vertical line, and also (B) after the primordia, P, have been formed. The apical dome indicated by the bracket in A gives rise to apical dome + primordia in B. Bar = 50 µm. (From Cunninghame & Lyndon, 1986.)
Cell division in shoot apices

Fig. 6. Distribution of periclinal divisions in TS *Pisum* apical dome. (A) When the primordium, P, is small few periclinal divisions occur in the I₁ region, the site of the next leaf primordium, but occur over the whole of the region forming primordium P. (B) Later in the plastochron, but before the primordium is formed, periclinal divisions appear in I₁ over the whole of the region which forms the next primordium. Bar = 50 μm. (From Cunninghame & Lyndon, 1986.)

Fig. 7. Distribution of periclinal divisions in TS *Silene* apical dome (A) almost one plastochron and (B) half a plastochron before (C) the formation of the next pair of primordia, P. The distribution of periclinal divisions associated with the young primordia is already present at least half a plastochron before they form. Periclinal divisions seem just as frequent at the left and right sides of the apex which are forming only the collar linking the pair of primordia. Bar = 50 μm. (From Cunninghame & Lyndon, 1986.)

Lyndon, 1986). We may suppose that periclinal divisions, which allow a reorientation of microtubules and cellulose microfibrils (Selker & Green, 1984), also allow the growth direction to change at the site of primordium formation, and may specify the maximum area over which this can occur. The occurrence of periclinal divisions may therefore be permissive of primordium formation which may then depend on other factors for its realization. A more rapid growth rate on the flanks of the apex (when there are already periclinal divisions, as in *Silene*) may also be a permissive rather than a causal factor in primordium formation if there is some overriding
structural constraint which does not allow outward growth despite the occurrence of periclinal divisions. Some such overriding constraint is implied by the data for Silene since the periclinal divisions seem to be at least as frequent, if not more frequent, at the sides of the apex (Fig. 7) which do not form the main parts of the primordia but only the bases of the primordial pair which meet at the sides of the apex to form a collar (Fig. 8).

Primordium formation may therefore depend on:

1. the occurrence of periclinal divisions to permit subsequent change in the direction of growth on primordium formation and to specify the maximum area over which this may occur,

2. a gradient in either the rate of growth and cell division or the frequency of periclinal divisions, or both, from the summit of the apical dome to a maximum on its flanks, and

3. a change at the time of primordium initiation in the structure(s) in the apex which orientate(s) the direction of growth; presumably this is a change in the plasticity of the walls of those cells in the region which grows out to form the primordium, so that it 'gives' and is able to bulge outwards, accompanied by a reorientation of cellulose microfibrils to provide hoop-reinforcement of the axis of the new organ (Green, 1980) and a local increase in the rate of cell division in the epidermis, as in Pisum (Lyndon, 1982).

On this view, one role of periclinal divisions would be to facilitate the

Fig. 8. SEM photograph of a vegetative Silene apex. There are vertical cell files at the sides of the apex where the bases of the opposite leaves meet to form a collar, but they are less obvious over the developing leaf primordium. Bar = 50 μm.
Cell division in shoot apices

reorientation of the microfibrils (Selker & Green, 1984), the orientation of
the latter possibly being an important factor in determining the axis of
growth of the new primordium. Since the distribution and occurrence of
periclinal divisions in Silene does not seem to be correlated in space or time
with the formation of the primordium, the factors determining the plane of
cell division and the outgrowth and axis of growth of the primordium are
presumably not the same. The bulging of the surface when a new axis
begins to be formed, the reorientation of the wall microfibrils, and the
change in the plane of cell division that precedes it may be controlled in
different ways. This is perhaps most clearly seen by comparing the forma-
tion of a new axis in Graptopetalum, in which a periclinal division may be
followed by a reorientation of the wall microfibrils, with the continued
growth of the root of Azolla in which no new axis is formed and a change
in division plane is not followed by a change in the orientation of the
microfibrils, these remaining transverse to the root axis (Green 1984).

Flower initiation

On initiation of the flower there is a decrease in the size of the primordia
relative to the apical dome at the time of their initiation (Table 1) (Lyndon,
1978b; Lyndon & Battey, 1985) so that whatever factors control primor-
dium size will also show changes on flowering. Periclinal cell divisions are
presumably a prerequisite for the outgrowth of the primordia in the flower
as in the vegetative apex. If it were the distribution of periclinal divisions
which dictates where the primordia will arise and over what area then we
might expect that because sepal primordia are smaller than leaf primordia
at initiation the distribution of periclinal divisions may become more
restricted to the positions of the incipient sepal primordia. In Silene this
does not seem to be so (Fig. 9); the occurrence of periclinal divisions seems,
as in the vegetative apex, more likely to be permissive of primordium
formation than to determine the position and area of the primordia. Peri-
clinal divisions seem to be sometimes more frequent in the developing
primordia but this is only to be expected in organs developing a new axis
of growth normal to the apical surface.

Together with a reduction in the size of the primordia at initiation when
flowers are formed there is also a reduction in the size at initiation of the
stem frustum which gives rise to the node and internode (Table 1) (Battey
& Lyndon, 1984; Lyndon & Battey, 1985). This implies that fewer cells are
assigned to the frustum in the same way as fewer cells are presumably
assigned to the primordium. Since the size of the frustum is determined by
its vertical height it may be expected that a smaller frustum would be
Fig. 9. Distribution of periclinal divisions in TS Silene apices at the level of sepal initiation. Sepals 1 and 2 are obvious, sepal 3 is just forming, and the sites of sepals 4 and 5 are shown in parentheses. AB is the axillary bud just below the flower. Periclinal divisions are present all round the apex and are not confined to the sites or potential sites of primordia. Bar = 50 μm.

Table 1. Sizes of primordia and stem frusta at initiation relative to the generating tissue in vegetative and flower meristems

<table>
<thead>
<tr>
<th>Plant</th>
<th>Primordium area</th>
<th>Stem frustum length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranunculus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>0.71</td>
<td>0.63</td>
</tr>
<tr>
<td>Sepals</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td>Stamens</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Impatiens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>0.41</td>
<td>0.35</td>
</tr>
<tr>
<td>Petals</td>
<td>0.26</td>
<td>0.17</td>
</tr>
<tr>
<td>Silene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Sepals</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Petals</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Stamens</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

Data from Meicenheimer, 1979 (Ranunculus); Battey & Lyndon, 1984 (Impatiens); Lyndon, 1978b (Silene).

associated with fewer transverse cell divisions at or just below the apex.

One of the striking things about the developing flower is that the petals grow more slowly than the sepals or stamens so that in the young flower the petals are less prominent (e.g. see examples in Sattler, 1973; Lyndon,
The relative growth rate of the petals is lower than that of the sepals from their inception (Lyndon, 1978b, 1979). This immediately poses the question of how the rate of cell division and growth is controlled locally and precisely in groups of cells on the apex which are only a few cells' width away from other groups with different rates of division. Again it may be that, what is controlled is growth rate and cell division rate is a consequence of growth rate.

The difference in growth rates between petals and other organs is only one facet of differences in growth and cell division rates throughout the developing flower. In the *Silene* flower the growth rate increases distally along the floral axis from the sepal node, in which the rate is lowest (Table 2). However, the floral organ primordia show a different trend. Since the parts of the flower originate in sequence, and the relative growth rates of the organs in the developing flower reflect the relative rates of growth and cell division at initiation of the primordia (Lyndon, 1978b, 1979), then the rate of growth and cell division in the apex changes during formation of the flower in *Silene* in the way shown in Table 3. There is an alternation between lower growth rates in the axillary bud, petals, and carpels and higher growth rates in the sepals and stamens. This could possibly represent an oscillation of rates of growth and cell division which is intrinsic to the sequential initiation of the different types of organs and

<table>
<thead>
<tr>
<th>Growth temperature</th>
<th>Pedicel</th>
<th>Sepal node</th>
<th>Petal/stamen node</th>
<th>Floral meristem</th>
</tr>
</thead>
<tbody>
<tr>
<td>13°</td>
<td>0.25</td>
<td>0.09</td>
<td>0.20</td>
<td>0.27</td>
</tr>
<tr>
<td>20°</td>
<td>0.29</td>
<td>0.13</td>
<td>0.24</td>
<td>0.37</td>
</tr>
<tr>
<td>27°</td>
<td>0.29</td>
<td>0.12</td>
<td>0.20</td>
<td>0.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Growth temperature</th>
<th>Axillary bud</th>
<th>Sepals</th>
<th>Petals</th>
<th>Stamens</th>
<th>Carpels (floral meristem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13°</td>
<td>0.33</td>
<td>0.47</td>
<td>0.39</td>
<td>0.49</td>
<td>0.27</td>
</tr>
<tr>
<td>20°</td>
<td>0.36</td>
<td>0.64</td>
<td>0.56</td>
<td>0.66</td>
<td>0.37</td>
</tr>
<tr>
<td>27°</td>
<td>0.43</td>
<td>0.57</td>
<td>0.26</td>
<td>0.34</td>
<td>0.26</td>
</tr>
</tbody>
</table>
would correspond to a periodicity of about one day (at 20 °C), about equal to the probable cell cycle time of 1 to 2 days (Lyndon, 1979).

There are clearly gradients of relative growth rate in the axis of the developing flower and possibly an alternation of higher and lower rates of cell division at the initiation of successive types of floral primordia. The growth rate is least in the sepal node, which marks the transition from iterative to sequential growth at the meristem. Whether, in the developing flower, the rate of cell division depends on the rate of growth, or vice versa, and whether or how these may be influenced by metabolites or growth substances produced by other floral organs or elsewhere in the plant is at present quite unknown.

The role of the epidermis: rates and planes of cell division

Green (1980) has pointed out that when a new axis is formed on the flanks of an existing one the major change in growth orientation will be found at the sides of the new axis where the major growth direction is reorientated through 90°. This is indeed what is found in Graptopetalum which forms pairs of opposite, decussate leaves which are initially more or less cylindrical (Green & Brooks, 1978; Hardham, Green & Lang, 1980). However, in Pisum there did not seem to be an obvious change in the orientation of the plane of cell division in the epidermis at the sides of the emerging leaf primordium (Lyndon, 1982). Cell divisions on the surface of the shoot apical dome of Pisum are normal to the main direction of growth and are predominantly transverse (Lyndon, 1976) and remain so during the initiation of the leaf primordium (Lyndon, 1982). This was interpreted as indicating that the Pisum leaf was essentially a simple outgrowth of the apical surface, and that the strong dorsiventrality of the leaf meant that relatively few cells constituted the sides or edges of the leaf.

SEM photographs of the epidermis of the Pisum apex indicate that the cell divisions in the I_1 region are almost entirely transverse during the first part of the plastochron. During the second part of the plastochron there is apparently an increased proportion of longitudinal divisions which seems to persist during the formation of the young primordium. The ratio of longitudinal/transverse divisions seems to increase at about the same time as periclinal divisions appear in the underlying cells but becomes no greater than about 0.6. This is in accord with the conclusion that the predominantly longitudinal polarity of growth of the epidermis decreases about half a plastochron before primordium formation but is nevertheless maintained throughout primordium initiation (Lyndon, 1982).

Whether, in Pisum, the orientation of the microtubules and microfibrils
changes in a more marked fashion, as in *Graptopetalum*, is not known. In *Pisum* the longitudinal polarity of growth of the epidermis of the apical dome, which was associated with the transverse orientation of cell division in the epidermis, seemed to become imposed on the underlying cells of the young primordium. In these latter cells the division orientation was random in the half plastochron before the formation of the primordium (Lyndon, 1970b; 1982). The axial polarity of the young *Pisum* leaf therefore seems to be derived from the longitudinal polarity (and predominantly transverse division) of the epidermis on the flanks of the apical dome.

In *Silene*, in the area over which a leaf primordium will form, the predominant orientation of the plane of new cell walls is transverse. As the apical surface begins to bulge to form a new primordium the proportion of longitudinal cell walls increases (Fig. 10 and Table 4). The predominant direction of growth will be normal to this and so will have a strong transverse component. The longitudinal direction of growth (as shown by the transverse cell walls) is maintained at the sides of the *Silene* apex where the opposite leaves of a pair meet to form a collar round the stem. These

Fig. 10. Distribution and orientation of the most recently formed cell walls in the epidermis of *Silene* at the site of the next leaf primordium. Early in the plastochron (A) the new cell walls are predominantly transverse. In mid- (B) and late- (C) plastochron there are roughly equal numbers of transverse and longitudinal divisions. As the next leaf pair begins to be formed, early in the next plastochron (D) on the sides of the apical dome longitudinal divisions predominate and this is maintained through mid- (E) and late- (F) plastochron as the young primordia begin to bulge out. The preponderance of longitudinal divisions indicates that the leaf of *Silene* begins to form by mainly transverse growth. (Transverse cell walls are those $<45^\circ$ to the horizontal; longitudinal, $<45^\circ$ to the vertical.) Bar = 50 $\mu$m. Combined data from 23 apices.
Table 4. Orientation of cell divisions (as shown in A–F, Fig. 10) on the flanks of the Silene shoot apex before (A,B,C) and during (D,E,F) primordium formation.

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Mid</th>
<th>Late</th>
<th>Early next</th>
<th>Early/Mid</th>
<th>Mid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td>Number of divisions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>36</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Transverse</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>16</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Ratio: Longitudinal/Transverse</td>
<td>0·2</td>
<td>1·1</td>
<td>0·8</td>
<td>2·3</td>
<td>3·0</td>
<td>2·6</td>
</tr>
</tbody>
</table>

Conclusions are consistent with the appearance of the vegetative Silene apex, which shows clear vertical cell files at the sides of the apex but less obvious files over the developing primordium (Fig. 8). The Silene primordium therefore appears to grow at first by lateral expansion to form the bulge, unlike Pisum in which longitudinal expansion predominates (Lyndon, 1982). Graptopetalum seems to be intermediate in that the longitudinal direction of growth is conserved over the centre of the putative primordium but transverse growth occurs at the sides (Green & Brooks, 1978). These differences between Pisum, Silene and Graptopetalum in the main direction of growth of the epidermis of the young primordium imply that the initial orientation of growth in the surface cells is not crucial to the actual formation of the primordium. It may be more concerned with the shape of the young primordium, which shows strong dorsiventrality in Pisum, which extends round the apex to join with the opposite primordium of the pair in Silene, and which is essentially cylindrical in Graptopetalum. In the formation of the primordium the orientation of growth, and by implication the orientation of cellulose microfibrils in the wall, may therefore be secondary to the changes in wall plasticity and yield threshold which allow the apical surface to bulge out in a new direction. A change in orientation of the wall microfibrils in the epidermal cells may not be essential for outward growth to occur but only to direct it into the appropriate shape when it does occur.

For a primordium to form at all, the surface of the apex must bulge and the epidermis must therefore grow faster locally (Lyndon, 1983). The crux of the problem is whether the change in shape is controlled by changes in the surface layer, to which the underlying layers accommodate themselves or whether the critical changes are in the inner layers and the epidermis merely acts like a skin, reacting to what is going on underneath (Lyndon, 1971). Since growth of plant cells is driven by turgor, changes in the ability
of the cells to expand in a particular direction imply changes in the properties of the cell wall and particularly in its extensibility (Cleland, 1977; Taiz, 1984). The simplest assumption would be that it is the outer wall of the epidermis which is a restraining layer and that it is alterations in the properties of this layer which will determine where the surface bulges.

The control of the shape of the expanding apical surface during organogenesis need not be entirely under the influence of the epidermis. It could perhaps be that the whole meshwork of cell walls, especially in the outer cell layers, provides the restraint on the directions of growth. While the pattern of stresses thus imposed on the cells may be concerned with orientating the new cell walls, which appear to be often in the plane of least shear (Lintilhac, 1974), it would also presumably be possible for stresses to be relieved locally deep within the tissues as long as the surrounding walls were unaffected. If so, the general shape of the apical system need not be altered even though there might be a change in the strain pattern at a particular locus within the tissues. A change of this nature would be consistent with the possibility that, in *Pisum*, cells within the apex may be affected by some metabolite or growth substance so that division planes become random, and so periclinal divisions appear (Lyndon, 1970a; Cunninghame & Lyndon, 1986), predominantly in the corpus cells but without a concomitant change in shape of the surface.

During initiation of the flower the arrangement of the primordia changes from that in the vegetative shoot, suggesting that the polarity and surface structure of the apex may also change. In *Clethra* the radial polarity of the epidermal cells in the vegetative apices is lost in the flowering apex (Hara, 1971, 1977). The longitudinal files of cells seen on the flanks of the vegetative *Silene* apex are clearly visible in the flowering apex at the time of sepal initiation. However, this longitudinal polarity of the epidermis is lost once the sepals have been initiated. This may be due to the loss of the predominantly transverse orientation of cell division when the apex expands during flower development. Since stamens are initiated towards the summit of the apical dome rather than on the flanks (Sattler, 1973; Lyndon, 1978a; Tucker, 1984) they may therefore be initiated from that part of the apical dome which lacks longitudinal polarity. The appearance of files of cells in young primordia may therefore be partly a consequence only of the position on the apical dome that the primordia arise. However, radial files of cells may later be seen again in young carpels which arise near the summit of the apical dome.

The possible involvement of the epidermis in flowering is evident in a chimaera of *Camellia* where the formation of stamens and carpels is apparently under epidermal control (Stewart, Meyer & Dermen, 1972).
C. japonica has flowers with many petals but no stamens or carpels. C. sasanqua has flowers with stamens and carpels as well as petals and sepals. The graft chimaera with a sasanqua epidermis and with all underlying cells japonica has stamens and carpels. Thus it is the nature and genetic constitution of the epidermis which determines in Camellia whether or not stamens and carpels are formed. In this chimaera the degree of epidermal control over primordium initiation apparently alters as the flower develops, suggesting that the initiation of stamens and carpels differs in some way from the initiation of other primordia. If stamens and carpels are formed only from that part of the apex in which the epidermis lacks polarity, then it could perhaps imply that only in the absence of orientation of cell division (absence of polarity) in the epidermis, does the epidermis control whether or not primordia are formed.

The existence of polarity in the growth and cell division planes in the epidermis raises the problems of how the polarity is maintained, and whether it is of importance for primordium formation. The early changes in the Graptopetalum epidermis (Hardham et al. 1980), the apparent imposition of epidermal polarity on the developing leaf primordium (Lyndon, 1982) and the control of primordium initiation in Camellia (Stewart et al. 1972) are differing lines of evidence all pointing to an important role for the epidermis in primordium formation. Perhaps the epidermis could be the locus at which the supposed morphogens involved in primordium initiation (Thornley, 1975) might act.

Control of rates and planes of cell division

Because cell size stays roughly constant in apices and, in the eumeristem, cell shape also remains more or less constant, the implication is that cell division and growth go hand in hand. It is therefore difficult to ascribe a special role to cell division other than that of maintaining the cellular structure of the tissue. For short periods at least apical meristems can function without cell division. The protrusion of a leaf primordium can begin in γ-irradiated seedlings in which division has been suppressed (Foard, 1971). The bulging occurs in the epidermal cells, suggesting that the properties of at least the outermost cell walls of these cells become altered to allow the new growth direction. In root apices treated with hydroxyurea cell division is suppressed but growth can continue so that cells become elongated in the direction of growth (Barlow, 1969) and the main orientation of growth in different parts of the apex can thus be observed directly. Furthermore, in root caps treated with colchicine or hydroxyurea to suppress cell division, differentiation of the cells continues
showing that cell division is not always an essential precursor of development (Barlow, 1977, 1981). This has also been the conclusion drawn from experiments with stem tissues showing that differentiation can occur without the cells dividing beforehand (Turgeon, 1975; Hardham & McCully, 1982).

The converse is that not all cell division in apices is necessarily just a consequence of growth in volume. The regions in which procambium developed in the pea apex could first be detected as regions with a higher frequency of cell divisions (Lyndon, 1970a). It is not clear whether there is any special significance in the mapping out of the procambium in this way— it may simply be that the first stages of procambium differentiation involve cell division with cells becoming narrower or reduced in volume. If so, then the higher frequency of cell division (perhaps only temporary) may be characteristic of the early stages of many different cell types.

It seems unlikely, however, that cell wall formation is only a response to the direction of growth in the shoot apex. Apart from the formation of the procambium, the occurrence of periclinal divisions with equal frequency all round the Silene meristem and perhaps at higher frequency at the sides at 90° to the primordia suggests that there must be some other control on the plane of division. This is also suggested by the pea shoot apex in which periclinal divisions are absent from much of the apical dome until half a plastochron before a leaf primordium is formed, and the appearance of periclinal divisions is marked neither by an increase in the rate of cell division in that part of the apex (Lyndon, 1970a) nor by a change in shape of the apex. In the pea, there is a hint that the orientation of division might be associated with a change of metabolism in the apex because at this same time there is a change in the amounts of starch accumulating in the plastids (Lyndon & Robertson, 1976). The mechanism for determining whether or not a primordium forms could perhaps depend on the concentrations of morphogens in the apex (Thornley, 1975). In considering the positioning of primordia in the developing flower (Lyndon, 1978b) it seemed necessary to postulate two sets of factors, 'one governing the positions at which primordia will arise and a second set persisting for only two plastochrons, governing the sequence in which primordia are initiated'. Perhaps the first set of factors, governing the positioning of primordia is not chemical but physical in nature and comprises the pattern of wall reinforcement in the apical epidermis recently shown to be associated with primordium arrangement (Green, 1985). Chemical morphogens may perhaps then determine whether or not the surface would bulge to form the primordium at a site predetermined by the structure of the apical surface.

Clearly there are several aspects of cell division in relation to apical
growth, and growth in general, which we need to understand (Furuya, 1984). First, we need to understand what controls the plane of cell division especially in circumstances where stress does not seem important, for instance in the formation of stomata (Palevitz & Hepler, 1974) and in the formation of vascular strands, where flow of morphogen through the cells seems important (Sachs, 1975). Mechanical stress is however almost certainly also important (Lintilhac, 1974). This may be another example of the possibility of the plant making use of back-up systems to achieve the same end and thus conferring homeostatic stability on the developing system. Second, we need to understand how the rate of cell division is controlled and how this may affect the plane of cell division. For example if growth is rapid in relation to cell wall synthesis and growth, does this affect the plane of cell division differently from when growth is slower and cell walls perhaps have more rigidity at the time of the next cell division? Third, we need to understand the relationship between the orientation of microtubules and the orientation of the preceding and succeeding cell plates and the relationship between the orientation of microtubules/microfibrils and the direction of growth. It is not clear whether this orientation has to change in order for the direction of growth to change or whether it is a consequence of changes in the plane of cell division and in growth direction. It should be possible for bulging of the surface to occur without a change in orientation of the microfibrils if there is a sufficient increase in extensibility of the walls, which is presumably the determining factor in altering growth direction. It may be in these initial stages of primordium initiation that morphogens are produced which alter the physical properties of the cell walls in specific regions of the apex (Green & Poethig, 1982). If the reorientation of microfibrils follows from, rather than is causal to, initial changes in the direction of growth this would explain why in Azolla the orientation of microfibrils does not change when the plane of cell division changes in the root and the direction of growth remains unaltered (Gunning, Hardham & Hughes, 1978; Green, 1984).

We can see that, even if changes occurred in the epidermis but the underlying cells could not grow out (or vice versa), then changes in the directions of growth and the formation of primordia would not occur. Clearly the apical system is acting as a whole. There is the danger that, in trying to focus on specific aspects of apical growth or the involvement of cell division in it, we lose sight of the fact that the apex grows as a unitary system. Also there may be several different processes contributing to a given end result so that when the system is perturbed it is not disrupted, because where one aspect of the system may be inactive another may nevertheless achieve the same end though in a different way.
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The Cell Cycle in Vegetative and Floral Shoot Meristems Measured by a Double Labelling Technique

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Summary. A double labelling technique was used to measure the length of the cell cycle in the shoot apical meristem of Silene coeli-rosa L. plants kept in short days, in which they remained vegetative, or exposed to 7 long days, which induced flowering. The length of the cell cycle in the vegetative plants (those in short days throughout) was about 18 hrs. It was also 18 hrs, or somewhat longer, in plants which had been exposed to long-day conditions for 5 days, by which time 90% of the plants are committed to flower. When plants had been exposed to the full inductive period of 7 long days and had been transferred back again to non-inductive (short day) conditions, floral morphogenesis had just begun and the cell cycle had almost halved, to 10 hrs. The cell cycle was therefore unaltered during floral induction and shortened only at the onset of the growth of the flower itself.

Introduction

Measurements have shown when the apical meristem of a shoot begins to initiate flowers the rate of cell division is higher than when the shoot was vegetative. The rate of division has been measured in the shoot meristem of Datura (Corson, 1969) and Sinapis (M. Bodson, 1975) by the technique of metaphase accumulation in the presence of colchicine. The increased percentage of colchicine metaphases in the floral shoot apices indicated that the rate of division was higher than in the vegetative apices. The cell doubling time, measured from counting cell numbers in a series of apices in Lupinus and in vernalised rye (Secale), shortened on transition to flowering (Sunderland, 1961). Assuming all, or most, of the apical cells were dividing, this indicates a faster rate of cell division at the onset of floral initiation.

These experiments do not allow the distinction to be made between the events occurring in the apex during floral induction, when the apex is still producing leaves, during floral evocation, when the apex becomes committed to flower, and during realisation, when flower morphogenesis itself begins. The occurrence of peaks of mitotic index during induction (Bernier, 1971) is often interpreted as indicating an increased rate of cell division during induction.

The length of the cell cycle in the shoot apex can theoretically be measured by techniques involving labelling with radioactive substances. In practice there are several difficulties with the shoot apical meristem. Firstly, the cell cycle is relatively long especially in the slowly growing cells at the summit of the apical dome, so that even in experiments which last for several days the cell cycle of some of the apical cells cannot be measured (Michaux, 1969; Jacqmard, 1970).
Secondly, applied solutions are not readily absorbed by the intact shoot apex and usually each apex has to be exposed by dissection before label can be successfully administered (Bernier and Bronchart, 1963). This seriously limits the number of plants that can be used for an experiment and so allows only a small number of samples. Thirdly, the mitotic index is characteristically low in the shoot apex so that methods which depend on scoring the percentage of mitotic figures require many samples. Despite these difficulties Jacquemard (1970) succeeded in measuring the cell cycle and its component phases in most parts of the vegetative apex of \textit{Rudbeckia}. However it is obvious that a more convenient method would be useful for measuring the cell cycle in the shoot apex, where the cell cycle is relatively long—often a day or more (Lyndon, 1973)—and the number of plants available for sampling may be limited. A method was therefore devised, similar to that of Wimber and Quastler (1963), which depends on labelling with $^{14}$Cthymidine followed by $^{3}$Hthymidine and which allows measurements to be made for several days with a minimum of samples.

The principle of the method is to label a cohort of cells, which are in the S phase of the cell cycle, with a pulse of $^{14}$Cthymidine and then to allow these cells to proceed through the cell cycle. The progress of these cells is monitored by applying $^{3}$Hthymidine at intervals as a terminal label. If the $^{3}$Hthymidine is applied at the same time as the $^{14}$Cthymidine then the cells will become doubly labelled with both $^{14}$C and $^{3}$H. If there is a sufficient interval between the application of $^{14}$Cthymidine and $^{3}$Hthymidine then all the $^{14}$C-labelled cells will have moved out of S and into G2, M, or G1 and the $^{3}$H will therefore label other cells, which are in S at that time and no doubly labelled cells will be found. When the $^{14}$C-labelled cohort has completed a whole cell cycle and again reaches S, then the application of $^{3}$Hthymidine will also label them and they will be observed as doubly labelled cells. The length of the cell cycle is therefore the interval between the application of $^{14}$Cthymidine (at the beginning of the experiment) and this new occurrence of doubly labelled cells.

This method was used to measure the length of the cell cycle in the shoot apical meristem of \textit{Silene} plants during vegetative growth, floral induction and the early stages of floral morphogenesis. \textit{Silene} was chosen because, on induction, the apical meristem transforms directly into a terminal flower.

\textbf{Materials and Methods}

The plant used for this work was \textit{Silene coeli-rosa} (L.) Godron (supplied as \textit{Viscaria cardinalis} by Stewart's Ltd., Seedsmen, Edinburgh). This is a qualitative long-day plant in the conditions under which it has been grown in these experiments. Plants were routinely maintained in a short-day regime in a growth room at a constant temperature of 20°C. High light intensity (90–100 W m$^{-2}$ fluorescent light and 15 W m$^{-2}$ tungsten light) was given during 8 hrs daily from 9 a.m. to 5 p.m. For long-day conditions low intensity illumination (tungsten light, 2 W m$^{-2}$) was continued from 5 p.m. to 9 a.m. The plants were sown and all were reared in short-day conditions for 28 days. A uniform population of plants was obtained by retaining only those plants in which the 3rd leaf pair was between 20 and 30 mm long. These plants had just initiated, or were on the point of initiating, the 7th pair of leaf primordia. All experimental treatments were subsequent to this sampling of 28-day old plants. Plants were induced to flower by growing them for 7 days (days 28 to 35) in long-day conditions; the plants were then returned to short days.
The labelling experiments were performed on plants on the 33rd and 36th day of growth (Fig. 1). On day 33 the plants in short days were vegetative; the plants being induced in long days were still making leaves although by this time 90% of them are committed to flower and will subsequently do so if returned to short days at this point. On day 36 the plants in short days were vegetative. The plants which had been in long days, and which had now been returned to short days, had become induced to flower and the very early stages of flower morphogenesis were about to commence. At this stage the apex is beginning to enlarge prior to flower formation. During the 33rd and 36th day of growth the plants to be used were carefully dissected to remove the young leaves near the shoot apex so that the apex was exposed. As each apex was prepared, a drop of water was placed on the apex to prevent it drying out while further plants were being prepared. The $^{14}$C thymidine was applied to each apex after careful removal of the drop of water which had been previously placed on the apex. 0.2 μCi $^{14}$C thymidine (specific activity 59 Ci.mol$^{-1}$) was applied to each apex as an aqueous drop of 0.05 ml. After 2 hrs, excess $^{14}$C thymidine was carefully blotted off and the apex was washed with distilled water. A drop of water, held in place by a small piece of cotton-wool, was left on the apex to keep it moist until the application of $^{3}$H thymidine. $^{3}$H thymidine was applied at intervals as a terminal label. 2 μCi of $^{3}$H thymidine (specific activity 22 Ci. mmol$^{-1}$) in an aqueous drop of 0.05 ml was applied to each apex for 2 hrs. The $^{14}$C thymidine was in every case initially applied to the plants at 5 p.m. at the end of the high intensity light period and the $^{3}$H thymidine applied at intervals up to 24 hrs later. $^{14}$C thymidine was applied as the first isotope since fewer of the higher energy β particles emitted by the $^{14}$C isotope would terminate inside the nucleus than would be the case for the lower energy β particles emitted by tritium. Since tritium was applied as a terminal label any deleterious effects would be minimised. A vegetative plant and an induced plant which had been treated with both radio-isotopes were allowed to continue growth. The vegetative plant continued to produce leaves and the induced plant subsequently flowered showing that the apical meristem continued growth after treatment.
Fig. 2A and B. Autoradiographs of labelled nuclei. A and B are the same field of view at different focal planes. When the lower emulsion is in focus (A) the autoradiographs of the nuclei labelled with $^3$H are clear and autoradiographs of $^{14}$C-labelled nuclei are indistinct. At a higher focal plane (B) the autoradiographs of the $^3$H-labelled nuclei are now indistinct but the $^{14}$C-labelled nuclei are clear. The $^{14}$C also tends to produce tracks. Nuclei 1 and 2 are singly labelled with $^3$H; nucleus 3 is singly labelled with $^{14}$C; nuclei 4 and 5 are interpreted as being doubly labelled.

The labelling was terminated by fixing the plants in 80% aqueous ethanol. The material was dehydrated, embedded in wax, and longitudinal serial sections 5 μm thick were cut through each apex. The sections were mounted on "subbed" slides (Rogers, 1967).

For autoradiography the slides were dewaxed with xylene and hydrated. They were then coated with Ilford K2 emulsion which records tritium emissions with high efficiency, but due to its relative insensitivity records very few of the higher energy $^{14}$C β particles. The K2 emulsion was applied as a 50% aqueous mixture which resulted in an emulsion thickness of approximately 3 μm. About 1 hr after the K2 emulsion had dried a second layer of emulsion, this time Ilford L4, was then applied to the slides as a 75% aqueous mixture which gave a relatively thick layer of emulsion. Ilford L4 emulsion is more sensitive than K2 and will record β particles from $^{14}$C as tracks of silver grains. Since β particles emitted by tritium travel a maximum of 3 μm, relatively few penetrated the K2 emulsion layer. After 1 week's exposure the emulsions were developed in Ilford ID19 developer, washed and then fixed in Ilford Hypana fixative. The sections were then stained through the emulsion with methyl green/pyronin (Casselman, 1959).

The isotope source was identified on the autoradiographs by the presence of silver grains in the different emulsion layers, which appear in different focal planes under a high power objective, and also by the more diffuse scatter of grains caused by a $^{14}$C source (Fig. 2). A further aid to source identification was that the L4 silver grains were slightly smaller than those in the K2 emulsion layer and tended to appear as tracks. The numbers of nuclei, singly labelled with either $^{14}$C or $^3$H, or doubly-labelled with both $^{14}$C and $^3$H, were counted in the apical dome of each apex. Usually several non-adjacent, approximately median sections were scored for each plant.

Results

The values for the percentage of labelled cells which are doubly labelled for the experimental series are shown in Fig. 3. The values for the samples obtained
Fig. 3a—d. The percentage of doubly labelled cells at intervals after the application of $[^{14}C]$thymidine at 0 hrs. (a) Plants in short days, day 33 (vegetative). (b) Plants in long days, day 33 (induction completed for most plants). (c) Plants in short days, day 36 (vegetative). (d) Plants which have received 7 long days, day 36 (floral morphogenesis beginning).

on day 33 are shown in Fig. 3a and b, and on day 36 in Fig. 3c and d. In each case the values fall from a high value, presumably 100% at 0 hrs, to a minimum some hrs later. The values then rise to a new maximum, the period between the beginning of the experiment and the peak of the new maximum being the length of the cell cycle. In the vegetative plants on day 33 (Fig. 3a) the second maximum occurs at 18-19 hrs. The length of the cell cycle is therefore approximately 18-19 hrs. In the plants being induced in long days on day 33 (Fig. 3b) the values rise to a new maximum but the data are insufficient to say where the peak of this maximum occurs. It is however at least 20 hrs and the cell cycle in these plants undergoing induction was therefore at least as long as in the comparable vegetative plants in short days.

In the plants sampled at 36 days, those which had been in short days throughout and were vegetative (Fig. 3c) showed a new maximum of doubly labelled cells at about 18 hrs. The length of the cell cycle was therefore about 18 hrs. In the plants which had received 7 long days and had been returned to short days by the 36th day (Fig. 3d) the new maximum was attained very much sooner, at about 10 hrs. There was a second maximum at 21 hrs. The length of the cell cycle in these induced plants was therefore about 10 hrs, little more than half that of the comparable vegetative plants (Fig. 3d and c). The length of the cycle therefore appears to be similar, about 18–20 hrs, for vegetative plants on both the 33rd and 36th day of growth and for plants undergoing induction on their 33rd day of growth. In
contrast the plants which have been induced and are now committed to flower and in which floral morphogenesis is about to begin (Fig. 3d) have a cell cycle which is about one half of this, about 10 hrs. These results demonstrate that there is a shortening of the cell cycle in the shoot apex of *Silene* but only after induction is completed and only when floral morphogenesis is beginning.

**Discussion**

These values for *Silene* confirm the indications obtained from *Datura* (Corson, 1969), *Sinapis* (Bodson, 1975) and lupin and rye (Sunderland, 1961) that the length of the cell cycle in the shoot apex becomes shorter at the transition to flowering. However, in *Silene* the consequent speeding up of the growth rate is clearly a phenomenon associated with the formation of the new organs of the flower itself rather than with the processes which lead up to this.

It should be possible to calculate from the data not only the length of the whole cell cycle, but also the length of its component phases. The length of S is given by the distance between the ascending and descending limbs of the peak of doubly labelled cells, at the points midway between peak and trough values, when the 2 hrs labelling period is subtracted. This gives a value of about 4 hrs for S for both sets of short-day plants (Fig. 3a and c) and a value of about 2.5 hrs for the long-day plants on day 36 (Fig. 3d). Such a shortening of the length of S when the cell cycle shortens has been observed in vegetative apices of other plants (Lyndon, 1973).

The time at which mitosis occurs in the cohort of $^{14}$C-labelled cells should be marked by the appearance of $^{14}$C-labelled mitotic figures and also by a sudden doubling in the ratio of $^{14}$C/$^3$H singly labelled cells. However, labelled mitotic figures were not unequivocally identified on the autoradiographs and the variations in the observed ratio of $^{14}$C/$^3$H singly labelled cells were too great to allow meaningful conclusions to be drawn. The ratio in many samples was greater than 2, which is not theoretically possible, suggesting that there was an excess of $^{14}$C-labelled cells, perhaps because the absorbed $[^{14}$C]thymidine was not adequately removed by the washing procedure, but persisted in the tissues so that there was a continued labelling of cells with $^{14}$C after the initial period of application.

From the theory of the method it would be expected that when the $^{14}$C-labelled cells once again reach S and become labelled with $^3$H as well, the percentage of labelled cells which are doubly labelled in this new peak should reach 100%. It clearly does not do so in any of the samples measured (Fig. 3). One reason for this may be that 100% doubly-labelled cells would only be observed if there were no cell-to-cell variation in the length of the cell cycle, which presumably there is. A further reason that the doubly-labelled cells may not reach 100% could be the overestimation of singly labelled cells, for the reasons already discussed.

Despite the inadequacies of the present technique for measuring the phases of the cell cycle, it has proved satisfactory for measuring the length of the whole cell cycle. The double labelling method has considerable advantages for measuring the length of the cell cycle in tissues in which division is relatively slow and the
mitotic index is low, as in the shoot apex, and in tissues which are difficult to label. Relatively small numbers of plants may be used, since all the nuclei which are labelled contribute to the data and not only those which happen to be in mitosis. Also, sampling does not have to be so close as with methods which require the scoring of the percentage of labelled mitotic figures. Although there is some subjectivity involved in determining whether or not a nucleus is doubly labelled, the technique could possibly be improved by modification of the autoradiographic procedure so that cells, singly labelled with $^{14}$C or $^3$H, or doubly labelled, could be more easily distinguished from each other.

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References


Rates of Growth and Cell Division in the Shoot Apex of *Silene* During the Transition to Flowering

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ABSTRACT

The growth rates of the shoot apex during and after floral induction were measured in *Silene*, a long-day plant. Plants were induced to flower with 4 or more long days (LD) but not with 3 long days or with short days (SD). The rate of increase of cell number in the apical dome, above the youngest leaf pair, was exponential and in plants given 3 LD remained the same as in plants in SD. In plants induced to flower with 7 LD, until the end of the inductive period the rate of increase of cell number in the apical dome remained the same as in plants in SD. Only when the apex began to enlarge as the first stage in the formation of the flower did the growth rate of the apical dome increase. The rates of increase of cell numbers in the apex corresponded to mean cell generation times of 20 to 33 h for plants in SD, for plants given 3 LD, and during the 7 days of induction for plants given 7 LD, and 6 to 8 h for induced plants when flower formation was beginning.

The distribution of cell division in the apex was examined by treating plants with colchicine and noting in sections the positions of the resulting metaphases. In vegetative apices and also in apices undergoing transition to flowering the whole of the apical dome appeared to consist of cells dividing at a similar rate.

The rate of leaf initiation during induction was the same as in vegetative, non-induced plants.

INTRODUCTION

On the transition from vegetative to floral growth the shoot apex stops producing leaves and instead becomes transformed into a flower or inflorescence. The floral apex is usually much larger than the vegetative apex and the speed of the transition indicates that growth of the apex is rapid at this time. Sometimes this rapid growth has been observed directly, as in *Chrysanthemum*, in which the area of the apical dome increases 400-fold, most of this increase occurring in only a few days (Schwabe, 1959). The rapid increase in size of the apex could be due either to an increase in the rates of cell division and growth, or to an increase in the number of growing cells without there necessarily being an increase in the rate of division, or to changes in the distribution of growth within the apex.

Direct measurements of the growth rates of vegetative and floral shoot apices have been made in a few species. In *Datura* (Corson, 1969) and *Sinapis* (Bodson, 1975) the rates of cell division in vegetative and floral apices have been compared by measuring the rate of accumulation of colchicine metaphases, which in both

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cases was greater (about double) in the floral apices compared to the vegetative ones, showing that the cell division rate had approximately doubled on transition. Although the rate of division increased in the cells of the central zone at the summit of the apex, the differential between the rates of division in the central zone and the rest of the apex was maintained, so that the central zone cells were still dividing more slowly than the cells on the flanks of the floral apex. Measurements which involved counting the numbers of cells in the apices of lupin and rye (Sunderland, 1961) showed that the mean cell generation time in the apex decreased during the vegetative phase and increased transiently on transition to flowering. An increase in the rate of growth on transition to flowering in the shoot apex has often been assumed because an increase in the mitotic index is usually observed (Nougarède, 1967; Bernier, 1971). This evidence is less conclusive because it is not always clear how to interpret mitotic indices. These may indicate an increase in synchrony rather than a permanent increase in the rate of cell division. An increase in the degree of synchrony has in fact been observed in *Sinapis* (Bernier, Kinet, and Bronchart, 1967; Jacqmand and Milksche, 1971).

All the quantitative data on growth rates have been obtained from plants where the vegetative apex transforms into an inflorescence apex. This is essentially a continuation of the vegetative mode of growth, since in the floral apex the primordia are still formed as before except that it is the axillary portion of each primordium which grows out to form the floral shoot, and the foliar part which is inhibited, in contrast to what happens in the vegetative apex. In plants such as *Chrysanthemum* and *Sinapis* it is clear that a large part of the increased growth rate is concerned with the enlargement and increased growth of the axis. Whether or not the increased growth rate is concerned with the morphogenesis of the flower itself is less clear. This aspect of the process has been studied in very much less detail. It was to examine the changes in growth rate associated with the morphogenesis and development of the flower itself, in which the transition of the apex is from a vegetative mode of growth to a new, floral mode of growth, that the present work was undertaken.

To examine the initiation and growth of the flower as distinct from the initiation and growth of the floral axis as a whole it is necessary to use a plant with a terminal flower where the apical meristem itself transforms directly from a vegetative apex producing leaves into a floral apex producing floral parts instead of leaves. Other desirable properties of the plant would be that it was photoperiodically sensitive so that flowering could be experimentally induced and comparison could be made with vegetative plants of the same age. A distichous or decussate arrangement of leaves would also be convenient, allowing accurate orientation of the apex for sectioning so that changes occurring regularly during the plastochronic functioning of the apex could be clearly separated from changes occurring as a result of the inductive process.

The plant selected for this investigation was therefore *Silene coeli-rosa* which has these various properties. The inflorescence is a cyme and the apex transforms directly into the first-formed flower of this inflorescence, the growth of the inflorescence itself being carried on by the buds below the flower. Under the conditions
in which it was grown in this investigation *Silene* was a qualitative long-day plant, requiring at least 4 long days for induction. The leaves are opposite and decussate.

The purpose of the experiments to be described was to examine the changes in growth rate of the apex and to see whether these could be experimentally separated from changes occurring as a result of floral induction. *Silene* has the advantage that, because of the relatively long inductive period, measurements of the rates of cell division can readily be done at different times during induction and during the transition to floral growth.

**MATERIALS AND METHODS**

The plant used was *Silene coeli-rosa* (L.) Godron, supplied as *Viscaria cardinalis* by Stewart’s Ltd., Seedsmen, Edinburgh. The seeds were sown in Levington’s Seedling Compost and the plants were grown in controlled-environment rooms at a constant temperature of 20 °C. The plants were maintained in short day (SD) conditions with a daily light/dark cycle of 8 h light/16 h dark. The lights were on from 0900 to 1700 h daily. Illumination was provided by Philips 65–80 W warm white fluorescent tubes supplemented by 40 W tungsten bulbs. This gave a light intensity of 87–105 W m⁻². The pots of plants were kept moist by adding Hoagland’s solution into the trays in which they were standing.

Plants which had been grown under these conditions were selected when they were 28 d old. The plants grew fairly uniformly so that by selecting plants where the third-oldest leaf pair was between 20 and 30 mm long a population of plants was produced in which almost all had just initiated the 7th pair of leaf primordia. The plants which did not meet these specifications were carefully cut off so that the selected plants were left as undisturbed as possible in the pots. The plants were then placed under the appropriate experimental conditions. Those to be kept in SD were returned to the conditions already outlined. Those plants to be induced to flower and given long days (LD) were placed in another growth room in which the growing conditions were precisely the same as already outlined with the addition of low intensity tungsten light (2 W m⁻²) for the period corresponding to darkness in the SD conditions (1700 to 0900 h). This light intensity was only about 2 per cent of that given during the high intensity light period and was therefore insufficient to result in appreciable photosynthesis. Induction was continued under these conditions for 7 d by which time all the plants were induced (Table 1). They were then returned to SD conditions in which they ultimately flowered. Standard induction conditions were therefore 7 LD. It can be seen from Table 1 that 98 per cent of the plants given 3 LD did not flower. It was therefore possible to compare the effect of SD and 3 LD, in which the plants remained vegetative, 7 LD which resulted in 100 per cent induction, and continuous LD in which the plants were maintained in LD after the 7 d of induction has been completed.

**Table 1. Number of long days required for induction**

<table>
<thead>
<tr>
<th>Number of long days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants forming flowers (per cent)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>66</td>
<td>90</td>
<td>93</td>
<td>100</td>
</tr>
</tbody>
</table>

Plants were grown in short days, then given 1 to 7 long days and returned to short days. After a further 3 weeks the apices of at least 50 plants from each treatment were examined.

The 28th day of growth, when selection of the plants was done, and when the inductive LD treatments were begun, will be designated day 0. A sample of three plants from each experimental treatment was taken at 1000 h daily from day 0 to day 12 (i.e. the 40th day after sowing). The plants were fixed in ethanol:acetic acid (3:1, by vol.) for at least 2 h, then dehydrated through an alcohol series and embedded in wax. Serial longitudinal sections, 10 μm thick, were stained with Heidenhain's haematoxylin (Johansen, 1940).

A camera lucida drawing, at a standard magnification, was made of every section of each apex examined. The cell number in the median section of each apex was counted by scoring the number of nuclei present. Each nucleus was recognized by the presence of one or more
darkly staining nucleoli. The volume of the median section was calculated from the weight of the camera lucida drawing. The apical volume for the whole apex was similarly calculated from the total weight of the camera lucida drawings of all the serial sections. Cell number per apex was then calculated by assuming that the mean cell number per unit volume in the median section was similar to that in the apex as a whole. Cell numbers calculated by this procedure were on average within 8 per cent of the value derived by the longer procedure of counting every cell in each apex directly. The cell number was calculated for that part of the apex distal to a line joining the axes of a pair of leaves. Thus values for the numbers of cells above the 7th, 8th, and 9th pairs of leaves were obtained.

RESULTS

In plants subjected to 7 or more LD the rate of leaf initiation was until day 7 the same as in SD and was one new leaf pair about every 4 days (Fig. 1). The development of the apex of plants subjected to 7 LD is shown in Plate 1 (E–H) with apices of plants of the same ages but maintained in SD for comparison (A–D). After 4 LD the apices of plants in LD and SD were morphologically similar (Plate 1A and E). Between days 0 and 8 all plants initiated two leaf pairs. By day 8 the apices of 7 LD plants had begun to enlarge (Plate 1B and F) prior to the formation of the sepals on days 9 to 11 (Plate 1G) and the initiation of stamen and petal primordia in the next day or so (Plate 1H).

The growth of the apex, measured as increases in the cell numbers in the different treatments, is shown in Figs 2–4. Until day 3, when the 8th leaf pair was formed, the apex above the 7th leaf pair consisted only of the apical dome. Similarly, from days 4 to 7 the apex above the 8th leaf pair also consisted only of apical dome. The

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**Fig. 1.** Rate of initiation of primordia in plants in SD (●—●), and in plants given 7 LD (○—○). Each value is a mean of 20 plants. At the time that e.g. the 8th leaf pair was just being initiated, half the plants would have 7 leaf pairs (14 leaves) and half 8 leaf pairs (16 leaves) so that the mean number of leaves plus primordia would be 15.
growth of the apical dome is therefore represented by the exponential increase in cell number from days 0 to 3 above leaf pair 7 (Fig. 2), days 4 to 7 above leaf pair 8 (Fig. 3), and days 7 to 10 (in SD and 3 LD apices) above leaf pair 9 (Fig. 4). The rate of growth of the apical dome in all treatments from days 0 to 7 (Figs 2 and 3) and in SD and 3 LD plants from days 7 to 10 (Fig. 4) was similar, as shown by the similarity in the slopes of the lines, which corresponded to mean cell generation times of 20 to 33 h (Table 2). Above the 9th leaf pair, in 7 LD plants or in continuous LD (Fig. 4), when the apex was enlarging and the flower was beginning to form (Plate 1F–G) the rate of increase of cell number became much greater than in the other treatments (Fig. 6) and at days 7 to 8 corresponded to a mean cell generation time of 6 to 8 h (Table 2). The rate of growth of the apical dome was therefore similar in all plants except those in which floral induction had been accomplished and the flower was beginning to form, in which plants the rate of growth was approximately doubled or trebled.

In plants in SD, the apical dome above the youngest leaf pair (above A–A in Fig. 5) is a meristem in which the rate of cell division was relatively uniform. This is shown by the relatively even distribution of accumulated metaphases in the apical domes of plants which had been treated with colchicine (Fig. 5). When another leaf pair is initiated on the apical dome, the apex above the original leaf pair (above B–B in Fig. 5) now includes not only the young leaf primordia, in which
Fig. 3. Number of cells in the apex above the 8th leaf pair for plants in SD (— —), and plants given 3 LD (△— △), or 7 LD (■— ■), or continuous LD (□— □).

**Table 2.** Mean cell generation times for the apical meristem distal to the youngest pair of leaf primordia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period (days)</th>
<th>Mean cell generation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0–3</td>
<td>24</td>
</tr>
<tr>
<td>SD</td>
<td>4–7</td>
<td>22</td>
</tr>
<tr>
<td>SD</td>
<td>7–10</td>
<td>20</td>
</tr>
<tr>
<td>3 LD</td>
<td>0–3</td>
<td>20</td>
</tr>
<tr>
<td>3 LD</td>
<td>4–7</td>
<td>33</td>
</tr>
<tr>
<td>3 LD</td>
<td>7–10</td>
<td>20</td>
</tr>
<tr>
<td>7 LD</td>
<td>0–3</td>
<td>20</td>
</tr>
<tr>
<td>7 LD</td>
<td>4–7</td>
<td>24</td>
</tr>
<tr>
<td>7 LD</td>
<td>7–8</td>
<td>8</td>
</tr>
<tr>
<td>Continuous LD</td>
<td>7–8</td>
<td>6</td>
</tr>
</tbody>
</table>
cell division is relatively rapid, but also the incipient pith in the central part of the axis, in which cell division is ceasing in most cells. Because of the presence of these cells in which division is slowing down the overall rate of increase of cell number in apical segments larger than the apical dome, i.e. above leaf pairs other than the youngest, will be lower than in the apical dome itself. The fall in the rate of increase in cell number in the apex above leaf pair 7 after day 3 (Fig. 2), above leaf pair 8 in plants in SD and 3 LD after day 7 (Fig. 3), and above leaf pair 9 in plants in SD and 3 LD after day 10 (Fig. 4) is interpreted not as a reduction in the rate of cell division in the apical dome but as a consequence of the maturation and slowing down of division in the cells in the stem axis between the newly initiated pair of leaf primordia and distal to the leaf pair above which cell number was counted.

In 7 LD plants and in continuous LD the rate of increase of cell number above leaf pair 8 did not decrease after day 7 as in SD and 3 LD plants but remained the
same as before (Fig. 3). This was because the reduction of the rate of increase of cell number in the incipient pith, between the newly initiated 9th pair of leaves, was compensated by the increase in growth rate of the apical dome above the 9th leaf pair (Fig. 4). The decrease in the growth rate above the 9th leaf pair in 7 LD plants and in continuous LD after day 8 (Fig. 4) was presumably the result of the slowing
down of division in the central part of the apex below the more rapidly dividing cells of the apical dome (Fig. 6). Despite the formation of incipient pith in which division was ceasing, the increased growth rate in the apical dome resulted in an overall rate of growth equal to that of the cells of the apical dome alone in non-induced SD and 3 LD apices (Fig. 4).

The values for cell numbers were derivative in that they depended upon measurements of the volumes of the apices. The rates of increase of apical volumes were very similar to the rates of increase of cell numbers and the calculated volume doubling times were exactly the same as the cell doubling times.

DISCUSSION

The values for the increase in cell numbers (Figs 2–4) show that during induction of flowering with 7 LD there was no sustained increase in the growth rate of the apical meristem. The growth rate increased only at the beginning of flower morphogenesis when the apex was enlarging just before the formation of the sepals. The calculated values for the mean cell generation times (Table 2) have been confirmed by independent measurements, made by a double-labelling technique (Miller and Lyndon, 1975) which gave values for the length of the cell cycle in apices of plants in SD or during and after induction in 7 LD which were almost identical to those in Table 2. In Silene, in which the apex transforms into a terminal flower, there is therefore no increase in the growth rate of the apical meristem which can be related to growth of the stem axis before the flower is formed. Since 90 per cent of the plants were induced after 5 LD (Table 1) and were therefore committed to flower by then, the apices continued growing for at least another 2 d at the same rate as apices of vegetative (SD) plants. For 66 per cent of the plants there were at least 3 d after commitment to flower in which the growth rate of the apex remained unchanged. Other experiments (Miller and Lyndon, 1976) show that there is an increase in the concentration of RNA, in apices of plants in LD, about 3 d before the increase in growth rate occurs. The increase in growth rate seems to be part of the process of morphogenesis of the flower in Silene and is apparently one of the later events of evocation rather than one of the first.

In the stem below the apical meristem there are undoubtedly changes in the growth rate in plants in long days before changes occur in the apical dome. The internodes begin to elongate and the uppermost axillary buds may begin to grow faster. There may therefore be increases in the rate of cell division in the axillary positions before changes occur in the apical dome.

One of the first results of the arrival of the floral stimulus at the shoot apex is an increase in mitotic index (Bernier, 1971) which has sometimes been assumed to indicate an increased rate of cell division but is more likely to indicate an increased degree of synchrony of the cells (Bernier et al., 1967; Jacqmard and Miksche, 1971). If the synchrony is achieved by a speeding up of the cell cycle of some of the cells the result would be a transient increase in the rate of cell division. The data of Figs 2–4 suggest that this might have occurred in Silene. A consistent feature is that from days 1 to 7 the number of cells in the apical dome was slightly higher in plants
exposed to 7 LD than in the corresponding plants in SD (Fig. 2, days 1–3; Fig. 3, days 4–7). The cell number in the apical dome of plants given 3 LD also tended to be higher than that of the plants in SD on days 4 to 6 (Fig. 3). However, in all cases the rates of increase of cell number, after day 1, were similar. The differences in cell numbers, if indeed they are real ones, could perhaps be accounted for by an increase in cell number during the first day of exposure to LD. If such a transient increase were to occur it would presumably be associated with an increased degree of synchrony in cell division in the apex and a temporary increase in mitotic index which would not necessarily lead to floral induction. In Sinapis it has been shown that the initial rise in mitotic index can occur without necessarily being followed by floral induction (Bernier, Bronchart, and Kinet, 1970).

The number of cells in the apices of plants in continuous LD tended to be higher than in plants given only 7 LD (Figs 3 and 4), suggesting an effect of LD in addition to that which results in evocation. The high values for plants in continuous LD could be associated with a further increase in the rate of growth of the apices, since observation of plants which have been grown on to flowering shows that continuous LD do in fact hasten the growth of the flowers.

It has been suggested that a stimulation of the rate of leaf initiation, as a preliminary to floral initiation, is a general phenomenon (Langer and Bussell, 1964). Clearly this is not so in Silene (Fig. 1). This might be because the apex in Silene transforms directly into a flower, whereas in the plants mentioned or examined by Langer and Bussell (1964) the apices all grew to produce inflorescences. This again emphasizes the need to distinguish between these two modes of apical growth during the transition to flowering.

ACKNOWLEDGEMENT
We are grateful to the Agricultural Research Council for the award of grant AG 15/81 in support of this work, during which M.B.M. held a Research Assistantship.

LITERATURE CITED

EXPLANATION OF PLATE
PLATE 1
Growth of shoot apices of plants in short days (A–D) and in plants given 7 LD (E–H), on days 4 (A, E), 8 (B, F), 11 (C, G), and 12 (D, H) after the start of the LD treatment. 8 = 8th pair of leaf primordia; 9b = base of 9th pair of leaf primordia; 10 = 10th pair of leaf primordia; S = sepal; St = stamen. (×200).
PLATE 1
INTERACTING PROCESSES IN
VEGETATIVE DEVELOPMENT AND IN THE
TRANSITION TO FLOWERING
AT THE SHOOT APEX

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The shoot apex may be regarded as a multi-component system in which the different components continually interact. The formation of leaf primordia at the shoot apex leads to the partitioning of the apical meristem into component parts – the primordia, the axial tissue of the stem, and the apical dome that remains – all of which may grow at different rates even though they are each part of the same meristem (Sunderland & Brown, 1976). These derivatives of the apical meristem also follow different developmental pathways. For the leaf primordium the pathway to be followed has already become fixed, one plastochron after the primordium has been formed (Sachs, 1969). The nodal and internodal tissue, which is also formed from the apical meristem, follows another developmental pathway and the cells quickly enlarge and vacuolate and take on characteristics of their own (Lyndon & Robertson, 1976). The various components of the apical meristem have different metabolic characteristics (Sunderland, Heyes & Brown, 1956, 1957) and may respond to stimuli, external and internal, in different ways. For example, when gibberellic acid is applied to the shoot a characteristic response is a large increase in mitotic activity in the subapical meristem leaving the terminal meristem unaffected (Sachs, Lang, Bretz & Roach, 1960).

The formation of each component may itself be regarded as the result of the interaction of several growth processes. Processes which can be identified and measured include (1) growth, the rate of which can vary; (2) leaf initiation, the rate of initiation being more conveniently measured as its reciprocal, the length of the plastochron; (3) the partitioning of the meristem, which determines the size of the primordia on initiation in relation to the size of the apical dome and the axial tissue; and (4) changes in polarity, which can be observed as changes in the frequency of anticlinal and periclinal divisions. These growth processes can be examined and measured in relation to the development of the apex in an attempt to
discover their role in the overall growth of the apical system. This has been done with some success in the study of the formation and positioning of leaf primordia.

The formation of a primordium occurs in that region of the meristem where the rate of division and growth is greatest (Lyndon, 1973) and is preceded by a change in the polarity of growth in this same region of the apex. The orientation of growth in the apical dome in the first half of a plastochron is restricted so that only anticlinal divisions occur. In the second half of a plastochron the orientation of growth appears to be unrestricted so that there are divisions in all planes and periclinal divisions occur (Lyndon, 1972). These observations have led to the view that the initiation of a primordium is the result of the lifting of a restriction on the polarity of growth so that the tissues where the volume growth rate was fastest could bulge out, forming a primordium. It has been suggested that the characteristically low growth rate at the tip of the apical dome means that even if the restriction on the polarity of growth might be temporarily lifted in this region the growth rate is insufficient here to result in a visible deformation of the surface of the apex at the tip in the course of a plastochron (Lyndon, 1976). The formation of a leaf primordium is therefore more readily understood by attempting to analyse it in terms of its component processes.

The interaction of different processes in the meristem is also apparent from observations of phyllotaxis. It can be shown that the phyllotactic pattern depends on the radial relative growth rate of the apical surface, the size of a leaf primordium on initiation, and the rate of leaf initiation (Richards, 1948, 1951). The formation of new primordia at positions which lead to very precise arrangements has led to the hypothesis that young leaf primordia interact with each other and with the positions on the meristem at which new primordia are about to arise (Richards, 1948; Thornley, 1975). In the flower also, the primordia occupy very precise positions in relation to each other, especially the primordia of one whorl in relation to the primordia of another. The production of growth substances by primordia, for example by the primordia of the anthers and the ovary (Nitsch, 1965; Murakami, 1975), carries with it the implication that the development of one primordium or type of primordium may influence the development of other primordia and the development and growth of the apical meristem itself.

The processes which bring about growth will themselves be the result of interactions of metabolites and metabolic systems. However, relatively little is known so far about this aspect of the growth of the shoot apex and it will not be considered in this paper. Our concern here will be to examine the transition from vegetative to floral growth in an attempt to understand the
in terms of changes in the interactions of the component parts of the meristem and the interactions of the processes which determine their patterns of growth.

CHANGES IN THE APICAL MERISTEM ASSOCIATED WITH THE TRANSITION TO FLOWERING

Some of the first events which occur at the shoot apex during floral induction and evocation are (1) an increase in the mitotic index, which may indicate either an increase in the degree of synchrony of cell division (Bernier, Kinet & Bronchart, 1967) or an increase in growth rate, (2) an increase in the concentration of RNA and protein, and then (3) an increase in the size of the apex (Bernier, 1971). The increase in RNA and protein can be measured histochemically (Jacqumard, Miksche & Bernier, 1972) and is evident as an increase in the concentration of ribosomes, as seen in electron micrographs (Nougarède, 1967). The enlargement of the apex is most striking in the case of plants such as Chrysanthemum, in which the area of the apex increases about 400-fold in a few days during the formation of the capitulum (Schwabe, 1959), or in the grasses and cereals, in which the apical dome may double in length before flowering (Kirby, 1974). From such observations it has been inferred that there is an increase in the growth rate of the apex at the time of transition to flowering and this has been confirmed by measurements of the rates of cell division in vegetative and floral apices of Datura (Corson, 1969) and Sinapis (Bodson, 1975).

These observations have all been made on plants which form terminal inflorescences, or in which the terminal meristem forms the last flower in the inflorescence (e.g. Pharbitis). Apices of this sort are essentially continuing the same mode of growth as when vegetative, and continue initiating primordia on their flanks, after the transition to flowering, in much the same way as before. There tends to be a large amount of axial growth, and growth of the apex is indeterminate, at least until the formation of the terminal flower. The flowers themselves are formed by the meristems of the side shoots and not by the terminal meristem. On the other hand the growth of a meristem which itself forms a flower (e.g. Silene) is somewhat different. Here the apex ceases producing foliar primordia or side shoots and instead produces primordia which develop into the floral members and organs. The apex in this case is determinate and progresses through a strict sequence of development from the formation of the sepals to the formation of the carpels. The internodes become shortened and almost non-existent. There is no obvious requirement for a large amount of rapid growth.
This raises the question of whether the changes which have been observed in apices during floral induction and evocation are essential for the process of flower morphogenesis or whether they may be more closely related to the increase in growth which usually accompanies flowering but which may be mainly concerned with the growth of the inflorescence axis. To attempt to answer this question, it is desirable to examine a plant in which the terminal apex itself transforms into the first flower, before the formation of an inflorescence. This would also allow a direct comparison between the initiation of leaf primordia and the initiation of the primordia of the floral members. With these points in mind the transition to flowering has been examined in the long-day plant *Silene coeli-rosa*, in which the first flower is formed by direct transformation of the terminal meristem. The inflorescence is a cyme and its growth is carried on by the axillary buds below the flower.

**INCREASES IN THE GROWTH RATE AND RNA CONTENT OF THE APEX AND THEIR RELATION TO FLOWERING**

In *Silene* the growth rate of the apical meristem was determined by measuring daily the change in volume and cell number in the apical dome as it grew between the initiation of successive pairs of leaf primordia (the leaves are produced in opposite, decussate pairs). This was possible because the plastochron at 20 °C was about 4 days. The doubling times for volume and cell number were the same (because mean cell volume remained essentially constant) and were about 20 h in non-induced plants in short days and also in plants undergoing induction in long days. Only when the apex began to enlarge, immediately before the formation of the sepals, did the doubling time become reduced, to about 10 h or less (Miller & Lyndon, 1976). This was confirmed by measuring the length of the cell cycle by a double-labelling technique. The cell cycle was about 20 h in short days and during induction in long days, and shortened to about 10 h at the onset of flower morphogenesis (Miller & Lyndon, 1975). In *Silene* there is therefore an increase in growth rate which is associated with flower morphogenesis, but not with induction and evocation. However, when plants were transferred from short days at 20 °C (which did not induce flowering) to long days at 13 °C (which did) the growth rate of the apex remained approximately the same (Miller, 1976). This suggested that an increase in growth rate of the apex was not obligatory for the transition to flowering to occur.

The concentration of RNA (measured histochemically) in the apex of *Silene* increased by about 30% during induction as it does in many other
plants (Miller, 1976). The RNA concentration could also be increased above the short-day level by exposure to 3 long days, which was non-inductive for 98% of the plants. This increase in RNA was ephemeral and there was no increase in the growth rate of the apex (except for a possible transient increase in the first long day (Miller & Lyndon, 1976)) and essentially no flowering. Similarly, when plants were given fractional induction treatments, in which sub-inductive numbers of long days were separated by periods of short days, the RNA level increased ephemeral in those treatments in which induction did not occur but showed a sustained increase in those treatments in which induction did occur. A very much greater increase in RNA – the concentration could be doubled – was produced by treatment with gibberellic acid although this did not measurably affect either the degree of induction or the growth rate of the apex. The gibberellic acid treatment raised the RNA concentration in short-day plants to a higher level than that in untreated plants in long days, but the plants in short days did not flower whereas those in long days did. The RNA content of the apex could therefore be increased experimentally without an associated increase in growth rate and without flowering. Conversely, when plants were induced by transferring them from short days at 20 °C to long days the increase in RNA was negligible and the growth rate did not increase and yet flowering occurred readily (Miller, 1976).

In these experiments with *Silene* it therefore proved possible to increase the concentration of RNA in the apex without altering the growth rate of the apex and without inducing flowering, and to induce flowering without an increase in RNA or in growth rate at the apex. What was not found was an increase in growth rate without an increase in RNA. We may therefore conclude that the increase in RNA which is generally observed in apices about to flower is probably more closely associated with the increase in growth rate which usually accompanies flowering than with the actual process of flower initiation itself. The same conclusion may be drawn from experiments with *Chenopodium rubrum*, in which flowering was stimulated in plants treated with 6-azauridine, which inhibited the accumulation of RNA (Seidlová & Krekule, 1973). Strong supporting evidence comes from experiments on the growth of *Cicer arietinum* buds (Usciati, Codaccioni & Guern, 1972). Axillary buds were released from inhibition by the application of cytokinin and within a few hours changes in the apex occurred, including an increase of mitotic index, an increase in the synthesis and amounts of DNA and RNA, and an increase in starch. These are all changes which occur in apices of plants induced to flower. However, these *Cicer* bud apices were vegetative and grew out to form leafy shoots. Many of the events occurring in apices on induction are therefore the same as
those occurring during stimulation of vegetative growth and are not peculiar to the flowering process.

It was noticed that when *Silene* plants were transferred to 13 °C the rate of leaf initiation fell (Miller, 1976). This suggested that what may be important in the transition to flowering is not so much an absolute increase in the rate of growth of the apical dome but an increase relative to the rate of leaf initiation. In the plants at 13 °C in long days this would have been achieved not by increasing the growth rate of the apex but by reducing the rate of leaf initiation. The result would be an enlargement of the apical dome. With these considerations in mind, a series of measurements was made on *Silene* apices during induction and the transition to flowering in order to explore the interactions of the processes which are involved in controlling the size of the apical dome, the enlargement of which seems to be a universal feature of the transition to flowering. But first it is necessary to consider briefly the nature of these processes involved in controlling apical dome size.

**PROCESSES INVOLVED IN CONTROLLING THE SIZE OF THE APICAL DOME**

Because of the initiation of a leaf (or, as in *Silene*, a pair of leaves) the apical dome is redefined at the beginning of each plastochron and its growth can be represented by the saw-toothed graph in Fig. 1. The apical dome may be assumed to grow exponentially between the initiation of leaves (Schüepp, 1938) and there is some direct evidence that this is so (Lyndon, 1968). The ordinate on Fig. 1 is therefore logarithmic. The growth of the apical dome between the initiation of successive leaves or leaf pairs is given by the increase in volume between A and B, B and C, and so on. The relative growth rate in volume per day ($R_{\text{day}}$) is the increase in log$_e$ volume per day and is represented by the slope of the line between successive days. The relative growth rate in volume per plastochron ($R_p$) is given by the increase in log$_e$ volume per plastochron. $R_{\text{day}}$ and $R_p$ are obviously related since $R_p = R_{\text{day}} \times$ plastochron in days.

The volumes of the apical dome at the end of successive plastochrons ($D_{\text{max}}$) are joined by the dashed line in Fig. 1. If $D_{\text{max}}$ increases from plastochron to plastochron (as shown in Fig. 1) the rate of increase depends on $R_p$ (the slope between A and B, B and C) but it also depends on the volume of the apical dome immediately after leaf initiation, at the beginning of a plastochron ($D_{m1n}$). This in turn depends on the fraction of the volume of the apical dome at the end of a plastochron ($D_{\text{max}}$) which is used to form the new apical dome ($D_{m1n}$). The ratio of the volume of the
apical dome at the end of a plastochron \((D_{\text{max}})\) to the volume that is re-invested in the new apical dome \((D_{\text{min}})\) at leaf initiation represents the multiple by which the apical dome volume must increase in the plastochron just beginning to restore it to the same size as it was at the end of the previous plastochron \((D_{\text{max}})\). The natural logarithm of this ratio is:

\[
V = \log_e (D_{\text{max}}) - \log_e (D_{\text{min}})
\]

and is represented by the vertical distance between \(D_{\text{max}}\) and \(D_{\text{min}}\) in Fig. 1. This value, \(V\), is therefore the increase in \(\log_e\) (apical volume) which is required for \(D_{\text{max}}\) at the end of the plastochron (at B) to be the same as at A.

The size of the apical dome at the end of each plastochron \((D_{\text{max}})\) will remain constant if \(R_p = V\) from plastochron to plastochron, where \(V\) is calculated at the initiation of a leaf (or leaf pair) and \(R_p\) is the value for the growth in the plastochron of which this event marks the beginning. But if either \(R_p\) or \(V\) changes so that \(R_p \neq V\) then \(D_{\text{max}}\) will change in succeeding plastochrons. If \(R_p > V\) (as shown in Fig. 1) then the apical dome \((D_{\text{max}})\) will enlarge, and if \(R_p < V\) it will decrease. \(V\) alters if the ratio \(D_{\text{max}}/D_{\text{min}}\) changes. If the proportion of the apical dome \((D_{\text{max}})\) which is reinvested as \(D_{\text{min}}\) increases, then \(D_{\text{max}}/D_{\text{min}}\) decreases, as does \(V\). Conversely, if the proportion of \(D_{\text{max}}\) reinvested as \(D_{\text{min}}\) decreases then \(V\) increases. Changes in \(R_p\) can be brought about by changes in either \(R_{\text{day}}\) or in the length of the plastochron, or both. In Fig. 1 \(R_p\) is greater than is necessary to restore the volume of the apical dome at B to the value \(D_{\text{max}}\) had at A, and so \(D_{\text{max}}\) increases from plastochron to plastochron. If
the plastochron were lengthened, without any change in $V$ or $R_{day}$, growth would continue longer before initiation of the next leaf (or leaf pair) and so $D_{\text{max}}$ would increase faster than in Fig. 1. The same would happen if the plastochron remained the same and $R_{day}$ increased. In Fig. 1, $R_{day}$, $R_p$ and $V$ are all shown as constant.

The growth of the apical dome of *Silene*, particularly during the transition to flowering, will now be examined in the light of these parameters.

**GROWTH OF THE APICAL DOME OF *SILENE***

Plants of *Silene coeli-rosa* (L.) Godron were grown and induced to flower at three temperatures, 13, 20, and 27 °C, so that a comparison could be made of apical meristems with different growth rates and different rates of leaf initiation. *Silene* is a long-day plant requiring three or more long days for induction and remaining vegetative in short days. The plants were reared in short days and were selected for developmental uniformity when they were 28 days old (Miller & Lyndon, 1976). The measurements to be described began on this twenty-eighth day, which was when the plants were transferred to the experimental treatments. The number of days required for the same degree of induction increased with increasing temperature (Fig. 2). Plants were induced by exposing them to 7 long days at 13 or 20 °C and 12 long days at 27 °C, and were then transferred back to short days. Since flower initiation began about 12 days after the beginning of induction at 13 °C, after about 9 days at 20 °C and 11 days at 27 °C, development of the flowers took place in non-inductive conditions.

**Length of the plastochron**

The rates of initiation of primordia were established so that the beginning and end of each plastochron could be timed precisely (Figs. 3–5). Since the leaves are in opposite, decussate pairs, there are two leaf primordia initiated simultaneously at the beginning of each plastochron. The beginning of a plastochron was taken as the time that the leaf pair $n+1$ was initiated and this was given by the time at which there were $n + \frac{1}{2}$ leaf pairs ($= 2n + 1$ leaves + primordia), i.e. 50% of the plants had initiated leaf pair $n + 1$. The time at which each plastochron began was read from Figs. 3–5 and is shown on Figs. 6–11 by an arrow. The lengths of the plastochrons are summarised in Table 1.

In short days at 13 and 20 °C the plastochron remained constant, being 5.9 days at 13 °C and 3.9 days at 20 °C (Figs. 3, 4, Table 1). In short days at 27 °C the plastochron was initially 2.9 days but lengthened to 7.5 days after the first 4 days of growth at 27 °C (Fig. 5, Table 1). In fact the plants...
Interacting Processes at the Shoot Apex

Fig. 2. Number of long days required for floral induction in Silene. Plants were examined for flower buds after 5 weeks at 13 °C (○) and after 3 weeks at 20 °C (●) and 27 °C (×). Each point is a mean for at least 10 plants.

Fig. 3. Initiation of primordia in Silene at 13 °C. Plants in short days (SD) (●); plants exposed to 7 long days (LD) from days 0–7 (○). The long-day treatment has apparently synchronised leaf initiation. Each point is a mean for at least 10 plants.
Fig. 4. Initiation of primordia in *Silene* at 20 °C. Plants in short days (SD) (●); plants exposed to 7 long days (LD) from days 0–7 (○). Each point is a mean of at least 10 plants.

did not grow well at 27 °C, which was obviously near the limit of their tolerance. In long days the first leaf pair to be initiated at each temperature was initiated at the same time in long- and short-day treatments. However, the next leaf pair in the long-day treatments was in each case initiated about 0.5 or more of a day sooner than in short days (Figs. 3–5, Table 1). At 27 °C this did not represent a shortening of the plastochron compared to the plants in short days, as it did at 20 and 13 °C, but simply a maintenance of the length of the plastochron in long days compared to the plants in short days at 27 °C in which the plastochron lengthened.

In all cases the plastochron shortened markedly at the onset of flowering. As soon as the first sepals had been formed the plastochron reduced to one quarter or less of its original value (Table 1). The reduction was greatest at 27 °C at which flower growth was fastest and least at 13 °C at which flower growth was slowest.
Fig. 5. Initiation of primordia in *Silene* at 27 °C. Plants in short days (SD) (●); plants exposed to 12 long days (LD) from days 0–12 (○). Each point is a mean for at least 10 plants.

Table 1. *Length of the plastochron in Silene*

<table>
<thead>
<tr>
<th>Number of pairs of leaves + primordia</th>
<th>Days until initiation of next pair of primordia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 °C</td>
</tr>
<tr>
<td>Short days</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.9</td>
</tr>
<tr>
<td>6</td>
<td>3.9</td>
</tr>
<tr>
<td>7</td>
<td>5.9c</td>
</tr>
<tr>
<td>8</td>
<td>4.7</td>
</tr>
<tr>
<td>9</td>
<td>3.1</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>II</td>
<td>1.0</td>
</tr>
<tr>
<td>12</td>
<td>.</td>
</tr>
</tbody>
</table>

The horizontal bars mark the transition to flowering.

a First 4 days at 27 °C.

b After 4 days at 27 °C.

c These values are derived from the rate of leaf initiation after transfer to 13 and 27 °C, respectively. The actual period between the initiation of the seventh and eighth pairs of primordia was 5.7 days at 13 °C and 3.1 days at 27 °C; this was because the plants were not transferred to 13 or 27 °C until 1.1 days after the initiation of the seventh pair of primordia.
Fig. 6. Growth of the apical dome of *Silene* plants in short days (SD) at 13 °C. Apical dome above seventh (●), eighth (○), and ninth (▲) leaf pairs. Horizontal bars show size of apical dome at the end and the beginning of each plastochron. Each arrow marks the initiation of a pair of primordia. Each point is the mean for 3 plants.

*Relative growth rate* ($R_{\text{day}}$)

The volume of the apical dome was measured each day from serial sections (Miller & Lyndon, 1976). The values for $R_{\text{day}}$ (Tables 2, 3) for each plastochron were derived from the regression coefficient of the appropriate line in Figs. 6-11. These lines represent the regression of log$_e$ (apical dome volume) on time.

In short days, at 13 and at 20 °C, $R_{\text{day}}$ was constant in the three plastochrons examined but at 13 °C was reduced to about half the value of that at 20 °C (Figs. 6 and 7, Table 2). At 27 °C, $R_{\text{day}}$ was initially similar to that at 20 °C but then fell to a much lower value (Fig. 8, Table 2). Plants kept in short days at 27 °C usually stopped growth after about a month.

In plants transferred to long days at 20 °C the growth rate and hence $R_{\text{day}}$ was not significantly different for the first two plastochrons from that of plants in short days. Only after initiation of the ninth leaf pair (on day 6) did the growth rate increase (Fig. 9, Table 3). On initiation of the sepals (day 9) the growth rate seemed to decrease to the former level. Although this decrease was not significant statistically, further data (R. F. Lyndon, unpublished) show that after the formation of the sepals the relative growth rate, $R_{\text{day}}$, for the apical meristem of the flower above the sepals at 20 °C decreased to about 0.38. In plants transferred to long days at 27 °C,
Fig. 7. Growth of the apical dome of *Silene* plants in short days (SD) at 20 °C. Because the plants were growing in the same environment as they had been since sowing it is permissible to extrapolate back from day 0 to find the size of the apical dome \((D_{\text{min}})\) just after the seventh leaf pair was initiated. This in turn was fixed by extrapolation backwards in Fig. 4 and was on day \(-1.1\), or 26.9 days after sowing. The value for the size of the apical dome \((D_{\text{max}})\) at this same time but at the end of the sixth plastochron was estimated by adding the value for the size of the apical dome at the beginning of the seventh plastochron to the value for the size of the seventh apical unit on day \(-1.1\) which was derived by extrapolation from the regression line which fitted its growth (Table 6). The values for \(D_{\text{max}}\) and \(D_{\text{min}}\) at the initiation of the seventh leaf pair are shown, for convenience, at day 0, i.e. displaced 1.1 days to the right of their true position. The difference between \(\log_e(D_{\text{max}})\) and \(\log_e(D_{\text{min}})\) at a given time is the value for \(V\).

Table 2. Relative growth rates per day \(\left(R_{\text{day}}\right)\) of the volume of the apical dome of *Silene* plants in short days (non-inductive)

<table>
<thead>
<tr>
<th>Number of pairs of leaves + primordia</th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.30</td>
<td>0.68</td>
<td>0.59</td>
</tr>
<tr>
<td>8</td>
<td>0.34</td>
<td>0.67</td>
<td>0.17 a</td>
</tr>
<tr>
<td>9</td>
<td>0.28</td>
<td>0.69</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Significantly different from value for previous plastochron \((P < 0.05)\).

\(R_{\text{day}}\) remained similar to that in short days at 20 °C. The growth rate did not appreciably decline for three plastochrons (Fig. 10, Table 3). However, in the initiation of the tenth leaf pair (day 8) the growth rate increased markedly, so that \(R_{\text{day}}\) almost trebled in value. The values for \(R_{\text{day}}\) for the
Fig. 8. Growth of the apical dome of *Silene* plants in short days (SD) at 27 °C. Key: as for Fig. 6.

Fig. 9. Growth of the apical dome of *Silene* plants exposed to long days (LD) from days 0–7, at 20 °C. Key: as for Fig. 6. Apical dome above sepals (□).
Fig. 10. Growth of the apical dome of *Silene* plants exposed to long days (LD), from days 0–12, at 27 °C. Apical dome above seventh (●), eighth (○), ninth (▲) and tenth (□) leaf pairs, and above eleventh (◆) or twelfth (▼) leaf pairs or sepals. Otherwise, as for Fig. 6.

Table 3. Relative growth rates per day ($R_{\text{day}}$) of the volume of the apical dome for *Silene* plants exposed to long days (inductive)

<table>
<thead>
<tr>
<th>Number of pairs of leaves + primordia</th>
<th>$13\degree C$</th>
<th>$20\degree C$</th>
<th>$27\degree C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.44</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>8</td>
<td>0.46</td>
<td>0.75</td>
<td>0.53</td>
</tr>
<tr>
<td>9</td>
<td>0.50</td>
<td>1.06</td>
<td>0.51</td>
</tr>
<tr>
<td>10</td>
<td>0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54</td>
<td>1.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>.</td>
<td>.</td>
<td>0.54</td>
</tr>
<tr>
<td>12</td>
<td>.</td>
<td>.</td>
<td>0.50</td>
</tr>
</tbody>
</table>

The horizontal bars mark the transition to flowering.

<sup>a</sup> Significantly different from value for previous plastochron ($P < 0.05$).

<sup>b</sup> Significantly different from value for previous plastochron ($P < 0.01$).
eleventh and twelfth plastochrons (day 10 onwards) at 27 °C are complicated by the fact that there is considerable scatter in the points and so the relative growth rates are not certain. Also about half the plants initiate the flower after the tenth leaf pair and half after the eleventh leaf pair so that the values for the eleventh and twelfth plastochrons are derived from a mixture of plants at various stages of flower initiation. Again, however, further data (R. F. Lyndon, unpublished) show that the relative growth rate \( R_{\text{day}} \) for the apical meristem of the flower above the sepals reduces to about 0.43 after the sepals have been initiated.

In plants transferred to long days at 13 °C, \( R_{\text{day}} \) was reduced compared to plants at 20 °C in short days but was higher than in plants maintained in short days at 13 °C (Fig. 11, Table 3). In long days at 13 °C the growth rate was maintained without significant difference during induction; there was no rapid increase as was seen in a single plastochron at 20 and 27 °C. A further increase in growth rate was observed only after the first sepals were initiated, but again further data (R. F. Lyndon, unpublished) show that \( R_{\text{day}} \) declined to about 0.23 once the sepals had been initiated.

From cell counts made in a number of apices it was found that cell number was proportional to apical volume, and that \( 10^6 \, \mu\text{m}^3 \) corresponded to about 2500 cells. Since the changes in volume result from changes of cell number and not cell size, the values of \( R_{\text{day}} \) are therefore also values for the relative rates of increase of cell numbers.
Size of the apical dome

The sizes of the apical dome at the beginning and end of each plastochron are indicated by the horizontal bars on Figs. 6–11. Where necessary the lines representing the growth of the apical dome were extrapolated back to obtain estimates of dome size. The difference between two vertically displaced bars at the same point in time represents the growth increment that the apical dome must make in the next plastochron if it is to remain the same size. The difference between the natural logarithms of these values is \( V \). Since all plants, whatever the subsequent treatment, were growing in short days at 20 °C at the time of initiation of the seventh leaf pair, the estimated value for \( V \) at the beginning of the seventh plastochron was the same for all treatments (Tables 4 and 5). (For the derivation of this value of \( V \) see legend to Fig. 7.)

In short days at 20 °C the proportion of the meristem that was reinvested in the apical dome at the beginning of each plastochron remained constant (Fig. 7) and so \( V \) remained constant (Table 4). In short days at 27 °C, the changes in \( V \) were associated with the readjustment of the apex to a new lower growth rate (Fig. 8). At 13 °C in short days, the proportion of the meristem invested in the new apical dome increased (Fig. 6) and so \( V \) decreased (Table 4).

In long days at 20 °C, \( V \) remained very much the same as in short days (Table 5) and at 13 °C it fell to much the same value as in short days at 13 °C (Table 4). At 27 °C in long days (Fig. 10) a greater proportion of the meristem was reinvested in the new dome, at least until the tenth plastochron (day 8) and so \( V \) decreased (Table 5); but when the flower was about to be initiated (eleventh plastochron, day 10) a relatively large proportion (almost half) of the meristem was used to form the new apical dome. To summarise, at 20 °C about one-tenth, and at 13 °C about one-fifth, of the old apical dome was used in forming the new one at the beginning of the next plastochron, and at 27 °C it varied from these values up to one half.

Interaction of \( R_{\text{day}} \), plastochron and \( V \) in controlling the size of the apical dome in Silene

The interaction of the plastochron and \( R_{\text{day}} \) is assessed as \( R_p \), the relative growth rate per plastochron. For plants growing in short days (Table 4), at 20 °C \( R_p \) was constant and so was \( V \), but since \( R_p > V \) the apical dome enlarged slightly in successive plastochrons (Fig. 7). The reduction in \( R_p \) on transfer from 20 to 13 °C in short days (Table 4) implies that the longer plastochron at 13 °C (Table 1) was not sufficient to compensate for the reduction in \( R_{\text{day}} \) (Table 2). At 13 °C the size of the apical dome was
Table 4. Relative growth rates per plastochron ($R_p$) of the volume of the apical dome; and the increase in $\log_e$ (apical dome volume) necessary to restore the apical dome to the same size as at the end of the previous plastochron ($V$); for Silene plants in short days (non-inductive)

<table>
<thead>
<tr>
<th>Number of pairs of leaves + primordia</th>
<th>$13 , ^\circ C$</th>
<th>$20 , ^\circ C$</th>
<th>$27 , ^\circ C$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_p$</td>
<td>$V$</td>
<td>$R_p$</td>
</tr>
<tr>
<td>7</td>
<td>1.76$^a$</td>
<td>2.44</td>
<td>2.63</td>
</tr>
<tr>
<td>8</td>
<td>2.01</td>
<td>1.73</td>
<td>2.61</td>
</tr>
<tr>
<td>9</td>
<td>1.63</td>
<td>1.27</td>
<td>2.69</td>
</tr>
</tbody>
</table>

$^a$ The value for $R_p$ at $13 \, ^\circ C$ in plastochron 7 is artificially low because it does not take into account the more rapid growth which occurred at the very beginning of the plastochron when the plants were still at $20 \, ^\circ C$.

maintained, and indeed increased (Fig. 6), only because a larger proportion of the meristem was invested as apical dome at the beginning of successive plastochrons, resulting in a reduction in $V$ (Table 4) so that $R_p > V$ and the apical dome enlarged. Transfer from 20 to $27 \, ^\circ C$ in short days also resulted in a reduction in $R_p$ (Table 4), although this remained remarkably constant considering the large fall in $R_{\text{day}}$ (Table 2) and the lengthening of the plastochron (Table 1). At $27 \, ^\circ C$ in short days in the ninth plastochron, $R_p < V$ (Table 4), implying a shrinking apical dome, and visual observations confirm this.

On transfer to long days at $20 \, ^\circ C$, $R_p$ and $V$ both remained very much as they were in short days (Table 5) and decreased only after the transition to flowering. There was therefore no obvious alteration in the relationship between $R_p$ and $V$ on transfer from short days to long days and so the apex continued to enlarge gradually (Fig. 9). The increase in $R_{\text{day}}$ in the ninth plastochron, at day 8 (Fig. 9, Table 3) was not entirely offset by the decrease in the plastochron (Table 1) so that the apex enlarged at the transition to flowering. This enlargement was attributable solely to the increase in $R_{\text{day}}$ at this time.

In the plants transferred to long days at $27 \, ^\circ C$, $R_p$ decreased (Table 5) compared with plants at $20 \, ^\circ C$ in short days, but was about the same as in plants at $27 \, ^\circ C$ in short days. This was despite the fact that the plastochron was quite different in long and short days at $27 \, ^\circ C$ (Table 1). In long days $R_p$ doubled in the tenth plastochron (Table 5) because the large increase in $R_{\text{day}}$ (Table 3) more than offset the decrease in the plastochron (Fig. 10). The increase in size of the apical dome at the end of each plastochron until day 8 (Fig. 10) was attributable only to the reduction in $V$ in plastochron
Table 5. Relative growth rates per plastochron (Rp) of the volume of the apical dome; and the increase in loge (apical dome volume) necessary to restore the apical dome to the same size as at the end of the previous plastochron (V); for Silene plants exposed to long days (inductive)

<table>
<thead>
<tr>
<th>Number of pairs of leaves + primordia</th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rp</td>
<td>V</td>
<td>Rp</td>
</tr>
<tr>
<td>7</td>
<td>2.61</td>
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<td>2.74</td>
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<td>2.15</td>
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<td>12</td>
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</tbody>
</table>

The horizontal bars mark the transition to flowering.

8 and 9 (Table 5). At the beginning of the tenth plastochron, V in fact increased so that the increase in size of the dome, just before the transition to flowering and the initiation of the first sepals on day 10, was entirely due to the increase in Rd, as it was at 20 °C.

In the plants transferred to long days at 13 °C a different picture emerges. After transfer from 20 °C, Rp decreased (Table 5) because the plastochron did not lengthen sufficiently to compensate for the fall in Rd. Since a greater proportion of the meristem was reinvested in the apical dome, V also decreased (Table 5), but because Rp > V the apex enlarged (Fig. 11). In these plants at 13 °C, Rp decreased before flowering, and the enlargement of the apical dome was therefore entirely the result of the fall in V.

When the transition to flowering had been made, Rp decreased at 20 and 27 °C (Table 5) because Rd decreased about this time (Table 3) and the plastochron also decreased as the floral parts were initiated (Table 1), but since Rp < V the apical dome decreased in size. The same almost certainly happens at 13 °C but after initiation of the third and later sepals. It therefore seems that the apical dome enlarges up to the time that the apex is actually in the process of making the transition from vegetative to floral growth, and thereafter diminishes in size. The growth rate also seems to reach a maximum about the time of transition to flowering and may then decrease (Table 3).

**Processes involved in the transition to flowering in Silene**

The apical dome in Silene approximately doubled in volume before flower initiation. At 20 and 27 °C this increase was immediately before initiation of the sepals (Figs. 9, 10; about day 8) and at 13 °C it was a plastochron
At all three temperatures the apical dome reached a size of about $1.4-2 \times 10^6 \mu m^3$ at the time of flower initiation whereas the largest volume of the apical dome measured in non-induced plants was $< 10^6 \mu m^3$. These observations prompt several questions. First, what is the significance of the increase in size of the apical dome preceding flowering? Secondly, can the transition to flowering occur only when the apical dome has reached a certain absolute size? Thirdly, why does flowering not occur in short days, since the apex is enlarging gradually under these conditions? Because the change from vegetative to floral growth occurred only with the stimulus of long days, this poses a related question. What is the nature of the change in the growth of the apex which long days bring about? Let us now attempt to provide some answers.

The significance in the increase in the size of the apical dome on the transition to flowering in *Silene* may perhaps be found by examining the arrangement of primordia at the apex. In the vegetative apex the primordia are opposite and decussate. The first pair of sepal primordia are formed almost opposite each other but they are slightly displaced from the midline towards the axillary bud, which arises immediately below the flower. The next primordium, the third sepal, arises on the opposite side of the apex from the axillary bud and displaced to one side of the midline. This heralds the change from the opposite and decussate arrangement of the leaves to the spiral arrangement of the primordia of the flower. The sepals, stamens and carpels are initiated singly, in sequence, in positions which are predictable on the basis of Fibonacci phyllotaxis. The petals appear to be initiated as a whorl at the same time as the first few stamens are initiated. In *Silene*, therefore, the first change which is apparent after the apex enlarges is a change in phyllotaxis. This may be facilitated by the enlargement of the apical dome to the point where an extra primordium can be accommodated thus allowing the change to a new phyllotactic arrangement. This is normally followed by development of the floral parts into sepals, petals, stamens and carpels. However, an interesting condition has been observed in *Silene* plants growing in the garden, apparently mutants, in which this development does not occur. A small proportion of the plants (about 1%) formed inflorescences but, instead of forming flowers, initiated leaves in a spiral sequence with a characteristic Fibonacci phyllotaxis. After some weeks of growth like this and the initiation of many leaves, carpels, often aberrant, were formed. In these plants the transition to flowering had involved the change in phyllotaxis but without the subsequent differentiation of sepals, petals and stamens. These observations support the suggestion that the first stage in floral initiation is a change in phyllotaxis but that very soon afterwards other changes normally occur, perhaps...
involving changes in gene expression which result in the differentiation of the characteristic floral parts.

The observation that at each temperature the apical dome had reached a size greater than $10^6 \mu \text{m}^3$ at the time of transition (Figs. 9–11) suggests that perhaps the apex had to reach a certain absolute size for the change in phyllotaxis and the transition to flowering to occur. However, the axillary buds which continue the growth of the inflorescence also produce flowers and these are initiated when the apices of the axillary buds are very often only about half the size of the apex which forms the terminal flower. Clearly then absolute size itself is not the determinant. In these smaller flower buds, since they have a perfectly normal appearance, presumably the primordia are also smaller. This would suggest that it is the size of the apical dome relative to the size of the primordia and the axial tissue which is being formed which is important, rather than absolute size.

In short days, in which the plants remain vegetative indefinitely, the apical dome enlarged steadily (Figs. 6–8). Measurements were not carried out to see whether the apex continued to enlarge or whether it remained at a constant size, but smaller than that of apices of plants induced to flower. At 20 °C the maximum size of the apical dome measured in short days was not very much less than the size attained at the transition to flowering. Unless this was the maximum possible size for the apical dome in the whole of its vegetative growth, in a few more plastochrons it would have reached the same size as it was at transition in the induced plants. Again it seems more likely that it is not the absolute size reached by the apical dome that is related to flowering but the size of the apical dome in relation to some other parameter of apical growth (e.g. the size of the primordia at initiation), and that one effect of long days is to achieve an alteration in this relationship.

The original postulate was that the transition to flowering at 13 °C was achieved by a change in the relationship between the growth rate of the apical dome and the rate of leaf initiation and that this was brought about by a lengthening of the plastochron so that effectively the growth rate of the apical dome relative to the rate of leaf initiation would be increased. However more detailed analysis has shown that this is not so. At 13 °C the values for $R_p$ (Table 5) – which is an expression of this relationship – in fact decreased in long days, showing that the rate of growth of the apical dome decreased relative to the rate of leaf initiation. The apical dome increased in size at 13 °C only because a larger proportion of the meristem was used to form the new apical dome. It therefore seems that for transition to flowering the growth rate of the apical dome does not have to be increased relative to the rate of leaf initiation.

A consistent effect of long days was to increase the growth rate of the
Perhaps the effect of long days was to stimulate the growth of the apical dome more than the growth of the apical unit (consisting of the pair of primordia and their associated axial tissue formed at the beginning of the plastochron) thus shifting the emphasis of growth to the apical dome from the apical unit. Values for the volumes of the apical units were measured and plotted in the same way as were the volumes of the apical dome. Graphs similar to those of Figs. 6–11 were obtained, but with different slopes. The relative growth rates in volume per day per apical unit ($R_{ap}$) in short days and long days are given in Tables 6 and 7, respectively. In short days, and in long days before the transition to flowering, the relative growth rate of the apical unit was almost always less than the relative growth rate of the corresponding apical dome ($R_{day}$). This is most easily seen from the ratio $R_{ap}/R_{day}$ (Table 8). The increase in the ratio after flower initiation reflects the increased growth rate of the sepals and their associated axial tissue relative to the apical dome of the flower. At 20 and 27 °C plants in long days tended to have a higher value of the ratio than plants in short days. This shows that at these temperatures the effect of long days was not to shift the emphasis of growth to the apical dome—quite the contrary, it was shifted slightly towards the apical unit of primordia and axial tissue. The increase in growth rate of the apical dome just before flower initiation at 20 and 27 °C (Table 3) was also reflected in an increase in the growth rate of the apical unit at this time (Table 7). Indeed at 20 °C the increase in the growth rate of the apical unit appears to have preceded that in the apical dome by a plastochron. The stimulation of the growth of the primordia and axial tissue is consistent with the observation that the growth of leaves is stimulated on induction (Thomas, 1961a, c) and that the incorporation of precursors into RNA is stimulated in the leaves as well as in the apex itself (Arzee, Zilberstein & Gressel, 1975). Some of the growth of the apical unit in Silene would represent axillary bud growth, which is stimulated at nodes near the shoot apex on transition to flowering, as it is in other plants (Thomas, 1961b). At 13 °C, however, the picture is somewhat different. In long days, $R_{ap}$ falls to the point where the ratio $R_{ap}/R_{day}$ is less in long days than in short days (Table 8) showing that induction at this temperature does shift the growth rates in favour of the apical dome. Since the shift was in the opposite direction at 20 and 27 °C it cannot be correlated with the occurrence of flower initiation. However, it may be noted that the values for the ratio $R_{ap}/R_{day}$ before the transition to flowering were lowest in long days at 13 °C and highest in long days at 27 °C. This ratio appears to be proportional to the number of long days required for complete induction or for 50% induction (Fig. 2) so that the
Table 6. Relative growth rates per day of the volume of the nth apical unit ($R_{ap}$), consisting of the nth leaf pair and its associated axial tissue, for Silene plants in short days (non-inductive)

<table>
<thead>
<tr>
<th>$n$</th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.17</td>
<td>0.27</td>
<td>0.45</td>
</tr>
<tr>
<td>8</td>
<td>0.23$^a$</td>
<td>0.30</td>
<td>0.13$^a$</td>
</tr>
<tr>
<td>9</td>
<td>0.23</td>
<td>0.27</td>
<td>0.21</td>
</tr>
</tbody>
</table>

$^a$ Significantly different from value for previous plastochron ($P < 0.01$).

Table 7. Relative growth rates per day of the volume of the nth apical unit ($R_{ap}$), consisting of the nth leaf pair and its associated axial tissue, for Silene plants exposed to long days (inductive)

<table>
<thead>
<tr>
<th>$n$</th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.32</td>
<td>0.27</td>
<td>0.66</td>
</tr>
<tr>
<td>8</td>
<td>0.24$^a$</td>
<td>0.52$^b$</td>
<td>0.55$^a$</td>
</tr>
<tr>
<td>9</td>
<td>0.18</td>
<td>0.63</td>
<td>0.37$^b$</td>
</tr>
<tr>
<td>10</td>
<td>0.61</td>
<td>0.68</td>
<td>1.16$^b$</td>
</tr>
<tr>
<td>11</td>
<td>$\cdot$</td>
<td>$\cdot$</td>
<td>0.39</td>
</tr>
<tr>
<td>12</td>
<td>$\cdot$</td>
<td>$\cdot$</td>
<td>0.64</td>
</tr>
</tbody>
</table>

$^a$ Significantly different from value for previous plastochron ($P < 0.05$).

$b$ Significantly different from value for previous plastochron ($P < 0.01$).

The horizontal bars mark the transition to flowering.

Table 8. Ratio of $R_{ap}$ to $R_{day}$; for the nth apical unit ($R_{ap}$), and the apical dome of apices bearing n pairs of leaves + primordia ($R_{day}$) in Silene

<table>
<thead>
<tr>
<th>$n$</th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>LD</td>
<td>SD</td>
<td>LD</td>
</tr>
<tr>
<td>7</td>
<td>0.55</td>
<td>0.73</td>
<td>0.39</td>
</tr>
<tr>
<td>8</td>
<td>0.67</td>
<td>0.53</td>
<td>0.44</td>
</tr>
<tr>
<td>9</td>
<td>0.84</td>
<td>0.35</td>
<td>0.39</td>
</tr>
<tr>
<td>10</td>
<td>$\cdot$</td>
<td>0.76</td>
<td>$\cdot$</td>
</tr>
<tr>
<td>11</td>
<td>$\cdot$</td>
<td>$\cdot$</td>
<td>$\cdot$</td>
</tr>
<tr>
<td>12</td>
<td>$\cdot$</td>
<td>$\cdot$</td>
<td>$\cdot$</td>
</tr>
</tbody>
</table>

The horizontal bars mark the transition to flowering.

SD = short day; LD = long day.
lower the ratio the more easily did induction occur. This suggests that there may indeed be some connection between the difference in the relative growth rates of the apical dome ($R_{\text{day}}$) and the primordia and axial tissue immediately below it ($R_{a_p}$) and the transition to flowering, but exactly what this connection is, is not clear from the present data.

In the present analysis the sizes of the primordia themselves have not been measured at the moment of initiation. If the relationship between the size of the primordia and the size of the axial part of the apical unit changes at the beginning of successive plastochrons then it is quite possible that the primordia become smaller at initiation, relative to the apical dome, and that the transition to flowering is associated with a change in this particular relationship. That this is indeed the case is suggested by the change in phyllotaxis which occurs on the initiation of the sepals in *Silene*, and which implies a change in the ratio of the size of the apical dome to the size of the primordia on initiation (Richards, 1951). If the size of the primordia at initiation remains constant, and the absolute distance between primordia also remains constant then an enlargement of the apical dome will lead to a change to a higher order of phyllotaxis, which is what occurs in *Silene*. The present measurements were designed to investigate the changes in the size of the apical dome. It is clear that a further set of measurements is needed to investigate the size of the primordia at initiation in relation to the size of the apical dome during the transition to flowering. This could well throw light on what is happening at the apex as a result of photoperiodic induction.

**GROWTH OF THE APICAL DOME IN OTHER PLANTS**

The relative growth rate of the apical dome, the size of the apical dome, the plastochron, and the proportion of the apical dome invested in the primordia or the new apical dome each plastochron are known for the vegetative growth of a few plants up to the transition to flowering.

A detailed set of measurements is available for maize (*Zea mays*) (Abbe, Phinney & Baer, 1951). During vegetative growth $R_{\text{day}}$ increased but the plastochron shortened so that $R_p$ remained constant. The constant difference in the logarithms of the values for the maximum and minimum sizes of the apical dome at the moment of leaf initiation shows that $V$ also remained constant. The increase in size of the apical dome from plastochron to plastochron occurred because $R_p > V$ (which is clear from Fig. 3 of Abbe *et al.*). About three plastochrons before differentiation of the tassel $R_{\text{day}}$ doubled but there was no sudden increase in the size of the apical
dome because the proportion of the meristem reinvested in the new apical dome apparently decreased (and \( V \) increased), as it did in *Silene* at 27 °C in long days (Fig. 10, Table 5, plastochron 10). In maize the apex was therefore not in a steady state during vegetative growth (\( R_{\text{day}} \) and the plastochron were both changing, only \( V \) was constant) but flowering was preceded by a rapid increase in the relative growth rate of the apical dome, as it was in *Silene* at 20 and 27 °C.

In another two plants, tobacco (*Nicotiana tabacum*) and flax (*Linum usitatissimum*), the proportion of the meristem reinvested in the apical dome was not measured and so no direct estimate of \( V \) can be obtained. However, the area ratio was measured in these plants (Williams, 1975). The area ratio is the ratio of the mean area of the apical dome to the area of the primordium on initiation (Richards, 1951). The concepts of \( V \) and the area ratio are obviously related, \( V \) being proportional to the reciprocal of the area ratio.

In tobacco, \( R_{\text{day}} \) fell during growth as shown by the fall in plastochron ratio (Williams, 1975). The plastochron remained constant until after the transition to flowering, when it shortened. \( R_p \) therefore decreased throughout. The area ratio increased, suggesting that the primordia probably remained about the same size on initiation, as the apex enlarged from plastochron to plastochron, and \( V \) probably decreased. If there were changes restricted to one or two plastochrons just before the transition to flowering this analysis did not reveal them.

In flax, \( R_{\text{day}} \) was apparently constant during vegetative growth (Williams, 1975). The plastochron decreased and so \( R_p \) decreased. The area ratio increased, suggesting that \( V \) probably decreased. The 20-fold enlargement of the apical dome indicates that \( R_p > V \) although both were decreasing, as in tobacco. The increase in the area ratio resulted entirely from the increased size of the apical dome, since the actual area of a primordium on initiation did not change. Measurements stopped before flowering.

A change to a higher order of phyllotaxis, which was measured in tobacco and flax, may well be universal during the transition to flowering. Even in those plants (such as flax) which have a spiral leaf arrangement and in grasses, in which the distichous arrangement of primordia may be continued into the flowers (Sharman, 1947), a change to a higher order of phyllotaxis may occur without there necessarily being a change in the divergence angle between successive primordia.

Enlargement of the apical dome with age or as a prelude to flowering occurs in many, if not all, plants. The data for maize, tobacco, flax and *Silene*, summarised in Table 9, show that this enlargement can occur with
Table 9. Growth of the apical dome during vegetative growth and just before the transition to flowering. Parameters are shown as increasing (+), decreasing (−) or constant (0).

<table>
<thead>
<tr>
<th>Vegetative</th>
<th>$R_{\text{day}}$</th>
<th>Plastochron</th>
<th>$R_p$</th>
<th>$V$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize (Zea mays)</td>
<td>+</td>
<td>−</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tobacco (Nicotiana tabacum)</td>
<td>−</td>
<td>0</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Flax (Linum usitatissimum)</td>
<td>0</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Silene 20 °C (short days)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Just before transition to flowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize (Z. mays)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Silene 20 °C (long days)</td>
<td>+</td>
<td>−</td>
<td>+$^b$</td>
<td>−$^b$</td>
</tr>
</tbody>
</table>

$^a$ For definition of $R_{\text{day}}$ etc. see text.

$^b$ This increase and decrease were small.

either $R_{\text{day}}$, the plastochron, or $V$, or all three being constant, or all three changing simultaneously. Changes in the mean size of the apical dome could therefore result from changes in any or all of these parameters. The processes responsible for enlargement of the apical dome in any plant must therefore first be determined before the effect of environmental or experimental variables can be properly understood.

It is of interest that only in the plants growing in non-inductive conditions and not progressing towards flowering (Silene) was the apex growing in a balanced state, with all three parameters constant. This suggests the possibility that at least one difference between photoperiodically sensitive plants and day-neutral plants is that in the former the apex may grow in a balanced state under non-inductive conditions and that a stimulus is required to remove this balanced state. The implication is that plants in which the apex is not in a balanced state during vegetative growth may be able to progress to flowering without any further stimulus. This leaves open the question of whether a flowering hormone is required to bring about a change in the relationships of the various growth parameters at the apex, these changes resulting in flowering, as implied by Evans (1971), or whether the changes in the growth parameters bring about a change in the relationships between the components of the apical system that allows the flowering hormone to act and result in the differentiation of flowers. The fact that changes in the growth pattern of the apex at the transition to flowering could be brought about by changes in any one or more of several processes perhaps indicates why flowering may be induced in so many ways (Evans, 1969). One of the most important requirements may well be the need to release the apical dome from the influence of the leaf primordia and any morphogens they may produce, before the initiation of the flowers can occur.
CONTROL OF APICAL GROWTH

So far the growth of the apical dome has been considered as the result of the interaction between the relative growth rate, the length of the plastochron, and the proportion of the meristem that is used to form the new apical dome. An obvious question is how the magnitude of these parameters is controlled. There are published data and observations of the effects of various experimental treatments on the size of the apical dome, but since this is the change in mean size from plastochron to plastochron it depends on the magnitude of the difference between $R_p$ and $V$. The mean size of the apical dome of *Agropyron repens* increases with increasing nitrogen level and decreasing temperature and can apparently be regulated by the nutrition of the plant (Rogan & Smith, 1975a, b). More specifically the apical dome may compete with the young leaves for nutrients and metabolites. Removal of young leaf primordia in the tomato (*Lycopersicon esculentum*) increased the growth rate of the apical dome and the rate at which it increased in mean size, and hastened flowering (Hussey, 1963).

It is not known what the chemical nature is of the substances which can control apical growth in this way in intact plants. In excised apices the growth rate may be controlled by sugar concentration (Ball & Soma, 1965) or growth substances (Hussey, 1971). The length of the plastochron may also be affected by the nutritional status of the plant (Rogan & Smith, 1975b) or by defoliation, which presumably alters the nutritional status of the apex (Fulford, 1965). We do not know what factors may affect the proportion of the meristem used to form the apical dome, but it seems that the size of a primordium on initiation may be altered by disturbing the auxin balance of the apex. In *Chrysanthemum* the phyllotaxis could be altered from spiral to distichous by the action of the auxin antagonist, triiodobenzoic acid (Schwabe, 1971). The area ratio decreased (i.e. relative to the apical dome the primordia on initiation were larger) in the treated plants with distichous phyllotaxis. This coincided with a lengthening of the plastochron but there was no change in the relative growth rate of the apical dome.

There is, then, some evidence that the growth rate of the apical dome and the length of the plastochron may be altered by nutritional factors such as carbohydrate supply, and that the size of a primordium on initiation and the plastochron may be affected by alterations in the auxin metabolism of the apex. Since the rate of growth of the apical dome, the length of the plastochron, the proportion of the meristem forming the apical dome, and the size of a primordium on initiation are all closely inter-related, it may not always be possible to single out any one parameter as being the one...
primarily affected by changes in the nutrition or environment of the plant. But it may be helpful to consider the possibility that changes in the growth pattern of the apex may be brought about by different factors, such as carbohydrate level and auxin level, each affecting primarily only one of the growth processes occurring at the apex.

So far almost no work has been done in trying to understand the behaviour of the apex in terms of the interacting processes within it which determine its growth. A start has been made in this direction with experiments, on the effect of indole acetic acid (IAA) on the growth of the apex during and after induction, that showed that there were differential effects on the growth of the apical dome and the growth of the axillary buds, which altered according to when the IAA was applied (Seidlová & Khatoon, 1976). We also need to know how the levels of metabolites such as sugars and growth substances, which are known to affect apical growth, may alter in the shoot apex as a result of changes in photoperiod or nutritional status of the plant which alter the growth pattern of the apex. To do this will require the development of techniques for measuring the levels of such metabolites in minute pieces of tissue or even in single cells. Only by such an approach, by measuring the effects of experimental treatments on the different components of the apex and the interacting processes which control their growth, will a proper understanding be achieved of the growth of the shoot apex and the way in which it is switched from vegetative growth to flowering.

I am grateful to Ken Parker for his expert technical assistance and to the Agricultural Research Council who supported this work through Grant AG 15/105.

REFERENCES


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Changes in RNA Levels in the Shoot Apex of *Silene* during the Transition to Flowering

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Department of Botany, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH, U.K.

Abstract. Changes in RNA concentration in the shoot apical meristem during induction and the transition to flowering were measured histochemically in *Silene coeli-rosa* (L.) Godron, a long-day plant. In the apices of plants induced by 7 long days the RNA concentration increased to about 25 per cent higher than in non-induced plants. Three long days did not induce flowering but resulted in a transient rise in RNA concentration. When plants were given long days interrupted by varying numbers of short days successful induction was accompanied by a sustained increase in RNA concentration but those treatments which were not inductive gave only transient increases in RNA. Gibberellic acid had no effect on induction or apical growth rates but increased the RNA concentration by 50 per cent or more in both induced and non-induced plants. Plants induced to flower at 13°C had the same RNA concentration and growth rate at the apex as in non-induced plants at 20°C. Since changes in RNA concentration in the apex could occur without changes in growth rate and without flowering, and induction could occur without a change in RNA concentration or growth rate, it is suggested that the increase in RNA and growth rate which normally occur at the transition to flowering might not be essential for the formation of a flower but may be more closely related to the rapid growth associated with the formation of the inflorescence.

Key words: Floral induction — Gibberellic acid — RNA — Shoot meristem — *Silene*.

Introduction

When a plant is induced to flower the first observable change at the shoot apex during the process of evocation (Evans, 1969) is an increase in the rate of synthesis of RNA and this is followed by an increase in the RNA content of the cells of the apical meristem (Bernier, 1971). The increase in the rate of RNA synthesis occurs in *Lolium* (Evans et al., 1970) and *Sinapis* (Bronchart et al., 1970) at about the time that the floral stimulus is presumed to arrive at the apex, or even slightly earlier in *Pharbitis* (Gressel et al., 1970). A rise in mitotic index also occurs at this time or a few hours later (Bernier, 1971) and is associated with an increased degree of synchrony in cell division (Bernier et al., 1967) but whether or not this signifies a permanent increase in division rate is not clear. The increased rate of cell division which has been measured in floral apices of *Datura* (Corson, 1969) and *Sinapis* (Bodson, 1975) may not begin until the second rise in mitotic index, which occurs later in the process of evocation (Bernier, 1971).

These events which occur at the shoot apex as a result of floral induction have usually been observed in plants in which the apex forms the whole inflorescence axis before it transforms into a flower itself. On the transition to flowering in such plants the apex continues forming primordia on its flanks but these develop into bracts instead of leaves and the axillary primordia grow out either into flowers or flowering shoots. The apex may continue growing for quite some time in this way before it finally transforms into a terminal flower. Many of the events occurring at the shoot apex as an immediate result of induction could therefore be concerned with the increased growth of the inflorescence axis and its branches rather than with the initiation of the flowers themselves, a process in which the primary apex may not be immediately involved.

The events at the shoot apex on transition to flowering were therefore examined in *Silene*, a long-day plant in which the inflorescence is a cyme and so the apex transforms directly into a flower, which is
the first to develop, and the growth of the inflorescence is continued by the growth of axillary buds below the terminal flower. The rates of growth and cell division did not alter until several days after induction had been completed and the apex had become committed to flowering. The rate of cell division increased only when the flower itself was beginning to be formed (Miller and Lyndon, 1975, 1976).

In the experiments to be described the RNA content was measured in the shoot apical meristems of *Silene* plants during and after induction so that the relationships between changes in RNA content and changes in growth rate could be examined. In order to try and separate the events associated with increased growth rate of the apex from those associated with floral induction the plants were exposed to various experimental treatments which either altered the growth rate or the RNA content of the apex without affecting induction or else prevented induction but still resulted in changes in RNA content.

**Materials and Methods**

The plants used were *Silene coeli-rosa* (L.) Godron, a qualitative long-day species when grown at 20°C in controlled environments as previously described (Miller and Lyndon, 1976). All plants were maintained in short-day (SD) conditions (cycles of 8 h light, 16 h dark) until 28 days after sowing, when they were selected for developmental uniformity. Since all experimental treatments began on this 28th day after sowing it will be convenient to refer to it as day 0 when describing the experiments. Long-day (LD) conditions were the same as SD except for low intensity tungsten light during the period corresponding to darkness in the SD regime. Plants transferred to continuous LD were maintained in LD. Plants exposed to 7 LD (7LD plants) or 3 LD (3LD plants) were returned to SD. Other plants were maintained in SD throughout the experiments. These experimental procedures have been described more fully elsewhere (Miller and Lyndon, 1976). Plants which were subjected to lower temperature (13°C) were transferred from 20°C SD conditions to SD or LD at 13°C on the 28th day after sowing (day 0) and were maintained at this temperature of 13°C for the rest of the experiment. Plants transferred back from LD at 13°C to SD remained at 13°C.

Gibberellic acid was applied to the apical bud as a drop of aqueous solution (about 0.05 ml) at a concentration of 0.75 mg ml⁻¹. Applications were made at the end of the high intensity light period on each of seven successive days.

Samples of plants were taken at daily intervals throughout the experiments, in all cases 1 h after the beginning of the high intensity light period. The samples were fixed in ethanol:acetic acid (3:1) for at least 2 h, then dehydrated in an alcohol series and embedded in wax. Longitudinal serial sections at a thickness of 10 μm were cut in the plane of the youngest pair of leaf primordia (the leaves are in opposite decussate pairs). The sections were stained with gallocyanin-chrome alum, which quantitatively stains cell nuclei (Mitchell, 1967). Since measurements showed no change in mean cell size (Miller and Lyndon, 1976) and assuming the rate of cell division did not alter until several days after induction, the growth rate or the RNA content of the apex could be followed by measuring the changes in apical volumes by the methods already described (Miller and Lyndon, 1976).

**Results**

**Effects of 3LD and 7LD on RNA Concentration**

All plants which were maintained in SD, and 98% of those given 3LD, did not flower, but all those given 7LD were induced and subsequently flowered when returned to SD. The growth rate of apices of plants in SD or given 3LD remained unchanged but in plants given 7LD the growth rate doubled about day 7 or 8 when flower formation was just beginning (Miller and Lyndon, 1976).

For the first 3 days after the start of the treatments the RNA concentration in the apices of 3LD and 7LD plants was the same as in plants in SD (Fig. 2, days 0 to 3). From day 4 onwards the RNA concentration in the apices of the 7LD plants was higher than in comparable SD plants and remained so for
Table 1. Effect of long-day treatments, interrupted by short days, on flowering

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Floral</td>
</tr>
<tr>
<td>3LD/2SD/3LD</td>
<td>18</td>
</tr>
<tr>
<td>3LD/4SD/3LD</td>
<td>18</td>
</tr>
<tr>
<td>4SD/6LD</td>
<td>19</td>
</tr>
<tr>
<td>3LD/6SD/3LD</td>
<td>3</td>
</tr>
<tr>
<td>9SD/3LD</td>
<td>2</td>
</tr>
</tbody>
</table>

The plants were scored when flower buds in the induced plants were about 5 mm long.

The relationship between the rise in RNA concentration in the apices increased on days 4 and 5, as it did in the 7LD plants, but on day 6 it had fallen and by day 7 it had once more reached the value for plants in SD (Fig. 2). This experiment was repeated, with very similar results. The sustained rise in RNA concentration in the apices of the 7LD plants was followed by an increase of growth rate and by flowering but the ephemeral rise in the 3LD plants was followed by neither.

The changes in the RNA concentration in the apex in the course of these experimental treatments are shown in Figure 3. With 3LD the RNA concentration increased to a maximum on day 6 and then fell to the original level. This confirms the effect of 3LD shown in Figure 2. With two periods of 3LD separated by 6SD (3LD/6SD/3LD), the RNA concentration increased transiently for a second time on days 12 and 13 but then fell once more. Thus in both these treatments, which did not induce most plants, the increase in RNA was ephemeral. Where the two periods of 3LD were separated by only 2SD, which resulted in most plants being induced, the increase in RNA was maintained, as it was with 7LD (Fig. 2). With 3LD/4SD/3LD a transient increase in RNA concentration was followed by a sustained increase on days 11 to 13. Flower initiation was delayed by about 3 days (3LD/2SD/3LD) or 6–7 days (3LD/4SD/3LD) compared to plants given 7LD. In these experiments successful floral induction was associated with a sustained increase in the concentration of RNA, but not with an ephemeral increase.

Treatment with Gibberelic Acid

In spite of numerous application regimes, on no occasion did gibberelic acid result in flowering. The application regime finally adopted was one which gave in plants in SD a similar degree of internode elongation to that resulting from 7LD treatment in the absence of gibberelic acid (Table 2). In the control plants not treated with gibberelic acid, from day 8 onwards the growth rate of the apex of 7LD plants
Table 2. Effect of short days, 7 long days and gibberellic acid on internode length

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3rd internode</th>
<th>4th internode</th>
<th>5th internode</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>3.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SD+GA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>11.0</td>
<td>16.6</td>
<td>1.7</td>
</tr>
<tr>
<td>7LD</td>
<td>11.3</td>
<td>13.7</td>
<td>-</td>
</tr>
<tr>
<td>7LD+GA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>17.0</td>
<td>19.7</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 3. Growth rate of the apex in the presence and absence of gibberellic acid

<table>
<thead>
<tr>
<th>Days after start of long day treatment</th>
<th>Mean apical volume (10&lt;sup&gt;5&lt;/sup&gt; μm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Above leaf pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>21.3, 14.4, 12.1, 13.7, 6</td>
<td></td>
</tr>
<tr>
<td>SD+GA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>32.1, 31.0, 27.8, 32.2, 6</td>
<td></td>
</tr>
<tr>
<td>7LD</td>
<td>3.6, 4.9, 12.4, 13.7, 8</td>
<td></td>
</tr>
<tr>
<td>7LD+GA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>8.1, 19.1, 38.8, 41.8, 8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11.4, 11.5, 79.4, 67.8, 9</td>
<td></td>
</tr>
</tbody>
</table>

had increased dramatically compared to the plants in SD (Table 3), confirming similar previous experiments (Miller and Lyndon, 1976). Treatment of plants in SD, and 7LD plants, with gibberellic acid had no apparent effect on the growth rates of the apex compared to the untreated plants (Table 3).

Application of gibberellic acid did however have a marked effect on the RNA concentration in the apices of plants in SD and in plants given 7LD (Fig. 4). In both cases the gibberellic acid applications resulted in a rapid increase in RNA concentration which was much greater in the plants exposed to 7LD. Although the RNA concentration in plants in SD and in 7LD plants fell somewhat over the course of the experiment, even in the plants in SD it remained at least as high as in any untreated plants. The gibberellic acid treatment therefore resulted in sustained high concentrations of RNA which were not associated with increases in the growth rates of the apices and were not followed by flower initiation unless the plants were also exposed to LD.

Growth at 13°C

When plants were grown at 13°C during the exposure to 7LD and held at 13°C when subsequently returned to SD, all plants in a sample of 25 flowered. Although induction was apparently unaffected at 13°C the growth rate of the apical meristem (Table 4) and the RNA concentration in the apex (Fig. 5) of plants given 7LD at 13°C did not increase appreciably above the values for plants maintained in SD at 20°C. Floral induction at 13°C was therefore associated with growth rates and RNA concentrations in the apex which were very similar to those of plants in SD at 20°C, which remained vegetative. Even though flower initiation was delayed and the first stage in flower formation, the enlargement of the apex, did not occur until day 12 at 13°C, compared with day 8 in 7LD plants at 20°C, there was still no evidence of an increased growth rate of the apices by day 13 (Table 4). Although there was no absolute increase
in the growth rate or the RNA concentration in the apex of plants induced by 7LD at 13°C there was an increase relative to plants in SD at 13°C because of the drop in growth and RNA concentration in the latter (Table 4; Fig. 5). At 13°C (as at 20°C) induction by 7LD was therefore associated with a higher growth rate and a higher RNA concentration in the apex than in plants in SD.

Discussion

The transient rise in RNA as a result of 3LD and the sustained increase in RNA concentration in plants treated with gibberellic acid in SD show that the RNA concentration in the apex can be increased, and even maintained at levels comparable to those in induced plants, without an increase in growth rate and without flower initiation. Conversely, from the experiment in which flowering was induced at 13°C it can be argued that successful induction and flowering does not depend on an absolute increase in either the RNA concentration or the growth rate in the apex. Although an increase in the RNA concentration in the apex did not necessarily lead to an increase in the growth rate or to flower initiation in no case was there an increase in the growth rate of the apex which was not preceded by an increase in RNA concentration, and when the growth rate decreased, as it did in plants in SD at 13°C (Table 4), the RNA concentration in the apex also decreased somewhat (Fig. 5). A sustained increase in the rate of RNA synthesis may be necessary to allow an increased growth rate but neither of these may be essential for flowering. A similar conclusion may be drawn from experiments with Chenopodium in which an increase in the concentration of RNA in the apex was prevented by the application of 6-azauridine whereas flowering was stimulated (Seidlová and Krekule, 1973).

An increase in RNA and in growth rate in the apex is not peculiar to floral induction since they may also occur in vegetative shoot apices. When axillary apices of Cicer were stimulated into (vegetative) growth by the application of a cytokinin the rate of incorporation of uridine into RNA increased within 90 min and this was accompanied by an increase in growth rate of the buds within 2 h and a measurable increase in RNA concentration in the cells after 6 h (Usciati et al., 1972). These changes are remarkably similar to those occurring as a result of floral induction and lend support to the view that at least some of the changes which have been observed in some shoot apices on transition to flowering (Bernier, 1971) may not be specifically concerned with the formation of flowers instead of leafy shoots, but may be a reflection of the increased growth which is normally associated with the formation of the flower and the inflorescence.

There may also be changes in the shoot apex which are a direct result of the treatments used to induce flowering but which are themselves incidental to flowering. The increase in the rate of RNA synthesis in Pharbitis which occurs before the floral stimulus is presumed to reach the apex (Gressel et al., 1970) and which occurs in the young leaves as well as in the apex (Arzee et al., 1975) could either be a first step in evocation or could possibly be an independent event resulting from the short-day treatment. Another event is the initial rise in mitotic index which, in Sinapis, indicates an increased synchrony of cell division but which can still occur in plants which are not induced (Bernier et al., 1970). A possible similar event was observed in Silene, in that there were indications that the growth rate of the apex might have been stimulated transiently by only one long day (Miller and Lyndon, 1976). A transient increase in the rate of cell division, which also occurs as a result of inductive treatments (Bernier, 1971) might therefore be either an event of evocation or possibly in some plants a direct result of exposure to long or short days.

The experimental findings discussed in the present paper all point to the possibility that these and perhaps other events, such as changes in amounts of starch (Bernier, 1971), which are observed in the shoot apex as a result of an inductive treatment may not necessarily be directly concerned with the subsequent transformation of a meristem into a flower, but may perhaps be more related either to direct effects of changes in photoperiod or to the changes in growth rates in the apex which usually, but not always, accompany flowering.
We are grateful to the Agricultural Research Council for the award of grant AG 15/81 in support of this work, during which M.B.M. held a Research Studentship.

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Early Effects of Floral Induction on Cell Division in the Shoot Apex of Silene

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Abstract. The changes in cell number, the relative proportions of interphase nuclei with different amounts of DNA, mitotic index and labelling index have been investigated in the shoot apex of Silene coeli-rosa L. (a long-day plant) during the first long day of photoinduction, and compared with the corresponding changes in plants in short days. 3 h after the start of induction the proportion of nuclei in the G2 phase of the cell cycle had increased, the mitotic index tended to be higher, and the labelling index was lower than in plants in short days. 8-9 h later the values for plants in the long day had become similar to those for plants in short days. No evidence was obtained for a synchronisation of cells in one phase of the cell cycle as a result of photoinduction. The results obtained were consistent with a temporary shortening of the cell cycle in the induced apices over the first long day which resulted in a greater increase in cell number by the end of the first day of photoinduction than in plants in short days.

Key words: Cell cycle — Cell division — DNA — Photoinduction — Silene.

Introduction

One of the first events of evocation in the shoot apex as a result of induction is an increase in mitotic index which has been observed in several plants (Bernier, 1971). This increase in mitotic activity has been particularly studied in Sinapis, a long-day plant in which the mitotic index in the induced plants reached a value about five times higher than that in the non-induced controls in short days. The increase in mitotic index reached its maximum within 16 h of the beginning of induction (Bernier et al., 1967) and before the increase in the proportion of cells synthesising DNA, indicating that there was a sudden release of meristematic cells from G2 into mitosis (Kinet et al., 1967). These findings suggested that there was a synchronisation of the cells in the cell cycle and direct evidence of this was obtained by Jacqumard and Miksche (1971) who showed that 30 h after the beginning of induction the cells in the Sinapis apex were predominantly in the G1 phase of the cell cycle compared with the controls in which cells were found in both G1 and G2. These findings were all consistent with the hypothesis that in the apex of Sinapis the cells became synchronised in the G1 phase of the cell cycle by 30 h after the beginning of induction.

In plants of Silene coeli-rosa which were subjected to one or more LD the cell number after the first LD was 50% higher than in comparable plants in SD. The subsequent rate of growth, during the period of induction and until floral initiation, was essentially the same in both induced plants in long days and in non-induced plants in short days (Miller and Lyndon, 1976). An increase in cell number at the beginning of induction is what might be expected if there were a synchronisation of cells in the apex which occurred at the beginning of the inductive period. However, were synchronisation to occur in Silene its significance to flower initiation is not so obvious as in Sinapis. In Sinapis floral initiation occurs about 60 h after the beginning of induction (Bernier et al., 1967) which is about one cell cycle after the synchronisation observed 30 h from the beginning of induction (Bodson, 1975). In Silene the period between the first long day and floral initiation is approximately 8 days (Miller and Lyndon, 1976) and since the cell cycle is of the order of 20 h in induced and non-induced plants this means there would be approximately 8 or more cell cycles between the presumed synchronisation and the initiation of the flowers (Miller and Lyndon, 1975). The question then arises, if

Abbreviations: LD = long day; SD = short day
there is synchronisation in Silene does it occur only on the first day, or is it maintained until flower initiation takes place? If it is not maintained then what, if any, is its significance to the flowering event?

The purpose of the present experiments was therefore to examine the apex of Silene during the first long day, during which time it is known that the cell number increases (Miller and Lyndon, 1976) and to see whether there is any evidence for synchronisation of cell division during this period. This can be sought by looking for an increase in mitotic index but this might be sufficiently transient to be easily missed except with very close sampling procedures. If synchronisation occurs it should be more easily observed by microdensitometric measurements of the proportions of cells with different DNA contents, or by measuring the labelling index, which would indicate the proportion of the cells engaged in DNA synthesis at the time of labelling.

**Materials and Methods**

**Conditions of Growth**

The plant used was Silene coeli-rosa L. Godron. The seeds were sown and grown under those conditions used by Miller and Lyndon (1975, 1976). Plants were selected when they were 28 days old using the method described previously and either kept under SD or transferred to LD conditions (Miller and Lyndon, 1976). Three seedlings were fixed in either 80% (v/v) ethanol or 3:1 absolute ethanol-glacial acetic acid (v/v) at intervals over the 24 h of the long day. The LD conditions were the same but with the addition of low intensity tungsten light from 1700 h on day 0 to 0900 h on day 1 (Miller and Lyndon, 1975, 1976).

**Preparation of Sections and Squashes**

Median longitudinal sections, 10 µm in thickness, were cut from plant material fixed in 80% ethanol to make measurement of individual nuclei easier (Lyndon, 1973) and embedded in wax. The sections were hydrolysed in 5 N HCl at 25°C (25 min) (Fox, 1969), stained with Feulgen for 2 h at 25°C (Francis and MacLeod, 1977) and mounted in Canada balsam.

Squash preparations were made from apices of plants fixed in 3:1 absolute ethanol-glacial acetic acid. The six oldest pairs of leaves were carefully cut off under a dissecting microscope, leaving the 7th pair of leaf primordia attached to the exposed apex. The apices were then hydrolysed and stained with Feulgen as detailed above. The apical dome was then carefully removed and a permanent squash preparation made.

**Cell Number**

The number of cells above the 7th leaf pair was determined as already described (Miller and Lyndon, 1976) in plants which were fixed at 1200 h on the 28th day of growth and 24 h later after exposure to either a LD or a SD.

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**DNA Measurements**

The amount of DNA per interphase nucleus was measured using a Vickers M85 Scanning Microdensitometer at a wavelength of 560 nm. The absorbances were measured of all measurable intact interphase nuclei in the apical dome, above the 7th pair of leaf primordia in all sections of each of three apices for each treatment at each sampling time. Care was taken to ensure that nuclei were not overlapping other nuclei. In the squashes, the first 50 interphase nuclei encountered in a series of transects across the width of the coverslip were measured. The DNA values 2C and 4C were assigned to the mean absorbance values for 10 half-aphase and 10 prophase nuclei respectively for each slide. This provided a scale which enabled the C value for any interphase nucleus on this slide to be determined.

Of the interphase nuclei measured, the fractions in the G1 and G2 phases of the cell cycle are those with the 2C and 4C amounts of DNA respectively, minus those nuclei in the S phase of the cycle. Since the number of nuclei in S could not be accurately determined by microdensitometry alone these were partitioned equally to the 2C and 4C populations by calculating the percentage of cells with <3C and >3C values. In this paper these are called G1 and G2 populations respectively. Any differences in the ratios of G2/G1 therefore tend to be underestimates.

**Mitotic Index**

At each fixation time mitotic index (the sum of prophase, metaphase, anaphase and telophase figures expressed as a percentage of all cells) was measured above the 7th leaf pair in all of the sections, and in a series of transects across the width of the coverslip in the squashes. In the latter, all nuclei in small apices, and 500 nuclei per slide in larger apices, and 3 slides per fixation time were scored.

**Labelling Index**

The labelling index (the percentage frequency of cells labelled with radioactive thymidine) was measured in squashes of apices which were made for other purposes and which were doubly labelled with [3H]- and [14C]thymidine (Francis and Lyndon, unpublished).

Plants were partially defoliated as described by Miller and Lyndon (1975). Tritiated-[methyl-3H]thymidine was applied to each apex as an aqueous drop (c. 0.05 ml) containing 5 µCi (S.A. 15,000-30,000 mCi/mmol) which was left on the apex for 2 h. The apices were then fixed in 3:1 (v/v) ethanol:acetate acid. The samples treated at 1700 h were given [methyl-14C]thymidine (0.2 µCi in 0.05 ml; S.A. >50 mCi/mmol) instead of 3H-thymidine. Labelling index was taken as the percentage of labelled nuclei (14C at 1700 h and 3H thereafter) in all the nuclei in small apices and in larger apices the first 500 nuclei encountered in a series of transects across each slide, 3 slides per fixation time being scored.

**Results**

**Cell Number**

After exposure to 1 LD the cell number in the apical dome, above the 7th leaf pair, was almost three times that of comparable plants kept in SD (Table 1). This compares with the 50% greater cell number after 1 LD.
Table 1. Mean cell number (± standard error) in the apical dome, above the 7th leaf pair, on day 0 at 1200 h (SD), and on day 1 at 1200 h in both SD and LD conditions

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>SD</td>
<td>213±112</td>
</tr>
<tr>
<td>1</td>
<td>SD</td>
<td>563±189</td>
</tr>
<tr>
<td></td>
<td>LD</td>
<td>1542±714</td>
</tr>
</tbody>
</table>

Table 2. The proportions of interphase nuclei with a DNA content of < 3C (G1) and > 3C (G2), and the ratio of G2 to G1 nuclei, in the sectioned apical domes above the 7th leaf pair, during the first 24 h, in SD and LD conditions. (Data from Fig. I)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>G1</th>
<th>G2</th>
<th>G2/G1</th>
<th>G1</th>
<th>G2</th>
<th>G2/G1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G2/G1</td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Day 0</td>
<td>1200</td>
<td>0.51</td>
<td>0.49</td>
<td>0.96</td>
<td>0.32</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>0.49</td>
<td>0.51</td>
<td>1.04</td>
<td>0.34</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.41</td>
<td>0.59</td>
<td>1.44</td>
<td>0.38</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>0.50</td>
<td>0.50</td>
<td>1.00</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>Day 1</td>
<td>0400</td>
<td>0.55</td>
<td>0.45</td>
<td>0.90</td>
<td>0.53</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>0800</td>
<td>0.58</td>
<td>0.42</td>
<td>0.72</td>
<td>0.53</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>0.53</td>
<td>0.47</td>
<td>0.89</td>
<td>0.53</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Proportions of Cells in G1 and G2

Measurements on sections showed that in SD there were roughly equal proportions of cells in G1 and G2 (as specially defined—see Methods) throughout the experimental period (Fig. 1, Table 2). Induction was begun at 1700 h for the plants put into LD and by 2000 h there was already an increase in the proportion of cells in G2 compared with the plants in SD (Fig. 1, Table 2). This was despite the fact that the control series in SD at 2000 h showed a higher proportion of cells in G2 than any other SD sample. The increased proportion of cells in G2 in the LD was maintained until 0400 h. By 0800 h and at 1200 h of the next day the proportions of cells in G1 and G2 were essentially the same as in the SD controls (Fig. 1, Table 2).

This experiment was repeated using squash preparations which obviated the difficulties of measuring nuclei in sections. In the SD controls throughout the experiment there tended to be a lower proportion of cells in G2 than in G1 (Fig. 2, Table 3). In the plants in the LD, by 2000 h the proportion of cells in G2 had increased and this increase was maintained until 2400 h (Fig. 2, Table 3) after which it decreased to a value similar to that in the SD controls (Fig. 2, Table 3).

These experiments are consistent in showing an increase in the proportion of cells in G2 as a result of exposure of plants to LD conditions. This difference was maintained in both experiments for 4 to
Table 3. The proportion of interphase nuclei with a DNA content of <3C (G1) and >3C (G2), and the ratio of G2 to G1 nuclei, in the squashed apical domes above the 7th leaf pair, during the first 24 h, in SD and LD conditions. (Data from Fig. 2)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>SD</th>
<th>LD</th>
<th>G1</th>
<th>G2</th>
<th>G2/G1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1200</td>
<td>0.63</td>
<td>0.37</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>0.49</td>
<td>0.55</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.55</td>
<td>0.45</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>0.61</td>
<td>0.39</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0400</td>
<td>0.62</td>
<td>0.38</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0800</td>
<td>0.67</td>
<td>0.33</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>0.50</td>
<td>0.50</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

8 h, after which the plants in LD were indistinguishable from the plants in SD. These experiments therefore suggest that transfer to LD induces a transient, partial synchronisation of the cells of the meristem. Overall, the microdensitometric data obtained from the sections (Fig. 1) were different from those obtained from the squashes (Fig. 2). On a sample to sample basis there tended to be more nuclei in G1 in the squashes (Fig. 2) than there were in the sections (Fig. 1). We cannot say whether this was because of the use of sections and then squashes or whether in fact there were real differences between plants in the two experiments.

Mitotic Index

The mean mitotic index for the sections of apices of plants in SD was $1.90 \pm 0.13$ (Fig. 3a). The variations from one sampling time to another were not statistically significant, as shown by a two-way analysis of variance ($F=2.17, 4$ and $20$ d.f.). In the plants transferred to long days the mitotic index from 2000 h to 0400 h was higher than the corresponding SD values, but neither these values nor the mean mitotic index, $2.42 \pm 0.27$, were statistically distinguishable from the SD values (Fig. 3a; $p > 0.1$).

In the second experiment, with the squashes preparations, the mean mitotic index in SD was $2.01 \pm 0.15$. The variations between sampling times in SD were not statistically significant (Fig. 3b; $p > 0.1$) as shown by a two-way analysis of variance ($F=2.01, 4$ and $20$ d.f.). In the plants transferred to LD the mitotic index again tended to be higher than in the SD controls, and in this experiment the difference seemed to be maintained throughout the whole of the sampling period. However again the mean value for LD ($2.60 \pm 0.17$) was not statistically significantly dis
Labelling Index

The labelling index in both SD and LD apparently fluctuated somewhat (Fig. 4) but the mean value for the whole period in LD (12.6 ± 0.8) was significantly lower than the mean value in SD (16.2 ± 0.7) (p < 0.001) and this difference was confirmed by a two-way analysis of variance. The analysis of variance showed a significant effect of daylength on the labelling index ($F = 14.28$, 1 and 11 d.f.), but not of sampling time ($F = 1.49$, 11 and 11 d.f.). In LD the labelling index tended to be lower than in SD over the first 12 h but from 0700 h onwards it seemed to increase to about the same value as in SD (Fig. 4).

Discussion

After one LD the increase in cell number in the shoot apex of Silene plants was about 50 per cent greater than in plants in SD (Miller and Lyndon, 1976). The more limited data presented in Table I also show that the cell number in the apex 24 h after the start of photoinduction was higher than in plants in SD.

Since cell number increases faster over the first LD than in SD this implies faster growth and a shorter cell cycle in LD than in SD. This could be brought about either by shortening only some phases of the cell cycle, which may result in synchronisation of the cells in the other phases, or by equal shortening of all phases which would not give synchronisation. What has been observed is an increase in the proportion of cells in G2 in the apices of LD plants 3 h after the beginning of photoinduction (Figs. 1–2; Tables 2–3). This increase was accompanied by a decrease in the proportion of cells in G1, a possible increase in the mitotic index (Fig. 3) and a decrease in the labelling index (Fig. 4) in LD plants relative to the SD ones. There was however no shift of all or most of the cells to one phase of the cell cycle and consequently there was no subsequent displacement of all or most of the population through the cell cycle together (Figs. 1 and 2). The fact that these differences are maintained for only a further 4 to 12 h (Figs. 1–4), suggests that, at best, there is only a partial, transient synchronisation. These results are consistent with a shortening of the cell cycle but proportionately more shortening of G1 and S than G2 and M. From day 1 onwards (until about day 8) cell number in the apices of LD plants increased at
the same rate as in SD plants (Miller and Lyndon, 1976). This suggests that the shortening of the cell cycle (and faster growth) in the first LD is followed by a subsequent lengthening of the cell cycle to its original length by the second LD, consistent with the reduction in the growth rate, for the rest of the inductive period, to the same as that in SD.

In Sinapis the synchronisation of the cells during floral induction was inferred from the peak in mitotic index 26 h after the beginning of induction, followed by a second peak (at flower initiation) about 36 h later (Bernier et al., 1967) which was exactly one cell cycle later (Bodson, 1975). The first peak was followed by an increase in the proportion of cells in G1 (Jacqumar and Miksche, 1971) and an increase in labelling index (Bernier et al., 1967; Kinet et al., 1967).

Table 4. The proportion of interphase nuclei with a DNA content of <3C (G1) and >3C (G2), and the ratio of G2 to G1 nuclei, in Sinapis and Xanthium at various times after the start of inductive treatment.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Sinapis*</th>
<th>Xanthium*</th>
</tr>
</thead>
<tbody>
<tr>
<td>start of LD</td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>0</td>
<td>0.24</td>
<td>0.76</td>
</tr>
<tr>
<td>22</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>30</td>
<td>0.67</td>
<td>0.33</td>
</tr>
<tr>
<td>34</td>
<td>0.68</td>
<td>0.32</td>
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<tr>
<td>46</td>
<td>0.46</td>
<td>0.54</td>
</tr>
<tr>
<td>70</td>
<td>0.73</td>
<td>0.27</td>
</tr>
</tbody>
</table>

a The data concerning Sinapis are taken from Jacqumar and Miksche (1971) and
b those concerning Xanthium from Jacqumar et al. (1976)

When the microdensitometric measurements for Sinapis (Jacqumar and Miksche, 1971) are analysed in the same way as for Silene i.e. by referring those nuclei <3C to the G1 population and >3C to G2 (see Methods) (Table 4), exactly the same conclusions could be drawn as were drawn by the authors, that most of the nuclei shifted from the 4C (G2) to the 2C (G1) level and the cell population at the 4C (G2) level remained low until flower initiation. In Silene however, it was the proportion of cells in G2 which increased (Table 2 and 3). This implies that the speeding up of the cell cycle is brought about in different ways in Sinapis and Silene. This difference is confirmed by the lack of a clear peak of mitotic index (Fig. 3) and by the fall in labelling index (Fig. 4), in Silene, in contrast to Sinapis.

In Xanthium, too, the recurring peaks of mitotic index (Jacqumar et al., 1976) may be taken as evidence for synchronisation of cells at the beginning of floral induction. Since the first rise in mitotic index preceded the rise in labelling index it was concluded that cells were released from G2, as in Sinapis. However, when the proportions of cells in G1 (<3C) and G2 (>3C) are calculated from the data of Jacqumar et al. (1976) it would appear that in Xanthium the proportion of cells in G2 increases for 4 or more hours (Table 4). In this respect, Silene (LD plant) therefore appears to be more like Xanthium (SD plant) than like Sinapis (LD plant) (Table 4).

Although the cell cycle apparently shortens at the beginning of photoinduction in Sinapis, Xanthium and Silene, the synchronisation of cells which is found in Sinapis and Xanthium is not found in Silene. However, we must point out that in Sinapis flower initiation is achieved about 60 h, or less than 2 cell cycles, after the start of photoinduction whereas in Silene flower initiation does not take place until the 9th day, or about 10 cell cycles after the start of induction (Miller and Lyndon, 1975, 1976). Therefore we cannot rule out the possibility of a synchronisation of cell division taking place just before flower initiation, perhaps on the 8th LD, when the cell cycle is known to decrease to about 10 h (Miller and Lyndon, 1975, 1976). The question therefore remains open whether the temporary increase in division rate (and shortening of the cell cycle) in Silene during the first LD is an integral part of the flowering process or is an independent phenomenon resulting from change in photoperiod.

As changes were observed in the Silene shoot meristem only 3 h after the beginning of the extended light period, the plants must have begun to respond to the change in photoperiod before this. Although the critical daylength for Silene coelii-rosa is not known, 8–11 h would be rather short for a long-day plant. This again suggests that the effects observed at the shoot apex of Silene during the first 12 h of induction could be either direct effects of photoperiod or perhaps result from a change in the spectral quality of the light.

The mitotic index, over the first 12 h of photoinduction, was about 40% higher in the apices of plants in LD relative to those in SD (Fig. 3). It has been shown in the shoot apices of a number of species that the length of mitosis remains constant in both vegetative and flowering conditions (Denje, 1966; Corson, 1969; Lyndon, 1973; Bodson, 1975) and that the mitotic index would therefore be expected to be approximately inversely proportional to the length of the cell cycle. Assuming this is also the case in Silene, then the 40% greater mitotic index of plants in LD compared to plants in SD (Fig. 3) would be
consistent with a cell cycle during the first LD about 70% of the length of the cell cycle in SD. Since the cell cycle in SD is about 20 h (Miller and Lyndon, 1975, 1976) this would mean the cell cycle in the first LD would be reduced to about 12 to 14 h (i.e. from 1700 h on day 0 to about 0500-0700 h on day 1). It would then revert to its former length of 20 h. Over the first 24 h from the beginning of induction this would correspond to about 1.6 cell cycles (1 cell cycle of 12 h and 0.6 cell cycle of 20 h) which would result in an increase in cell number of 203%. In the plants in SD 1.2 cell cycles (of 20 h) would be accomplished during this 24 h, giving an increase in cell number of 130%. This would therefore result in a 56% greater increase in cell number in the plants in LD than those in SD. This is consistent with a greater cell number in plants given one LD than in plants in SD (Table 1; Miller and Lyndon, 1976) and suggests that the explanation we have given is a plausible one. Direct measurements of the length of the cell cycle and its phases during the first day of induction are in progress.

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The Cell Cycle in the Shoot Apex of *Silene* During the First Day of Floral Induction

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Summary

The length of the cell cycle was measured in the shoot apical meristem of *Silene coeli-rosa* during the first day of an inductive photoperiod. The length of the cell cycle in the shoot apex of vegetative controls (those in short days) was about 18–20 hours. Exposure of plants to the long day resulted in an immediate shortening of the cell cycle to about 13 hours, roughly two thirds of that in short days. Measurements of the component phases of the cell cycle revealed that the shortened cycle in long days was the result of a decrease in the length of G1 and perhaps S, whilst G2 and M remained constant.

1. Introduction

An increase in mitotic index seems to be one of the earliest events of evocation in the shoot apex during floral induction (Bernier 1971). In *Sinapis* and *Xanthium* this increase in mitotic index apparently results from a synchronization of cells into one particular phase of the cell cycle (Bernier, Kinet, and Bronchart 1967, Kinet, Bernier, and Bronchart 1967, Jacqmard and Miksch 1971, Jacqmard, Raju, Kinet, and Bernier 1976). An increase in the rates of growth and cell division at the transition to flowering (Sunderland 1961, Saint-Côme 1969, Corson 1969, Bodson 1975, Miller and Lyndon 1976) and the synchronization may therefore be brought about by a differential shortening of the phases of the cell cycle. In *Sinapis*, for example, the G2 phase appears to be speeded up resulting in synchronization in G1 (Kinet et al. 1967, Jacqmard and Miksch 1971).

In *Sinapis*, and perhaps also in other plants requiring only a single photoperiod for induction, only about one or two cell cycles elapse between the beginning of induction and the rise in mitotic index which indicates synchronization and the initiation of flowers (Bodson 1975). In *Silene coeli-rosa*, however, there are about 10 cell cycles between the beginning of induction and flower initiation (Miller and Lyndon 1975, 1976). In *Silene* there is an increase in the growth rate of the shoot apex over the first long day (LD).
resulting in a higher cell number on day 1 in the induced plants in LD compared with vegetative plants in SD (Miller and Lyndon 1976, Francis and Lyndon 1978). For the rest of the inductive period the growth rate reverts to that of non-induced plants (Miller and Lyndon 1976). If synchronization of cells in a particular phase of the cell cycle were an early feature of evocation in Silene as it appears to be in such plants as Sinapis (Bernier et al. 1967) and Xanthium (Jacqmaard et al. 1976) then we may expect it to occur in Silene during this first long day.

In fact, we did not find any clear evidence for synchronization at this time (Francis and Lyndon 1978). The transient changes in the mitotic index, labelling index and the proportions of cells in G1 and G2 were consistent with a shorter cell cycle in induced apices relative to non-induced ones over this first long day (Francis and Lyndon 1978). It was the purpose of the present investigation, therefore, to make direct measurements of the length of the cell cycle and its component phases in Silene over the first day of floral induction using the double labelling technique described by Miller and Lyndon (1975).

2. Materials and Methods

The plant used was Silene coeli-rosa L. Godron. The seeds were sown and grown in short days (SD) under those conditions used by Miller and Lyndon (1975, 1976). 28 days following the start of germination (day 0) plants were selected using the method described previously and either kept under SD (non-inductive) or transferred to long days (LD; inductive) (Miller and Lyndon 1976). Plants in SD were maintained in 8 hour days from 9.00-17.00 hour each day (fluorescent + tungsten light = 300-400 μE/m²/sec). The LD conditions were the same but with the addition of low intensity tungsten light (5-10 μE/m²/sec) from 17.00 hour of day 0 to 9.00 hour of day 1 (Miller and Lyndon 1975, 1976).

Plants in LD were partially defoliated, as described by Miller and Lyndon (1975) and at 17.00 hour an aqueous drop (c. 0.05 ml) containing 0.2 μCi (specific activity > 50 mCi/mmol) of [methyl-14C]-thymidine was supplied to each plant for 2 hours. At 19.00 hour each drop of label was removed with filter paper and each apex was thoroughly washed with distilled water in order to remove excess 14C-thymidine. Three of these labelled plants were subsequently fixed in 3:1 (v/v) absolute ethanol : glacial acetic acid. For all but 3 of the remaining partially defoliated plants a piece of moistened cotton wool was carefully inserted over the apex to prevent desiccation. The three remaining exposed apices were then given a terminal label with tritiated [methyl-3H] thymidine applied to each apex as an aqueous drop (c. 0.05 ml) containing 5 μCi (specific activity 15,000-30,000 mCi/mmol), which was left on the apex for 2 hours. At 21.00 hour these three terminally labelled apices were fixed and 3 more plants were then taken and labelled with 3H-thymidine for 2 hours, and so on for every 2 hours of the experimental period. Comparable plants in SD were treated in the same way.

Squash preparations of Feulgen-stained apices were prepared as described previously (Francis and Lyndon 1978), but incorporating the freeze-dry method of Conger and Fairchild (1953) to remove the coverslips. Permanent autoradiographs were subsequently prepared by the method of Miller and Lyndon (1975). The numbers of nuclei, singly labelled with either 14C or 3H, or doubly labeled with both 14C or 3H, were counted in the apical dome of each apex. The rationale of the double labelling method has been described previously (Miller and Lyndon 1975).
3. Results

The length of the cell cycle is measured as the interval between the initial labelling with $^{14}$C-thymidine and the maximum percentage of doubly labelled cells, which occurs when the cells labelled with $^{14}$C at the beginning of the experiment again reach the S phase of the cell cycle (Miller and Lyndon 1975).

![Graph](image)

Fig. 1. The % of doubly labelled cells at intervals after the application of $[^{14}C]$-thymidine at 17.00 hour, in apical domes, above the 7th leaf pair, in SD. Each point was derived from 2-3 slides, where not < 60 and not > 200 labelled nuclei were scored per slide.

The values for the percentage of labelled cells which were doubly labelled are shown in Figs. 1 and 2. It may be assumed that at 17.00 hour 100% of the cells would be doubly labelled. For apices kept in SD (Fig. 1) the percentage of doubly labelled cells reached a minimum approximately between 3.00-9.00 hour of day 1 and rose to a maximum about 11.00-13.00 hour of day 1, which corresponds to a cell cycle of 18-20 hours. For the apices in LD (Fig. 2) the peak of doubly labelled cells was between 3.00 and 9.00 hour of day 1 which corresponds to a cycle time of 10-16 hours or approximately 13 hours. Thus on transfer to LD there is an immediate shortening of the cell cycle in the shoot apex to about two-thirds of that in SD (Figs. 1 and 2). From these curves of the percentages of doubly labelled cells the only phase of the cell cycle that can be measured is the length of S (the period of DNA synthesis) (Miller and Lyndon 1975) which is equivalent to the interval between the mid points of the ascending and descending limbs of the peak, from which the labelling time is subtracted. This gives values for S of 6*
approximately 3 hours for plants in SD (Fig. 1) and 5.5 hours in LD (Fig. 2).

The lengths of the other phases of the cell cycle may be found if the time is known at which the $^{14}$C-labelled cells reach mitosis (somewhere between the beginning of the experiment and the peak of doubly labelled cells in Figs. 1 and 2). The percentages of mitoses that were singly labelled with $^{14}$C-thymidine are given in Tables 1 and 2. In SD and in LD $^{14}$C-labelled mitoses first appeared 6 hours from the initial labelling, suggesting that G2 in both SD and LD was about 4–6 hours. The length of G1 + M may be obtained as the interval between the initial appearance of $^{14}$C-labelled mitoses and the beginning of the increase in the percentage of doubly labelled nuclei (i.e., the beginning of S). For apices in SD this is approximately from 23.00 hour of day 0 (Table 1) to 7.00–9.00 hour of day 1 (Fig. 1), giving approximately 8–10 hours for G1 + M. For apices in LD the corresponding times are from 23.00 hour of day 0 (Table 2) to about 24.00 hour of day 0 (Fig. 2) giving a value of only about 1 hour for G1 + M. The tentative conclusion is that the shorter cell cycle in LD is likely to be the result of a shortening of G1.

These estimates, especially of the lengths of G1 and S depend very much on the detailed shape of the graphs of doubly labelled cells (Figs. 1 and 2) and the number of mitoses which can be scored on a slide (Tables 1 and 2). Calculations which are based on more data may be made from the values for labelling index, mitotic index and the proportion of cells with $\geq 3$ C and
<3 C amounts of DNA (Francis and Lyndon 1978). Using these values the proportion of cells in S, M, G1 and G2 can be obtained (Table 3). The lengths of each of these phases can then be calculated (Table 3) by correcting for the age gradient in a population of cells whose number is increasing exponentially (Nachtwey and Cameron 1968) assuming that at the time

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Total</th>
<th>14C-labelled</th>
<th>% 14C-labelled</th>
</tr>
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<tr>
<td>17.00</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19.00</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21.00</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23.00</td>
<td>7</td>
<td>2</td>
<td>28.6</td>
</tr>
<tr>
<td>1.00</td>
<td>9</td>
<td>4</td>
<td>44.4</td>
</tr>
<tr>
<td>3.00</td>
<td>3</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>5.00</td>
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<td>3</td>
<td>30.0</td>
</tr>
<tr>
<td>9.00</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>11.00</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.00</td>
<td>5</td>
<td>1</td>
<td>20.0</td>
</tr>
<tr>
<td>15.00</td>
<td>8</td>
<td>4</td>
<td>50.0</td>
</tr>
</tbody>
</table>

1 No mitoses, labelled or unlabelled, observed.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Total</th>
<th>14C-labelled</th>
<th>% 14C-labelled</th>
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<td>17.00</td>
<td>6</td>
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<tr>
<td>15.00</td>
<td>7</td>
<td>5</td>
<td>71.4</td>
</tr>
</tbody>
</table>
that the measurements were taken the cell cycle was in a steady state. Estimated in this way the lengths of M and G2 were essentially constant in both SD and LD (Table 3). In apices maintained in LD, however, S and G1 were shortened to approximately half of those for apices in SD (Table 3).

Table 3. Duration (hours) of the Component Phases of the Cell Cycle in the Apical Domes Above the 7th Leaf, Calculated From the Corresponding Proportions of Nuclei in the Cell Cycle Phases at 20.00 hour of Day 0, Assuming That the Length of the Cell Cycle in SD = 20 hours and in LD = 13 hours

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G1</th>
<th>S</th>
<th>G2</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short day</td>
<td>0.46</td>
<td>0.16</td>
<td>0.36</td>
<td>0.02</td>
</tr>
<tr>
<td>duration (hours)</td>
<td>7.50</td>
<td>3.27</td>
<td>8.66</td>
<td>0.57</td>
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<tr>
<td>Long day</td>
<td>0.35</td>
<td>0.13</td>
<td>0.49</td>
<td>0.03</td>
</tr>
<tr>
<td>duration (hours)</td>
<td>3.57</td>
<td>1.62</td>
<td>7.35</td>
<td>0.55</td>
</tr>
</tbody>
</table>

1 From Francis and Lyndon (1978).

Both the estimates, and calculations made, are consistent in showing that on transfer to LD G2 and M remained constant in length but G1 and perhaps S are shortened accounting for the shorter cell cycle in apices in LD relative to those in SD.

4. Discussion

For Silene apices in SD the length of the cell cycle was 20 hours (Fig. 1) a value very similar to that previously obtained for apices of Silene which were growing vegetatively in SD (Miller and Lyndon 1975). On transfer to LD (Fig. 2) the cell cycle became shortened to 13 hours or about two-thirds of the corresponding cell cycle in shoot apices in SD (Fig. 1). Over the first 12–13 hours of this first LD the mitotic index of induced apices increased to 140% of the value for non-induced (SD) plants (Francis and Lyndon 1978). On the assumption that the length of mitosis remained constant this was consistent with a cell cycle of 12–14 hours and this is the value which has been obtained in the present experiments (Fig. 2). The greater increase in the cell number which takes place in the apices in the first LD compared with apices in SD (Miller and Lyndon 1976, Francis and Lyndon 1978) is also consistent with one cell cycle of 13 hours followed by a return to the longer cycle of 20 hours for the rest of the inductive period.

Exposure of shoot apices of Silene to one long day resulted in an increase, for 8–12 hours, in the proportion of cells in G2 compared with non-induced apices (Francis and Lyndon 1978). It could perhaps be reasoned that this was a consequence of a transient synchronization of cells in G2 because of
an absolute increase in the length of G2. However the present data indicate that G2 was the same in induced (LD) and non-induced (SD) apices (Table 3) although the cell cycle was shortened in the induced apices (Fig. 2). The increase in the proportion of cells in G2 (FRANCIS and LYNDON 1978) is therefore the result of a reduction in the length of the rest of the cell cycle other than G2, especially G1 and perhaps S (Table 3 and see Results). It is unclear whether these initial effects of floral induction on changes in the rate of cell division, and the length of the cell cycle in Silene are related to a change in photoperiod or to flower initiation, which does not take place until another 7 days and 8–9 cell cycles have elapsed (MILLER and LYNDON 1975, 1976). Experiments are in progress to investigate this point. The shortened cell cycle in the induced apices could perhaps be a reflection of the change in light quality which takes place in LD at 17.00 hour. The change from fluorescent + tungsten light to tungsten only may have an effect on the length of the cell cycle in the shoot apex of Silene. Red and far-red light have a marked effect on the rate of cell division in rice shoot apices (ROLINSON and VINCE-PRUE 1976). However, this was on transfer to light after growth in darkness, whereas in Silene the transfer is from one set of lighting conditions to another. Changes in spectral quality of light, however, have been found to influence cell division in roots (BROWN and KLEIN 1973) and may be worthy of closer attention in the shoot apex.

Acknowledgement
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Flower Development in Silene: Morphology and Sequence of Initiation of Primordia

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ABSTRACT

The initiation and development of the flower of Silene coeli-rosa was followed by examining apices by scanning electron microscopy. The sepals, stamens and carpels are initiated in a spiral sequence, the direction of the spiral being the opposite of the acropetal helix of unequal axillary buds at the nodes below the flower. The petals are initiated almost simultaneously and at the same time as the first few stamens. The change in phyllotaxis from opposite and decussate in the vegetative shoot to spiral in the flower occurs with the displacement of the first two sepals away from the mid-line of the apex and towards the axillary bud at the node below the flower. The sizes of the sepals and stamens are a function of their age since initiation but the petals grow more slowly. The Silene flower can be interpreted as a shoot bearing primordia with associated axillary primordia, some of the latter being precocious in their development.

Key words: Silene coeli-rosa, flower initiation, flower development, phyllotaxis, primordia.

INTRODUCTION

In an attempt to disentangle the processes intrinsic to the formation of the flower from the processes involved in the growth of the inflorescence the chosen plant was Silene coeli-rosa because, in common with other members of the Caryophyllaceae, the transition from vegetative to floral development occurs first at the terminal meristem to form a terminal flower. The inflorescence is a cyme and the growth of the inflorescence is carried on by the axillary bud at the node below the flower.

The growth rates and RNA levels in the apex during induction and evocation could be varied experimentally in such a way that it was concluded that the changes in the apex usually accompanying the transition to flowering were likely to be more closely associated with the changes in growth rate which normally accompanied flowering rather than with the formation of the flower itself (Miller and Lyndon, 1977). This prompted a closer look at the nature of the flowering process itself in which the growth of the apex during the transition to flowering (Lyndon, 1977) and the growth of the flower (Lyndon, in press) were examined.

The growth of the flower could be followed with precision because in Silene the positions of the primordia in the flower are related to the positions of the vegetative axillary buds. The leaves in Silene coeli-rosa are opposite and decussate, and therefore in pairs. At each node one axillary bud in the axil of one leaf of the pair develops at first much more than the other. The large buds, one at each node, form a helix up the stem, as in some other species with opposite, decussate leaves (Champagnat, 1965; Cutter, 1966). In Silene the positions of the primordia of the floral members of the terminal flower can be identified and their sequence predicted by relating their positions to those of the axillary buds at the upper-most two nodes. Flower development can therefore be measured precisely...
and in detail because each floral member can be identified individually and consistently from flower to flower.

The purpose of this paper is to document the morphological events and the sequence of initiation of the primordia during flower development in *Silene coeli-rosa*.

**MATERIALS AND METHODS**

Plants of *Silene coeli-rosa* (L.) Godron were grown from seed in controlled environments at 20 °C and as already described (Miller and Lyndon, 1976). After growth in short days they were induced to flower by 7 long days at either 20° or 13 °C (Lyndon, 1977) and were then returned to short days in which flower development took place. The development of the flower was observed by examining plants daily with a dissecting microscope. Observations were repeated many times until a total of about 1000 plants had been examined, and at least 60 plants (and usually many more) for each day during flower initiation and development. The conclusions reached about the sequence of initiation of primordia were confirmed by examining plants at various stages of development with a Cambridge Stereoscan scanning electron microscope (S.E.M.). To do this, leaves were excised from growing plants to expose the apex which was then cut off and mounted, fresh and with no further treatment, by the use of water-soluble glue ("Expandabond") to the mounting stub. The apex was then placed in the microscope and could be viewed and photographed for about 5 min before shrivelling. This simple method gives the most satisfactory results (Falk, Gifford and Cutter, 1971; Emino and Rasmussen, 1975).

**RESULTS**

In the vegetative plant the leaves are initiated in pairs giving opposite, decussate phyllotaxis (Plate 1A). During induction there are usually two pairs of leaves initiated before the apical dome enlarges and sepals are initiated (Miller and Lyndon, 1976; Lyndon, 1977). The first two sepals are initiated in much the same way as a pair of leaves but are displaced to one side of the mid-line (i.e. diameter) of the apex towards the axillary bud which is the uppermost of the helix traceable up the stem (Plate 1B). The sepal which is in the position which continues the helix of axillary buds tends to be initiated slightly earlier than the second sepal and is therefore often a little larger (Plate 1B). All subsequent primordia (except the petals), i.e. the remaining sepals, the stamens, and the carpels are initiated singly in spiral sequence.

The 3rd sepal primordium is always initiated on the side of the apical dome furthest away from the last axillary bud (Plate 1C) and is displaced towards the 1st sepal (Plate 1B). These first three sepals are therefore initiated in a spiral (anti-clockwise in Plate 1B and Fig. 1) which is the reverse direction of the spiral of large axillary buds (clockwise in Plate 1B and Fig. 1). The direction of these two spirals is always opposite although the occurrence of clockwise and anti-clockwise spirals of axillary buds (and therefore the direction of the spiral of primordia in the flower) is equally frequent in each direction. The 4th sepal is formed on the opposite side of the apical dome from the 3rd, approximately midway between the 1st and 2nd sepals (Plate 1B). The 5th sepal is formed in the gap between the 2nd and 3rd (Plate 1B). The sepals are therefore initiated in a spiral sequence which begins by the displacement of the first two sepals away from the mid-line of the apical dome. The sepals become joined into a whorl by growth of the edges of the sepals and the intervening parts of the apical surface, and the axils of the sepals become well-marked (Plate 1E). At this stage there is an interval of about half a day (Lyndon, in press) during which the apical dome above the sepals is apparently free of primordia (although the stamens and petals soon appear as incipient bulges) (Plate 1E).

The next primordium to be initiated usually appears to be the first stamen (Plate 1F) which is on almost the same radius as the 1st sepal (Plate 2A). Stamens 2–5 are initiated in
FIG. 1. Diagram showing the sequence of initiation of primordia in the flower of *Silene coeli-rosa*. The sepals (shaded) and stamens (circles) and carpels (C1 etc.) are numbered according to the sequence of initiation in each whorl. Stamen 1 is initiated after sepal 5, and carpel 1 after stamen 10. Petals are indicated by shaded half circles. The acropetal helix of unequal axillary buds (AB) is indicated by the dashed line. The penultimate pair of leaves is shown at the sides, and the last pair above and below, the apex.

sequence on approximately the same radii as sepals 2–5 (Plate 2A; Fig. 1). These stamens are therefore antesepalous. The remaining stamens (6–10) are initiated in spiral sequence in which the next stamen is always initiated in the gap which is bordered by two of the stamens 1 to 5, and which is (a) furthest from the last initiated stamen, and (b) is not adjacent to the penultimately-initiated stamen.

The petal primordia originate as pendulous bulges low on the sides of the apex (Plates 1F and 2B) and are therefore less easily distinguished than the stamen primordia which are more discrete and tend to look more like pimples when they first appear (Plate 2B). The petals are initiated simultaneously or in very quick succession when the first few stamens are being initiated (Plate 1F). The petals are therefore all approximately the same size (Plate 2A, B, C) even though the antepetalous stamens, 6–10, lying opposite the petals, are of quite different sizes (Plate 2A, C). The petals and stamens 6–10 are therefore not initiated together even though they may sometimes have the appearance of a double primordium (Plate 2B). Although the petals are at first larger than the antepetalous stamens (Plate 2A), the stamens have grown larger by the time the carpels are formed (Plate 2D).

The carpels are first seen as flat areas on the apical dome which become indentations, presumably by the faster upward growth of the edges of the carpels (Plate 2D, E). The carpels also appear to be initiated in a spiral sequence which continues that of the stamens (Fig. 1).
The unequal sizes of the members of the sepals and stamens persist into the older flower (Plate 2E, F) although the inequalities are less obvious as the flower matures (Plate 2F).

DISCUSSION

The primordia of the sepals and the stamens of Silene coeli-rosa are initiated in spiral sequence on the apex. The subsequent appearance of whorls is presumably because there is very little internodal growth between members of the same whorl. Only the petals are initiated almost simultaneously but sequential initiation of the petals becomes apparent at lower temperatures (Lyndon, in press). A sequential initiation of primordia in some Caryophyllaceous flowers has also been observed by Payer (1857), Rohrbach (1968) and Sattler (1973).

The spiral of the sequence of initiation of the primordia of the floral members in Silene coeli-rosa is in the opposite direction to the acropetal helix of unequal axillary buds. This change in direction appears to originate from the displacement of the first two sepals towards the axillary bud immediately below the flower which could occur in one of two ways. The sepal primordia could be displaced at the moment of initiation towards the axillary bud; this would imply some interaction between the axillary bud and the positions on the apical dome at which the sepal primordia arise. Alternatively, the displacement could arise, after the initial determination of the positions of the sepal primordia at 180° from each other, by greater growth of the apical dome on the side away from the axillary bud than on the side adjacent to it. The circumference of the apical dome would have to increase by about 15 per cent, at the level of the sepal primordia, for this to result in the 156° divergence angle between the two sepal primordia which is observed (Plate 1b and Lyndon, 1978a) and this in turn could be achieved by there being a difference in growth rates of this order between the sides of the apical dome nearest and farthest from the axillary bud. A plausible hypothesis is that the growth of the axillary bud results in a reduction of the growth rate on the adjacent side of the apical dome relative to the far side.

The position of the 3rd sepal primordium nearer the older (1st) than the younger (2nd) is what would be expected if it is governed by the positions of the existing primordia in the same way as proposed for leaves (Richards, 1948, 1951; Thornley, 1975). The approximate positions of the remaining sepals and the stamens (Fig. 1) are also predictable if it is assumed that the same rules apply as for the positioning of leaf primordia, i.e. that the next primordium arises on the apical dome as far away as possible from the previous primordium and that its position relative to the two nearest primordia is such that its distance from each of these primordia is in inverse ratio to their ages in plastochrons (Richards, 1948, 1951). The initiation of the first antepetalous stamens at about the same distance from the centre of the apical meristem as the last initiated antesepalous stamens does, however, suggest that the positioning of a stamen primordium may be governed principally by the positions of the previous two primordia rather than the previous three or more, and also that the apex is expanding radially only slowly, relative to the rate of initiation of primordia, at this stage of flower development.

If the positions of the primordia in the flower are governed by the same sorts of processes as govern the positions of leaves in spiral phyllotaxis then there should logically be an arrangement of the floral members with no orthostichies, which clearly there are since the sepals and the antesepalous stamens are on the same radii, as are the petals and the antepetalous stamens. These pairs of primordia, have the same phyllotactic relationship as a leaf and its axillary bud. Also the 1st stamen occurs in the position where the next large axillary bud would be expected in helical sequence (Fig. 1).
If the stamens are more comparable with axillary buds the comparison with the positioning of leaves may not be exact. The positions of successive unequal buds are explicable as being determined by the positions of existing buds (Cutter, 1967, 1972). Surgical experiments have also shown that the positioning of axillary buds is governed by the positions of existing leaves and axillary buds (Snow, 1965). Axillary buds may be initiated and develop almost simultaneously with their subtending leaves, as tends to happen in apices just before flowering (Thomas, 1961) or several plastochrons later, as in many vegetative shoots (Esau, 1953).

The *Silene* flower is therefore understandable as a series of primordia corresponding to leaves (the sepals and the petals) and axillary buds (the stamens) with the latter being initiated after the former and the positions of new primordia being determined by the positions of existing primordia. The probable correspondence of carpels to leaves initiated in sequence is suggested by the structure of modified *Silene* flowers in which all stages of the transition from leaves to united carpels have been observed (Lyndon, in press).

In general the sizes of the primordia of the floral members of the same whorl are proportional to their ages, i.e. the time elapsed since their initiation (Plate 2A, c) and the sepals are always larger than the stamens (Plate 2A, b, c). This implies that the growth rates of all the sepals and stamens are similar and measurements have confirmed this (Lyndon, in press). The petals begin by being larger than the antepetalous stamens (Plate 2A) but the stamens appear to grow faster (Plate 2D), suggesting that the growth rate of the petals is lower than that of the other floral members. Again, measurements have confirmed that this is so in *Silene* (Lyndon, in press) and observations suggest that a relatively slow growth rate is common to the petals of many plants (Sattler, 1973). The different growth rates of the petals and the antepetalous stamens are paralleled by the different growth rates of axillary buds and their subtending leaves when apical dominance is removed.

The quantitative aspects of flower development in *Silene* will be considered in detail in subsequent papers (Lyndon, 1978, in press).

**ACKNOWLEDGEMENTS**

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**LITERATURE CITED**


Lyndon—Flower Development in Silene


EXPLANATION OF PLATES

PLATE 1
Scanning electron micrographs showing the early development of the flower up to the initiation of the first stamens.

A. Vegetative apex with apical dome and two pairs of leaf primordia. (Older leaves have been removed.) x<sub>c</sub> 300.

B. Apex with the last pair of leaves and the first two sepals. The 3rd sepal appears as a small bump just below and to the left of the black mark on the apex. The first two sepals are displaced from the midline of the apex towards the axillary bud (at the right) at the node below the flower. This is the uppermost of the helix of axillary buds. The second uppermost axillary bud is visible (top centre, to the left of the 2nd sepal). x<sub>c</sub> 220.

C. Side view of the same apex as in 1B. The uppermost pair of leaves enclose the axillary bud (at right of apex). The 1st sepal is towards the reader and the 3rd sepal is the small bump on the left side of the apex. x<sub>c</sub> 220.

D. Apex with 5 sepals, and possibly stamens just beginning to be initiated. The axillary bud immediately below the flower is very large, which was characteristic of plants induced at 13°, as this one was. The next lower axillary bud is also visible and shows the helix of buds to be anti-clockwise in this apex. x<sub>c</sub> 180.

E. Terminal pair of leaves partially enclosing an apex with all sepals initiated but the apical dome apparently free of other primordia. The sepals have grown together forming a whorl. x<sub>c</sub> 180.

F. Side view with sepals (as a whorl), petals (the bulges alternating with the sepals, at the base of the apical dome at the front and at the right) and a stamen, at the left of the apex, opposite a sepal. (Leaves have all been removed.) x<sub>c</sub> 200.

PLATE 2
Scanning electron micrographs showing the later development of the flower. All leaves have been removed.

A. Apex, from above, with 5 sepals, 5 petals, 5 antepetalous stamens (1–5) and stamens 6 and 7 present, with stamens 8 and 9 just being initiated and stamen 10 not yet initiated. x<sub>c</sub> 180.

B. Side view of same apex as 2A, showing sepals, petals and stamens. Stamens 1–5 are opposite the sepals. Three petals are visible, alternating with the sepals. Stamen 7 is the bump just above the petal at the right of the apex and stamen 8 has just been initiated and can be discerned just above the petal on the left of the apex, between stamens 3 and 5. x<sub>c</sub> 180.

C. Apex from above, with all sepals, petals and stamens present but carpels not yet initiated. x<sub>c</sub> 150.

D. Apex with sepals removed showing the disparate sizes of the stamens. At the front of the apex, the two petals which are visible are now smaller than the antepetalous stamens they subtend. The carpels have been initiated and are seen as indentations or depressions on the apical surface. x<sub>c</sub> 120.

E. Same apex as 2D, seen from above and showing petals (just visible), stamens and carpels. (The pattern of marks at the top of stamen 3 can be seen in both photographs.) x<sub>c</sub> 120.

F. An older flower showing only the sepals which now form the calyx tube, although the calyx members are still different sizes. The rest of the flower is enclosed within the calyx. x<sub>c</sub> 60.
Phyllotaxis and the Initiation of Primordia During Flower Development in Silene

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ABSTRACT

The measured divergence angles between successive primordia in the developing flower were compared with angles expected on several hypotheses concerning primordial initiation. The results led to the conclusion that the position and sequence of initiation of the younger sepals is determined by the older ones but that the influence of an older primordium lasts for only two plastochrons. The petals and carpels are apparently positioned by the sepals. The positions of the stamens are consistent with their being determined by the sepals (antesepalous stamens) or petals (antepetalous stamens), but their sequence of initiation is consistent with its being determined, like the sepals, by the two youngest primordia. The data indicate that there are two sets of factors governing the initiation of the primordia subsequent to the sepals; one governing the positioning of the primordia and resembling the factors governing the positions of axillary buds, and the other governing the sequence of primordia and resembling the factors which determine the initiation of leaves. Measurements of the plastochron ratios were used to calculate the sizes of the sepal, petal and stamen primordia at initiation. Measurements of the plastochron ratios were used to calculate the sizes of the sepal, petal and stamen primordia at initiation. At the moment of initiation the sepals were about one third, and the petal and stamen primordia about one sixth, of the size of the leaf primordia. In its early development the Silene flower therefore resembles a condensed leafy shoot with precocious axillary buds but with primordia which are small compared to leaf primordia.

Key words: Silene coeli-rosa, flower development, primordia, phyllotaxis.

INTRODUCTION

In the development of the flower of Silene coeli-rosa the primordia of the sepals, stamens and carpels, and also possibly the petals, are initiated in spiral sequence. The positions of the 3rd and subsequent sepals, and the stamens, suggested that the same sorts of rules could be governing the positioning and sequence of the sepals and stamens as govern the positioning of leaves (Lyndon, 1978). The positioning of leaves in a plant having Fibonacci phyllotaxis is consistent with the hypothesis that the leaf primordia produce a substance which results in an inhibitory field around the young primordium and which decreases in effectiveness as a function of time (Richards, 1948, 1951; Thornley, 1975).

An examination of the phyllotaxis of a flower in which the floral members develop sequentially should, in the same way, provide information about the possible factors governing the positioning of the floral members. It would also be of interest to know how the sizes of the primordia of the members of the different floral whorls might differ at the time of their initiation. This is difficult to measure directly but it can be estimated as the reciprocal of the area ratio (which is the ratio of mean transverse apical dome area to the transverse area of the primordium on initiation) which in turn can be derived from the plastochron ratio (Richards, 1951).

Accurate pictures of the surface of the developing flower can be obtained by scanning electron microscopy (Lyndon, 1978) and this allows accurate measurement of phyllotactic parameters in the young flower. The phyllotaxis of the developing flower of Silene coeli-rosa.
*Silenecoeli-rosa* was therefore examined to obtain information about the factors which may govern the positioning of the primordia of the floral members and hence govern the morphogenesis of the flower.

**MATERIALS AND METHODS**

Flower apices of *Silene coeli-rosa* (L.) Godron grown at 20 °C, at all stages of their development, were photographed with a Cambridge Stereoscan scanning electron microscope (Lyndon, 1978). Photographs taken from vertically above the developing flowers were traced. The positions of the primordia were marked as the central point on each visible primordium (Fig. 1). The divergence angles were measured as the angle subtended by successive primordia at the centre of the apex, this point being determined subjectively. The plastochron ratios were also measured from this same central point, the plastochron ratio being the radial distance of a primordium from the centre of the apex divided by the radial distance of the next primordium to be initiated (Richards, 1948, 1951).

Apical radii were half the diameters measured on median longitudinal sections. The diameter at the insertion of the sepals was measured at the axils of the sepals in those apices with sepals, but no petals and no or few stamens. The diameter at the insertion of the petals was measured at the petal axils in apices with incomplete numbers of petals. The diameter at the insertion of the stamens was measured at the stamen axils in those apices which had no carpels.

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**Fig. 1.** Tracing of a S.E.M. photograph of a developing flower of *Silene*. The presumed centres of the primordia are shown for the sepals (large black circles), stamens (small black circles) and petals (open circles). The sepals and stamens are numbered in the sequence of their initiation. The estimated centre of the apex is starred. The position of the uppermost axillary bud is indicated by a dotted outline.
RESULTS

Sizes of primordia on initiation

An estimate of the sizes of the primordia on initiation may be obtained from measurements of the plastochron ratio, \( r \), which is the ratio of the distances of two successive primordia from the centre of the apex (Table 1). The radial relative growth rate per plastochron is represented by \( \log_r \), and the area relative growth rate per plastochron by \( 2 \log_e r \). If the mean area of the apical dome during the plastochron is given as 1, then the increase in the area of the apex associated with the initiation of a primordium is

\[ \frac{\text{Area of primordium}}{\text{Area of apical dome}} = 2 \log_e r \] (Richards, 1951).

This value gives the area of the primordium at initiation relative to the mean transverse area of the apical dome. While this relationship is valid for a flat or disc-like apex, it must be modified if the apex is dome-shaped, as it is in *Silene* in which the primordia are initiated on the flanks of the dome. Assuming that the surface of the apical dome at the level of the initiation of primordia approximates to the surface of a cone, then the angle of the cone, \( \theta \), is the apical angle, which can be measured from longitudinal sections. The revised value which gives the actual area of the primordium on the apical surface relative to the transverse area of the apex is \( 2 \log_e r' \) and can be calculated because \( \log_e r' = \log_e r / \sin \theta \) (Richards, 1951). Absolute areas of the primordia on the apical surface can then be calculated when the apical radius is known since absolute primordial area = \( 2 \log_e r' \times \) transverse apical area (Richards, 1951).

Values for the primordial area are given in Table 2, which shows that the plastochron ratio decreases on flowering, that on initiation the primordia of the sepals are only one

### Table 1. Plastochron ratios of successive primordia in the flower of *Silene coeli-rosa*

<table>
<thead>
<tr>
<th>Primordia</th>
<th>Plastochron ratio</th>
<th>s.e.</th>
<th>Number of apices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepals 1/2</td>
<td>1.034</td>
<td>0.021</td>
<td>12</td>
</tr>
<tr>
<td>2/3</td>
<td>1.090</td>
<td>0.024</td>
<td>12</td>
</tr>
<tr>
<td>3/4</td>
<td>1.010</td>
<td>0.034</td>
<td>9</td>
</tr>
<tr>
<td>4/5</td>
<td>1.019</td>
<td>0.025</td>
<td>9</td>
</tr>
<tr>
<td>Sepal 5/Stamen 1</td>
<td>1.126</td>
<td>0.046</td>
<td>8</td>
</tr>
<tr>
<td>Stamen 1/2</td>
<td>1.034</td>
<td>0.024</td>
<td>10</td>
</tr>
<tr>
<td>2/3</td>
<td>1.052</td>
<td>0.039</td>
<td>8</td>
</tr>
<tr>
<td>3/4</td>
<td>1.031</td>
<td>0.041</td>
<td>7</td>
</tr>
<tr>
<td>4/5</td>
<td>1.032</td>
<td>0.027</td>
<td>7</td>
</tr>
<tr>
<td>5/6</td>
<td>0.933</td>
<td>0.041</td>
<td>7</td>
</tr>
<tr>
<td>6/7</td>
<td>1.068</td>
<td>0.035</td>
<td>7</td>
</tr>
<tr>
<td>7/8</td>
<td>1.050</td>
<td>0.033</td>
<td>7</td>
</tr>
<tr>
<td>8/9</td>
<td>0.995</td>
<td>0.042</td>
<td>7</td>
</tr>
<tr>
<td>9/10</td>
<td>1.044</td>
<td>0.040</td>
<td>7</td>
</tr>
<tr>
<td>Sepal 5/Petal 6</td>
<td>1.177</td>
<td>0.041</td>
<td>7</td>
</tr>
<tr>
<td>Petal 6/7*</td>
<td>1.034</td>
<td>0.030</td>
<td>7</td>
</tr>
<tr>
<td>7/8</td>
<td>1.047</td>
<td>0.043</td>
<td>6</td>
</tr>
<tr>
<td>8/9</td>
<td>0.985</td>
<td>0.070</td>
<td>5</td>
</tr>
<tr>
<td>9/10</td>
<td>1.005</td>
<td>0.055</td>
<td>6</td>
</tr>
<tr>
<td>Stamen 10/Carpel 1</td>
<td>2.112</td>
<td>0.189</td>
<td>2</td>
</tr>
<tr>
<td>Carpel 1/2</td>
<td>1.041</td>
<td>0.089</td>
<td>2</td>
</tr>
<tr>
<td>2/3</td>
<td>0.938</td>
<td>0.018</td>
<td>2</td>
</tr>
<tr>
<td>3/4</td>
<td>1.021</td>
<td>0.021</td>
<td>2</td>
</tr>
<tr>
<td>4/5</td>
<td>0.959</td>
<td>0.002</td>
<td>2</td>
</tr>
</tbody>
</table>

* The petals are numbered to correspond with the stamens they subtend.
**Table 2. Apical dimensions, mean plastochron ratios and areas of primordia at initiation**

<table>
<thead>
<tr>
<th>Primordia</th>
<th>Apical radius, $R$ ($\mu$m)</th>
<th>$\theta$</th>
<th>Mean plastochron ratio, $r$</th>
<th>$\log_e r$</th>
<th>$\log_e r'/\sin \theta$</th>
<th>$2 \log_e r'$</th>
<th>Absolute primordial area on apical surface ($\mu$m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves*</td>
<td>58</td>
<td>28°</td>
<td>1.180</td>
<td>0.1655</td>
<td>0.3525</td>
<td>0.705</td>
<td>7451</td>
</tr>
<tr>
<td>Sepals</td>
<td>75</td>
<td>28°†</td>
<td>1.038</td>
<td>0.0372</td>
<td>0.0792</td>
<td>0.1584</td>
<td>2799</td>
</tr>
<tr>
<td>Petals</td>
<td>77</td>
<td>25°</td>
<td>1.018</td>
<td>0.0175</td>
<td>0.0414</td>
<td>0.0828</td>
<td>1543</td>
</tr>
<tr>
<td>Stamens</td>
<td>62</td>
<td>43°</td>
<td>1.039</td>
<td>0.0382</td>
<td>0.0560</td>
<td>0.1120</td>
<td>1352</td>
</tr>
</tbody>
</table>

* From data of Lyndon (in press).
† Assumed to be the same as for the leaves.
Values for apical radius and $\theta$ are means of 5 (leaves), 4 (sepals), 7 (petals) or 13 (stamens) plants.

**Table 3. Relative volume growth rates derived from plastochron ratios**

<table>
<thead>
<tr>
<th>3 $\log_e r'$ (Relative growth rate per plastochron)</th>
<th>Plastochron (days)†</th>
<th>Relative growth rate per day (3 $\log_e r'/\text{plastochron in days}$)</th>
<th>Relative growth rate measured directly†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves*</td>
<td>1.0575</td>
<td>1.95</td>
<td>0.54</td>
</tr>
<tr>
<td>Sepals</td>
<td>0.2376</td>
<td>0.375</td>
<td>0.63</td>
</tr>
<tr>
<td>Petals</td>
<td>0.1242</td>
<td>0.225</td>
<td>0.55</td>
</tr>
<tr>
<td>Stamens</td>
<td>0.1680</td>
<td>0.225</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* From plastochron ratios in Lyndon (in press).
† Data from Lyndon (in press).
‡ This value is the relative growth rate of the apical dome distal to the youngest leaves and not that of the leaves themselves, for which data are not available.

third the size of leaf primordia and that the petals and stamens are about one half the size of the sepals.

The values for the plastochron ratio also allow calculation of the relative growth rate of the apex in the region of primordial initiation since the volume relative growth rate is $3 \log_e r'$. The values so obtained (Table 3) show good agreement with those for the relative growth rates measured directly for the changes in tissue volumes (Lyndon, in press).

**Positioning and sequence of primordia: divergence angles**

The divergence angle between successive pairs of leaves in the vegetative plant is 90° with the two leaves of a pair being separated by 180°. The first two sepals deviate from this pattern (Fig. 1) by being displaced from the mid-line of the apex towards the axillary bud which is immediately below the flower (Lyndon, 1978a). Their divergence angle is 156.1° (Table 4). The divergence angles for the three succeeding sepals average 141.7°. This is not very different from the ideal angle for spiral phyllotaxis, 137.5° (Richards, 1948, 1951). If the sepals are positioned by the same rules as apparently govern the positioning of leaves (Richards, 1948) then it should be possible to predict the divergence angles of the sepals. In spiral phyllotaxis the position of a new primordium is (a) as far away from the previous primordium as possible on the apical dome and (b) in relation...
Table 4. Comparison of the actual divergence angles (± s.e.) of the sepals 3–5 with the angles predicted on two hypotheses. The hypothetical angles are predicted assuming that a new primordium (a) arises as far away as possible on the apical dome from the previous primordium and (b) is positioned by the adjacent primordia (Prediction A), or by only the two previously initiated primordia (Prediction B), such that it is nearer the older than the younger in the inverse ratio of their ages in plastochrons. (The influence of the leaves is assumed to be nil.) Number of apices measured given in parentheses. The divergence angles for successively initiated carpels are similar to those of the corresponding sepals.

<table>
<thead>
<tr>
<th>Sepals</th>
<th>Divergence angle °</th>
<th>Actual</th>
<th>Prediction A</th>
<th>Deviation from actual</th>
<th>Cumulative deviation</th>
<th>Prediction B</th>
<th>Deviation from actual</th>
<th>Cumulative deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>156·1 ± 3·1 (12)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>135·9</td>
<td>+3·5</td>
<td>135·9</td>
</tr>
<tr>
<td>2/3</td>
<td>132·4 ± 1·8 (12)</td>
<td>135·9</td>
<td>+3·5</td>
<td>—</td>
<td>135·9</td>
<td>+3·5</td>
<td>—</td>
<td>+3·5</td>
</tr>
<tr>
<td>3/4</td>
<td>147·4 ± 2·0 (9)</td>
<td>161·7</td>
<td>+14·3</td>
<td>+17·8</td>
<td>149·4</td>
<td>+2·0</td>
<td>+5·5</td>
<td>—</td>
</tr>
<tr>
<td>4/5</td>
<td>145·2 ± 2·7 (9)</td>
<td>143·9</td>
<td>-1·3</td>
<td>+16·5</td>
<td>140·4</td>
<td>-4·8</td>
<td>+5·5</td>
<td>+0·7</td>
</tr>
<tr>
<td>Stamen 10/Carpel 1</td>
<td>175·5 ± 0·5 (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>135·9</td>
<td>+3·5</td>
<td>135·9</td>
</tr>
<tr>
<td>Carpel 1/2</td>
<td>150·5 ± 6·5 (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>135·9</td>
<td>+3·5</td>
<td>135·9</td>
</tr>
<tr>
<td>2/3</td>
<td>135·5 ± 1·5 (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>135·9</td>
<td>+3·5</td>
<td>135·9</td>
</tr>
<tr>
<td>3/4</td>
<td>146·5 ± 1·5 (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>135·9</td>
<td>+3·5</td>
<td>135·9</td>
</tr>
<tr>
<td>4/5</td>
<td>146·5 ± 3·5 (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>135·9</td>
<td>+3·5</td>
<td>135·9</td>
</tr>
</tbody>
</table>
Table 5. Comparison of actual divergence angles (±s.e.) of presumed successive petals with angles predicted on three hypotheses. For basis for Predictions A and B see Table 4. Prediction C: divergence angle if petals on radii midway between adjacent sepals. Predicted values are based on the actual angles for the sepals and the predicted angles for the petals. Petals are numbered to correspond with the stamens they subtend. Numbers of apices measured are given in parenthesis.

| Primordia | Divergence angles ° | | | | | | | |
|-----------|---------------------|----|----|-----------------|----|----|-----------------|----|-----------------|
|           | Actual              | Prediction A | Deviation from actual | Cumulative deviation | Prediction B | Deviation from actual | Cumulative deviation | Prediction C | Deviation from actual | Cumulative deviation |
| Sepal 5/Petal 6 | 102-7 ± 3-8 (7) | 112-1 | +9-4 | +9-4 | 143-2 | +40-5 | +40-5 | 103-2 | +0-5 | +0-5 |
| Petal 6/7  | 153-3 ± 3-8 (6)    | 152-8 | -0-5 | +8-9 | 144-5 | -8-8 | +31-7 | 151-8 | -1-5 | -1-0 |
| 7/8       | 135-2 ± 3-1 (9)    | 137-2 | +2-0 | +10-9 | 143-7 | +8-5 | +40-2 | 138-9 | +3-3 | +2-3 |
| 8/9       | 150-0 ± 3-1 (5)    | 143-5 | -6-5 | +4-4 | 144-2 | -5-8 | +34-4 | 143-2 | -6-8 | -4-5 |
| 9/10      | 153-0 ± 3-1 (6)    | 149-4 | -3-6 | +0-8 | 143-9 | -9-1 | +25-3 | 150-7 | -2-3 | -6-8 |
to the adjacent primordia is such that it is nearer the older than the younger in the inverse ratio of their ages in plastochrons (Richards, 1948, 1951). On this basis the divergence angle for successive sepal primordia can be predicted, given that the divergence angle between the first two sepals is 156° (Prediction A, Table 4).

The prediction agrees reasonably with what is observed except for the positioning of the 4th and 5th sepals, which are in actuality about 17° nearer the 1st and 2nd sepals respectively than is predicted. In other words the repelling effect of the 1st and 2nd sepals is less than would be expected. If instead it is assumed that each sepal is positioned only by the two younger ones a better fit is obtained (Prediction B, Table 4). This model assumes that the position of the 4th sepal is not affected by the presence of the 1st, nor the 5th by the 2nd, and agrees better with what is found.

It is somewhat problematical which primordium is the next to arise after the 5th sepal. Logically the 1st petal would be expected but the first primordium which is seen is the 1st stamen. This may be because the petals arise as pendulous bulges at the sides of the apex and may therefore be more difficult to distinguish than the stamens, which tend to consist of well marked, localized protuberances.

The position of the presumed 1st petal, i.e. that which subtends stamen 6, can be predicted on the same bases as for the sepals (Table 5). The prediction which gives the best fit is that which assumes this petal to be positioned not by the sepals as a function of their TABLE 6. Comparison of actual divergence angle of stamen 1 from last sepal or petal with angles predicted on four hypotheses. For basis of Predictions A and B see Table 5. The last primordia to be initiated before stamen 1 are assumed to be either (i) sepals or (ii) petals

<table>
<thead>
<tr>
<th>Previous primordia</th>
<th>(i) Sepals</th>
<th>(ii) Petals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successive primordia</td>
<td>Sepal 5/Stamen 1</td>
<td>Petal 10/Stamen 1</td>
</tr>
<tr>
<td>Divergence angles:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual</td>
<td>144.5</td>
<td>171.4</td>
</tr>
<tr>
<td>Prediction A</td>
<td>112.1</td>
<td>149.3</td>
</tr>
<tr>
<td>Deviation from actual</td>
<td>-32.4</td>
<td>-22.1</td>
</tr>
<tr>
<td>Prediction B</td>
<td>143.2</td>
<td>138.3</td>
</tr>
<tr>
<td>Deviation from actual</td>
<td>-1.3</td>
<td>-33.1</td>
</tr>
<tr>
<td>Predicted if on same radius as sepal 1</td>
<td>138.9</td>
<td>—</td>
</tr>
<tr>
<td>Deviation from actual</td>
<td>-5.6</td>
<td>—</td>
</tr>
<tr>
<td>Predicted if on radius midway between adjacent petals 6 and 9</td>
<td>—</td>
<td>141.5</td>
</tr>
<tr>
<td>Deviation from actual</td>
<td>—</td>
<td>-3.0</td>
</tr>
</tbody>
</table>

TABLE 7. Positions of stamens in relation to sepal and petal radii. (Values for stamens 1–5 are means of 27 measurements and for stamens 6–10 means of 20 measurements, ±s.e.)

Mean angular deviation of

<table>
<thead>
<tr>
<th></th>
<th>±s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stamens 1–5 from sepal radii</td>
<td>+5.7 ± 0.9</td>
</tr>
<tr>
<td>Stamens 1–5 from radii midway between adjacent petals</td>
<td>+3.5 ± 0.6</td>
</tr>
<tr>
<td>Stamens 6–10 from petal radii</td>
<td>+3.8 ± 1.0</td>
</tr>
<tr>
<td>Stamens 6–10 from radii midway between adjacent sepals</td>
<td>+5.8 ± 1.0</td>
</tr>
<tr>
<td>Stamens 6–10 from radii midway between adjacent stamens 1–5</td>
<td>+0.4 ± 0.7</td>
</tr>
</tbody>
</table>
Table 8. Comparison of the actual divergence angles (±s.e.) of stamens 2–10 with the angles predicted on two hypotheses. For basis of Predictions A and B see Table 4. For stamens 2–5 prediction A based on actual positions of sepals and stamen 1, predicted positions of stamens 2–4. Prediction B based on actual positions of sepals, predicted positions of stamens 1–4. Predicted positions of stamen 6 based on actual positions of stamens 1–5. Predicted positions of stamens 7–10 based on actual positions of stamens 1–6 and predicted positions of stamens 7–9. Number of apices measured given in parentheses.

<table>
<thead>
<tr>
<th>Primordia</th>
<th>Divergence angles °</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual</td>
<td>Prediction A</td>
<td>Deviation from actual</td>
<td>Cumulative deviation</td>
<td>Prediction B</td>
</tr>
<tr>
<td>Sepal 5/Stamen 1</td>
<td>144·5 ± 3·7 (8)</td>
<td>120·4</td>
<td>-33·2</td>
<td>-33·2</td>
<td>143·2</td>
</tr>
<tr>
<td>Stamens 1/2</td>
<td>153·6 ± 2·3 (10)</td>
<td>137·2</td>
<td>+5·8</td>
<td>-27·4</td>
<td>144·5</td>
</tr>
<tr>
<td>2/3</td>
<td>151·4 ± 1·9 (8)</td>
<td>143·4</td>
<td>+4·9</td>
<td>-32·3</td>
<td>144·2</td>
</tr>
<tr>
<td>3/4</td>
<td>148·6 ± 2·5 (7)</td>
<td>149·4</td>
<td>+5·1</td>
<td>-29·2</td>
<td>143·9</td>
</tr>
<tr>
<td>4/5</td>
<td>146·3 ± 1·3 (7)</td>
<td>112·5</td>
<td>+8·6</td>
<td></td>
<td>142·5</td>
</tr>
<tr>
<td>5/6</td>
<td>150·9 ± 1·6 (7)</td>
<td>160·2</td>
<td>+9·1</td>
<td>+9·1</td>
<td>170·6</td>
</tr>
<tr>
<td>6/7</td>
<td>151·1 ± 3·5 (7)</td>
<td>136·9</td>
<td>0·0</td>
<td>+9·1</td>
<td>139·3</td>
</tr>
<tr>
<td>7/8</td>
<td>146·3 ± 1·0 (7)</td>
<td>147·1</td>
<td>+1·4</td>
<td>+10·3</td>
<td>147·1</td>
</tr>
<tr>
<td>8/9</td>
<td>149·9 ± 2·0 (7)</td>
<td>149·1</td>
<td>-0·8</td>
<td>+9·5</td>
<td>141·9</td>
</tr>
</tbody>
</table>
ages but by its being midway between the adjacent sepals. All the petals lie within 6·8° of the radii midway between adjacent sepals. On the next best hypothesis the first petal would be more than 9° away from its true position. This would mean that although the divergence angles for the next two primordia would be correct these primordia would also be displaced anodically (i.e. uphill on the genetic spiral) by about 9° from their true positions (Table 5).

The position of the 1st stamen might be governed by the positions of the petals (if they have been initiated at this time) or else by the sepals (Fig. 1). The position of the 1st stamen agrees most closely with the assumption that it is positioned by the sepals, and in the same way as the sepals—by the previous two primordia (Table 6). Almost equally good agreement is given by the assumption that the 1st stamen is positioned on the radius midway between adjacent petal primordia.

The divergence angle between the first two stamens is 153·6°, which is similar to that (156·1°) between the first two sepals (Table 8), and suggests that the position of these stamens is determined by the sepals. Since stamens 1–5 are antesepalous it might be expected that they would lie on the same radii as the sepals but they seem to be consistently anodic to the sepals by about 6° (Table 7). They lie closer to the radii midway between adjacent petals, being anodic to these by only about 3·5° (Table 7). It is therefore not clear whether stamens 1–5 are positioned exclusively by the sepals or by the petals or by both.

The possible factors governing the sequence of initiation of the stamens may be examined by comparing the predicted positions of successive stamens with their actual positions, given that the divergence angle between the first two stamens is 153·6° (Table 8). The assumption that the sequence of initiation of stamens 1–5 is governed by only the previous two primordia (Prediction B, Table 8) does not give a precise fit to the observed values but is sufficiently close to define the sequence in which the stamens would be initiated. An alternative assumption is that the sequence of initiation of stamens 2–5 is determined by the adjacent sepal and stamen primordia (Prediction A, Table 8). This model gives a poor fit to the observed values because of the poor prediction of the position at which the 2nd stamen would arise.

The antepetalous stamens, 6–10, arise at positions which lie on the radii which bisect the angles subtended at the apical centre by adjacent older stamens, 1–5 (Table 7). The position of stamens 6–10 may also be set by their being on the same radii as the petals (Table 7) but this is not quite such a good fit as assuming they lie on the radii midway between adjacent stamens. The sequence of stamens 6–10 is more closely defined on Prediction A than on Prediction B (Table 8), i.e. the sequence is consistent with its being determined by the adjacent older stamens.

The divergence angles for successive carpels (Table 4) were derived from only two plants. However, a comparison of their divergence angles with those for the corresponding sepals suggests that the positions of the sepals are reflected in the positions of the carpels. This implies that the positions of the carpels are determined by the sepals on whose radii they lie.

**DISCUSSION**

The effect of the leaves on the positioning of the sepals seems to be small or non-existent. If the leaf below and the 1st sepal were both exerting a repelling effect on the 3rd sepal and this was in inverse ratio to their ages in plastochrons the expected divergence angle between the 2nd and 3rd sepals would be 142·7°, i.e. 10° nearer the 1st sepal than its observed position. Similarly, if the opposite leaf had affected the position of the 4th sepal it would be expected in a position approximately 20° nearer the 1st sepal. The
simplest interpretation, that the positions of sepals 2–5 are influenced to a negligible extent by the leaves, is consistent with the suggestion that one of the necessary events of the transition to flowering is the reduction or removal of the influence of the leaves on the positioning of subsequent primordia (Lyndon, 1977).

The sepals were initiated at positions and in a sequence most consistent with the hypothesis that only the previous two sepals position a new sepal. This carries the implication that the effect exerted by a sepal lasts only two plastochrons and then ceases. This contrasts with the postulate that in the positioning of leaves in a spiral the inhibitory effect of a primordium persists, but with decreasing effectiveness, as it ages (Thornley, 1975). Which situation corresponds to the vegetative state in Silene coeli-rosa is not known, but since the leaves are opposite and decussate a reasonable hypothesis is that a leaf pair is positioned by only the previous leaf pair, i.e. that the effect of a pair of leaves would last only for the time that a new pair of leaves were initiated, which corresponds to two plastochrons during the initiation of single primordia such as the sepals. The suggestion is, therefore, that the positioning and sequence of the sepals is governed by the same sorts of factors, acting in the same way, as govern the positioning of the leaves. However, the mean time between the initiation of successive single leaf primordia was 1.95 days compared with only 0.375 days between successive sepals. The postulated inhibitory effect of the primordia therefore would last 3.9 days during leaf initiation but only 0.75 days, or about one fifth as long, during sepal initiation. This implies that the rate of formation of the postulated factor is reduced or its removal increased at the transition to flowering.

The cause of the change in phyllotaxis from opposite to spiral on the transition to flowering may also be sought in the change in the relationships between the size of the apex and the size and vertical spacing of the primordia (Lyndon, 1977). One aspect of this change is the reduction in size of the primordia at initiation when the sepals are initiated (Table 2) and the further reduction in primordial size when stamens and petals are initiated. Other measurements show that not only at 20°, to which the plastochron ratios in this paper refer, but also at 13° and 27° (Lyndon, in press) the size of the stamens on initiation is approximately half the size of the sepals.

While it is unknown what determines the size of a primordium at initiation, the reduction in size of the sepal primordia compared to the leaves suggests a reduction in the effectiveness of any factors which may increase or maintain primordial size. It may be noted that this parallels the postulated reduction in the persistence of the supposed inhibitory factors emanating from the primordia and leads to the speculation that the same factors may be responsible for both determining primordial size and inhibiting the formation of other primordia. Although it may be premature to speculate further on the nature of such an insubstantial factor it may be worth noting that auxin and auxin antagonists are known to affect both the positioning and size of primordia (Soma, 1968; Wardlaw, 1968; Schwabe, 1971).

The positions at which the petals arise agree best with the assumption that they are positioned by the adjacent sepals (Table 5) so that they are on or very near the radii midway between the adjacent sepals. In determining the site of a petal primordium the influence of adjacent sepals would therefore be equal. Since the petals are not initiated until all the sepals are present the petals are presumably positioned by some factor emanating from the sepals which persists equally from all sepals and persists longer than the factor determining sepal positioning, since this apparently persists only for the time to initiate two sepal primordia. It seems reasonable, therefore, to postulate two sets of factors associated with the sepals, one concerned with the positioning and sequence of the later sepals and another concerned with the positioning of the petals.

The positions of the successive antesepalous stamens (1–5) agree most closely with their being determined (like the sepals) by the positions of the two previously initiated pri-
mordia rather than because they lie on the sepal radii, from which they are in fact displaced anodically by almost 6° (Table 7). If this is so then the occurrence of antesepalous stamens would seem to be fortuitous, depending on the positions of the first initiated sepals. This is hardly a convincing explanation of antesepaly, which is of ubiquitous occurrence in a stamen whorl throughout the Angiosperms. More likely is the possibility that two sets of factors govern the sites of the stamens, one associated with the sepals and determining the positions of stamens 1–5 as axillary, or nearly so, and another set, associated with the younger primordia and persisting for 2 plastochrons after primordium initiation, which govern the sequence in which stamens arise at the predetermined sites.

In the positioning of the antepetalous stamens two sets of factors may again be invoked. The sequence of initiation of stamens 6–10 suggested that it was determined by the positions of the older, adjacent stamens (Table 8). However, this is not consistent with the previous conclusion that the factors determining the sequence of stamens 1–5, and associated with the sepals and these antesepalous stamens, lasted for only two plastochrons. There is a difference of at least three plastochrons between any antepetalous stamen and its antesepalous neighbours (Fig. 1). A more reasonable hypothesis is that the sequence of initiation of stamens 6–10 is also determined by only the two previously initiated primordia. Although this would be inadequate to position these stamens correctly since the error would be about 20° (Table 8) it might be sufficiently approximate to determine which of these stamens is next to be initiated. If the sites of these stamens are determined by the petals (or the adjacent stamens) the specification of the stamen positions would only have to discriminate between sites on average 72° apart, i.e. an error of anything up to 30° should not alter the observed sequence.

The initiation of primordia in the Silene flower is therefore understandable as the product of two sets of factors, one set governing the positions at which primordia will arise and a second set, persisting for only two plastochrons, governing the sequence in which these primordia are initiated. The positioning of all the primordia is ultimately traceable to the sepals, since these appear to determine the sites of the petals and, directly or indirectly, the stamens. The carpels also seem to be positioned with reference to the sepals.

The factors which determine the positioning of leaves are also thought of as determining the sequence of leaf initiation on the assumption that any position on the flanks of the apex is a potential leaf site (Richards, 1948, 1951; Thornley, 1975). The initiation of the sepals of Silene may also be thought of in this same way. But in the flower there are obviously and characteristically orthostichies, sepals and antesepalous stamens, and petals and antepetalous stamens being on the same, or very nearly the same radii. This is the same relationship as subtending leaves bear to their axillary primordia. Studies of phyllotaxis have usually been concerned with the positioning solely of the leaf primordia. A notable exception is the work of Snow (1965) who was able to show that the positioning of leaf and axillary primordia in Cucurbita could be affected by both other leaf and other axillary primordia. The phyllotaxis of the Silene flower can therefore be thought of as analogous to that of a leafy shoot with precocious axillary buds.

The close similarity of the relative growth rates of the apex at the points of initiation of the primordia of the floral members with the subsequent rates of growth of these primordia measured directly from changes in their volumes (Table 3) suggests that the growth rates of the primordia are determined at initiation. The same conclusion could be drawn about the growth rates of leaf primordia of Trifolium and Triticum in which the relative growth rates of successive leaf primordia were different and the differences were traceable back to the time of leaf initiation (Williams, 1975). These values (Table 3) show that it may be possible to learn much about the changes in growth rate during the transition to flowering by a careful study of the phyllotaxis, which is more easily achieved than making detailed growth measurements.
ACKNOWLEDGEMENTS

I am grateful to Ken Parker for his assistance, and to the Agricultural Research Council who made this possible through Grant No. AG 15/105.

LITERATURE CITED


Aberrations in flower development in *Silene*

R. F. Lyndon

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Pages 233–235
Introduction

In following the development of flowers of *Silene coeli-rosa* (L.) Godron (Lyndon 1977, 1978a, 1978b, 1978c) a significant proportion of flowers were found which had aberrant numbers of floral members. From casual inspection it was not obvious whether aberrations in one of the floral whorls (sepals, petals, stamens, or carpels) might be correlated with aberrations in other parts of the flower. If there were patterns or correlations these might be expected to provide some pointers to the developmental interrelationships of the various floral whorls. Since it was also obvious that the frequency of aberrant flowers varied with the growth temperature, it seemed possible that the pattern of aberrations might vary too and again might prove helpful in interpreting the growth of the normal flower.

Materials and Methods

Plants of *Silene coeli-rosa* (L.) Godron were grown and induced to flower at 13, 20, and 27°C (Miller and Lyndon 1976; Lyndon 1977). Young flowers were examined by dissection as soon as all the floral primordia had been initiated. Younger flowers with primordia still to be initiated were carefully excluded from the present analysis.

Results and Discussion

The reason for the occurrence of aberrant flowers is obscure. Their frequency was not directly related to the effectiveness of the inductive treatment, since induction was most effective at 13°C and least so at 27°C (Table 1) but the least proportion of aberrations occurred at 20°C. At 27°C more than a third of the flowers were aberrant (Table 1). It was thought that the frequency of aberrations might have been inversely related to the growth rate of the developing flowers, which was least at 13 and 27°C (Lyndon 1978c), but when the incidence of aberrations in the different whorls was tested against their growth rates there was no significant correlation ($r = -0.37; p > 0.1$). The most that can be said is that in *Silene*, as in other plants (Matzke 1929; Heslop-Harrison and Woods 1959; Meyer 1966; Garrod and Harris 1974), aberrancy is increased by relatively high and relatively low temperature during induction and flower development. About half the aberrant flowers were aberrant only in one of the floral whorls. At 20°C, at which the frequency of aberrant flowers was least,
TABLE 2. Frequency of flowers (percentage) with different numbers of aberrant floral whorls

<table>
<thead>
<tr>
<th>No. of whorls aberrant</th>
<th>13°C</th>
<th>20°C</th>
<th>27°C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>68</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>16</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>11</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

TABLE 3. Frequency of aberrations in different whorls. Values are given as a percentage of aberrant flowers with wrong number of members in whorl

<table>
<thead>
<tr>
<th></th>
<th>13°C</th>
<th>20°C</th>
<th>27°C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepals</td>
<td>53</td>
<td>5</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>Petals</td>
<td>53</td>
<td>37</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Stamens</td>
<td>62</td>
<td>68</td>
<td>85</td>
<td>73</td>
</tr>
<tr>
<td>Carpels</td>
<td>27</td>
<td>42</td>
<td>32</td>
<td>31</td>
</tr>
</tbody>
</table>

The proportion aberrant in more than one whorl was also least (Table 2).
The whorl which was most often aberrant was the stamens (Table 3) and as with the other floral members there was a tendency for a greater proportion of aberrant flowers with parts missing than with extra parts (Table 4). There were no consistent differences between plants grown at different temperatures. In about a third of the aberrant flowers only the stamens, and no other floral parts, were affected (Table 5). This suggests that the initiation of stamen primordia may be influenced by sepal, petal, and carpel primordia. This would be consistent with the supposition that the stamens are qualitatively different from the other floral parts and are basically axillary structures (Lyndon 1978a, 1978b) or analogous to them (Dengler 1972), whereas the other floral whorls are not. In Stellaria, however, the carpels were more often aberrant than even the stamens (Matzke 1929).

In very few plants were there aberrant numbers of sepals without there also being aberrant numbers in at least one other whorl (Table 5). This suggests the possibility that aberration in one whorl may lead to aberrations in subsequent whorls. Aberrations in more than one whorl are more likely to be connected if the aberration is quantitatively the same in both whorls. A comparison of the numbers of parts in pairs of whorls, of which at least one was aberrant, is made in Table 6. It is clear that for five of the six comparisons the numbers of parts in different whorls are not usually related. The only exception is the sepals and petals, since almost half the flowers in which at least one of these whorls was aberrant had the same aberration in the other whorl too. This suggests that the numbers of petals and sepals are more closely linked than the numbers of parts in any other pair of whorls and is consistent with the hypothesis that the positions of the sepals determine the positions of the petals (Lyndon 1978b). However, the occurrence of some plants with an aberrant number of sepals but a correct number of petals or vice versa (Table 5) shows that the number of petals is not entirely dependent on the number of sepals.

The data from the aberrant flowers therefore provide further evidence consistent with the following hypothesis: (a) usually the positions of the petal primordia are determined by the sepals, (b) the number of primordia in a floral whorl can vary independently of the number of primordia in the preceding whorl, and (c) the physiological factors determining the positions of the stamens may differ qualitatively from those determining the positions of the members of the other floral whorls, which would be consistent with the stamens being essentially axillary structures in contrast with the other floral members.

The different frequencies of aberrant flowers in different experimental environments suggest the possibility that this may also be a feature of some plants in the field growing at the edges of their range or exposed to unusual weather conditions. Under such circumstances some aberrations, such as increased numbers of stamens, might well become adaptive (Stebbins 1974). The possibility also presents itself of further manipulation of the numbers

TABLE 4. Frequency of aberrant flowers (number of flowers) with missing or extra parts

<table>
<thead>
<tr>
<th></th>
<th>13°C</th>
<th>20°C</th>
<th>27°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepals</td>
<td>29</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Petals</td>
<td>30</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Stamens</td>
<td>25</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Carpels</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

The whorl which was most often aberrant was the stamens (Table 3) and as with the other floral members there was a tendency for a greater proportion of aberrant flowers with parts missing than with extra parts (Table 4). There were no consistent differences between plants grown at different temperatures. In about a third of the aberrant flowers only the stamens, and no other floral parts, were affected (Table 5). This suggests that the initiation of stamen primordia may be influenced by sepal, petal, and carpel primordia. This would be consistent with the supposition that the stamens are qualitatively different from the other floral parts and are basically axillary structures (Lyndon 1978a, 1978b) or analogous to them (Dengler 1972), whereas the other floral whorls are not. In Stellaria, however, the carpels were more often aberrant than even the stamens (Matzke 1929).

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The different frequencies of aberrant flowers in different experimental environments suggest the possibility that this may also be a feature of some plants in the field growing at the edges of their range or exposed to unusual weather conditions. Under such circumstances some aberrations, such as increased numbers of stamens, might well become adaptive (Stebbins 1974). The possibility also presents itself of further manipulation of the numbers
of organs in floral whorls in order to investigate experimentally the physiological relationships of the different parts of the flower during development.

Acknowledgments

I am grateful to Ken Parker for technical assistance and to the Agricultural Research Council for making this possible through grant No. AG 15/105.


**Erratum**


For: "This suggests that the initiation of stamen primordia may be influenced by sepals, petal and carpel primordia."

Read: "This suggests that the initiation of stamen primordia may be influenced by factors which are less effective in influencing the sepals, petal and carpel primordia."
Synchronisation of Cell Division in the Shoot Apex of *Silene* in Relation to Flower Initiation

Dennis Francis* and Robert F. Lyndon

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Abstract. In plants of *Silene coeli-rosa*, induced to flower by 7 LD, synchronisation of cell division in 20 per cent or more of the cells in the shoot apical dome was found on the 8th and 9th days after the beginning of induction, during the plastochron before sepal initiation. Synchronisation was inferred from the changes in the proportions of cells with the 2C and 4C amounts of DNA, and changes in mitotic index and labelling index. From the peaks of mitotic index a cell cycle of 10 h was measured for the synchronised cells, half that of cells in the apices of uninduced plants in short days. The faster cell cycle and synchronisation in the induced plants was associated with a shortening of both G1 and G2, suggesting two control points, while S and M remained unchanged. These results are compared with those from other plants in which synchronisation occurs at the beginning rather than the end of evocation.

Key words: Cell cycle – DNA – Flower initiation – Shoot apex – *Silene*.

Introduction

The occurrence of successive peaks of mitotic index in the apices of plants undergoing floral induction (Bernier, 1971) suggests that synchronisation of cells into a particular phase of the cell cycle may be an important feature of evocation in the shoot apex. This conclusion may be drawn particularly from observations on *Sinapis alba*, a long day plant, where there was a significant increase in the mitotic index 22 h after the beginning of induction (Bernier et al., 1967) and a subsequent increase in the percentage of cells synthesising DNA 18 h later (Kinet et al., 1967). Moreover 30 h after the beginning of induction cells in the *Sinapis* apex were predominantly in G1 (Jacqmard and Miksche, 1971). A second increase in the mitotic index 60 h after the start of induction (Bernier et al., 1967) was thought to be a result of the synchronised population of cells in G1 moving on to a second mitosis (Bernier, 1969). This second peak of mitotic index occurred immediately before flower initiation and roughly one cell cycle after the initial increase in mitotic index observed 30 h from the beginning of induction (Bodson, 1975).

In *Sinapis* floral induction is achieved with a single 20 h day, which is shorter than one cell cycle, and flower initiation takes place less than two days later which is less than 2 cell cycles later (Bernier et al., 1967; Bodson, 1975). In *Silene* there are 9 days and about 10 cell cycles between the beginning of floral induction and flower initiation (Miller and Lyndon, 1976). No clear evidence was obtained for a synchronisation of cell division in the shoot apex of *Silene* during the first LD (Francis and Lyndon, 1978a) although the growth rate of the shoot apex was faster in induced plants during the first LD than in non-induced plants in SD. Thereafter the growth rate of apices in LD was similar to that of apices in SD (Miller and Lyndon, 1976). Although synchronisation could not be detected on the first L.D, it seemed possible that it might occur when the growth rate again increases about the 8th day after the beginning of induction (Miller and Lyndon, 1975, 1976). This faster rate of growth takes place during the plastochron before sepal initiation (Miller and Lyndon, 1976; Lyndon, 1977). If synchrony is a regular feature of the transition to flowering then we may perhaps expect it to occur on this eighth day in *Silene*. It seems...
possible that the synchronisation in *Sinapis* might occur as part of inflorescence formation. In *Silene* the apex transforms into the first flower and so we ought to be able to distinguish whether synchronisation is likely to be a feature of all flowering apices or occurs only in those in which the apex is concerned with making the inflorescence axis.

It was the purpose of the present investigation, therefore, to examine photoinduced apices of *Silene* for evidence of synchronisation during the eighth day after the start of floral induction, using the techniques we used during exposure of plants to 1 LD (Francis and Lyndon, 1978a, 1978b).

Materials and Methods

**Conditions of Growth**

Plants of *Silene coeli-rosa* L. Godron were grown in SD at 20°C and selected for developmental uniformity when they were 28 days old as described previously (Miller and Lyndon, 1975, 1976). The seedlings were then either maintained in SD, exposed to 7 LD (which induced 100% of the plants) and transferred back to SD, or exposed to 8 LD. On the eighth day after the start of floral induction measurements began. Under each set of conditions three seedlings were fixed in 3:1 (v/v) absolute ethanol-glacial acetic acid at 2 h intervals over the 24 h period of the experiment beginning at 0900 h of day 8. Plants in SD were maintained in 8 h days with light from 0900 h to 1700 h each day. The LD conditions were the same but with the addition of low intensity tungsten light from 1700 h to 0900 h of the following day (Miller and Lyndon, 1975, 1976).

**DNA Measurements and Mitotic Index**

The amount of DNA per interphase nucleus was measured, using a Vickers M85 scanning microdensitometer, in Feulgen-stained squash preparations of apical domes above the 9th leaf pair, each slide being prepared, scored and calibrated as detailed before (Francis and Lyndon, 1978a).

The proportion of cells with <3C and >3C amounts of DNA were calculated and are referred to as G1 and G2 populations respectively. Any differences in the ratios of G2/G1 therefore tend to be underestimates (Francis and Lyndon, 1978a).

At each fixation time the mitotic index (sum of prophase, metaphase, anaphase and telophase figures expressed as a percentage of all cells) was measured in a series of transects across the width of the coverslip; 500 nuclei per slide and 3 slides per treatment at each fixation time were scored.

**Labelling Index**

The labelling index (the percentage frequency of nuclei labelled with ³H]thyidine) was measured in Feulgen-stained squashes of apical domes above the 9th leaf pair. Plants were partially defoliated as described by Miller and Lyndon (1975). Methyl[³H]thyidine (S.A. 5000 mCi/mmol) was applied to each apex for 2 h, every 2 h of the experiment as described previously (Francis and Lyndon, 1978a). Apices were then fixed in 3:1 (v/v) ethanol-glacial acetic acid. Permanent autoradio-

Results

**Proportions of Cells in G1 and G2**

In SD the proportion of cells in G1 was roughly twice that in G2 (as specially defined – see Methods) over the period from 0900–2300 h of day 8 (Figs. 1a–c and 2). During the next 6 h values fluctuated somewhat such that at 0300 h of day 9 the G2 proportion was greater than the proportion in G1 (Figs. 1e–d and 2). By 0700 h, however, the pattern reverted to that found on the previous day (Figs. 1d and 2). Whether this transient oscillation is a random fluctuation or perhaps part of an endogenous rhythm is not known but the accompanying fluctuations in mitotic index and labelling index were not statistically significant (see below).

In the plants exposed to 7 LD and transferred back to SD the proportions of cells in the apical dome in G1 and G2 varied from one fixation time to another (Figs. 1a–d and 2). Thus the G2/G1 ratio decreased from 1.13 at 0900 h to 0.56 at 1300 h rising to 2.13 at 2300 h of day 8 and subsequently falling again to 0.39 at 0300 h of day 9 (Fig. 2). Thus, the ratio was constantly changing in the apices of 7 LD plants whereas in SD it remained relatively constant. This would be consistent with a synchronised population of cells in the apices of 7 LD plants moving through the cycle more or less together whereas in SD cells were cycling asynchronously. If this is so, the synchronised population in LD would tend to be predominantly in G1 between approximately 1300–1500 h of day 8, in G2 between approximately 2100–2300 h of day 8 and in G1 again about 0300 h of day 9.

**Mitotic Index and Labelling Index**

In SD, the variation in the mitotic index from one sampling time to another (Fig. 3), was not statistically significant at the 0.05 level as shown by a three-way analysis of variance (F = 2.0; 12 and 51 d.f.). There was a significant interaction, however, between time of day and the effect of 7 LD (F = 2.27; 12 and 51 d.f.). In the 7 LD plants the mitotic index peaked at 1500 h of day 8 and again at 0100 h of day (Fig. 3). There was a possible third peak at 0700 h of day 9.
In SD, labelling index fluctuated over the experimental period (Fig. 4) but again the variation from one sampling time to another was not statistically significant at the 0.05 level as revealed by a three-way analysis of variance ($F=1.81; 12$ and $51$ d.f.). Once more there was a significant interaction between time of day and 7 LD ($F=2.10; 12$ and $51$ d.f.). In the 7 LD plants the labelling index peaked at about 1900 h of day 8, approximately midway between the major peaks of mitotic index.

These results for mitotic index (Fig. 3) and labelling index (Fig. 4) suggest that in the apical domes of plants given 7 LD a synchronised population of cells was in mitosis at approximately 1500 h of day 8, about the middle of S (the period of DNA synthesis) at approximately 1900 h of day 8 and in mitosis once again at 0100 h of day 9. This would give a cell cycle length of approximately 10 h for the synchronised population.

**Lengths of the Component Phases of the Cell Cycle**

The lengths of S, M, G1 and G2 were calculated for cells in SD (Table 1) from the mean labelling index, mean mitotic index and the mean proportions of cells with <3C and >3C amounts of DNA respectively (corrected for the proportion of cells in S), calculated over the experiment as a whole (calculated from Figs. 2, 3 and 4 using the formulae of Nachtwey and Cameron (1968) and taking the length of the cell cycle in SD at day 8 to be 20 h (Miller and Lyndon, 1975)). Calculated in this way G1 and G2 are the longest phases, being about 10 h and 7 h re-
D. Francis and R.F. Lyndon: Synchronisation of Cell Division in Silene

Fig. 2. The relationship between the ratio of the proportions of cells in G2/G1 and time, in squashes of apical domes above the 9th leaf pair, on the 8th and 9th days after the beginning of induction maintained in SD (●) or exposed to 7 LD (○) or 8 consecutive LD(CLD) (△).

Since a synchronised population of cells was detected in the 7 LD plants (Figs. 1–4) it was not possible to calculate the lengths of the component phases of the cell cycle using the above method. We can, however, make an estimate of S as the interval between the mid-points of the ascending and descending limbs of the peak of labelling index for the 7 LD apices in Fig. 4, from which the labelling time of 2 h is subtracted. This gives a value of 2 h for S for the 7 LD apices. Since the peak of labelling index is at about 1900 h of day 8 this implies that S lasts from approximately 1800–2000 h. If we assume that mitosis is of constant length, as it is in the apices of other plants when they become floral (Corson, 1969; Bodson, 1975) and that it therefore takes 1 h as it does in SD (Table 1), then G1 would last from about 1530–1800 h (i.e. 2.5 h) and G2 from 2000–3000 h (i.e. 4.5 h). Thus, when we compare the lengths of the component phases of the cell cycle in apices of 7 LD plants with those in SD plants, both G1 and G2 are shorter in the 7 LD plants but there is proportionately more shortening of G1 whilst S and probably M remain constant in length (Table 1).

Degree of Synchrony

Since neither mitotic index nor labelling index rose to 100% at any time (Figs. 3 and 4) there was
Table 1. Length (h) of the cell cycle (C) and its component phases on day 8 in the apical dome above the 9th leaf pair in apices in SD or exposed to 7 LD. Values for SD calculated from the corresponding mean proportions of nuclei in the cell cycle phases at days 8–9, assuming that the length of the cell cycle = 20 h, and that the age gradient is exponential

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GI</th>
<th>S</th>
<th>G2</th>
<th>M</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0.57</td>
<td>0.11</td>
<td>0.29</td>
<td>0.03</td>
<td>20</td>
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<tr>
<td>SD</td>
<td>9.8</td>
<td>2.2</td>
<td>7.1</td>
<td>0.9</td>
<td>20</td>
</tr>
<tr>
<td>7 LD</td>
<td>2.5</td>
<td>2.0</td>
<td>4.5</td>
<td>1.0</td>
<td>20</td>
</tr>
</tbody>
</table>

a From Figs. 1–4. S = proportion of cells labelled with [3H]thy midine (from labelling index); G1 = proportion with < 3C amount of DNA minus half the proportion in S; G2 = proportion with > 3C amount of DNA minus half the proportion in S

b Derivation as described in text

presumably only a partial synchronisation of cells in the meristem. What is the degree of synchronisation? The lowest mitotic index value for the 7 LD apices (2.35%) was recorded at 2100 h of day 8 (Fig. 3). This value could be taken as a measure of the percentage of cells in mitosis that are not part of the synchronised population. Since the proportion of cells dividing asynchronously must be subject to some variation a better indication of the true proportion of cells dividing randomly in the 7 LD apices can be obtained from the mean value for mitotic index of apices in SD over the experimental period (3.03 ± 0.78%). The area of the 7 LD graph in the peaks above 3.81 would represent the synchronised population and was calculated as a minimum value of approximately 22% of the total cells in the 7 LD apices whereas the lowest mitotic index of 7 LD (2.35%) gave a value of 53%. The maximum mitotic index observed in the synchronised population of cells was about 8%. Clearly, if 22% of the cells are synchronised we should observe a mitotic index of 22% above the value for the asynchronous population. However, if the length of mitosis itself remains unchanged, and is 1 h as it is in uninduced apices (Table 1), then the width of the mitotic peaks in the synchronised population and their non-rectangular shape (Fig. 3) indicates that synchrony is not precise within each peak, and is consistent with a lower peak mitotic index.

Proportions of Cells in G1 and G2 in 8 Consecutive LD

The synchronisation of cell division in the apical dome observed when plants are exposed to 7 LD and returned to SD (Figs. 1a–d) could have resulted from the change in photoperiod at this time. To test this plants were maintained in 8 consecutive LD to avoid the change in photoperiod. It was found that the proportions of cells in G1 and G2 changed with time over the 12 h of this experiment (Figs. 5 and 2) in the same way as the plants given 7 LD and then transferred back to SD (Figs. 1 and 2). Thus, the lowest value for the G2/G1 ratio in 8 LD was recorded at 1500 h of day 8 which was similar to that obtained at 1300 h of day 8 in the 7 LD plants (Figs. 1b and 2), and the ratio then increased in 8 LD as it did in 7 LD plants. These results indicate that there was a synchronisation of cells in the apical dome after 8 consecutive LD, showing that the synchronisa-
Discussion

Synchronisation of cell division in the shoot apex of *Silene* has been observed during the 8th day after the beginning of induction, which is also the day before sepal initiation. The cell cycle length was 10 h as measured between the peaks of mitotic index at 1500 h of day 8 and 0100 h of day 9 (Fig. 3). A second cycle of 6 h between 0100 h and 0700 h of day 9 is less certain because a 2nd peak of labelling index would have been expected somewhere between 0100 h–0700 h of day 9. The labelling index in LD only peaked once, however, at about 1900 h on day 8 (Fig. 4), although the decreases in the G2/G1 ratio at 0300 h of day 9 (Fig. 2) and its subsequent slight rise at 0500 h would be consistent with such a 6 h cycle of less well synchronised cells.

It was calculated that at least 22% of cells in the apices of 7 LD plants were probably part of a synchronous population leaving up to 78% distributed around the cell cycle asynchronously. The rate of increase in cell number in apices of *Silene* 8 days from the beginning of induction was consistent with a mean cell cycle length of 6–8 h (Miller and Lyndon, 1976). Thus, both the synchronous and asynchronous populations in apices given 7 LD (Fig. 3) have cell cycle lengths of 10 h or less.

In *Sinapis* synchronisation of cells in G1 follows a sudden release of meristematic cells from G2 into mitosis (Kinet et al., 1967). It may be inferred that in *Sinapis* there is a shortening of the G2 phase of the cell cycle which would result in the synchronisation in G1. In *Silene* estimates of the lengths of the component phases of the cell cycle indicate both G1 and G2 were shortened in apices in LD compared with those in SD (Table 1) with proportionately more shortening of G1 than G2 whilst S and M remained constant. The shortening of both G1 and G2 would effectively synchronise cells in the meristem and also result in a shorter cell cycle in LD than in SD (Fig. 3; Table 1). This is different from the shortening of the cell cycle after exposure of *Silene* plants to 1 LD. During the first LD a shortened cycle of 13 h was the result of decreases in the length of G1 and possibly S whilst G2 and M remained constant (Francis and Lyndon, 1978b). Thus, there appear to be at least two control points, one in G1 and one in G2, required for the shortening of the cell cycle and the synchronisation on day 8 whereas during the first LD the control point for the shortening of the cell cycle was probably only in G1 (Francis and Lyndon, 1978a, 1978b).

Bernier (1971) has described an increase in mitotic index in the shoot apex of *Sinapis* as one of the early events of evocation. Since the time interval between the beginning of induction and flower initiation in *Sinapis* is rather short (about 3 days; Bernier et al., 1967) it is perhaps difficult to say whether the apparent synchrony is part of evocation or flower initiation, or both. In *Silene* synchronisation of cell division in the shoot apex is not an early feature of evocation (Francis and Lyndon, 1978a) but occurs on days 8–9 (Figs. 1–4), which is just before the initiation of the flower on the 9th day after the beginning of induction (Miller and Lyndon, 1976).

The present measurements were done only on days 8 and 9 and we do not know when synchronisation begins or how long it lasts. Synchronisation presumably begins when the growth rate increases after day 7 (Miller and Lyndon, 1976) and might be expected to disappear when the growth rate decreases, when the sepals are initiated (Lyndon, 1977). The absence of a peak in the G2/G1 ratio (Fig. 2) just before the first peak of mitotic index (at 1500 h; Fig. 3) might suggest that the cells are just becoming synchronised at this time by a speeding up of G2 (which would tend to decrease the G2/G1 ratio), as happens in *Sinapis*. Also, the lack of a second peak in labelling index (Fig. 4) after the second peak of mitotic index at 0100 h of day 9 (Fig. 3) might further suggest that synchrony is becoming lost by this time and that therefore the cells are synchronised only for approximately one cell cycle, on day 8.

The data indicated that there could be two populations of cells in the shoot apex, one synchronous and the other asynchronous. It would clearly be of interest to know whether these populations are mixed together in the apex or whether synchronisation is localised, perhaps to the sites where floral organs are to be initiated.

The occurrence of synchronisation of cell division during the transition to flowering could perhaps be an incidental by-product of changes in nutrient or growth regulator levels within the apex. Alternatively it could be an essential part of the flowering process. If a substance essential to flowering was synthesised predominantly in one part of the cell cycle a synchronisation might be necessary to allow a critical threshold amount of this hypothetical substance to accumulate within a limited time. Another possibility is that synchronisation is necessary in order to have a critical number of cells in mitosis, for example, perhaps to allow changes in polarity of growth in the meristem. In any case it is of obvious interest to know the nature of the substances which cause
synchronisation for it is likely that they are key substances in bringing about the transition from vegetative to floral growth.

We are grateful to the Agricultural Research Council for support through Grant No. AG 15/138.

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Rates of Growth and Primordial Initiation During Flower Development in *Silene* at Different Temperatures

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Accepted: 19 June 1978

**ABSTRACT**

The growth of the flower and its constituent parts was measured in *Silene coeli-rosa* plants, induced at 3, 20 and 27 °C, in order to try and identify those processes which consistently occurred and would therefore be more likely to be essential for flower formation.

The increased growth rate of the apical dome just before or about the time of sepal initiation was not maintained in the flower, the growth rate of which was comparable to that of a vegetative apex until all the carpels had been initiated, when it decreased further. The primordia of the same whorl all had similar growth rates so that the relative sizes of the primordia reflected their relative ages since their initiation. The relative growth rate of the stamens was the same (13 and 20 °C) or less (27 °C) than that of the sepals, but the relative growth rate of the petals was lower than either. The growth rate of the flower axis was least at the sepal node and increased both distally and proximally from this region.

The plastochron during sepal initiation was shorter than for leaf initiation and tended to be shorter still during initiation of stamens and petals. Increasing temperature increased the rate of primordial initiation but at 27 °C the growth rates of the primordia were lowest although the rates of primordia initiation were highest. The form of the flower, as exemplified by the relative sizes of the primordia at the moment when all carpels had been initiated, was constant despite the differing growth rates and sizes of the primordia on initiation in different temperatures. It is concluded that neither the initiation of the primordia in the flower nor the form of the flower is determined primarily by the relative growth rates of its component parts.

Key words: *Silene coeli-rosa*, flower development, primordia initiation, growth.

**INTRODUCTION**

The transition from vegetative growth to flowering at the shoot apex is usually accompanied by increases in the RNA and protein content of the apical cells, and by an increase in the rate of growth and cell division (Bernier, 1971). However, flowering may be induced without these accompanying changes as in *Chenopodium* (Seidlova and Krekule, 1973) and *Silene* (Miller and Lyndon, 1977). Conversely, increases in mitotic activity, RNA content and growth rate occur also in meristems stimulated into vegetative growth (Usciai, Coddaccioni and Guern, 1972) and so these changes are not peculiar to the transition to flowering and may be related to changes in growth rate at the apex rather than to the morphogenetic change which occurs on flowering (Lyndon, 1977).

If many of the cytological and physiological changes which are seen at the shoot apex when flowering is induced are not necessarily linked to flowering one may then ask: what are the processes which occur at the shoot apex which are intrinsic to flowering? One approach, which is adopted in this present work, is to induce flowering under different environmental conditions and to follow the growth of the apex during transition from vegetative to floral growth and during early flower development. If there are characteristic changes, in the rate of growth in different parts of the apex or in the rate of primordium
initiation, which always occur these are therefore more likely to be directly concerned with the transition to flowering than changes which are not consistently found.

*Silene* plants have therefore been induced to flower by long days at three different temperatures, 13, 20 and 27 °C (Lyndon, 1977), which would be expected to result in different growth rates and different rates of primordial initiation. The changes in these growth rates of the apex during induction and evocation at these temperatures have already been described (Lyndon, 1977) as have the morphological changes during the transition to flowering and during early flower development (Lyndon, 1978a). In the present paper the growth rates of the apex and primordia, and the rates of initiation of primordia, during early flower development at 13, 30 and 27 °C are compared.

**MATERIALS AND METHODS**

Plants of *Silene coeli-rosa* (L.) Godron were grown in short days at 20 °C for 28 days before being selected for developmental uniformity and then induced to flower by exposure to long days (Miller and Lyndon, 1975, 1976). The day on which induction in long days was begun was designated day 0. Plants were induced to flower by 7 long days at 13 or 20 °C and 12 long days at 27 °C, 13 °C being most and 27 °C least effective. The plants were then transferred back to short days at the same temperature as the long-day treatment had been (Lyndon, 1977). Since flower initiation and development did not begin until after (13 and 20 °C) or near (27 °C) the end of the inductive treatment (Lyndon, 1977) the development of the flower essentially took place in non-inductive photoperiods.

**Initiation of primordia**

Plants were examined daily with a binocular dissecting microscope. The number of leaf pairs and the number and type of primordia which were visible in the developing flower were recorded. Measurements were repeated on successive sowings of plants until for each day a total of 30 plants had been recorded with 9 leaf pairs below the flower at 13 °C, with 9 leaf pairs at 20 °C or with 11 leaf pairs at 27 °C.

**Measurement of tissue volumes**

Duplicate plants were taken each day and were fixed [in ethanol:acetic acid, 3:1 (v/v)] embedded in wax, serially sectioned longitudinally at 10 μm, and stained. Duplicate outline tracings of each section were made, using a camera lucida, and divided into consistently recognizable but arbitrary regions (Fig. 1). One set of tracings, representing an apex, was stapled together, in order, to make a simple three-dimensional reconstruction of the apex which was an invaluable aid in identifying the individual primordia. The primordia could be identified individually and consistently because of the constant relationship of the order in which the primordia were initiated to the direction of the helix of the unequal axillary buds below the flower (Lyndon, 1978a). The duplicate set of tracings, made on standard paper, was cut up and weighed so that the volumes of the regions could be calculated (Miller and Lyndon, 1976). In some apices the number of nuclei were counted in all sections of each apex to give the number of cells in each region.

A complication was that all plants did not initiate flowers after the same number of leaves had been produced. Variation from this source was eliminated by measuring tissue volumes and cell numbers only in those apices which had the modal number of leaves below the flower. These were plants which on flowering had 9 leaves at 13 or 20 °C and 11 leaves at 27 °C.
RESULTS

Initiation of primordia

The mean time at which a given primordium was initiated was taken as the time at which this primordium was present in 50 per cent of the plants (Table 1). These times were read from graphs showing the percentage of plants which had achieved a given stage of development at a given time and from them the rate of initiation of primordia can be calculated. This is expressed more conveniently as the plastochron, which is the mean time between the initiation of successive primordia (Table 1).

At all temperatures the rate of primordial initiation increased, and the plastochron decreased considerably, at the transition from vegetative to floral growth (Table 1). The plastochron generally decreased further at the initiation of the stamens and petals. The plastochron during carpel initiation was about the same as during stamen initiation. In all plants there was a single longer plastochron between the end of stamen initiation and the beginning of carpel initiation.

The general effect of increasing temperature was to speed up the rate of initiation of primordia with a consequent decrease of the plastochron. The mean plastochron decreased from 0.52 days at 13 °C to 0.25 days at 27 °C (Table 1).

Temperature affected the rate of initiation of all the types of primordia, but the effects were least on the sepals. The rates of sepal initiation at 13 and 27 °C were similar. This implies that the effect of temperature was mainly on the rate of initiation of the other primordia in the flower. With increasing temperature the plastochron shortened most during the initiation of the petals and the stamens (Table 1).

Growth rates

The increase in the growth rate of the apical dome which occurs just before flower initiation (20 and 27 °C) or just after (13 °C) (Table 2) was not sustained by the meristem of the flower, for the growth rate fell to a value which is less than in the apical dome before flower initiation and approaches or is lower than that of the apical meristem in vegetative, non-induced plants.
Lyndon—Growth Rates in Developing Silene Flowers

Table 1. Day after the beginning of induction that 50 per cent of the plants had reached the given stage of development: and the lengths of the plastochron (i.e. days between initiation of successive primordia) in developing Silene flowers, grown at 13, 20 or 27 °C

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Increase in no. of primordia</th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative growth*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beginning of induction to 1 sepal†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 sepal</td>
<td></td>
<td>12-9</td>
<td>9-5</td>
<td>11-4</td>
</tr>
<tr>
<td>5 sepals</td>
<td></td>
<td>15-5</td>
<td>10-3</td>
<td>13-6</td>
</tr>
<tr>
<td>1 stamen</td>
<td></td>
<td>16-1</td>
<td>10-5</td>
<td>13-8</td>
</tr>
<tr>
<td>10 stamens</td>
<td></td>
<td>21-0</td>
<td>13-8</td>
<td>15-4</td>
</tr>
<tr>
<td>1 stamen – 10 stamens (not including petals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 petal</td>
<td></td>
<td>17-2</td>
<td>11-4</td>
<td>14-3</td>
</tr>
<tr>
<td>5 petals</td>
<td></td>
<td>18-0</td>
<td>11-8</td>
<td>14-4</td>
</tr>
<tr>
<td>10 stamens – 1 carpel</td>
<td></td>
<td>24-4</td>
<td>15-6</td>
<td>16-0</td>
</tr>
<tr>
<td>1 carpel</td>
<td></td>
<td>25-3</td>
<td>17-1</td>
<td>17-3</td>
</tr>
<tr>
<td>1 sepal – 5 carpels</td>
<td></td>
<td>24</td>
<td>0-52</td>
<td>0-32</td>
</tr>
</tbody>
</table>

* Data from Lyndon, 1977.
† Assuming all plants had on average 7-3 leaf pairs at the beginning of induction (Lyndon, 1977).
‡ Final number of leaf pairs minus 7-3; multiplied by 2; plus 1S.
§ Petals were also initiated at this time.

Table 2. Mean volume relative growth rates (per day) (± s.e. where values are means of individual primordia) of the apical dome and of the floral members (as delimited and lettered according to Fig. 1), and the ratios of the relative growth rates of the floral members

<table>
<thead>
<tr>
<th>Growth temperature</th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical dome in vegetative plants*</td>
<td>0-31</td>
<td>0-67</td>
<td>0-19</td>
</tr>
<tr>
<td>Apical dome at beginning of floral induction*</td>
<td>0-44</td>
<td>0-70</td>
<td>0-51</td>
</tr>
<tr>
<td>Fastest growth rate of apical dome, just before (20, 27 °C) or just after (13 °C) sepal initiation*</td>
<td>0-80</td>
<td>1-06</td>
<td>1-49</td>
</tr>
<tr>
<td>(F) Sepals</td>
<td>0-47±0-01</td>
<td>0-64±0-02</td>
<td>0-57±0-01</td>
</tr>
<tr>
<td>(G) Petals</td>
<td>0-39±0-01</td>
<td>0-56±0-03</td>
<td>0-26±0-01</td>
</tr>
<tr>
<td>(H) Stamens</td>
<td>0-49±0-02</td>
<td>0-66±0-05</td>
<td>0-34±0-04</td>
</tr>
<tr>
<td>(E) Floral meristem (distal to stamens)</td>
<td>0-27</td>
<td>0-37</td>
<td>0-26</td>
</tr>
<tr>
<td>(D) Axis of stamen and petal nodes</td>
<td>0-20</td>
<td>0-24</td>
<td>0-20</td>
</tr>
<tr>
<td>(C) Axis of sepal node</td>
<td>0-09</td>
<td>0-13</td>
<td>0-12</td>
</tr>
<tr>
<td>(B) Stem (pedicel)</td>
<td>0-25</td>
<td>0-29</td>
<td>0-29</td>
</tr>
<tr>
<td>(A) Axillary bud</td>
<td>0-33</td>
<td>0-36</td>
<td>0-43</td>
</tr>
<tr>
<td>Ratios of relative growth rates of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petals/stamens</td>
<td>0-80</td>
<td>0-85</td>
<td>0-76</td>
</tr>
<tr>
<td>Petals/sepals</td>
<td>0-83</td>
<td>0-88</td>
<td>0-46</td>
</tr>
<tr>
<td>Stamens/sepals</td>
<td>1-04</td>
<td>1-03</td>
<td>0-60</td>
</tr>
</tbody>
</table>

* Data from Lyndon, 1977.
The increases in volume of representative parts of the flowers are shown in Figs 2-4. At each temperature the growth curves for the same types of floral member (e.g. sepal, stamen or petal) were similar in shape and so the data given in Figs 2-4 are representative of those floral members not shown there. At 13 °C there appear to be several stages in the growth curves, which are best exemplified by the data for sepal 1. There was a phase of rapid growth from day 13, when this 1st sepal is just initiated (Table 1), until about day 17 by which time all the sepals were present. There was then a phase of slow growth, from days 17–20, corresponding to the period during which the stamens and petals were initiated (Table 1). This was followed by another phase of rapid growth, until day 25, when initiation of the carpels had just been completed. The periods of rapid growth therefore correspond to the periods when sepals were being initiated and the period after all the stamens had been initiated and which ends with the initiation of the carpels. These growth curves are clearest for the sepals and for the axial parts of the flower, principally the stamen node (the axis at the level of insertion of the stamens) and the stem (the axis below the level of insertion of the sepals, i.e. the pedicel). These phases are not so obvious for the sepal node (the axis at the level of insertion of the sepals) or for the stamens and the petals, but since these latter were not in general initiated before day 17 the first period of rapid growth was over before they originated. The phases in the growth of the meristem distal to the stamens (and which included the developing carpels from day 24 onwards) do not clearly coincide with other events in flower development.

However, the growth curves for all parts of the flower suggest a decrease in growth rate when the carpels had all been initiated (day 25 onwards). This was also found from day 17 onwards at 20 °C (Fig. 3), but there is no evidence at 20 or 27 °C for the other growth phases which seemed to occur at 13 °C. This is probably because the rapid growth at 20 and 27 °C occurred before sepal initiation (Lyndon, 1977) and the plastochron between the last stamen and the first carpel was shorter than at 13 °C. At 27 °C there is a greater scatter in the data and there is no clear evidence for any differences in growth.
FIG. 3. Growth of representative parts of the flower at 20 °C. Key: see Fig. 2.

FIG. 4. Growth of representative parts of the flower at 27 °C. Key: see Fig. 2.
rate with time. Since there seemed to be a decrease in growth rate after carpel initiation was completed the growth rates given below refer only to the growth of the flower up to this point, i.e. up to and including day 25 at 13 °C, and day 17 at 20 and 27 °C. These were the days by which 50 per cent of the plants had five carpels (Table 1).

The growth rates of the different parts of the flower up to this point may best be compared by comparing the regression coefficients of the regressions of log_e volume on time (Table 2). The growth rates of sepals and stamens were similar (except at 27 °C where the stamen growth was slower than that of the sepals) but the growth rate of the petals was lower. The growth rates of all the primordia (except for the petals at 27 °C) were higher than the growth rate of the floral meristem distal to the youngest primordia (Table 2). In the axis of the flower the lowest growth rate was in the sepal node (the axial tissue at the level of insertion of the sepals) and the growth rate increased both distally (to the stamen node and the flower meristem) and proximally (to the pedicel and axillary bud) from this point. With the exception of the petals at 27 °C, at all three temperatures (a) the primordia of the floral members (sepals, petals and stamens) had a higher growth rate than the apical meristem of the flower (the floral meristem) and the axial tissue, (b) the growth rate of the petals was lower than that of the sepals and stamens, and (c) there was a gradient of increasing growth rate both distally and proximally along the axis from the level of insertion of the sepals.

The effect of increasing temperature, from 13 to 20 °C, was to increase the growth rate of all parts of the flower by about 30 per cent (Table 2). Increasing the temperature to 27 °C resulted in a growth rate which was lower than 20 °C and which was comparable to that at 13 °C, with the difference that at 27 °C the growth rates of the stamens and petals were lower than at 13 °C. The growth rates of the lower parts of the flower axis (sepal node, pedicel and axillary bud) were higher than at 13 °C and about the same as at 20 °C. This high temperature, 27 °C, at which the plants did not grow very well, therefore did not increase the rates of growth above those at 20 °C and resulted in a reduction in the growth rate of all those parts of the flower distal to the sepals (Table 2). However, the period from the initiation of the sepals to the initiation of all the carpels was only about 6 days at 27 °C compared to about 12 days at 13 °C (Table 1). The rate of primordial initiation was therefore not a function of the growth rate of the flowers.

The relationships of the growth rates of the sepals, petals and stamens are shown in Table 2. The ratios are the same at 20 as at 13 °C showing that the absolute growth rates of sepals, petals and stamens, although different, were all increased to exactly the same extent by increase in temperature. At 27 °C the growth rates of the petals and stamens were both reduced compared to the sepals but not relative to each other. These data suggest that at 13 and 20 °C the growth rates of sepals, petals and stamens may all be controlled by the same factors but that at 27 °C the growth rate of the petals and stamens may be controlled by factors different from those controlling the growth rate of the sepals.

Sizes of primordia

The sizes of the primordia of the sepals and stamens were proportional to their ages since initiation (Lyndon, 1978a) suggesting that (at a given temperature) their growth rates were all similar. The measurements showed that this was so and have confirmed that the sizes of the carpels and sepals at a given stage of flower development (when 50 per cent of the plants have formed five carpels) were in general proportional to their age, the oldest being the largest (Table 3). The form of the flower as exemplified by the relative sizes of the primordia at this stage of development was constant regardless of the temperature at which it was grown and regardless of differences in relative growth rates between different parts of the flower at different temperatures (Table 4). This implies
Lyndon—Growth Rates in Developing Silene Flowers

Table 3. Sizes of primordia ($10^3 \times \mu m^3$) at the time when all carpels have just been initiated (i.e. when 50 per cent plants have $\geq 5$ carpels). Primordia numbered in order of initiation. These values were calculated from the equations for the regression of loge volume against time, the values for the regression coefficients being the relative growth rates for the individual primordia.

<table>
<thead>
<tr>
<th></th>
<th>13°C (Day 25)</th>
<th>20°C (Day 17)</th>
<th>27°C (Day 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5936</td>
<td>3747</td>
<td>2803</td>
</tr>
<tr>
<td>2</td>
<td>6758</td>
<td>4353</td>
<td>3130</td>
</tr>
<tr>
<td>3</td>
<td>4669</td>
<td>3257</td>
<td>1824</td>
</tr>
<tr>
<td>4</td>
<td>4441</td>
<td>2561</td>
<td>2037</td>
</tr>
<tr>
<td>5</td>
<td>3938</td>
<td>2295</td>
<td>1096</td>
</tr>
<tr>
<td>Mean</td>
<td>5148</td>
<td>3243</td>
<td>2178</td>
</tr>
<tr>
<td>Stamens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1374</td>
<td>871</td>
<td>670</td>
</tr>
<tr>
<td>2</td>
<td>1151</td>
<td>888</td>
<td>613</td>
</tr>
<tr>
<td>3</td>
<td>838</td>
<td>415</td>
<td>432</td>
</tr>
<tr>
<td>4</td>
<td>730</td>
<td>502</td>
<td>365</td>
</tr>
<tr>
<td>5</td>
<td>638</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>6</td>
<td>423</td>
<td>310</td>
<td>196</td>
</tr>
<tr>
<td>7</td>
<td>391</td>
<td>260</td>
<td>176</td>
</tr>
<tr>
<td>8</td>
<td>354</td>
<td>208</td>
<td>65</td>
</tr>
<tr>
<td>9</td>
<td>325</td>
<td>232</td>
<td>143</td>
</tr>
<tr>
<td>10</td>
<td>258</td>
<td>122</td>
<td>102</td>
</tr>
<tr>
<td>Mean</td>
<td>648</td>
<td>405</td>
<td>300</td>
</tr>
<tr>
<td>Petals*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>228</td>
<td>146</td>
<td>135</td>
</tr>
<tr>
<td>7</td>
<td>272</td>
<td>146</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>254</td>
<td>173</td>
<td>129</td>
</tr>
<tr>
<td>9</td>
<td>275</td>
<td>181</td>
<td>137</td>
</tr>
<tr>
<td>10</td>
<td>310</td>
<td>149</td>
<td>128</td>
</tr>
<tr>
<td>Mean</td>
<td>268</td>
<td>159</td>
<td>121</td>
</tr>
<tr>
<td>Axillary bud</td>
<td>9035</td>
<td>3940</td>
<td>3324</td>
</tr>
</tbody>
</table>

* Presumed order of initiation; numbered to correspond with the stamens they subtend.

Table 4. Mean sizes of primordia relative to the sepals (= 1.00) at each temperature at the time when all carpels have just been initiated (data from Table 3).

<table>
<thead>
<tr>
<th></th>
<th>13°C</th>
<th>20°C</th>
<th>27°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepals</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Petals</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Stamens</td>
<td>0.13</td>
<td>0.12</td>
<td>0.14</td>
</tr>
</tbody>
</table>

that the different relationship between the growth rates of the sepals and the rest of the flower at 27°C compared to 13 and 20°C (Table 2) was compensated for by a different relationship between either the times of initiation of sepals, petals and stamens or the sizes of the primordia at the moment of initiation. The sepals, petals and stamens were largest at 13°C and smallest at 27°C so that for any individual floral member the size was in the order 13 > 20 > 27°C (Table 3). Since the growth rates were greatest at 20°C (Table 2) this also implies that the sizes of the primordia at initiation may have been different at different temperatures.

The sizes of the primordia at initiation (Table 5) can be estimated from the regressions...
of primordial size against time (Tables 2 and 3) when the time of initiation of the primordia is known (Table 1). At any given temperature, at initiation the primordia in the same floral whorl were in general similar in size (Table 5). This is consistent with the subsequent sizes of the primordia being a function of their age. At 27 °C the mean size of the floral members of each whorl at initiation was larger than that of the corresponding primordia at 13 and 20 °C (Table 5). The smaller final size of the primordia at 27 °C (Table 3) is a reflection of both the lower growth rates (Table 2) and the shorter period for flower

**Table 5. Sizes of primordia at initiation (10³ x μm³).** Values for each primordium were calculated in the same way as those in Table 3, except that the times for the initiation of the primordia were obtained from Table 1, with the assumption that the primordia not listed in Table 1 were initiated at equal intervals between the first and last primordia of each whorl. As the primordia within each whorl seemed to be approximately the same size, only mean values (± s.e.) are given

<table>
<thead>
<tr>
<th></th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sepals</strong></td>
<td>36±4.6</td>
<td>36±4.1</td>
<td>158±7.3</td>
</tr>
<tr>
<td><strong>Stamens</strong></td>
<td>26±2.6</td>
<td>16±2.2</td>
<td>106±12.9</td>
</tr>
<tr>
<td><strong>Petals</strong></td>
<td>16±1.4</td>
<td>8±1.0</td>
<td>61±5.0</td>
</tr>
<tr>
<td><strong>Axillary bud</strong></td>
<td>166</td>
<td>257</td>
<td>300</td>
</tr>
</tbody>
</table>

* At time when 1st sepal was initiated, i.e. day 12.9 (13 °C), day 9.5 (20 °C), day 11.4 (27 °C) (from Table 1).

development (Table 1) than at 20 or 13 °C. The larger final size of the primordia at 13 than at 20 °C (Table 3) is because the lower growth rate at 13 °C (Table 2) was apparently more than offset by the longer time for the development of the flower (12.4 days between the initiation of the first sepal and the last carpel at 13 °C compared to 7.6 days at 20 °C; Table 1).

The axillary bud at the base of the flower was also largest at 13 °C and smallest at 27 °C (Table 3) even though the size of the axillary bud early in flower development (Table 5) and its growth rate (Table 2) were least at 13 °C and greatest at 27 °C. This is again possible because of the longer period over which primordia were initiated at 13 °C and the much shorter period at 27 °C (Table 1).

**Cell numbers**

At each temperature the number of cells in each region of the apex was counted for two apices early in flower development and one late in development. The relationship of cell number to tissue volume appeared to be the same for all regions of the flower and for all three temperatures. The log/log plot of cell number as a function of tissue volume covered a 10000-fold range in each case. The correlation coefficient was 0.9693 (n = 168) and the regression could be expressed by the equation:

\[
\text{cell number} = \exp[1.06 + 0.94 \log_e (\text{tissue volume})],
\]

where the tissue volume is expressed in 10³ μm³.

Since the regression coefficient is < 1 the implication is that the cells gradually enlarge throughout the developing flower. Since the regression is maintained down to very small volumes and cell numbers this implies that the enlargement begins when the cells are part of the apical meristem. From the equation it can be calculated that a doubling of cell number corresponds to a volume increase of 2.09 x, which means that cell volume increases, on average, in each cell cycle by 4.5 per cent.
The average cell size in the meristem and in a primordium on initiation can be calculated as about 350 μm^3. When the sepals, for instance, had enlarged and had undergone about 7 cell cycles by the time that all the carpels had just been initiated, the average cell size had increased to about 500 μm^3.

The above equation allows conversion of the values for volumes of the different parts of the apex (Table 3) into cell numbers. It also allows the calculation of the relative growth rate for cell number, by multiplying the corresponding growth rates for tissue volume (Table 2) by 0.94. From this the cell doubling times are obtained:

\[
\text{cell doubling time (h)} = \frac{24 \log_e 2}{0.94 \text{ (relative growth rate for tissue volume)}}
\]

and these values are given in Table 6. If most or all of the cells in the developing flower divide then these represent the lengths of the cell cycle. They are of the order of 1–3 days, which is comparable with the length of the cell cycle in other vegetative and floral apices (Lyndon, 1973, 1976). After the carpels had been initiated, when the relative growth rate fell (Figs 2–4), the cell cycle presumably lengthened to about a week or so.

### Table 6. Cell doubling times (h). Parts of the flower lettered as in Fig. 1

<table>
<thead>
<tr>
<th></th>
<th>13 °C</th>
<th>20 °C</th>
<th>27 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical dome:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above youngest leaf pair in vegetative plants</td>
<td>57</td>
<td>26</td>
<td>93</td>
</tr>
<tr>
<td>Above youngest leaf pair in induced plants</td>
<td>35</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>Above sepals</td>
<td>22</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>(E) Floral meristem (distal to stamens)</td>
<td>66</td>
<td>48</td>
<td>68</td>
</tr>
<tr>
<td>(F) Sepals</td>
<td>38</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>(G) Petals</td>
<td>45</td>
<td>32</td>
<td>68</td>
</tr>
<tr>
<td>(H) Stamens</td>
<td>36</td>
<td>27</td>
<td>52</td>
</tr>
<tr>
<td>(D) Axis of stamen and petal nodes</td>
<td>88</td>
<td>74</td>
<td>88</td>
</tr>
<tr>
<td>(C) Axis of sepal node</td>
<td>197</td>
<td>136</td>
<td>147</td>
</tr>
<tr>
<td>(B) Stem (pedicel)</td>
<td>71</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>(A) Axillary bud</td>
<td>54</td>
<td>49</td>
<td>41</td>
</tr>
</tbody>
</table>

### DISCUSSION

The increased growth rate which occurs in the apical dome just before (20 and 27 °C) or at (13 °C) flower initiation in *Silene* is not maintained by the apical dome of the developing flower (Lyndon, 1977). Nor is it maintained by any part of the growing flower (Table 2), the growth rates of which are comparable with apices undergoing induction or apices which are vegetative. The relative growth rates have also been measured during flower initiation in *Chrysanthemum* (Jeffcoat and Cockshull, 1972) and wheat (Williams, 1966). In both cases the growth rate declined during initiation of the floral members. The increase in the growth rate of the shoot apex which occurs in many plants at or about the time of floral initiation (Sunderland, 1961; Corson, 1969; Saint-Côme, 1971; Bodson, 1975; Miller and Lyndon, 1975, 1976) may therefore not be necessary to maintain the growth of the flower when primordia are being initiated rapidly. The significance of the increased growth rate which may occur in most plants at or before floral initiation must therefore be sought not in the growth and development of the flower but in the process of flower initiation itself and the transition from vegetative to floral growth. It has been suggested that it is primarily concerned with altering the relationships between the growth of the different parts of the apex so that the change in primordial arrangement which is associated with flowering is brought about (Lyndon, 1977).
The temperature coefficients \(Q_{10}\) for the rates of initiation of the primordia of the floral members were 1.88 (13–20 °C), 1.71 (20–27 °C) and 1.79 (13–27 °C). These are comparable with the \(Q_{10}\)'s which can be calculated from the data of Lyndon (1977) for the rates of leaf initiation, and which were 1.81 (13–20 °C), 1.53 (20–27 °C) and 1.66 (13–27 °C). This is consistent with the hypothesis that the factors which govern the sequence of initiation of the floral members are the same or similar to those governing the initiation of leaves (Lyndon, 1978b).

The rate of initiation of primordia in the flower was not a simple function of growth rate, as shown by the more rapid initiation of petals and stamens at 27 °C than at 20 or 13 °C (Table 1) although the relative growth rates of these floral members and the flower as a whole (Table 2) were lower at 27 °C than at 20 or 13 °C. For rapid initiation to occur with a lower growth rate, at 27 °C either the size of the primordia at initiation would be expected to be smaller relative to the apical dome or the apical dome would be used up by the initiation of primordia and would therefore decrease in size. The estimates of the sizes of the primordia at initiation (Table 5) show that at 27 °C the primordia are much larger than at 20 or 13 °C. The rapid initiation of primordia at 27 °C is therefore presumably possible only by a progressive reduction in the size of the apical meristem relative to the primordia and this is indeed what occurs (Table 7). The floral meristem distal to the stamens increases in volume by about 11-fold during the initiation of the stamens and petals at 13 and 20 °C but only doubles at 27 °C. The rate of initiation of primordia seems therefore to be controlled by metabolic processes, the rate of which is increased by temperature over the range 13 to 27 °C, but which are not the same processes as control the rates of growth and cell division.

The lack of correlation between the growth rates of the parts of the flower and the rate of initiation of primordia at different temperatures suggests the possibility that the form of the flower may be modified by temperature, but this was not the case (Table 4). The constancy of the relative sizes of the sepals, petals and stamens when the carpels are initiated (Table 4) suggests that the time at which the initiation of carpels is completed is determined by the petals and stamens reaching a particular size relative to the sepals. If this is so, the flower form would result from the size relationships of the floral parts irrespective of how these were achieved or the time taken. This might be possible if the concentrations of morphogens produced by the primordia in the flower were a function of their size. This postulate is in contrast to that made for a model of leaf initiation which depended on the source strength of a morphogen decreasing as a function of time (Thornley, 1975). In modified, proliferous flowers carpels can form, however, without the prior differentiation of petals and stamens but after a variable number of leafy organs have been formed at the apex (Lyndon, 1979).

The smaller size at initiation of the petal and stamen primordia compared to the sepal primordia (Table 5) confirms the measurements made from phyllotactic parameters and
which showed a decrease in the size of the primordia on flower initiation and a further decrease when petals and stamens were initiated (Lyndon, 1978b). What has been called the ‘spatial competence’ of the primordia (Thornley, 1975) therefore alters on flower initiation and during flower development in *Silene*.

The growth rates of the sepals, petals and stamens at 20 °C (Table 2) were virtually identical with the values for growth rates at initiation measured from the plastochron ratio (Lyndon, 1978b). This indicates that the growth rate of the primordia is determined at their initiation, a conclusion also reached for *Nigella* and *Lycopersicon* flowers on the basis of the allometric relationships of petal and stamen lengths (Greyson and Sawhney, 1972). Also comparable are the findings for the leaves of other plants that the different growth rates of successive leaves are traceable back to the moment of their initiation (Williams, 1975). The growth rates of the primordia which are determinate in their growth in the vegetative and the floral shoot may therefore be programmed into them at the time they are initiated.

It has been proposed that the cytological and metabolic changes which occur in the shoot apex during induction and evocation of flowering (Bernier, 1971), and which are similar to the changes occurring in vegetative buds released from apical dominance (Usciati et al., 1972), may be concerned with the increased growth rate of the apex on transition to flowering rather than with the morphogenetic change involved in the formation of flowers instead of leafy shoots (Miller and Lyndon, 1977). The apparent lack of obvious connexion in *Silene* between increased growth rates and the rate of flower development or the form of the developing flower suggests that when the changes in growth rate do occur they are primarily involved in (a) the actual transition from a vegetative to a flower apex and (b) the change in branching pattern which results in the formation of the inflorescence and which arises from a release of apical dominance (Thomas, 1961).

The measurements given in this paper are consistent with the hypothesis that the processes intrinsic to flowering are a change in primordial arrangement (phyllotaxis) followed by subsequent differentiation of the primordia into sepals, petals, stamens and carpels (Lyndon, 1979), that the changes in absolute growth rate of the meristem which usually accompany flowering are not a fundamental part of this process (Miller and Lyndon, 1977; Lyndon, 1977) and that the first steps in the flowering may involve changes in the correlative effects of primordia on the apex and vice versa which may be most easily observable as changes in their relative size, and which may be brought about in different ways even within the same plant (Lyndon, 1977).

**ACKNOWLEDGMENTS**

I would like to thank Ken Parker for his expert assistance, and the Agricultural Research Council for their support through Grant No. AG 15/105.

**LITERATURE CITED**


--- Lyndon—Growth Rates in Developing Silene Flowers


A Modification of Flowering and Phyllotaxis in *Silene*

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ABSTRACT

Modified proliferous flowers arose spontaneously in a small proportion of plants of *Silene coeli-rosa* growing in garden plots. The modified flowers consisted of leaves, arranged spirally with a mean divergence angle of 138.4° instead of the pentamerous arrangement of the normal flower, and sometimes also carpels which ranged from open structures with exposed ovules to follicle-like structures, free or fused, to fully fused carpels with free-central placentation. In the modified flowers petals and stamens were not formed. The primordia at initiation were intermediate in size (relative to the apical dome) between normal leaf and normal sepal primordia but were the same absolute size as the latter. The structure of these anomalous flowers is discussed in relation to the normal flowering process.

Key words: *Silene coeli-rosa*, flowering, phyllotaxis.

INTRODUCTION

When plants of *Silene coeli-rosa* (L.) Godron were grown out of doors at Edinburgh in the summer of 1976 approximately 1 per cent or less of the plants, instead of forming flowers, formed heads which consisted of spirally arranged leaves, usually culminating in an anomalous gynoecium, and which could be regarded as proliferous flowers. The structure of the inflorescence appeared to be of the normal cymose type. These proliferous flowers appear to be an anomaly very similar, if not identical to the 'pinecone' mutation of the carnation (Holley and Baker, 1963; Emino, 1966). The purpose of this note is to describe these proliferous flowers and the change in phyllotaxis accompanying their formation, and to show how this anomalous growth may be related to the normal processes of transition from vegetative growth to flowering.

OBSERVATIONS

Structure of the proliferous flowers and the shoots bearing them

The proliferous flowers consisted of heads of spirally arranged leaves (Plate 1A) and were borne terminally on leafy shoots which developed from axillary buds in the way typical of normal plants in which the inflorescence is a cyme. The axillary shoots which bore a proliferous flower also bore leaves which were arranged in the opposite, decussate manner typical of this species and with an axillary bud apparent in the axil of one of the leaves. This again was typical of this species in which the axillary buds at each node are unequal. Above the opposite, decussate leaves were 1–3 whorls of, usually, tricussate leaves (with 1–3 axillary buds per whorl) before the proliferous flower itself. Sometimes there were up to 6 leaves per whorl. A similar anomaly occurs in abnormal *Dianthus* shoots (Blake, 1962). The whorls of leaves below the proliferous flower were distinguished from the leaves making up the proliferous flower itself by (a) the elongation of the internode above...
each whorl of leaves and (b) the presence in the axil of most leaves of an axillary bud, which had an apparently normal structure. Some of these axillary buds had grown out to form a leafy side-shoot, itself terminating in a proliferous flower. In the proliferous flower the internodes had not elongated and axillary buds were usually reduced to filiform structures, which were smaller and less frequent in progressively more distal parts of the proliferous flower.

Most proliferous flowers terminated in an anomalous gynoecium (Plate I B). In many proliferous flowers there was a complete gradation from leaves to carpels, the intermediate forms next to (and just below) the carpels being leaves with their edges inrolled, sometimes with what were apparently ovules on the inrolled margins so that the ovules were exposed. Similar structures have been observed in the carnation (Blake, 1962), and similar phenomena in other plants have been listed and summarized by Meyer (1966). Distal to these were often similar leaves but with their margins fused to form tubular leaves or follicle-like structures. Some proliferous flowers terminated in clusters of follicle-like structures, fused to each other to varying extents, each follicle terminating in a style and stigma, as in other anomalous Silene flowers (Rohrbach, 1868). Sometimes the fusion was complete, giving a gynoecium with axile placentation, and in others the divisions between the carpels (for such they appeared to be) were not present and so the gynoecium was almost identical with the normal gynoecium showing free-central placentation. It was therefore possible to observe in a single plant a complete gradation between leaves, carpels and an ovary with free-central placentation.

Although carpels were formed, the other leaves constituting the proliferous flowers showed no signs of differentiation into sepals, petals or stamens, which were missing completely. It seemed possible that these floral members might be represented by the leaves in the proliferous flower. If so one might expect the number of leaves in each proliferous flower to be equal to the sum of the numbers of sepals (5), petals (5) and stamens (10) in the normal flower. Observations on 10 proliferous flowers (Table 1) show that this expectation was only very roughly fulfilled, the number of leaves in the proliferous flowers varying from 9 to 36 (compared to a total of 20 for sepals + petals + stamens in a normal flower) and the total number of floral parts ranging from 14 to 36 compared with 25 in the normal flower.

### Table 1. Numbers of modified leaves and carpels in ten proliferous flowers

<table>
<thead>
<tr>
<th>Replicate</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of leaves</td>
<td>16</td>
<td>13</td>
<td>30</td>
<td>36</td>
<td>12</td>
<td>9</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Number of carpels</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total number of 'floral' organs</td>
<td>21</td>
<td>19</td>
<td>30</td>
<td>36</td>
<td>18</td>
<td>14</td>
<td>23</td>
<td>20</td>
<td>14</td>
<td>18</td>
</tr>
</tbody>
</table>

### Changes in phyllotaxis

**Divergence angles.** In the normal vegetative plant the phyllotaxis is strictly opposite and decussate (Fig. 1) so that the divergence angle between each successive pair of leaves is 90° and the leaves of each pair are placed at 180° from each other. In the normal flower the formation of the sepals, stamens and carpels is in spiral sequence (Lyndon, 1978a) and the mean divergence angle of successive sepals is 145°, of successive stamens is 141°, of successive petals is 148°, and of successive carpels 145° (Lyndon, 1978b). The divergence angles between successively initiated floral members were best interpreted as indicating that the sequence of initiation was determined by only the two youngest
primordia but that the positions of the primordia were determined with respect to the radii on which older primordia lay.

In the proliferous flowers the divergence angle of the leaves, and carpels, which constituted them was measured for the youngest 11 or 12 floral members in five 'flowers' (Fig. 2). The mean divergence angle was $138.39 \pm 1.77^\circ$ ($n = 65$), indistinguishable from $137.57^\circ$ and suggesting that the phyllotaxis in these proliferous flowers is of the typical Fibonacci type in which the positioning and sequence of initiation of primordia is probably determined by the adjacent older primordia as a function of their ages in plastochrons (Richards, 1948, 1951; Thornley, 1975).

**Plastochron ratios.** Measurement of the plastochron ratio ($r$) allows the size of the primordia at initiation to be estimated. The ratio of the area of the primordium on
initiation to the mean transverse apical area is given by $2 \log_e r$ and is the reciprocal of the area ratio (Richards, 1951). To allow for the fact that the primordia are initiated on the flanks of a rounded apex the plastochron ratio must be corrected to $r'$ and this corrected value can be obtained from the equation: $\log_e r' = \log_e r / \sin \theta$, where $2\theta$ is the apical angle, i.e. the angle of the cone the surface of which is tangential to the surface of the apex at the point of initiation of the youngest primordium. The apical angle was measured on the reconstructed apical profiles (Fig. 3). Plastochron ratios were measured as follows. In apices which had been sectioned transversely at 10 $\mu$m, the most distal section in which each leaf was observable was noted and the radius of the section to one side of the leaf base was measured. The mean values for apical radius at the insertion (or more properly the axil) of each leaf were thus obtained. The plastochron ratio (Table 2) was obtained as the antilogarithm of the regression coefficient of log (apical radius) against leaf age, in plastochrons. For the normal vegetative apices the value so obtained was not the true plastochron ratio (per leaf) because each plastochron a pair of leaves was formed. The true value for the plastochron ratio (Table 2) was obtained as the square root of this initial value (Richards, 1951).

![Fig. 3. Reconstructions of the apical profiles of normal vegetative plants (a) and the modified flowers (b). The diameters of successive serial 10 $\mu$m transverse sections were plotted as a function of distance from the summit of the apex (Schwabe, 1971). Each point represents a measurement on one section. Data from 5 apices (a) and 3 apices (b) used to construct free-hand curves. The mean levels of the axils of the leaf pairs (a) or individual leaves (b) are shown by the bars.](image)

The proliferous flowers had a plastochron ratio intermediate between the normal vegetative apex and the normal flower (Table 2). The primordia on initiation were also intermediate in size between normal foliage leaves and normal floral primordia when measured in relation to the area of the apical dome ($2 \log_e r'$, the reciprocal of the area ratio) but were the same absolute size as sepal primordia (Table 2). This difference was because the apex in the modified, proliferous flowers was smaller than the apex in the normal flower. It was also slightly smaller than in the normal vegetative plant as the reconstructions of the apical profiles show (Fig. 3). Although the radius of the apex at the axil of the youngest leaf, i.e. at the base of the apical dome, was 58 $\mu$m in the normal
**Table 2. Comparison of the apical dome and primordia of the proliferous flowers with those of normal vegetative plants and normal flowers**

<table>
<thead>
<tr>
<th></th>
<th>Normal vegetative plants</th>
<th>Modified flowers*</th>
<th>Normal flowers*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sepals</td>
<td>Petals</td>
</tr>
<tr>
<td>Apical radius (μm), R, at axil of youngest primordium</td>
<td>58.4 ± 1.8†</td>
<td>48.3 ± 1.6‡</td>
<td>75</td>
</tr>
<tr>
<td>Apical angle, 2θ°</td>
<td>56</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td>Plastochron ratio (r)</td>
<td>1.180</td>
<td>1.074</td>
<td>1.038</td>
</tr>
<tr>
<td>Primordial area relative to transverse apical area, (2 logₐ r/sin θ)</td>
<td>0.705</td>
<td>0.315</td>
<td>0.158</td>
</tr>
<tr>
<td>Actual primordial area on apical surface (μm²); (nR².2 logₐ r/sin θ)</td>
<td>7451</td>
<td>2280</td>
<td>2799</td>
</tr>
</tbody>
</table>

* Data from Lyndon (1978b).
† Mean of 10.
‡ Mean of 3.

vegetative plants and only 48 μm in the proliferous flowers, the height of the apex above this axil was the same in both cases, 42 μm. The apex of the proliferous flowers was therefore narrower than in the normal plant.

The modified proliferous flowers were therefore intermediate between the normal vegetative apex and the normal flowering apex because (a) relative to the apical dome area the primordia were almost exactly intermediate between leaves and sepals in size and (b) the primordia of the modified flowers were the same absolute size as sepals, but developed as leaves.

**DISCUSSION**

In the modified flowers the achievement of phyllotaxis with a divergence angle indistinguishable from the Fibonacci angle of 137.5° is consistent with the hypothesis that the positioning of the modified leaves was by the adjacent primordia and that these older primordia had a continuing, though diminishing, effect as they aged, as has been postulated for the initiation of leaves (Thornley, 1975). This contrasts with the normal flower, in which the divergence angle more nearly approximates to 144°, which would be consistent with only the previous two primordia governing the position of a new primordium, the positioning effect of the existing primordia lasting for only two plastochrons after their initiation (Lyndon, 1978b).

The modified flower resembles the true flower in having primordia which are (a) initiated in spiral sequence and (b) of the same absolute size as sepals. It differs from the true flower in (1) having a narrower apex, (2) lacking differentiation into floral parts (except for carpels) and (3) lacking development of primordia in axillary positions, i.e. on the same radii.

The comparison of plants having modified, proliferous, flowers with normal plants allows the following conclusions to be drawn.

(1) A change in phyllotaxis similar to that which is associated with flowering (Lyndon, 1978a) can occur without being accompanied by the differentiation of sepals, petals or stamens, and sometimes without the differentiation of carpels. This suggests that the first change in the apex on flowering is a change in primordial arrangement and that changes in gene expression to give normal differentiation of the floral members are secondary rather than primary events of the flowering process.
(2) The formation of carpels without the formation of sepals, petals or stamens shows that carpel formation does not depend on the presence of these other floral members. If a relay system of the sort postulated by Heslop-Harrison (1959, 1963) normally operates then it would seem to be able to be short-circuited.

(3) The gradation, in a single plant, from leaves to an ovary with free central placentaion suggests that the differentiation of the ovary in the normal flower may be the result of a rapid increase in the amount of a substance or substances but that a gradually changing concentration can result in a graded effect on the primordia.

(4) The formation, in the plants with modified flowers, of a branching pattern which is similar to that of the normal cymose inflorescence shows that the release of axillary buds from apical dominance and the formation of the inflorescence can be dissociated from the differentiation of the floral members. This suggests that the stimulation of axillary bud growth which typically occurs on flowering may be associated primarily with the formation of the inflorescence structure rather than the formation of flowers.

These conclusions lead to the hypothesis that flowering normally consists of a temporal sequence of three phases: (1) a change in branching pattern, (2) a change in phyllotaxis, and finally (3) the differentiation of sepals, petals, stamens and carpels. A change in gene expression need be invoked only for the last of these three steps.

ACKNOWLEDGMENTS

I am grateful to Ken Parker for his assistance, to Jim Goodall for taking the photographs and to the Agricultural Research Council for support through grant no. AG15/105.

LITERATURE CITED


EXPLANATION OF PLATE

Plate 1

Scanning electron micrographs of modified flowers (for method see Lyndon, 1978a).

A. ‘Flower’ consisting entirely of spirally arranged leaves which are unlike normal leaves as they have not expanded very much but resemble the unfused tips of the normal sepals as illustrated elsewhere (Lyndon, 1978a). \( \times c. 70 \).

B. ‘Flower’ with some of the outer leaf-like structures removed to show carpels with the tips prolonged into stigmas. The three most centrally placed and visible carpels are fused together. \( \times c. 105 \).
Synchronisation of Cell Division During Transition to Flowering in *Silene* Apices Not Due to Increased Symplast Permeability

Brief Report

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2 Department of Botany, University of Edinburgh

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Summary

Seven long days induce flowering in *Silene coeli-rosa*. FRANCIS and LYNDON (1979) reported a strong synchronization of cell division in the apex on the eighth day. This could be due to a sharp fall in apical cell isolation, leading to syncytium formation or to increased cell isolation, leading to the derepression of cell division. To test the degree of cell isolation, a range of water soluble fluorescent probes was injected into either non-induced or induced apices just before, or at the time of the synchronous division. Probes of molecular size up to 665 daltons moved through vegetative apices. Those of size up to 536 daltons moved in the induced apices. Hence, as judged by this criterion the cells in the induced apices are more isolated than those in non-induced apices. Thus the induced synchronous division may be due to an increase in cell isolation.

Keywords: Flowering; Fluorescent probes; Intercellular communication; *Silene*; Synchronous cell division.

1. Introduction

Flowering causes major changes in the behaviour of the shoot apical meristem. These changes become difficult to reverse at about the time of sepal initiation. A striking event during this period of evocation is one or more cycles of synchronous cell division, found in *Sinapis* and possibly other species (BERNIER 1971), and in the long day plant *Silene* (FRANCIS and LYNDON 1979). The question arises, is synchrony due to a major increase in cell/cell permeability, so that a syncytium is formed, as in pollen mother cell development, and the cells coordinate one another in division. Alternatively, is there an increase in cell isolation analogous to that which LOEWENSTEIN has proposed as responsible for the unrestricted proliferation of certain cancerous cells (for a review see LOEWENSTEIN 1979). This study reports an investigation of the permeability of the *Silene* shoot apex to a range of fluorescent probes. Measurements were made on vegetative apices and on induced apices just before and during the period of the synchronous division.

2. Materials and Methods

2.1. Plant Material

*Silene coeli-rosa* L. Godron plants were grown in 8 hour days at 20 °C following MILLER and LYNDON (1975, 1976). At 28 days uniform plants were selected and either maintained in short days, or transferred to 7 long days and replaced in short days during flower initiation. The long days consisted of the same light regime as short days, supplemented for the remaining 16 hours with low intensity tungsten light. Seven long days induced approximately 100% of the plants to flower. The synchronous division occurred at 1500 hours on the eighth day, with 7-8% of the cells in mitosis (FRANCIS and LYNDON 1979). During the eighth day small numbers of 7 long day and short day plants were harvested, and the apical 2-3 cm cut off, stripped of leaves to expose the apical dome and first few leaf primordia, and then presented on a "blind" basis to the electrophysiologist. The apices were pinned in a horizontal position to a clear layer of elastomer (Sylgard 184), and covered with...
bathing buffer (Spanswick 1972) ready for injection of chemicals. Lissamine rhodamine B (LRB) and fluorescein isothiocyanate isomer I (F) conjugates with glutamic acid (GLU), glutamyl-glutamic acid [(GLU)]₂, hexaglycine [(GLY)₆], leucyl-diglutamyl-leucine (LGGL) and microperoxidase (MP); and electrophysiology, microscopy and preparation of electrodes were as described in Goodwin (1983).

2.2. Movement in Cell Walls

The dyes and dye conjugates have been shown not to pass the plasmalemma, at least in Elodea (Goodwin 1983). Thus injected compounds could move in either the symplast or the apoplast (the cell walls), but not between these. Since most cells in the apex are internal and so are shielded from the external solution, injected chemicals could move from cell to cell without loss by diffusion through the cell walls. The degree of movement in cell walls was assessed in two ways. Firstly the cuticle on the surface of the apex was broken by light cuts, and the apex soaked in 1.0 mM FMP or FLGGL for 30 minutes. Conjugates unable to move in the symplast were chosen for this test in order to exclude any possibility of entry to and movement in the symplast. Secondly, non-moving injections of all compounds were left for a further 1 hour, and then re-examined.

2.3. Injection of Chemicals

Injections were made to the side of the apical dome, into cells just below the tunica. Each apex was injected with a fresh needle, by iontophoresis at 170 volts (square wave, 500 msec per second) for 60 seconds, with the electrode left in the cell for a further 15 minutes. The apex was then cut off, placed under the incident light fluorescence microscope, and photographed using Kodak Ektachrome 400 Daylight film, rated at 1600 ASA. Movement was evaluated in the microscope, and confirmed from the photomicrographs.

3. Results

3.1. Diffusion in the Cell Walls

One hour after injection all non-moving injections still appeared strongly localized. In the soak test, although conjugates entered some of the damaged surface cells, they did not show generalized movement in the apex. Thus movement of these compounds via cell walls makes a very limited contribution to movement through the shoot apex. By way of contrast, all conjugates showed extensive movement via the cell walls in more mature tissues.

<table>
<thead>
<tr>
<th>Compound</th>
<th>LRB</th>
<th>FGLU</th>
<th>(GLU)₂</th>
<th>(GLY)₆</th>
<th>FLGGL</th>
<th>FMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (daltons)</td>
<td>559</td>
<td>536</td>
<td>665</td>
<td>749</td>
<td>874</td>
<td>2268</td>
</tr>
<tr>
<td>Non-induced apices</td>
<td>3/3</td>
<td>11/15</td>
<td>2/6</td>
<td>0/6</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Induced apices</td>
<td>3/3</td>
<td>2/14</td>
<td>0/8</td>
<td>-</td>
<td>-</td>
<td>0/1</td>
</tr>
</tbody>
</table>

P. B. Goodwin and R. F. Lyndon: Synchronisation of Cell Division

3.2. Movement of Injected Compounds

Movement was generally an all-or-none process: in any given injection the compound either moved freely through most of the apex, or remained localized at the point of injection. The results of the trials are summarized in Table I and Fig. 1. Compounds of MW 749 daltons and above failed to move in either induced or non-induced apices. However, FGLU, 536 daltons, moved in most of the non-induced apices tested, but in only 2/14 of the induced apices. LRB moved freely in both. It will be noted that in most cases F conjugates were not mobile in induced apices. This could be due to induced apices showing tissue sensitivity to these compounds, leading to closure of plasmodesmata. This was tested by injecting a mixture of FGLU and LRB. In 2 injections into vegetative apices both compounds moved. In 2 injections into induced apices the LRB moved, but the FGLU did not. Thus FGLU does not cause induced apices to become impermeable, or at least it does not cause them to become impermeable to LRB. The result also indicates that LRB does not move by virtue of making the apex permeable to molecules such as FGLU and LRB.

4. Discussion

The diffusion coefficient of fluorescein in water has been measured by Tyree and Tammes (1975). Using Graham's law, that the diffusion coefficient is inversely proportional to the square root of the molecular weight, the diffusion coefficients of the conjugates can be estimated. The concentration at a distance of a substance diffusing from a point source into an infinite volume can readily be calculated using equations provided by Crank (1956). If the shoot apex is regarded as a sphere, and the appropriate calculation carried out, it is found that even the largest conjugate (FMP) should diffuse through the apex within ten seconds. Since, except for the smaller conjugates, little movement occurs, there is evidently a selective barrier to
movement, a type of molecular sieve. Evidence that these conjugates are stable, and cannot pass the plasmalemma in *Elodea* has been presented (Goodwin 1983). Thus it is probable that the compounds are moving (or unable to move) in the shoot apex symplast and that the molecular sieving occurs at the plasmodesmata, the presumed mediators of cell/cell communication.

The largest molecule tested which could move freely in *Silene* apices was $F(\text{GLU})_2$ of 665 daltons. This is smaller than the size limit (874) in *Elodea* (Goodwin 1983). However, in *Elodea* probeds larger than $F(\text{GLU})_2$ showed restricted movement. Limited movement of dyes to nearby cells was not readily detectable in the *Silene* apex because of the solid block of small cells. Nevertheless, it is probably that the *Silene* apex

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**Fig. 1.** Location of fluorescent dyes after injection into *Silene* apices. Fig. 1 a, b, c, f: non-induced; d, e: induced apices respectively. Fig. 1 a: LRB injection ($\times$ 325), b: FGLU injection ($\times$ 260), c: F(\text{GLU})$_2$ injection ($\times$ 260), d: LRB injection ($\times$ 260), e: F(\text{GLU})$_2$ injection ($\times$ 260), f: FGLGL injection ($\times$ 260). Marker bars indicate 20 $\mu$m.
symplast has a lower exclusion limit than the *Elodea* leaf.

The main objective of this work was to test whether there is a sharp change in cell/cell communication associated with the synchronous cell division. There is no increase, at least as measured by the size of the molecules which can pass freely between cells. In fact the size limit falls. The fall may reflect a differential response to the trauma of leaf removal by induced and non-induced apices; apices showing any sign of direct injury being excluded. Such an injury response would have to develop rapidly, as apices were commonly injected within minutes of removal from the plant, and had the youngest 2-4 leaf primordia plus 2.3 cm of stem still present. Another possible explanation of the difference is that there is a change in the metabolism of the conjugates. Because of the limited apical tissue available it was not possible to study this. However, the simplest explanation of the results is that the symplast in induced apices is less permeable to molecules such as F(GLU) and F(GLU)₂.

One might speculate following Loewenstein (1979) that it is the greater isolation of the cells (in respect to molecules of a particular size) which triggers their division, for example by restricting the movement of a division inhibitor. Synchronization appears to be due to a common response to a signal from outside the dividing apical cells.

**Acknowledgements**

The work was supported by the Australian Research Grants Committee.

**References**


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Changes in protein composition of the shoot meristem during floral evocation in *Sinapis alba*

R. F. Lyndon, A. Jacqmand and G. Bernier


Shoot apical meristems of vegetative and induced plants of *Sinapis alba* L. were labelled with [%]$^{35}$S methionine for 2 h and the proteins were then separated by isoelectric focussing and polyacrylamide gel electrophoresis. Quantitative and possibly qualitative changes in the complement of proteins being synthesised during evocation were detected in the meristem, distal to the primordia, 50 to 52 h after the beginning of the inductive long day. This was before morphological changes in the meristem, and before the initiation of flower bud primordia.

Additional key words – Flower induction, polyacrylamide gel electrophoresis, protein synthesis, shoot apex.


Introduction

Floral induction takes place in the leaves and leads to evocation, which consists of those events in the shoot apical meristem which commit it to flowering (Evans 1971). On flowering, genes are expressed which are not expressed in the vegetative plant. Since genes determine the formation of proteins the expression of genes specific to flowering should be accompanied by the occurrence of proteins also specific to flowering and not found in the vegetative plant. In the tulip, the protein complements of sepals, petals, anthers and pistils are, indeed, different from each other and from those of the leaves (Barber and Steward 1968).

It is not obvious when such changes in the protein complement should first be expected. They could be some of the first events of evocation in the apical meristem and necessary for the subsequent transition of the apex to flowering and the formation of the floral organs. Alternatively, protein changes could be consequent on other, general, changes in the metabolic activity of the apical meristem, which might be the first events of evocation (Evans 1971). It may not be necessary to invoke changes in gene expression in order to bring about the first events of evocation such as changes in apical size, primordial arrangement, and primordial size (Lyndon 1978, 1979). The early flowering apex could perhaps differ from the vegetative apex quantitatively rather than qualitatively.

Qualitative changes in the protein composition of the apical meristem itself have been detected by histoimmunofluorescence techniques in *Sinapis* (Pierard et al. 1980) at the time the apex became committed to flowering – at the "point of no return" – before the first floral buds were initiated. The two proteins which appeared in the meristem were characteristic of the reproductive buds but were also found in vegetative parts of the plant. It was therefore pertinent to use the technique of two-dimensional electrophoresis (O’Farrell 1975) for parallel investigation of the changes in the protein complement of the *Sinapis* meristem during evocation to see whether the changes were limited to a few proteins or
were more extensive, and to see whether there were any new protein types formed before the formation of floral organs.

If new proteins are synthesised at specific times after the beginning of induction, they might not be observed by examining the whole protein complement since this could consist not only of new proteins but perhaps also any proteins which, although no longer being synthesised, were persisting from the vegetative apex. The technique which we have used is, therefore, to label the meristem proteins with [35S] methionine and to examine the resulting complement of radioactive proteins. The plant used was Sinapis alba which can be made to flower by exposure to a single long day (Bernier 1969). The meristem is committed to flowering 44 h after the beginning of induction (Kinet et al. 1971), although the first flower buds are not initiated for a further 14–16 h (Bernier et al. 1967). [35S] methionine was supplied for a period of 2 h to meristems which were either vegetative or had been induced, the long day starting 50 h before the application of label. In this way a comparison was made of the proteins synthesised during the experimental period in vegetative and evoked meristems.

Abbreviations — IEF, isoelectric focussing; SDS, sodium dodecyl sulphate.

Materials and methods

Plants of Sinapis alba L. were raised from seed in a controlled environment at a constant temperature of 15°C, relative humidity 70% and light provided by four fluorescent tubes giving a light intensity of 2.2–2.8 mW cm⁻² at the level of the top of the plants. The plants were maintained in 8 h short days in which they remained vegetative until 2 months after sowing. They were then induced to flower by exposure to a single 20 h long day (Bernier 1969), after which they were returned to short days. Other plants were kept vegetative in short days throughout. Ten induced and ten vegetative plants were used for each experiment.

Just before the application of the radioactive solution to the meristem, each plant was prepared for a careful excision of enough young leaves to expose the apex. A plastic collar was fitted around the meristem and held in place with lanolin (Bernier and Bronchart 1963). Fifty h after the beginning of induction 10 µl of radioactive solution containing 74 Bq [35S] methionine (1480 MBq mol⁻¹; Radiochemical Centre, Amersham) and 0.5 µg eptomycin, was placed on each apex. The plants were maintained in the light under normal growth conditions for 2 h, after which the apical buds (a few mm long) were excised into sterile distilled water. Each meristem (approx. 2 × 10⁻³ mm³ of tissue) was carefully excised (fig. 1), as far as possible freed of young primordia, transferred to 20 µl homogenization medium, immediately frozen in liquid nitrogen, and then kept frozen at −20°C.

On thawing, and while viewed through a dissecting microscope, the meristems were homogenized using a fine glass rod. The homogenization medium (A) consisted of 9.5 M urea, 2% Nonidet P-40 (BDH Chemicals, England), 2% ampholines (Serva, Heidelberg, F.R.G.), and 5% β-mercaptoethanol (O’Farrell 1975). The homogenate was centrifuged at 10 000 g for 20 min at 3°C. The whole supernatant was then applied as the sample to one of a set of IEF gels, which were then run as SDS gels in the second dimension by the methods of O’Farrell (1975) modified as follows. The IEF gels were run with 0.2% sulphuric acid (pH 4.5) in the top tank (anode) and 0.5% ethanalamine (pH 9.0) in the bottom tank (cathode) for 30 min at 200 V, 30 min at 300 V and 20 h at 400 V. In the second dimension the SDS gradient gels (10 to 17% acrylamide) were run at 15 mA per gel.

After the gels had been dried and mounted on absorbent paper they were exposed to X-Omat L Ray film for about 4 months and were then developed. The experiment was repeated on another set of plants. Both experiments gave the same results.

The protein complements of vegetative buds and reproductive buds excised 240 h after the beginning of induction, by which time the flower buds had formed, were also separated on gels but were then stained. Buds (0.1 g fresh weight) were ground at 0°C with 12.5 ml extraction medium (B) consisting of 50 mM tris(hydroxymethyl)-amino-methane (Tris) buffer pH 7.4, 250 mM sucrose, 1 mM MgCl₂, 50 mM KCl, and 1% β-mercaptoethanol. The homogenate was centrifuged (15 min, 0°C, 10 000 g), and the supernatant was re-centrifuged (90 min, 0°C, 100 000 g). To the final supernatant, 50 ml cold acetone was added. The mixture was allowed to stand until the protein precipitate

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Fig. 1. Longitudinal section of the apical shoot meristem of Sinapis alba showing the region of the meristem (M) which was excised for analysis. LP, leaf primordium; H, hair.
Fig. 2A–B. Autoradiograph of the gels showing proteins from (A) vegetative meristems and (B) evoked meristems 50–52 h after the beginning of induction. Circles: proteins which relative to the rest were synthesised to (A) a lesser extent and (B) a greater extent, on evocation. Arrows (B): proteins synthesised in the evoked meristem but having no or only faint counterparts in the vegetative meristem.

had flocculated. It was then recovered by centrifugation (10 min, 0°C, 3000 g), washed in cold 80% acetone, and resuspended in 0.2 ml of homogenization medium (A). Forty μl was applied to each replicate IEF gel before electrophoresis in SDS gels in the second dimension as described above. Gels were stained with 0.2% Coomassie blue (Sigma B0630) in 50% methanol with 7% acetic acid, destained in 25% ethanol with 8% acetic acid, mounted and dried.
Results and discussion

Approximately 400–500 proteins were detected in both vegetative and evoked meristems (Fig. 2). These are probably only the most abundant proteins. Presumably more sensitive methods for detection would reveal the presence of more proteins in both types of meristem. The pattern of proteins synthesised was similar in both types of meristem. In general the protein spots tended to be more abundant and also darker in the evoked

Fig. 3A–B. Gels stained with Coomassie blue showing proteins detectable in (A) vegetative buds and (B) reproductive buds 240 h after the beginning of induction. Arrows indicate 2 proteins which were found in the same region of the gel as 2 proteins which were synthesised in the evoked meristem (cf. Fig. 2B).
meristem. This could be for a variety of possible reasons, including a more rapid uptake of $[^{35}S]$ methionine, more rapid protein synthesis, smaller endogenous precursor pools resulting in a higher specific activity of the incorporated $[^{35}S]$ methionine, or more proteins being present in the evoked meristem, or any combination of these possibilities.

Most of the spots present in the evoked meristem were also present, though often much fainter, in the vegetative meristem. However, unequivocal quantitative changes were shown by spots which, in contrast to the general trend, were stronger in the vegetative meristem, indicating that for these proteins the amounts synthesised relative to the rest decreased during evocation (Fig. 2A, circles). Proteins which, during evocation, show an increase in their rates of synthesis relative to other proteins, are those which show a greater than average increase in density from the vegetative to the evoked meristem (Fig. 2B, circles). In the evoked meristem there were also about 16 or so proteins which seemed to have no, or extremely faint, counterparts in the vegetative meristem (Fig. 2B, arrows). Two of these proteins apparently correspond to two proteins which were detectable on stained gels and which were characteristic of the reproductive buds (Fig. 3B, arrows). Further work is needed to show whether synthesis of proteins characteristic of flower buds can be detected before the buds themselves are initiated.

These results show that there are quantitative and possibly qualitative differences in the proteins synthesised in vegetative and evoked meristems of *Sinapis*. Changes in the amounts of a few proteins have previously been demonstrated by immunological techniques (Pierard et al. 1980). Using antisera raised to extracts from vegetative and reproductive buds of *Sinapis*, the technique of histioimmunofluorescence was used to demonstrate the appearance of 2 proteins in evoked and reproductive meristems which were not present in the vegetative meristem. These and the present data show that changes in the pattern of protein synthesis occur during evocation and well before bud primordia are initiated. This lends support to the view that in evocation there is not simply a general activation of protein synthesis but that changes in genome expression have begun by the time the apex has become committed to flowering and before the initiation of the floral organs.

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References


Inhibition of growth and synchronised cell division in the shoot apex in relation to flowering in *Silene*

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Abstract. When plants of *Silene coeli-rosa* (L.) Godron were induced by seven long days, then exposed to darkness for 48 h before being returned to short days, they went on to initiate flowers with a delay of about 2 d. The synchronisation of cell division which normally occurs before flower initiation was suppressed, showing that it is not essential for flowering. Periods of darkness of up to 240 h inhibited apical growth and leaf initiation but did not prevent eventual flowering in short days. The commitment of the apex to flower was therefore maintained while apical growth was inhibited.

Key words: Cell division synchrony – Flowering – Mitosis – Shoot apex – *Silene*.

Introduction

The occurrence of successive peaks of mitotic index in the shoot apices of plants induced to flower (Bernier 1971) indicates that synchronisation of cell division may be an important feature of the transition from the vegetative to the reproductive state. Synchronisation of cell division during evocation has been shown most clearly in *Sinapis alba* (Bernier et al. 1967) and in *Silene coeli-rosa* (Francis and Lyndon 1979).

In *Sinapis* the first synchronous division occurs soon after the end of the 20-h inductive photoperiod and flower bud initiation begins one cell cycle later when the second synchronous division occurs (Bernier et al. 1967). *Silene* differs in that there are 9 d and about 10 cell cycles between the beginning of induction and flower initiation (Miller and Lyndon 1975, 1976). Synchronisation of cell division in *Silene* was observed on the 8th and 9th d after the start of induction (Francis and Lyndon 1979), after the initiation of the last leaf pair and before initiation of the sepals. In both *Sinapis* and *Silene*, therefore, synchronisation of cell division takes place just before flower initiation.

If synchronisation is a necessary precursor of flowering then it should be possible to delay or suppress flowering by delaying or suppressing synchronisation. When *Silene* plants were put into darkness for prolonged periods after induction to see if induction could be reversed, growth of the apex was temporarily inhibited and flowering was delayed but was not prevented. Plants were therefore examined to see if synchronisation was also delayed. It will be shown that in fact synchronisation was suppressed although the plants went on to flower.

Materials and methods

Plants of *Silene coeli-rosa* (L.) Godron were grown in short days (SD) until 28 d after sowing. They were then selected for developmental uniformity as described previously (Miller and Lyndon 1975, 1976), and transferred to long days (LD). The day of transfer is designated day 0. Seven LD normally induced flowering in all plants. The SD conditions consisted of 8 h light (09.00 h to 17.00 h; 80–100 W m⁻². provided by fluorescent tubes supplemented by tungsten bulbs) and 16 h darkness (17.00 h to 09.00 h). Long-day conditions were like SD but with low intensity light (2 W m⁻²; provided by tungsten bulbs only) from 17.00 h to 09.00 h (Miller and Lyndon 1975, 1976). At 09.00 h on day 7 (the 7th d after the start of induction) the plants were either replaced in SD or placed in a darkroom, for varying periods of time according to the experiment, before return to SD. Plants were maintained throughout in controlled-environment chambers at 20°C.

For measurement of DNA values and mitotic indices, plants were sampled at intervals and their apical buds cut off and fixed in ethanol:acetic acid (3:1, v/v). The fixed apices were stained with Feulgen reagent, the apical dome was dissected out under a dissecting microscope, and a squash prepara-
tion was made (Francis and Lyndon 1978). The amount of DNA per interphase nucleus was measured with a Vickers M85 scanning microdensitometer. In transects across each slide every tenth nucleus was measured. 2C and 4C values were established for each slide by taking the mean values for telophase and prophase nuclei, respectively. The proportions of nuclei with $<3C$ and $>3C$ values were determined and are referred to as the G1 and G2 populations, respectively. Nuclei in the S-phase of the cell cycle were assumed to be equally partitioned between the $<3C$ and $>3C$ populations (Francis and Lyndon 1978). Fifty nuclei per apex, and three apices per sample were measured, making 150 nuclei per sampling time.

In the same preparations, mitotic indices were measured; 500 nuclei per apex were examined and the sum of all prophases, metaphases, anaphases and telophases was expressed as a percentage of the total number of cells. Values are each the mean from three apices at each sampling time.

Results

Flowering and apical growth. After induction, exposure of plants to up to 240 h of continuous darkness delayed but did not prevent flowering when the plants were subsequently placed in SD (Table 1). The delay in flower development which was found consistently in plants which had been subjected to prolonged darkness was approximately the length of the dark period. When plants were given 48 h darkness the initiation of sepals, stamens and carpels were each delayed by about 2 d compared with the control plants which did not receive the dark treatment (Fig. 1). Except for this delay, the development and form of the flower was the same in treated and control plants and was completely normal. The number of leaf pairs below the flower was the same in the dark-treated (9.15 ± 0.11) and in the control plants (9.08 ± 0.09). It therefore seems likely that leaf initiation stopped during the dark period. Even in long periods of darkness (Table 1) leaf number below the flower was not altered.

The inhibitory effect of darkness on the rate of leaf initiation could be demonstrated in plants which had received three LD, which was not inductive (Miller and Lyndon 1976), followed by 144 h of continuous darkness (Table 2). Plants had seven or eight leaf pairs at the beginning of the dark period but all had eight leaf pairs after 144 h of darkness, showing that only those leaves about to be initiated were formed in darkness. When the plants were restored to SD, leaf initiation was resumed though at a slower rate than in the controls.

If darkness inhibits only leaf initiation, but not the growth of the shoot apex, then during a period of darkness the apical dome would be expected to enlarge more than the controls, but it does not do so (Table 3). In the control plants the apical dome increases in size (Table 3) as it begins to en-

### Table 1. Effect on flowering in *Silene* of exposure to periods of prolonged darkness given immediately after induction. Plants (20 per treatment) were subsequently returned to SD until they were scored for flowering 27–28 d after the beginning of induction.

<table>
<thead>
<tr>
<th>Inductive treatment</th>
<th>No. of 24-h dark cycles</th>
<th>% Flowering</th>
<th>Mean No. of leaf pairs below flower (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 LD</td>
<td>0</td>
<td>100</td>
<td>9.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>100</td>
<td>9.9 ± 0.1</td>
</tr>
<tr>
<td>5 LD</td>
<td>0</td>
<td>100</td>
<td>10.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>92*</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>100*</td>
<td>10.0 ± 0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100*</td>
<td>10.0 ± 0</td>
</tr>
</tbody>
</table>

* Of the 13 plants which survived

**Table 2. Effect of prolonged darkness on leaf initiation in vegetative *Silene* plants. Plants were given three LD, then transferred either to SD (controls –—) or to darkness for 144 h (6 d) before being returned to SD. Number of plants per sample = 20**

<table>
<thead>
<tr>
<th>Days from beginning of 3 LD</th>
<th>Mean No. of leaf pairs (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7.6 ± 0.2 (11)</td>
</tr>
<tr>
<td>9</td>
<td>9.1 ± 0.2 (10)</td>
</tr>
<tr>
<td>24</td>
<td>13.0 ± 0.1 (29)</td>
</tr>
</tbody>
</table>

* One plant flowered

Fig. 1. a–d. Flower development in *Silene* as shown by the percentage of plants with **a** two or more sepals, **b** five sepals, **c** one or more stamens, and **d** one or more carpels. Plants given seven LD, and then returned to SD at 09.00 h on day 7 (controls ——) or given 48 h of darkness before being returned to SD at 09.00 h on day 9 (dark-treated —•—). Number of plants per sample = 20
Table 3. Effects of prolonged darkness on apical dome size and initiation of leaves in *Silene*. Plants given seven LD before transfer to darkness. Volume for each apex calculated as \( \frac{2}{3} \pi \frac{d^2 h}{4} \), where \( d \) is the diameter of the chord at the base of the apical dome at the axis of the youngest visible primordia, and \( h \) is the height normal to this chord of the highest point on the apical dome. Measurements were made with an ocular micrometer in a dissecting microscope. Means ± SE. Number of plants per sample in parentheses.

<table>
<thead>
<tr>
<th>Days from beginning of induction</th>
<th>Controls returned to SD on day 7</th>
<th>Plants in darkness from 09.00 h on day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height (μm)</td>
<td>Diameter (μm)</td>
</tr>
<tr>
<td>7</td>
<td>79 ± 6</td>
<td>147 ± 12</td>
</tr>
<tr>
<td>8</td>
<td>108 ± 11</td>
<td>159 ± 9</td>
</tr>
<tr>
<td>9</td>
<td>105 ± 20</td>
<td>165 ± 8</td>
</tr>
</tbody>
</table>

Large immediately after induction (Miller and Lyndon 1976). The apparent subsequent reduction of the volume of the apical dome in plants exposed to darkness is consistent with the formation of those leaves which are on the point of initiation (Table 3) but without further growth of the apical dome. A repeat experiment gave very similar results. The conclusion is that prolonged darkness arrests apical growth.

A period of 48 h or more of darkness after induction therefore appears to temporarily slow down or stop apical growth and leaf initiation. Flower initiation and development, although delayed, takes place in an otherwise normal fashion when the plants are transferred back to conditions in which growth can be resumed.

**Synchronisation of cell division.** Since plants survived 48 h darkness, this was used as the standard length of the dark period which followed induction in experiments in which the effects on cell division were measured. If synchronisation of cell division were essential for flowering then it should still occur in plants exposed to darkness, and if linked to flower initiation rather than to evocation it might be expected to be delayed by about 48 h.

Plants were therefore given seven LD and then at 09.00 h on day 7 were transferred to darkness for 48 h before being placed in SD at 09.00 h on day 9. The control plants were also given seven LD but were then placed in SD at 09.00 h on day 7. The occurrence of synchronisation was monitored by measuring mitotic indices and G2/G1 ratios. In synchronised cells, peaks in the ratio of G2/G1 would be expected before the mitotic peaks. By measuring both in the same samples the chance of not observing synchrony because of insufficient sampling was minimised. Measurements were made about every 4 h throughout the whole period from the end of induction (day 7) until day 12, by which time the plants which had been in darkness were initiating sepals and the control plants were initiating stamens (Fig. 1). Because of the extended period of sampling, values were obtained from seven separate experiments and are shown as a composite graph (Fig. 2). Since each experiment spanned only a portion of the total period, some samples in each experiment were taken well before or after the main experimental period so that a general correspondence of all the experiments could be checked. For each experiment the dark-treated and control plants were strictly comparable at any given sampling time.

In the control plants given 7 LD and then replaced in SD, cell division was synchronised for two cell cycles as shown by the three main peaks of mitotic index and the two peaks of G2/G1 ratio on days 8 and 9. The main mitotic and G2/G1 peaks occurred at exactly the same times as, and the other peaks within a few hours of, those found in previous experiments (Francis and Lyndon 1979). The three mitotic peaks were about 10 h apart, corresponding to the known cell-cycle length at this time (Miller and Lyndon 1975). From the end of day 9 onwards, fluctuations in mitotic index continued but with decreasing amplitude, and with the distance between peaks increasing and corresponding to the measured times for the cell cycle in plants initiating sepals (Lyndon 1979). The maxima in the G2/G1 curve tended to occur just before the mitotic maxima, as would be expected if there was a residual but decreasing amount of synchrony.

In the plants exposed to 48 h darkness starting at 09.00 h on day 7, the mitotic index fell during day 8 and rose to its former level only after the plants had been transferred back into the light at 09.00 h on day 9. There was no evidence for successive peaks of mitosis during days 8 and 9. The broad fluctuations after day 9 each extended over
the period of about one cell cycle and provided no evidence for delayed synchrony. The values for the G2/G1 ratio remained at about the same level throughout in the plants exposed to the darkness and no peaks were found. There was no evidence of any synchronised division by day 12, at which time 70% of the plants had initiated at least two sepals and 40% had a full complement of five sepals (Fig. 1).

It might be argued that peaks of mitosis were missed because the sampling was not sufficiently frequent at crucial times. While possible, it seems extremely unlikely that peaks were, by chance, missed in both mitotic index and in G2/G1 ratios since these would have been expected to have been slightly displaced with respect to each other. Synchronisation would also have had to have been sharper than in the controls. Another possibility is that on exposure to darkness synchronisation of cell division still occurred in all plants but that the plants became desynchronised one with the other. If this had been the case one would have expected to find at certain sampling times both high and low values for mitotic index and G2/G1 ratios. However, this was not observed and values as high as the highest in the controls were never found in the dark-treated plants. The conclusion is that the exposure to darkness caused the suppression of synchronisation of cell division before sepal initiation.

Discussion

Synchronisation of cell division did not occur in the apices of plants exposed to darkness after induction and yet the plants went on to flower.
Synchronisation alone is therefore not a prerequisite for flowering. However, it probably occurs in many plants induced to flower by long or short days (Bernier 1971; Bernier et al. 1987; Jacqasmard et al. 1976; Francis and Lyndon 1979). Since synchronisation can be mimicked by application of cytokinins (Bernier et al. 1977) it may perhaps be indicative of the arrival of some substance, perhaps cytokinin, at the shoot apex after induction and before flower initiation. If so, the suppression of synchronisation by darkness would not necessarily imply that the stimulus causing it was also suppressed or was not essential for flowering. If synchronisation is a secondary side effect of a flowering stimulus, then its suppression by darkness could perhaps occur without necessarily impairing the principal action of the stimulus.

The remarkably consistent timing of the peaks of mitotic index and G2/G1 ratio from experiment to experiment (Fig. 2, and compare Francis and Lyndon 1979) indicates that the arrival of the presumed stimulus for synchronisation is at a very precise time after the beginning of induction. Since the apices of the dark-treated plants do not show a delayed synchronisation this would imply that the stimulus is transient, remaining in the apex for less than 2 d. This is consistent with the hypothesis that, as a result of induction, substances from the leaves arrive at the apex in sequence at precise times during evocation and early flower initiation (Bernier 1976).

In the Silene meristem only molecules of a size equivalent to about 700 dalton or less can move through the symplast (Goodwin and Lyndon 1983). When synchronous division is occurring, on day 8, this exclusion limit becomes smaller, about 550 dalton, but still big enough to allow the rapid access of molecules such as cytokinins through the symplast to all the cells. The main effect of the synchronising stimulus could perhaps be to bring about this increased physiological isolation of the cells which may be a prerequisite for a change in developmental pattern but which may at the same time, result in synchronisation of division if the cells are growing when the stimulus reaches them. If so, then the exclusion limit in dark-treated apices on day 8 would be expected to decrease in the same way as in the cells showing synchronous division, but whether this happens is not known.

Synchronisation is only one of a number of characteristic events of evocation and early flower initiation which may not individually be essential for flowering. Others are the increase in growth rate, in RNA content, and in apical dome size. The increase in growth rate which occurs just before sepal initiation does not occur in plants induced at 13° C (Lyndon 1977). An absolute increase in the amount of RNA does not occur in plants transferred to 13° C for induction, although the amount remains higher than in non-induced plants (Miller and Lyndon 1977). In Chenopodium, application of 6-azauridine inhibits the accumulation of RNA in the apex although flowering is stimulated (Seidlova and Krekule 1973). The increase in size of the apical dome which often seems to be a prerequisite for flowering (Lyndon 1977) does not occur in some plants, such as the hop, in which apical size decreases at flower initiation (Thomas and Schwabe 1970).

Some of the events of evocation, especially the increase in RNA content and changes in mitotic index are not specific to evocation although they are characteristic of it. They have also been shown to occur in apices of axillary buds of Cicer stimulated into vegetative growth by release from apical dominance (Usciati et al. 1972). If floral evocation does not always require an increase in growth rate then some of the cellular changes required for it may not always occur in evocation. Presumably the critical events of evocation which are essential for flowering may, in these cases, be reached by an alternative set of cellular events. For example, it has been shown that an increase in size of the apical dome can be achieved in different ways (Lyndon 1977). If there are several interacting sequences of events in evocation (Bernier 1976) then it may be possible for the plant to complete evocation successfully by a different combination of sequences if one particular sequence is blocked experimentally.

An alternative view is that some of the characteristic changes in evocation are effects of photoperiod not directly relevant to flowering. This would be more likely if plants with different photoperiodic requirements showed different evocational sequences. They do not seem to do so (Bernier 1971; Bernier et al. 1981). It is, therefore, more probable that the events characteristic of evocation normally lead to the critical events, of which we are at present uncertain, which are essential but which can be reached by more than one set of cellular changes. This would be consistent with the observation that many plants can be stimulated to flower by a variety of external factors and that in the absence of a specific stimulus flowering may eventually be inevitable.

Even after many days in the dark, following induction, the surviving plants went on to flower (Table 1). This shows that the plants retained the commitment to flower while the growth of the apex.
was suppressed. This could perhaps have been because the flowering stimuli continued coming from the leaves and the apex reacted by flowering once it had begun to grow again. However, when this was tested by defoliating Silene plants at the end of induction but before the 48-h dark period, the plants still went on to flower. Therefore, the apex itself had apparently become committed to flower and this commitment was retained while growth of the apex was suppressed.

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APPEARANCE OF SPECIFIC ANTIGENIC PROTEINS IN THE MATURING SEXUAL ORGANS OF SINAPIS FLOWERS

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SUMMARY

Three proteins specific to the flowering state were found in Sinapis by immunological techniques. Two of these are specific to the stamen and one to the pistil. By the use of a histoimmunofluorescence technique their localization in the developing flower primordia and in the apex was examined during the transition to flowering. These proteins are not detected in the apex at evocation. They all appear at a relatively late stage of stamen or pistil maturation. The stamen proteins are localized in both the intine and exine layers of pollen grains in stamens 2–3 mm long; at anthesis they are essentially in the exine. The pistil protein is found in the stigma and in the transmitting tissue of the style. All these proteins contain sugar residues. A possible implication of these proteins in the process of male–female recognition is discussed.

INTRODUCTION

The transition to flower initiation by shoot meristems might be viewed as requiring the expression of genes that are not expressed in vegetative plants (Zeevaart, 1962; Salisbury, 1963; Bonner, 1965; Searle, 1965). Alternatively, the transition to flowering (floral evocation) has been seen by some investigators as resulting simply from an unspecific activation of meristems without an early derepression of particular genes (Evans, 1975). According to this hypothesis, new genes come into play only at a late stage of the transition when they are required to specify the chemical and structural characteristics of the developing inflorescences and/or flowers.

Since genes determine the formation of proteins, the expression of genes specific to flowering should be accompanied by the occurrence of proteins also specific to flowering, and which are not found in vegetative plants. In the past, attempts to find qualitative changes in the protein complement of meristems at evocation have been generally unsuccessful (Marushige & Marushige, 1962; Nitsan, 1962; Stiles & Davies, 1976), but more recently changes in the protein composition of the apical bud or meristem of Sinapis and Rudbeckia during floral evocation have been described (Pierard, Jacqmard & Bernier, 1977; Pierard, Jacqmard, Bernier & Salmon, 1980; Milyaeva, Kovaleva, Lobova & Chailakhyan, 1979).

Using immunological techniques, two antigenic proteins characteristic of the

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reproductive bud have been shown to appear in the apical meristem of *Sinapis* relatively early during the transition to flowering. This supports the hypothesis that a change in gene expression occurs at evocation (Pierard et al. 1980). However, these two proteins were not specific to the flowering state since they were also found in some vegetative parts of the plant (Pierard, Jacqmard & Bernier, 1979).

Changes in the complement of proteins being synthesized were also detected in the meristem of *Sinapis* during evocation by two-dimensional electrophoresis (Lyndon, Jacqmard & Bernier, 1983).

The aim in the present work was to look for proteins specific to the flowering state. Such proteins might be expected in the floral parts themselves, particularly in the stamens and pistil because these sexual organs are specific to flowers. Proteins specific to floral parts have indeed been found in *Pharbitis* (Marushige & Marushige, 1962), *Tulipa* (Barber & Steward, 1968), *Mercurialis* (Durand-Rivière, 1969; Kahlem, 1975) and *Prunus* (Raff, Hutchinson, Knox & Clarke, 1979). We compared the antigenic-protein compositions of the different floral parts of *Sinapis* in order to find out if there were proteins characteristic of one or another floral part. The antigenic proteins specific to stamens and pistil were then localized by an histoimmunofluorescence technique in sections of flower primordia and apical buds during the transition to flowering to determine whether these proteins might be detectable before the flowers themselves were initiated.

**MATERIALS AND METHODS**

**Growing conditions**

Plants of the long-day plant *Sinapis alba* were raised from seeds in 8-h short days in the growth rooms of the phytotron of the Botany Department at Liège as previously described (Pierard et al. 1977). Flowering was caused by subjecting 2-month-old plants to continuous long days.

**Preparation of extracts**

The different floral parts – sepals, petals, stamens and pistil – were collected from flowers at anthesis and separately from flower primordia just before anthesis. The term ‘flower primordium’ refers to the flower bud from initiation to anthesis. Buds 3 mm long were also collected from 2-month-old vegetative plants and from reproductive plants after exposure to 10 successive long days. The vegetative buds contained, in addition to the meristem, a number of leaf primordia 2 mm long and the upper 1 mm of the stem. The reproductive buds did not contain any leaves but had a great number of flower primordia at various stages of development. Plant materials were then frozen in liquid nitrogen and stored at −20 °C until use. Buds and organs were homogenized in cold 10 mM-Tris-HCl buffer (pH 7.5), with 0.5 M-sucrose, 5 mM-MgCl₂, 50 mM-KCl and 5 mM-β-mercaptoethanol. After centrifugation at 19 000 g for 20 min the precipitate was discarded and the protein concentration of the supernatant extract was estimated by the Bio-Rad Protein Assay (Bio-Rad Laboratories).

**Preparation of antisera**

Antisera against protein extracts only of floral parts collected at anthesis were raised in rabbits by the procedure described previously (Pierard et al. 1977). Four injections of 0.2–0.8 mg of protein each were made during 5 months. The first injection was with complete Freund’s adjuvant while the others were with incomplete adjuvant. The production of antibodies was monitored by regularly checking the reaction of the antiserum against the plant extract. Antisera collected after the third
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Injection, which were the richest in antibodies, were mostly used in this study. ASE, APE, AST and API refer to the anti-sepal, anti-petal, anti-stamen and anti-pistil antisera, respectively.

**Ouchterlony double immunodiffusion.** Antiseras were compared by means of the double immunodiffusion agar plate and carried out according to the method described by Pierard *et al.* (1977). Cross reactions were tested by various antigen–antibody combinations, placing the antiserum in the centre well and the antigens in the outer wells. Diffusion was for 24 h at room temperature.

**Immunoelectrophoresis.** This technique was used to compare the electrophoretic and antigenic properties of the antigens and to check that the precipitating antibodies were immunoglobulins G (IgG). Immunoelectrophoresis was performed using the micro-slide method (Scheidegger, 1955). Antigen samples were analysed by electrophoresis in 0.85% agarose (Calbiochem Behring) or 1.5% agar (Difco-Noble) in 30 mM-barbital-acetate buffer (pH 8.6) at 30 V/cm for 45 or 50 min at room temperature.

Diffusion with antiserum placed in the trench was then allowed to proceed for 24 h in a moist chamber at room temperature. After washing in 0.85% NaCl with 0.02% azide, and drying the gel, the electrophoretograms were stained with 0.1% Amido Black in acetic acid/methanol (1:9, v/v) for 5 min and then destained in acetic acid/methanol.

**Crossed-immunoelectrophoresis**

The first-dimension electrophoresis of the antigen preparation was done as above in agarose on 7.5 cm × 7.5 cm slides pre-coated with 0.5% agarose as an adhesive. After this first electrophoresis, a cut was made in the gel parallel to the zone of protein migration, 4 mm clear of the sample hole. The unused gel beyond the cut was stripped from the plate and the agarose layer replaced by 0.9% agarose gel containing 10% antiserum (v/v). The separated proteins were then run, at right angles to the first direction into the antibody-containing gel. The second electrophoresis was performed at 4 V/cm for 5 h at room temperature. The slide was then washed and stained as above.

**Immunoadsorption**

To demonstrate the exclusive capacity of an extract to produce specific antibodies and therefore to contain specific antigens, immunoabsorption was carried out by incubating serum and extract at an adequate concentration (v/v) for 2 h at 37°C then overnight at 4°C followed by centrifugation at 750 g for 10 min.

**Antigen characterization**

Pronase was used to show the protein nature of antigens; the enzyme (E 70,000 PUK/g, Merck, 20 mg/ml in 10 mM-Tris-HCl, pH 7.4) was added to the protein extract to reach a final concentration of 2 mg/ml. The mixture was incubated 3 h at 37°C. Two controls for this reaction were done simultaneously: (1) incubation in the presence of a large amount of bovine serum albumin (BSA) (50 mg/ml, final concentration) as a competitor; (2) separate preincubations of extract and Pronase, followed by combining them just before immunodiffusion to test the eventual effect of Pronase on the antibodies during the immunodiffusion reaction itself.

Galactosyl, mannoxyranosyl or glucopyranosyl residues in antigens were tested for by immunoelectrophoresis and crossed-immunoelectrophoresis. Antigen extract was preincubated with soybean agglutinin (SBA) (Miles Yeda Ltd) or concanavalin A (ConA) (Calbiochem), with identical concentrations of protein and lectin for 2 h at 37°C then overnight at 4°C, and centrifuged at 750 g for 10 min.

**Histoimmunological techniques**

An indirect histoimmunofluorescence technique was used to test for the presence of specific stamen and pistil antigens during flower development. Plant material was immediately frozen and cut at 6 μm thickness using a cryostat at −20°C, according to the technique of Knox & Heslop-Harrison (1970). The sections were fixed for 5 min in methanol, dried and stained in 0.2% toluidine blue in methanol for 5 min. This staining step was necessary to remove much of the native fluorescence and to allow antigen localization. The dye should bind to phenolic compounds, which are present in *Sinapis* flowers as well as in most other plant tissues (Knox & Clarke, 1978; Knox, Vithanage & Howlett, 1980), and so prevent them reacting with rabbit IgG. IgG that contained the
specific antibodies were isolated from AST and API by DEAE-cellulose chromatography. Then AST IgG and API IgG were made specific with respect to stamen or pistil by absorption with a floral extract containing all the antigens common to stamen (ST) or pistil (PI) except the specific ones. AST IgG was so absorbed by a pistil extract leaving only antibodies against three (ST1, ST2 and ST3) antigens specific to stamens, and API IgG was absorbed by a sepal extract leaving only antibodies against two (PI1 and PI5) antigens specific to pistils (see Results). Control IgG solutions were also prepared: (1) AST IgG absorbed by stamen extract; (2) API IgG absorbed by pistil extract. The first control solution did not contain any antibodies against stamen antigens and the second one was free of antibodies against pistil antigens. Each tissue section was incubated with 10 μl of IgG (6 mg/ml) in a damp and dark chamber at 37 °C for 60 min. They were then washed in three changes, for 15 min each, of phosphate-buffered saline (PBS; 0-01 M-potassium phosphate, 0·15 M-NaCl, pH 7·2). Goat anti-rabbit IgG (Nordic Pharmaceuticals, Antwerp, Belgium), hereafter called GAR IgG, labelled with fluorescein (FITC), was used as the conjugate throughout. GARFITC IgG had about two fluorochrome molecules per protein molecule. It was made up at a concentration of 0·25 mg/ml and allowed to act upon the sections previously treated with the rabbit IgG solutions. Incubation with GARFITC IgG was for another period of 60 min in the dark and in a moist atmosphere in 37°C. Slides were then washed in PBS, drained and mounted in PBS/ glycerol (9/1). They were stored in the dark at 4°C until observation. Additional control sections incubated with GARFITC IgG alone were also made.

**Fluorescence microscopy and photography**

The sections were examined with a Leitz Ortholux fluorescence microscope equipped with a Plom Opak appliance. A xenon XBO arc (75 W) or a mercury arc (100 W) was used as the light source. S 370-3 mm, AL 405 and 433-20 exciting filters were chosen and a barrier filter adjustment filtered out light of wavelength greater than 495 nm (K 490—K 495 emission filters). The slides were observed using a 60x oil immersion objective (n.a. 1·3) and were photographed using Kodak Tri X Pan film (400 ASA).

**RESULTS**

*Organ-specific antigens*

The antigenic relationships between the various floral parts at anthesis were determined by immunodiffusion and immunoelectrophoresis, using both homologous and heterologous antisera before and after immunoabsorption. The results are summarized in Table 1.

**Table 1. Antigenic relationships between floral parts and with the reproductive bud of Sinapis**

<table>
<thead>
<tr>
<th>Antiser to:</th>
<th>Sepal</th>
<th>Petal</th>
<th>Stamen</th>
<th>Pistil</th>
<th>Reproductive bud</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepal</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Petal</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Stamen</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pistil</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Numbers refer to the number of antigens detected by a combination of immunodiffusion and immunoelectrophoresis, as described in the text.
also common to the pistils. Immunodiffusion reaction of the different floral parts with ASE confirmed that sepal antigens were in pistils (Fig. 2). Components with antigenic determinants similar to those precipitating in the innermost band were also present in the petals and possibly in the stamens (Fig. 2, arrow).

**Petal antigens.** The three precipitin bands (Fig. 3, arrows) given by a petal extract with its homologous antiserum cross-reacted with three pistil antigens, but with pistil extract the median and the outermost lines precipitated as one. In the stamens the innermost band only was present, while in sepals there were only the other two that precipitated in one band as with the pistils. There was therefore no precipitating antigen specific to the petals.

**Stamen antigens.** The homologous stamen—AST interaction showed essentially four different antigens in the stamens (Fig. 4, arrowheads), of which three were present only in the stamens. One (ST1) was a cathodic antigen with a high isoelectric point, above pH 8.6; the other two (ST2 and ST3) were anodic antigens. Antigen ST4 was common to pistils, sepals and petals. The agar plate of Fig. 5 provides confirmation that there was one antigen (the innermost band) common to the various floral parts and that there were two precipitin bands specific to the stamens (arrows). The absorption of AST with a pistil extract removed the capacity of the serum to precipitate the antigen common to the pistils and stamens as shown in Fig. 6 where the precipitin arc ST4 of Fig. 4 is absent. Immunoprecipitation of AST with a stamen extract resulted in the loss of all antibodies (not shown).

**Pistil antigens.** Immunoelectrophoresis of pistil antigens against API gave five arcs of precipitation (Fig. 7, arrowheads). Two of these arcs (PI1 and PI3) were developed as protracted lines and probably represented two families of antigens with identical immunological properties, but with different electrophoretic mobilities. Of the five pistil antigens, two were present only in the pistils: a major one, PI1, migrated towards the cathode and a minor one, PI5, towards the anode. The others were also common to the sepals and PI3 was also present in the petals and stamens. The immunodiffusion test of Fig. 8, although showing only four precipitin lines for the pistils, confirmed the occurrence of one antigen common to the different floral parts (arrowhead) and a strong precipitin line specific to the pistils (arrows). Assignment of two antigens as being characteristic of the pistils was supported by the following results. First, the absorption of API with a sepal extract suppressed the capacity of the serum to precipitate the three antigens common to the pistils and the sepals but did not affect the capacity of the serum to precipitate the two characteristic antigens (Fig. 9, arrows), while an immunoprecipitation of API with a pistil extract removed the capacity of the serum to precipitate all antigens (not shown). In addition, the two precipitin lines present with the pistil extract only did not appear with a sepal extract even when the protein concentration of the sepal extract was double (not shown).

**Antigen characterization**

Serological identification, by immunodiffusion of the stamen or pistil antigens with their homologous antisera, was greatly diminished by preincubating the floral antigens with Pronase even when BSA was added as a competitor (Figs 10, 11). Separate
Figs 1–8
preincubation of antigens and Pronase followed by combining them just before immunodiffusion did not alter the immunoprecipitation of the antigens (shown for ST in Fig. 10). This control indicated that the considerable loss of antigen–antibody precipitation caused by preincubation of antigens with Pronase was not due to Pronase-mediated inactivation of the homologous antibodies during the subsequent immunodiffusion test. Stamen and pistil antigens are thus proteinaceous.

Sugar residues in stamen and pistil antigens were tested for, using lectins, by immunoelectrophoresis and crossed-immunoelectrophoresis. Figs 12 and 15 show crossed-immunoelectrophoreses of stamen and pistil extracts with their homologous antisera. The pistil proteins all moved towards the anode because of the use of agarose (Figs 15, 16) rather than agar (Fig. 7). The peaks were numbered as their corresponding arcs in Figs 4 and 7. Peaks ST1, ST2 and ST3 corresponded to the antigens characteristic of the stamen (Fig. 12) and peaks P11 and P15 to the antigens characteristic of the pistil (Fig. 15). Preincubation of a stamen extract with SBA did not eliminate any peak but reduced the proportion of ST2 in the antigenic solution (compare Figs 13 and 12). Preincubation with ConA eliminated all antigens except ST2 (Fig. 14). Preincubation of a pistil extract with SBA did not precipitate any pistil antigen (Fig. 16). On the contrary preincubation with ConA eliminated all antigens except P14 (Fig. 17). Note that the strong precipitin arc on the cathodic side in Fig. 17 did not result from a precipitation of pistil antigens with corresponding antibodies but from a reaction between ConA and a glycoprotein of the serum. Thus among the three antigens characteristic of the stamens (ST1, ST2 and ST3), ST1 and ST3 were glycoproteins with manno- or glucopyranosyl residues and ST2 contained galactosyl residues.

As far as the antigens characteristic of the pistil (PI1 and PI5) were concerned, both were glycoproteins with manno- or glucopyranosyl residues.

**Appearance and localization of antigens specific to floral parts**

Since no antigen was specific to the sepals or the petals, histochemical localization of these in developing flower primordia was not attempted. Immunodiffusion reactions of bud extracts with ASE indicated that the vegetative and reproductive buds contained at least one antigen in common with the sepal (Fig. 2, arrow), but immunoelectrophoresis showed that there were in fact two common antigens (not shown). Using APE, extracts of reproductive (and vegetative) buds were shown to possess the three petal antigens (Fig. 3).

**Stamen-specific antigens.** ST1, ST2 and ST3 were localized together by histo-immunofluorescence using AST IgG absorbed by a pistil extract. The specific

Figs 1–8. Immunodiffusion tests and immunoelectrophoresis of antigens of sepals (SE), petals (PE), stamens (ST), pistils (PI), and reproductive (RB) buds against anti-sepal (ASE), anti-petal (APE), anti-stamen (AST) or anti-pistil (API) serum. In Fig. 6, AST was absorbed by PI (AST + PI). In immunodiffusion tests, the protein concentration of extracts was 1 mg/ml, except in the cases designated by (a) where the concentration was 2.5 mg/ml or (b) 5 mg/ml. In Figs 1, 7, 6 μg of protein were electrophoresed in agar gel; in Figs 4, 6, 10 μg were used in agarose gel. Arrows and arrowheads point to precipitin bands or arcs (see the text).
Figs 9–14
Specific proteins of sexual organs in Sinapis

Figs 15-21. Figs 15, 16. Crossed-immunoelectrophoresis in agarose gel of PI (50 µg of protein) alone or preincubated with SBA (PI + SBA) against API. Figs 17, 18, 19, 20, immunoelectrophoresis in agar gel of PI (30 µg of protein) preincubated with ConA (PI + ConA), PI, stigmas (SA), ovaries (O), RB (10 µg of protein), against API. Fig. 21, immunodiffusion test of PI, VB and RB (1 mg of protein/ml), VBa and RBa (2.5 mg of protein/ml) against API.

Figs 9-14. Fig. 9, immunodiffusion test of SE and PI (1 mg of protein/ml), VBa (vegetative bud) and RBa (2.5 mg of protein/ml) against API absorbed by SE (APISE). Figs 10, 11, immunodiffusion tests of ST and PI (2 mg of protein/ml) without incubation (ST, PI) or incubated with Pronase (STp, P1p), with Pronase and BSA (STpB, P1pB) or alone and mixed with Pronase just before immunodiffusion (STp). Figs 12-14, crossed immunoelectrophoresis in agarose gel of ST (50 µg of protein) alone or preincubated with soybean agglutinin (ST + SBA) or concanavalin A(ST + ConA) against AST.
solution so obtained, contained a higher proportion of antibodies against ST1 and ST2 than against ST3 (Fig. 6). In the stamens at anthesis, a much higher fluorescence was found in the exine layer of the pollen wall in sections incubated with specific AST IgG (Fig. 22) than in sections treated with AST IgG absorbed with stamen antigens (Fig. 23). On some occasions activity was also detected in the immediate vicinity of the exine surface suggesting that the antigens had diffused out of the pollen grains. The wall of the anther was also fluorescent but this fluorescence was no higher in sections treated with specific IgG than in those treated with non-specific IgG (Fig. 24) and was thus considered to be due to non-specific binding of rabbit IgG with some components of the wall. No fluorescence was exhibited by control sections incubated with GARFITC IgG alone (not shown). The presence of the antigens, ST1, ST2 and ST3, in pollen grains of flowers at anthesis was confirmed by an immunoelectrophoretic analysis of an extract of pollen grains alone against AST (not shown). In flower primordia 6 mm long, collected just before anthesis, the localization of the fluorescence was the same as in freshly opened flowers (not shown). In younger primordia, 3–4 mm long, a specific fluorescence was detected in about 50% of the stamens and was localized in the exine layer of pollen grain but was also found in the intine (Fig. 25). There was almost no fluorescence in the control section treated by non-specific IgG (Fig. 26). In stamens smaller than 2 mm (collected from flower primordia smaller than 3 mm), no fluorescence was exhibited by sections treated with either the specific (Fig. 27) or the non-specific IgG solution (Fig. 28), although exine and intine were already differentiated in stamens only 1.5 mm long. These last results were confirmed by immunodiffusion and immunoelectrophoresis of a reproductive bud extract against AST, since reproductive buds contained flower primordia of 2 mm long or less. Antigens ST1 and ST2 were absent but antigen ST3 was present (Fig. 4). Furthermore, the same pattern was found for the vegetative buds (not shown). The discordance concerning the minor antigen ST3, shown by immunoelectrophoresis and immunofluorescence, was probably due, in the latter technique, to the pretreatment with toluidine blue, which may mask some antigenic determinants. Thus, of the three antigens (ST1, ST2, ST3) present only in stamens of flowers at anthesis, ST3 was already present in the bud at all developmental stages. It was therefore not, in fact, specific to the stamens, in contrast to antigens ST1 and ST2, which were. ST1 and ST2 were not present in the shoot apical meristem before and during floral evocation but appeared exclusively in the pollen wall during stamen development.

**Pistil-specific antigens.** A rough localization of PI1 and PI5 antigens in the pistils at anthesis was first made by an immunoelectrophoretic analysis of stigmas, styles and ovaries separately. PI1 was found exclusively in the stigmas (Fig. 18, arrowhead 1); PI5 was present in the styles and ovaries (Fig. 19, arrowhead 5) and possibly proportionately less in the stigmas (Fig. 18). A more precise localization was obtained using histoimmunofluorescence although both antigens PI1 and PI5 were tested for together. Note, however, that the specific API IgG solution (i.e. API IgG absorbed by a sepal extract) contained a higher proportion of antibodies against PI1 than PI5 (Fig. 7). Sections of pistils at anthesis treated with specific API IgG showed a high
Figs 22–28. Localization of the stamen-specific proteins using histoimmunofluorescence. Fluorescence is represented by white areas in the photographs. Pollen grain (po); exine (e); intine (i); wall (w). Figs 22, 23, 24, stamen at anthesis. Figs 25, 26, stamen 2 mm long. Figs 27, 28, stamen 1.5 mm long. Figs 22, 25, 27, sections incubated with AST IgG1 and then with GARFITC IgG. Figs 23, 24, 26, 28, control adjacent sections of Figs 22, 25, 27, respectively, incubated with AST IgG1ST and then with GARFITC IgG. \( \times 760 \).
Figs 29–34. Localization of the pistil-specific proteins using histoimmunofluorescence. Cytoplasm (c); stigma (sa); transmitting tissue (tt); ovule (o); placenta (pl). Figs 29–33, pistil at anthesis. Fig. 34, pistil 1.5 mm long. Figs 29, 30, 31, 34, sections incubated with API IgG and then with GARFITC IgG. Fig. 32, control adjacent section of Fig. 29 incubated with API IgG and then with GARFITC IgG. Fig. 33, another control adjacent section of Fig. 29 incubated with GARFITC IgG alone. ×760.
level of fluorescence in the stigmatic tissue (Fig. 29) and some fluorescence in the transmitting tissue of the style (Fig. 30), but no fluorescence in the ovule and placenta (Fig. 31). In stigma cells the fluorescence was localized to the peripheral cytoplasm (Fig. 29). In the transmitting tissue more fluorescing material accumulated in the cytoplasm at the pole of the cells orientated towards the base of the pistil. Control sections of stigma (Fig. 32) and transmitting tissue treated with API IgG absorbed by pistil extract exhibited almost no fluorescence. Other controls incubated with GARFITC IgG alone were almost completely dark (Fig. 33). In younger flower primordia about 6 mm long, the pistil antigens, P11 and P15, were present in both the stigmatic and the transmitting tissues (not shown). In flower primordia 2–3 mm long, the pistil (1.5–2 mm long) was totally devoid of fluorescence even in the stigma (Fig. 34), although the latter was already well differentiated. Similarly, no fluorescence was found in smaller flower primordia. These last results were confirmed, in the same way as for the stamen antigens, by immunodiffusion and immunoelectrophoresis of reproductive bud extract against API. Antigen P11 was absent but antigen P15 was present (Figs 9, 20, 21). The situation was the same in the vegetative buds. The discordance concerning the minor antigen P15, shown by immunoelectrophoresis and immunofluorescence, can be explained as above for ST3. Thus of the two antigens, P11 and P15, found only in pistils of flowers at anthesis, P15 was already present in the bud at all developmental stages and was not specific to the pistil. Only the other antigen, P11, was specific to the pistil. It was not detectable in the shoot apical meristem before and during the floral transition but appeared exclusively in the stigma and the transmitting tissue of the style during pistil development.

DISCUSSION

The results described in this paper indicate that the antigenic-protein composition of the different floral parts in Sinapis is qualitatively different, from one class of parts to another. Three proteins are present only in the stamens and two others only in the pistil. Different protein patterns have also been found in the floral parts of other species as mentioned in the Introduction.

The three proteins present only in the stamens and the two proteins present only in the pistil were localized using a histoimmunofluorescence technique in the developing flowers and in the apex during the transition to flowering, in order to determine whether these proteins could be detected before the flowers themselves are initiated. The three stamen antigens were tested for together, but it was shown that our histoimmunofluorescence procedure detects only the two major ones, ST1 and ST2. It was also observed that the minor stamen protein ST3 is not specific to the stamens since it is already present in the bud. The two pistil antigens, P11 and P15, were also localized together but our histoimmunofluorescence procedure detects only the major one, P11. It was also established that P11 is the only antigen specific to the pistil. Although the monospecific antibodies with respect to the antigens to be localized were not used in the present study, our localization procedure seems reasonably specific since the control experiments have yielded very weak, if any, non-specific background
fluorescence (Figs 23, 32). In addition, several results obtained with the immuno-
fluorescence technique were supported by the analysis of extracts using immuno-
diffusion and immunoelectrophoresis.

None of the specific stamen or pistil proteins appears in the meristem during floral
evocation. They only appear in the stamens or pistil at a late stage of maturation. It
can be concluded that, whichever developmental stage is considered, the proteins
specific to sexual organ maturation are never present in the shoot apical meristem.
Thus, the changes in the protein complement that occur in the apical meristem of
Sinapis at evocation (Pierard et al. 1980; Lyndon et al. 1983) do not involve the
antigenic proteins that concern us here that are specifically related to maturation of
the sexual organs.

The two stamen antigens are first found in both the intine and exine layers of pollen
grains in stamens 2–3 mm long. Later on, they are localized essentially in the exine
layer of the mature pollen grain. The pistil protein is localized only in the stigma and
the transmitting tissue of the style. All these antigenic proteins of stamens and pistil
appear just before anthesis and contain sugar residues. Their late appearance, their
localization and their glycoproteinaceous nature suggest that they could be involved
in the process of male–female recognition. In Brassica oleracea, a closely related
species, specific glycoproteins appear in maturing stigmas concomitantly with the
development of the self-incompatibility system (Nishio & Hinata, 1977; Roberts,
Stead, Ockendon & Dickinson, 1979). In Gladiolus, similar observations were also
made (Knox, 1971; Clarke, Gleeson, Harrison & Knox, 1979). However, if the
antigens that appear specifically in the stamens of Sinapis are related to the
male–female recognition process, it is surprising to find them in the intine at the
beginning of stamen maturation, since the self-incompatibility system is spor-
ophytically controlled in Sinapis (Knox & Clarke, 1980). Further experiments are
needed to clarify this point.

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Specific proteins of sexual organs in Sinapis


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Changes in Apical Growth and Phyllotaxis on Flowering and Reversion in *Impatiens balsamina* L.

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ABSTRACT

When plants of *Impatiens balsamina* L. were subjected to 5 short days and then re-placed in long days, they began to form a terminal flower and then reverted to vegetative growth at this terminal shoot apex. The onset of flowering was accompanied by an increase in the rate of initiation of primordia, an increase in the growth rate of the apex, a change in primordium arrangement from spiral to whorled or pseudo-whorled, a lack of internodes, and a reduction in the size at initiation of the primordia and also of the stem frusta which give rise to nodal and internodal tissues. On reversion, parts intermediate between petals and leaves were formed, followed by leaves, although in reverted apices the size at initiation and the arrangement of primordia remained the same as in the flowering apex. The apical growth rate and the rate of primordium initiation were less in the reverted apices than in floral apices but remained higher than in the original vegetative apex. Since the changes in apical growth which occur on the transition to flowering are not reversed on reversion, the development of organs as leaves or petals is not directly related to the growth rate of the apex, or the arrangement, rate of initiation or size at initiation of primordia.

Key words: *Impatiens balsamina* L., flower reversion, evocation, phyllotaxis, shoot meristem.

INTRODUCTION

Evocation consists of the events in the shoot apex which lead to flowering (Evans, 1969). To understand how flowering is controlled, it is necessary to know how these events are linked to the subsequent initiation of flower parts. However, the mechanism by which an evocational event has its effect is unknown, because it is thought of as leading to commitment to flowering, and the nature of this commitment step is unknown. The time of commitment to flower has been established in plants such as *Sinapis alba*, in which a 'point of no return' can be demonstrated about 44 h after the start of the inductive long day (Kinet *et al.*, 1971). In plants in which reversion of the flower meristem to vegetative growth can occur after flower formation has started, it is obvious that there is no 'point of no return' before flower development begins. This suggests that in such plants there is no commitment, and we can then ask whether there is a direct relationship between the events of evocation and the events of flower formation in these cases. Furthermore, it should be possible to see whether reversion to vegetative growth involves reversal of all preceding evocational changes, or whether the apex that results is partially evoked. The response of a partially evoked apex to further induction should allow us to pinpoint which subsequent evocational changes correspond to particular events of flower formation.

Plants showing reversion have not been used to study evocation because the sporadic nature of reversion in the species in which it has been observed has, until now, not allowed an experimental approach. For this to be possible reversion must be sufficiently consistent and predictable in all or most of the plants. In order to produce an experimental system a preliminary study was made of some species in which reversion of flowers occurs –
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Pharbitis nil (King and Evans, 1969), Anagallis arvensis (Brulfert and Chouard, 1961) and Impatiens balsamina (Simon, 1973). Of these, only I. balsamina could be made to revert from flowering in a sufficiently predictable manner to allow experimentation.

I. balsamina is a short-day plant which reverts to leaf production if transferred back to long days (LD) during flower development (Krishnamoorthy and Nanda, 1968; Debraux and Simon, 1969). It forms zygomorphic lateral flowers and also a large actinomorphic terminal flower with many floral parts. It is easiest to study flowering and reversion at this terminal meristem. In this paper we first describe the development of experimental regimes for the induction of flowering and for reversion in this terminal flower of I. balsamina. Secondly, we compare, in flowering and reverting plants, the changes in apical growth rates and in the arrangement, size and rate of initiation of primordia, in order to establish whether such changes, which are usually associated with flowering (Bernier, Kinet and Sachs, 1981) are necessarily linked to the production of flower parts in I. balsamina. If they are, they would presumably be reversed on reversion, and would therefore warrant particular attention as possible essential, causal, events for the initiation of flower parts.

The results show that the reverted apex is not the same as either the original vegetative apex or the flowering apex; nor are the morphological changes characteristic of evocation in I. balsamina reversed on reversion.

MATERIALS AND METHODS

Growth of the Plants

Mixed seed of Impatiens balsamina L. (cv. Dwarf Bush Flowered) was obtained from Wm K. McNair, Portobello, Edinburgh, U.K. Batches of seeds were imbibed on moist filter paper in LD for 65 h. Then the seeds with emerged radicles 3–4 mm long were sown at a depth of 1 cm in moist 3:1 peat:sand mix (G.C.R.I. No. 1 Formulation). Twenty-four seeds were sown per 37 x 23 x 6 cm holed tray, and watered until the soil was thoroughly moist. The trays were placed in LD of 24 h, consisting of 8 h light, provided by Philips 65–80 W white fluorescent tubes supplemented by tungsten bulbs (total photosynthetic photon flux density 275–285 μmol m⁻² s⁻¹ at the top of the plants on day 0; see below), followed by 16 h light of low photosynthetic photon flux density (6 μmol m⁻² s⁻¹ at the top of the plants on day 0) provided by tungsten bulbs. Temperature was 22 ± 1 °C, and relative humidity 55–65 per cent. The soil was kept moist by application of 200 ml distilled water per tray every day, except every third day when 200 ml full-strength Hoagland’s solution (Hoagland and Arnon, 1938) was supplied.

Seven days after sowing the young seedlings were selected for developmental uniformity; all those in which the first leaf was not 4–8 mm long were discarded. The day on which this selection was carried out marked the beginning of treatments in inductive short days (SD), and was designated day 0. A sample of five or six plants was taken after selection to establish the total number of leaves plus primordia present. Plants to be induced to flower were transferred to SD in which the 8 h light period was followed by 16 h dark, at 21 ± 1 °C. Normal flowering controls remained in SD until dissection 6–7 weeks later. About 90 per cent of the plants formed a terminal flower; the remaining 10 per cent formed flowers only at the axillary meristems and could be distinguished by their modified phyllotaxis and/or the presence of axillary flowers. Only the plants forming terminal flowers were used in the analyses described here.

Reversion was brought about by transfer back to LD after varying numbers of SD; vegetative controls were plants that remained in LD from day 0. About a quarter of the plants which remained in LD showed either an anomalous form of terminal flower formation, or flowering at the axillary meristems only. Since these plants could be
distinguished from vegetative plants at an early stage, they were excluded from the analyses described here.

**Numbers of Leaves plus Primordia**

Groups of not less than five plants were sampled at intervals during the experimental period, dissected out under a dissecting microscope, and the total number of leaves plus primordia (excluding the cotyledons) counted on each plant. Values of total number of leaves plus primordia obtained during sampling of plants for the analyses described below were also included. Rates of primordium initiation per day were calculated as described in the results section.

**Developmental Stages**

Plants were grouped into developmental stages according to the total number of leaves plus primordia present at the time of sampling, in all the growth analyses to be described. These stages (see Tables 4–6) were chosen to correspond to distinct phases of development in all treatments, and allowed comparison between the different treatments, and different phases of development during the same treatment.

**Distances of Primordia below the Apical Surface**

The shoot apex of *I. balsamina* has the shape of a flat-topped cone; the flat top bears the young primordia and will be referred to as the apical surface. The distances of primordia below the apical surface were measured to provide an estimate of the vertical relative growth rate per plastochron of the apex, and of the size of the stem frustum on initiation. This frustum includes the stem tissue adjacent to the primordium, and the stem tissue between the abaxial insertion point of the primordium and the adaxial insertion point of the next oldest primordium. These tissues give rise to the node and internode, respectively, in the mature plant. To provide these measurements three to six plants were taken on days 8, 10 and 12 following selection, the nine oldest leaves and the cotyledons removed, and the remaining shoot plus primordia fixed in formalin–acetic acid–70 per cent ethanol (1:1:18 by volume) for at least 24 h. The fixed tissues were dehydrated through an ethanol series and embedded in wax. Serial transverse sections, 5 μm thick, were stained with 0.5 per cent Toluidine Blue O (O'Brien and McCully, 1981) or by the method of Sharman (1943). From these sections the developmental stage of each plant was first determined by counting the number of primordia in the first transverse section to graze the surface of the shoot apex, and adding to this the nine leaves removed before embedding, to give the total number of leaves plus primordia.

The distances of the successive leaves or primordia below the apical surface were then measured by recording, for each, the section in which connection between its base and the stem was half complete. Where the succession was in doubt (e.g. in pseudo-whorls), it was assumed that the primordia were initiated with successive angular separations of approximately 137°. In some cases (particularly in flowering or reverting plants) shoot growth was not straight, resulting inevitably in some skewed sections. In these cases the distances of the primordia below the apical surface were measured in relation to the youngest primordium on the same side of the apex.

The number of primordia on the apical surface was also estimated from these measurements. Primordia with a mean transformed distance, \( \log_e(x \, \mu m + 1) \), of less than \( \log_e(5 \, \mu m + 1) \) below the apical surface were defined as being on the apical surface (see also Fig. 3).
The Plastochron Ratio

The plastochron ratio—the ratio between the transverse distance of a primordium from the centre relative to that of the next primordium to be produced (Richards, 1951)—was measured on photographs of the shoot apex, taken with a Cambridge Stereoscan 250 scanning electron microscope (SEM). Six plants were taken on days 8, 10 and 12 following selection, the cotyledons and the nine oldest leaves were removed, and the remaining shoot plus young parts was frozen in liquid nitrogen. These stem tips were each placed in a screw-topped tube containing a small amount of drying agent (molecular sieve type 4A; BDH) and a large excess of absolute methanol, at —80 °C. They were then freeze-substituted in absolute methanol for 2 weeks at —80 °C, 1 week at —40 °C, 1 d at —20 °C, 15 h at 4 °C and 2 h at room temperature (method modified from Robards, 1978). After transfer to fresh absolute methanol the tissues were passed through a methanol–acetone series to absolute acetone. Following critical-point drying with carbon dioxide, the apices were mounted on stubs, and the older primordia were carefully removed to reveal the apical surface, whilst leaving clear outlines of the positions of the primordial bases around the apex. The dissected apices were gold-coated and viewed from directly above in the SEM. Photographs were taken at successive magnifications, so as to include all the young primordia and the bases of those removed during dissection. The outlines of the primordia were traced from the photographs, and the centre of each was estimated using circles of appropriate size and equalizing parts of the primordium bases outside the circles.

The plastochron ratio \((r)\) can be measured most easily by the method of Maksymowych and Erickson (1977), in which only the order of initiation of primordia need be known. In vegetative plants, and for those primordia on flowering and reverting meristems initiated before and during the transition to flowering, in which the order of initiation could be determined from the genetic spiral, this method was therefore used. The order of initiation of primordia becomes difficult to establish objectively after the transition to flowering, so the plastochron ratio was estimated from these primordia in the following way. Firstly, the position of the apical centre was fixed from the intersection point of the radii from the older primordia whose order of initiation was known, and whose distances from the apical centre could be calculated by the method of Maksymowych and Erickson (1977). The distances from this centre of those primordia initiated after the transition to flowering were then measured. The order of their initiation was taken to be a function of their radial distances, and the plastochron ratios calculated by the method of Richards (1951). The areas of primordia on initiation, relative to the area of the free apical surface, and area relative growth rates per plastochron \((2 \log_e r)\) of the apex, were calculated from the mean plastochron ratios derived from primordia nearer the centre than, and including, the first primordium below the apical surface.

Area and Vertical Relative Growth Rates of the Apex

These were obtained as area and vertical relative growth rates per plastochron respectively, multiplied by the rate of initiation of primordia per day.

RESULTS

Morphological Characteristics of Flowering and Reversion

Examination of over 200 plants has shown that in continuous SD the terminal flower is formed as follows. At the time of transfer to SD about eight or nine leaves plus primordia are present. Part 9 or 10 (numbering from the first leaf upwards) is usually
FIG. 1. Changes in morphology of successively initiated parts (A) of the normal flower (SD) and (B) of the reverted flower (5 SD + LD), R4 reversion type, of *I. balsamina*. Successive parts were removed from these mature structures and the outlines and clearly visible venation of each traced. Areas with pigment typical of petals are shaded. ▼ indicates the presence of a spur. Part 1 = the lowest leaf on the plants.

the first to show differences from normal leaves, since this and all subsequent parts lack axillary structures, and one or both of the basal pair of second-order veins are not fused to the main vein in the lamina, but retain an independent identity into the petiole (Fig. 1 A). Parts 10–14 usually show increasing degrees of such modified venation, the second pair of second-order veins being affected in a similar way to the basal pair. These parts also show progressive reduction in size compared with normal leaves, may have small
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| Table 1. Characteristics of reversion types obtained as a result of transfer of I. balsamina plants back to LD after three to 10 SD |
|---|---|
| Reversion type | Characteristics of plants on dissection 6 weeks after return to LD |
| R0 | Plants show no sign of flowering and subsequent reversion |
| R1 | The only sign of flowering is a zone of leaves with no, or modified, axillary structures (the reversion zone) |
| R2 | Zone of leaves with virecent lateral flowers of the terminal inflorescence, above which is a zone of leaves with no axillary structures |
| R3 | As above, but with reduction of internodes between the leaves with no axillary structures |
| R4 | As above, but the leaves of the reversion zone not separated by internodes, and some have modified venation and/or areas with pigment typical of petals; subsequent leaves in a whorled or pseudo-whorled arrangement are separated by internodes |
| R5 | As above, but a zone of petals occurs before return to leaf production |
| R6 | As above, but with a zone of stamens after the petals, followed by a return to leaves, or occasionally by primordia, either leaves or petals, whose exact nature cannot be determined by inspection |
| R7 | As above, but the zone of stamens is followed by a return to petals |

| Table 2. Effect of different numbers of SD on the proportion of plants of I. balsamina showing each reversion type |
|---|---|---|---|---|---|
| Number of plants | R4 | R5 | R6 | R7 | Total |
| Number of SD before return to LD | | | | | |
| 5 | 13 | 2 | 1 | 1 | 6 | 23 |
| 7 | 4 | 7 | 3 | 2 | 11 | 27 |
| 8 | 2 | 11 | 1 | 1 | 11 | 26 |
| 10 | 2 | 3 | 1 | 3 | 9 | 18 |

R4, R5,... = reversion type 4, 5...(no plant showed R0–R3 reversion types). Combined data from two experiments.

areas of coloured pigment characteristic of petals, generally lack a petiole, and may also have a spur. Those parts which are less than 50 mm long when mature, and which lack a petiole and/or have a spur, are referred to as bracts. The first petal is usually part 15, and is defined as the first part lacking a spur which has more than 50 per cent of its area pigmented. Successive petals become more pigmented until the maximum amount of pigmentation (about 85 per cent of the petal) is attained by about part 17. Petals are usually followed by stamens and carpels (Debraux and Simon, 1969), although in some plants carpels, or stamens and carpels, may not be formed. In these cases petals continue to be initiated instead.

The modified leaves and bracts are initiated during the first five SD. Petals are initiated from the fifth SD onwards, until the formation of the first stamen (about part 34–35) on about the 12th SD. Carpel formation begins on about the 19th SD (about part 56–57).
Transfer of plants from SD back to LD, before initiation of the terminal flower is complete, causes this flowering terminal meristem to revert to leaf production in most cases. The types of reversion obtained (Table 1) range from R1, in which the only sign of an effect of SD is a zone of leaves with no axillary structures, through R4, in which a zone of intermediate parts (leaves with some pigmented areas – see Fig. 1B) is followed by a return to leaf production in modified arrangement, to the R6–R7 types. In both R6 and R7 petals and stamens are formed and are followed by leaves in R6 or petals in R7.

How far flower formation progresses before reversion depends on the number of SD the plant has received (Table 2). With an increased number of SD there is a decreased proportion of reverting plants of the R4 type, and an increased proportion of R5 to R7 types. The 5 SD treatment causes the most uniform reversion response (Table 2). In the R4 type that results from this treatment in most cases, the lack of internodes in the reversion zone and the presence of intermediate parts is evidence that flowering has begun,
but leaf initiation and internodal development resume more quickly than in the R5–R7 reversion types. For these reasons five SD were used to bring about reversion in the experiments to be described. In these experiments, although most of the plants in the 5 SD treatment were R4 reversion types, the data will inevitably include other reversion types and non-reverting plants. However, those statements concerning the morphology of reversion refer to the R4 type exclusively.

**Changes in Apical Growth and Phyllotaxis on Flowering and Reversion**

**Rates of Primordium Initiation**  The rate of primordium initiation during vegetative growth in LD is constant (Fig. 2) and is 0·9 primordia d⁻¹ (Table 3). In continuous SD the rate of primordium initiation increases from the vegetative rate during the first eight SD, and between days 8 and 13, when petals and the first stamens are being initiated, increases further to 3·8 primordia d⁻¹. During the initiation of stamens (days 13–19) the rate declines to 1·3 primordia d⁻¹ and then increases to about 3·4 primordia d⁻¹ before carpel initiation begins on about day 19 (Fig. 2; Table 3). The most rapid rate of primordium initiation during flowering therefore accompanies the initiation of petals and the earliest and latest stamens.

In plants given 5 SD + LD the rate of primordium initiation has increased from the vegetative value by day 8, as in flowering plants. After day 8 it does not increase to the same degree as in SD (Table 3), since from day 10 onwards the mean number of leaves plus primordia from plants in 5 SD + LD is consistently lower than in SD plants (Fig. 2). The rate of primordium initiation in 5 SD + LD is 2·5 primordia d⁻¹ between days 8 and 13, and 2·2 primordia d⁻¹ between days 13 and 20 (Table 3). Therefore, when the reverting plants begin to make leaves again (about day 10) the rate of primordium initiation is not quite as rapid as during petal initiation in SD, but is more than twice as rapid as during leaf initiation in vegetative plants in LD. Clearly, there is not a particular rate of initiation associated with leaf production, and reversion does not result in a return to the original vegetative (LD) rate.

**Divergence Angles**  Vegetative plants always have spiral phyllotaxis with a mean divergence angle of 137·9° (1 s.e. = 0·6, n = 76). At about part 21, whorls or pseudo-whorls, each of about six parts, are formed in both the SD and the 5 SD + LD treatments, even though petals are formed in SD, but intermediate parts in 5 SD + LD. A pseudo-whorled or whorled arrangement persists during subsequent flowering in SD and
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FIG. 3. Estimation of the length of a frustum on initiation: illustration of the method used for vegetative (LD), flowering (SD), and reverting (5 SD + LD) plants of I. balsamina at different developmental stages. $\log_{10}(x \, \mu m$ below the apical surface + 1) of the insertion points of primordia from 10 plants growing vegetatively in LD is plotted as a function of primordium number (the youngest primordium visible in the transverse section grazing the apical surface = P1). The mean transformed distances of P1–P3 below the apical surface are less than $\log_{10}(5 \, \mu m + 1)$; to allow comparison with flowering and reverting apices these primordia are defined as being on the apical surface. The regression line was fitted for primordia P4–P12, and the regression coefficient estimates $\log_{10} v$, where $v$ is the relative distance of any two successive primordia from the apical surface. $\log_{10} v$ is an estimate of the length of the frustum on initiation, relative to the tissue distal to the first primordium below the apical surface (P4 in this case).

also on reversion to leaf initiation in 5 SD + LD – there is no immediate return to the spiral arrangement found in LD (vegetative) plants.

Area of the Apical Surface and Sizes of Primordia at Initiation (Table 4) In vegetative, flowering and reverting plants the youngest primordium is initiated at about the same distance from the apical centre; hence the bare area of the apical surface, $\pi \times$ (mean radial distance of the youngest primordium from the apical centre)$^2$, remains constant at about $9 \times 10^3 \, \mu m^2$ at all the stages measured. The total area of the apical surface, $\pi \times$ (mean radial distance of the first primordium below the apical surface)$^2$, also remains constant at about $41 \times 10^3 \, \mu m^2$ in the three treatments. This implies that the area of the annulus bearing the primordia on the apical surface is also constant, and this is what is found. However, the number of primordia occupying this area increases to about the same extent in both the flowering and the reverting apices, so that there are two to three times as many primordia on the apical surface as on that of a vegetative plant. The implication is that the primordia must be smaller in the flowering and in the reverting apices. Using
the plastochron ratio \(r\), the area of a primordium at initiation, relative to the area of the apex, can be calculated as \(2 \log_e r\) (Richards, 1951). In both the flowering and the reverting plants the area of a primordium at initiation relative to the area of the apical surface, calculated in this way, decreases significantly from that in the vegetative apex. The absolute area of a primordium on initiation also decreases on flowering and remains lower on reversion. Confirmation of this trend was obtained when the area of primordia on initiation was estimated from regression lines fitted to graphs of \(\log\) (basal area of the primordium) against plastochron age in plastochrons.

On flowering there is therefore no change in the area of the apical surface, but there is a reduction in the absolute area of the primordia initiated. This reduction in size is maintained when the plant reverts to leaf initiation, indicating that the size of the primordium at initiation does not determine its developmental fate.

**Lengths of Stem Frusta on Initiation (Table 5)** Just as the area of a primordium at initiation (relative to the area of the apex) is \(2 \log_e r\), where \(r\) is the plastochron ratio, by analogy the length of the frustum on initiation is \(\log_e v\), where \(v\) is the relative vertical distance of successive primordia from the apical surface. As Richards (1951) says of the plastochron ratio, ‘one may define how one pleases the size of the circle to represent the mean area of the apex, but once this is defined the “primordial area” is automatically defined also...In the present case the area taken to represent the “bare” apex at its mean size is that circle on whose circumference the geometrical centres of the primordia make their first appearance’ (our italics). Similarly, we define the frustum as being initiated when it can first be measured (i.e. between the first and second primordia below the apical surface), equivalent to defining the primordium as being initiated at the time it can first be seen. Just as the tissue generating the primordium is the tissue nearer the centre of the apex than the first primordium, the tissue generating the frustum is the tissue distal to the first frustum. The length of the tissue distal to the first frustum has already been defined as \(\log_e (5 \mu m + 1)\), because this is the level at which a primordium is defined as first being below the apical surface. The procedure used to estimate the length of the frustum on initiation (relative to the tissue generating it) is illustrated in Fig. 3.

The length of the frustum on initiation relative to the length of the tissue distal to it, measured in this way, decreases in the flower compared to the vegetative apex, and is also smaller in the reverted apex. The tissue distal to the first frustum is 6–11 \(\mu\)m long; the absolute length of the frustum on initiation, calculated as the product of this and the relative length, is also lower in the flowering and reverted plants than in the vegetative plants. The larger frustum on initiation in the vegetative plants, compared with the flowering plants, is correlated with the length of internodes at maturity: vegetative plants have long internodes separating their leaves, whilst flowering plants have no visible internodes separating the parts of the mature flower. Reverted plants at the ‘leaf’ stage in the 5 SD + LD treatment have initiated 30–34 parts, 10 of which are on the apical surface (Table 4). Therefore, estimates of frustum length at initiation, in plants at this stage, are made from parts 10–24. All these parts lie within the reversion zone of mature R4 plants, since the first internode above this zone occurs above part number 24 (1 s.e. = 0.7, \(n = 39\)). Consequently, the similar values for frustum length at initiation (relative or absolute) in SD and 5 SD + LD are consistent with the hypothesis that a smaller frustum length at initiation may result in a lack of internode development at maturity.

On reversion, then, at the time that intermediate parts and the first leaves are initiated, the apex initiates frusta of the same size as those found in plants flowering normally in SD. The formation of intermediate parts and leaves is therefore not accompanied by reversion of the apex to the vertical growth pattern found in vegetative (LD) plants. Further measurements would be needed to determine whether such a change eventually occurs to give rise to the internodes seen after the reversion zone.
**Table 4. Area of the apical surface and areas of primordia on initiation during different phases of development of vegetative (LD), flowering (SD), and reverting (5 SD+LD) plants of I. balsamina**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of apex</th>
<th>Stage of development (total no. of leaves + primordia)</th>
<th>Number of plants per sample</th>
<th>Mean distance from the apical centre of Area of annulus bearing primordia on apical surface (10^3 × μm^2) (± 1 s.e.)†</th>
<th>Area of primordium at initiation relative to area of apical surface (2 log r)§</th>
<th>Area of primordium at initiation (apical area × 2 log r) (10^3 × μm^2)</th>
<th>Absolute area of primordium at initiation (intercept at age 0 of regression of log (basal area of primordia) against primordium age in plastochrons) (10^3 × μm^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>Vegetative</td>
<td>Leaf</td>
<td>10</td>
<td>59.5 / 112.1</td>
<td>28.3 ± 2.7</td>
<td>0.41</td>
<td>4.7</td>
</tr>
<tr>
<td>SD</td>
<td>Flowering</td>
<td>Mid-petal (21–29)</td>
<td>3</td>
<td>48.9 / 103.2</td>
<td>29.1 ± 9.9</td>
<td>0.23</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late petal (30–38)</td>
<td>7</td>
<td>44.6 / 116.8</td>
<td>37.8 ± 5.4</td>
<td>0.26</td>
<td>1.7</td>
</tr>
<tr>
<td>Five</td>
<td>Reverting</td>
<td>Intermediate part/leaf (21–29)</td>
<td>7</td>
<td>60.5 / 112.4</td>
<td>27.5 ± 3.9</td>
<td>0.20</td>
<td>2.4</td>
</tr>
<tr>
<td>5 SD+LD</td>
<td></td>
<td>Leaf (30–34)</td>
<td>5</td>
<td>49.1 / 125.1</td>
<td>43.3 ± 6.9</td>
<td>0.20</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Geometric means presented; analysis of variance on log-transformed data: n.s.
† Analysis of variance on all five treatment stages: n.s.
‡ Data obtained from serial transverse sections; see Table 5 for number of plants per sample.
§ Analysis of variance on all five treatment stages: LD plants significantly different from remaining plants at \( P = 0.005 \).
All other data in this table were obtained from SEM photographs.
### Table 5. Lengths of stem frusta on initiation during different phases of development of vegetative (LD), flowering (SD), and reverting (5 SD+LD) plants of *I. balsamina*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of apex</th>
<th>Stage of development (total no. of leaves + primordia)</th>
<th>Number of plants per sample</th>
<th>Mean distance of the first primordium below apical surface from apical surface (length of the tissue generating the frustum) (μm) (1)*</th>
<th>Length of frustum at initiation relative to the length of tissue generating the frustum (log e v) (± 1 s.e.) (2)</th>
<th>Absolute length of the frustum at initiation [(1) x (2)] (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>Vegetative</td>
<td>Leaf</td>
<td>10</td>
<td>10.2</td>
<td>0.35 ± 0.02†</td>
<td>3.6</td>
</tr>
<tr>
<td>SD</td>
<td>Flowering</td>
<td>Mid-petal (21-29)</td>
<td>2</td>
<td>8.8</td>
<td>0.31 ± 0.06</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late petal (30-38)</td>
<td>4</td>
<td>6.7</td>
<td>0.17 ± 0.01‡</td>
<td>1.1</td>
</tr>
<tr>
<td>5 SD+LD</td>
<td>Reverting</td>
<td>Intermediate (21-29)</td>
<td>3</td>
<td>6.3</td>
<td>0.27 ± 0.02</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaf (30-34)</td>
<td>4</td>
<td>7.2</td>
<td>0.22 ± 0.02‡</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Geometric means presented; analysis of variance on log-transformed data n.s.
† Significantly different from ‡ at P = 0.001.

### Table 6. Area and vertical relative growth rates (d⁻¹) of the apical surface and frusta during different phases of development of vegetative (LD), flowering (SD), and reverting (5 SD+LD) plants of *I. balsamina*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of apex</th>
<th>Stage of development (total no. of leaves + primordia)</th>
<th>Area relative growth rate (2 log e r × rate of primordium initiation)*</th>
<th>Vertical relative growth rate (log e v × rate of primordium initiation)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>Vegetative</td>
<td>Leaf</td>
<td>0.37</td>
<td>0.32</td>
</tr>
<tr>
<td>SD</td>
<td>Flowering</td>
<td>Mid-petal (21-29)</td>
<td>0.87</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late petal (30-38)</td>
<td>0.99</td>
<td>0.65</td>
</tr>
<tr>
<td>5 SD+LD</td>
<td>Reverting</td>
<td>Intermediate (21-29)</td>
<td>0.50</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaf (30-34)</td>
<td>0.50</td>
<td>0.55</td>
</tr>
</tbody>
</table>

* Since both area and vertical relative growth rates are derived from values (see Tables 3, 4 and 5) which have error terms, standard errors cannot be calculated for the values presented.
Area and Vertical Relative Growth Rates of the Apex (Table 6)  At the time of petal initiation both the area and the vertical relative growth rates of the apex have increased about two- to threefold. In the reverting apex the area and vertical relative growth rates are intermediate between the rates in vegetative and flowering apices. The higher growth rate is therefore specific to petal initiation, since it does not occur during initiation of parts of the same plastochron age in the reverting apex. Even so, the reverted apex grows at a higher rate than the vegetative (LD) apex, indicating that reversion to leaf initiation does not involve complete reversal of the increase in growth rate accompanying flowering.

DISCUSSION

Although on reversion leaves are initiated instead of petals, the arrangement and size of the primordia at initiation are the same as in the flowering plant, and are therefore not necessarily linked to the way in which the primordia initiated by the apex develop. The rates of primordium initiation and apical growth decrease on reversion, but remain markedly higher than in the original vegetative apex. *I. balsamina* can therefore have two types of vegetative apex, each characterized by a particular primordium arrangement, rate of primordium initiation, and apical growth rate. These differences between the apex of the reverted and the original vegetative plant imply that there have been changes at the cellular and molecular levels. This might suggest that the apex on the reverted plant has been altered in such a way that its subsequent response to induction may be different from that of the original vegetative plant. If the subcellular changes are some of those which normally occur during evocation then the reverted apex could be regarded as partially evoked. These changes would presumably be concerned with altering the metabolic state of the apex, since in the reverted apex they are clearly not correlated with the formation of floral organs. By following cellular changes in the apex during induction and reversion it may now be possible to identify those partial processes of evocation which are specifically required for new types of organ to be formed, since we would expect these particular processes to be reversed during reversion of the apex.

At present we do not know how long the altered vegetative state of the reverted apex persists. However, the rate of primordium initiation appears to decrease from about day 20 onwards in the 5 SD+LD treatment (Fig. 2), suggesting that eventually the phyllotaxis and growth pattern of the apex may revert completely to that characteristic of the original vegetative apex. If this is so, we might also expect that eventually all evocational changes in the apex would be reversed and the apex would return to its original state.

There is evidence that the size of primordia at initiation decreases in flowers (Lyndon, 1978), and this is also found in *I. balsamina* (Table 4). Another very characteristic feature of flowers is a lack of internodes; this may occur either because the internodes are initiated but do not develop, or because on initiation they are much smaller than in the vegetative plant. This question does not seem to have been previously considered, although the absolute size of stem frusta (the future node plus internode) during vegetative growth has been measured (Sunderland and Brown, 1956; Berg and Cutter, 1969; Smith and Rogan, 1975). It has also been suggested that a change in the vertical spacing of primordia could be the crucial factor controlling the changes in phyllotaxis typically found at the transition to flowering (Schwabe, 1979). On flowering in *I. balsamina* the length of the frustum at initiation decreases (Table 5), and this is correlated with the lack of internodes in the mature flower. On reversion internodes eventually reappear, but we do not yet know whether, when this happens, the length of the frustum on initiation also increases, or whether it remains the same as in the flowering apex. Meicenheimer's (1979, 1982) measurements of vertical growth rate per plastochron during flowering in *Ranunculus*...
and *Epilobium* can be interpreted as estimates of frustum length at initiation, and also indicate that this decreases on flowering. The evidence available is therefore consistent with the idea that the absence of the internode at maturity is a function of decreased frustum length at initiation.

In the case of the plastochron ratio, only those primordia on the apical surface have been used to estimate the area of the primordium at initiation, and this estimate therefore reflects only the changes in growth of the apical meristem proper. On the other hand, when estimating the length of the frustum at initiation from the relative vertical positions of primordia below the apical surface, we must remember that at some point below the apical surface the growth of the sub-apical meristem becomes important, and cells of the stem frustae begin to elongate and differentiate. It is at this point that internode elongation is considered to begin in cauliflorous plants (Esau, 1977). Our measurements of the relative vertical spacings of primordia are based only on those primordia less than about 150 µm below the apical surface, and in interpreting these measurements as estimates of frustum length at initiation we have assumed that the tissues bearing these primordia belong to the apical meristem itself. Internode length at maturity is presumably also dependent on factors operating after initiation, such as photoperiod and concentrations of growth substances, which alter activity of the sub-apical, rather than the apical meristem (Sachs, 1965). Further measurements of vertical growth in *I. balsamina* show that in vegetative plants the exponential pattern of growth per plastochron continues in tissues more than 150 µm below the apical surface but that in flowering and reverted plants growth rate per plastochron declines in these tissues. This suggests that growth of the sub-apical meristem is less than in vegetative plants, and that mature internode length is dependent on this growth as well as on the length of the frustum at initiation.

Final internode size may also be controlled by the duration of internode growth. Investigation of this in relation to flowering and reversion would be of interest, since the final stages of cell elongation in the differentiating internode are known to be prevented by leaf removal, probably as a result of decreased auxin supply (Sachs, 1965). It may be, therefore, that one reason for the lack of internode development that accompanies flowering is the presence of floral parts (rather than leaves) which may not supply auxin needed for cell elongation in the internodes next to them.

The length of a stem frustum at initiation is closely related to the area of its associated primordium at initiation. Compared with the original vegetative plant, during flowering and reversion the sizes of the primordium and the frustum at initiation are smaller, both relative to the generating tissue and in absolute terms (Tables 4 and 5). The apical area growth rates and the vertical (frustum) growth rates are also higher in the flowering and reverting apices than in the vegetative apex (Table 6). These similarities between the growth of the apex in generating primordia and frustae may indicate that the generation of both is controlled by the same physiological processes. However, the initiation of the frustum (defined here as the time it first becomes visible) occurs at a variable time after primordium initiation. In the vegetative plant it occurs when the primordium is three plastochrons old (there are three primordia on the apical surface), whilst in flowering and reverted plants it occurs when the primordium is between seven and 10 plastochrons old. This variation in initiation time of the frustum presumably results from differences in the growth rate and number of cells allocated to the frustum at the time that it is determined. This determination may occur before the frustum becomes visible, as in the case of the leaf primordium which is determined before it becomes visible and therefore recognizable (Snow and Snow, 1933).

An unusual feature of flowering in *I. balsamina* is the lack of increase in apical area in the flower (Table 4). Further measurements have shown that the apical area also remains constant during the actual transition to flowering. In most, though not all, other plants the apex does increase in size on flowering (Bernier *et al.*, 1981). The attainment
of a critical apical size has been modelled as a causal event bringing about flowering in Chrysanthemum (Charles-Edwards et al., 1979). It may be, therefore, that the lack of increase in apical size on flowering is linked to the ability of I. balsamina to revert. It would be of interest to know whether other species showing flower reversion (e.g. Anagallis arvensis, Pharbitis nil) also retain a constant apical size on flowering.

ACKNOWLEDGEMENTS

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Chapter 13

THE RESPONSE OF THE SHOOT APEX TO LIGHT-GENERATED SIGNALS FROM THE LEAVES

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INTRODUCTION

Evocation has been defined as the initial events at the shoot apex in response to the arrival of the photoperiodic stimulus, which commit the plant to the subsequent formation of flower primordia (Evans, 1971). We shall address ourselves to three questions concerning evocation. First, what are the events in the apex which occur in response to light-generated signals from the leaves? Second, are all of the associated events in the apex concerned with committing it to flower and if not, which are associated, but non-essential events? Third, what do the events of evocation achieve and how do they result in the formation of flowers?

THE EVENTS OF EVOCATION

Since the earliest time of arrival of the floral stimulus at the apex is difficult to measure, any events occurring after the beginning of induction may be considered as part of evocation. The end of evocation is when the apex is committed to flower, and can be determined as the time at which inhibitors applied to the apex during or after induction can no longer prevent flowering (Kinet et al., 1971). For plants in which this point is not known, the end of evocation is taken as the time at which the first morphologically distinct signs of flowering occur (Vince–Prue, 1975).
Morphological Changes

The morphological changes preceding flower initiation (Table 1) are common, but not inevitable, concomitants of flowering in most plants, whether or not they need a stimulus to flower.

### TABLE 1. Common morphological changes preceding flower initiation.

1. Precocious axillary bud growth
2. Change in branching pattern
3. Internode extension
4. Change in leaf form (bract formation)
5. Change in phyllotaxis to more complex system
6. Enlargement of apical dome

An increase in the rate of initiation of leaf primordia (Langer and Bussell, 1964) is often associated with a change to a higher order of phyllotaxis, as in *Xanthium strumarium* (Erickson and Meicenheimer, 1977). In plants in which flowering follows very rapidly after a single inductive photoperiod, such as *Pharbitis nil* (King and Evans, 1969) and *Anagallis arvensis* (Brulfert, 1965), such changes may not occur. The most obvious increase in the rate of primordial initiation occurs later, during the initiation of the floral organs.

Often, axillary buds form precociously and closer to the apex in prefloral shoot apices as compared with vegetative ones (Thomas, 1961). This is associated with loss of apical dominance so that lateral branches grow out forming flowers or inflorescences, although buds from the base of the plant may also grow out but remain vegetative. Exceptions are mineral-starved *Silene coeli-rosa* in which the axillary buds do not grow, and a *Silene* mutant in which axillary bud outgrowth was suppressed, yet the plants flowered (Lyndon, unpublished results). The growth of axillary buds (tillers) is also suppressed in unicolum varieties of cereals (Kirby, 1973).

Rapid internode extension is often a characteristic of the prefloral phase, but not in plants such as daisies (*Bellis perennis*) and dandelions (*Taraxacum officinale*), which remain rosettes on flowering, elongation being restricted to the peduncle.

In most plants the size of the apical dome increases just before flower initiation, usually in diameter, but mainly in length in the grasses and cereals (Evans, 1960). In *Chrysanthemum morifolium*, the initiation of the first bract always
occurs when the apical dome reaches a diameter of 0.23-0.26mm (Horridge and Cockshull, 1979). However, in a few plants, such as Perilla nankinensis (Nougarede et al., 1964) and Humulus lupulus (Thomas and Schwabe, 1970), the apical dome clearly becomes smaller just before flowering.

Some of these prefloral morphological changes may be essential in some plants, such as the increase in apical dome volume seems to be in Chrysanthemum, or the outgrowth of axillary buds which always accompanies flowering in Kalanchoe blossfeldiana (Harder, 1948). In other plants these changes may be incidental and may be responses to changes in internal concentrations of metabolites or growth substances necessary for flowering, and may perhaps be symptoms of flowering rather than essential to it.

**Cytological Changes**

The cytohistological zonate pattern is usually lost on flowering (Nougarede, 1967). The distinction between the central and peripheral zones of the apical meristem tends to disappear as RNA and ribosome density increases throughout the apex (Nougarede and Bronchart, 1965; Lin and Gifford, 1976) and the distribution of enzyme activity also becomes more uniform (Jacqmbard, 1978). In Impatiens balsamina, in which reversion of the flower to vegetative growth can occur, the zonation persists (Simon, 1972) as it also does in some species in which the meristem continues a plastochronic-like functioning by forming lateral flowers (Bernier et al., 1981). An example is Sinapts alba, in which the meristem can also be made to revert to vegetative growth (Bagnard et al., 1972). A gradual loss of the zonate pattern and an increase in meristem size are also characteristic of ageing vegetative (intermediate) meristems. Thus some features associated with the transition to flowering may also occur during vegetative growth, independently of floral evocation.

Changes in the size and numbers of starch grains in the apical meristem are also characteristic of the transition to flowering (Bernier, 1971) and presumably indicate changes in carbohydrate metabolism perhaps associated with increased growth and cell division. Other cytological changes are an increase in nucleolar volume, which may be linked to the synthesis of rRNA, and an increase in dictyosome number, which is probably linked to changes in carbohydrate metabolism and the increased synthesis of cell wall material associated with increased rates of cell division and growth (Havelange, 1980).
Changes in Growth Rates

The rate of growth and cell division in the apex increases just before or at flower initiation (Table 2). In *Silene* apices induced at 13°C, although an increase in size of the apical dome was accomplished without an increase in growth rate, there was still an increase in growth rate after the sepals had begun to be initiated (Lyndon, 1977).

**Table 2.** Cell doubling times (h) in vegetative and early floral shoot apices.

<table>
<thead>
<tr>
<th></th>
<th>Vegetative</th>
<th>Floral</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lupinus</em></td>
<td>48</td>
<td>34</td>
</tr>
<tr>
<td><em>Secale</em></td>
<td>50</td>
<td>31</td>
</tr>
<tr>
<td><em>Datura</em></td>
<td>36</td>
<td>26</td>
</tr>
<tr>
<td><em>Sinapis</em></td>
<td>157</td>
<td>25</td>
</tr>
<tr>
<td><em>Silene</em></td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td><em>Triticum</em></td>
<td>41</td>
<td>22</td>
</tr>
</tbody>
</table>

The increase in the rate of cell division is in both the peripheral and central regions of the meristem although the differential between these regions, which is most marked in the vegetative apex, is retained in the reproductive apex in *Datura stramonium*, (Corson, 1969) and *Sinapis* (Bodson, 1975). In both of these species the cytohistological zonation persists. The growth rate of the apex therefore increases before or at flower initiation in all the plants that have so far been examined, whether photoperiodic or day-neutral.

Cellular Events

A number of events are apparently common to a variety of photoperiodically-sensitive plants and occur in a similar sequence in all of them (Bernier, 1971). The events are best documented for *Sinapis* (Table 3). Ten hours after the beginning of the inductive LD there is an increase in the rate of RNA synthesis. From 12 - 18 h there is an increase in the activity of several enzymes - invertase, phosphatase, and ribonuclease. At 18 h there is an increase in the number of mitochondria, soon followed by an increase in the activity of succinic dehydrogenase, suggesting an increase or changes in respiratory metabolism. There is a first peak in mitotic index at 26 h, when RNA synthesis is at a minimum. This is
followed by an accumulation of cells in the G₁ phase of the cell cycle at about 30 h, then a peak of DNA synthesis at 38 h, a G₂ maximum at 44 h - "the point of no return" (Kinet et al., 1971) - and a second peak of mitotic index at 62 h, when the first flower buds begin to become visible. Throughout, nucleolus volume gradually increases to a maximum about the time of the second mitotic peak, when the number of dictyosomes also reaches a peak (Bernier et al., 1967; Havelange and Bernier, 1974; Havelange et al., 1974; Pryke and Bernier, 1978a,b; Jacqmard, 1978; Jacqmard et al., 1972).

<table>
<thead>
<tr>
<th>TABLE 3. Sequence of events during evocation in Sinapis alba.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours after beginning of inductive LD</td>
</tr>
<tr>
<td>0 Most cells in G₂</td>
</tr>
<tr>
<td>10 Increase in RNA synthesis</td>
</tr>
<tr>
<td>12 Increase of invertase activity</td>
</tr>
<tr>
<td>14 Increase of phosphatase activity</td>
</tr>
<tr>
<td>18 Increase of ribonuclease activity</td>
</tr>
<tr>
<td>22 Increase in mitochondria</td>
</tr>
<tr>
<td>26 1st ribosome maximum</td>
</tr>
<tr>
<td>27 RNA synthesis at minimum</td>
</tr>
<tr>
<td>30 Most cells in G₁</td>
</tr>
<tr>
<td>36 RNA synthesis increased</td>
</tr>
<tr>
<td>38 Peak of DNA synthesis</td>
</tr>
<tr>
<td>44 Point of no return</td>
</tr>
<tr>
<td>46 Most cells in G₂</td>
</tr>
<tr>
<td>54 ER maximum</td>
</tr>
<tr>
<td>62 Nucleolar volume maximum</td>
</tr>
<tr>
<td>2nd ribosome maximum</td>
</tr>
<tr>
<td>2nd mitotic peak</td>
</tr>
<tr>
<td>Dictyosome number maximum</td>
</tr>
<tr>
<td>Mitochondria number maximum</td>
</tr>
<tr>
<td>Flower buds begin to differentiate</td>
</tr>
</tbody>
</table>

All these events are consistent with a synchronized cell cycle of about 34 h. The variations in the rate of RNA synthesis are what would be expected in the course of a cell cycle, with minima at the times of mitosis (Mitchison, 1971). The increases in the cytoplasmic matrix (probably indicative of an increase in ribosome number) to maxima just before the
two mitotic peaks (Havelange et al., 1974) are consistent with the release of ribosomes from the nucleolus when it is dispersed at mitosis. The increases in ER and dictyosome number about the time of the second mitotic peak are probably linked to the formation of the cell plate and the new cell wall during cytokinesis.

A similar sequence of events, though not known in such detail, and with minor modifications, is also found in the Xanthium, Pharbitis, and Chenopodium rubrum, and in the LDP Lolium temulentum (Bernier, 1971).

Synchronization of cell division also occurs in apices of Silene but as a final event of evocation, on the 8th and 9th days after the beginning of induction, immediately before sepal appearance (Francis and Lyndon, 1979). The occurrence of two mitotic peaks during evocation in all the photoperiodic plants examined, irrespective of the number of inductive cycles required, implies that synchronization is an important event of floral evocation.

In Silene there is also a smaller increase in mitotic index in the apical dome at the very beginning of induction (Francis and Lyndon, 1978a). This is associated with a reduction in the length of the cell cycle from 20 to 13 h during the first LD (Francis and Lyndon, 1978b), and an increase in the proportion of cells in the G phase of the cell cycle, measurable only 1 h after the beginning of induction (Francis, 1981a). This is a phytochrome effect, as shown by its R/FR reversibility, although FR does not simply reverse the effect of R but itself causes changes to the cell cycle (Francis, 1981b). This initial effect does not result in synchrony and is clearly separated in time and in its effects from synchrony, which occurs 7 days later. Since changes in the Pfr/Ptot ratio during the first few hours of induction are characteristic features of photoperiodically sensitive plants (Vince-Prue, 1975) they could be associated with very early changes in the cell cycle. If so, these would be difficult to distinguish from the events of synchronization in plants which require only a single inductive photoperiod.

Increases in the rate of RNA synthesis are not related to a synchronous cell cycle. In Silene, the RNA concentration increases from the 4th day of induction (Miller and Lyndon, 1977) whereas synchronous cell division does not occur until the 8th day (Francis and Lyndon, 1979). A universal observation is that the concentration of RNA increases in preflorescent apices and is most marked in the outer layers of the apex, corresponding to the meristematic mantle which develops in the reproductive apex (Nougarede, 1967), and in the target tissues e.g. where spikelets will develop in Lolium (Knox and Evans, 1966). It is less marked and may be ephemeral in the pith and...
the pith rib meristem (Miller, 1976). The bulk of this new RNA is undoubtedly rRNA, as shown by the increase in density of ribosomes in the peripheral regions of the apex on evocation (Nougarede, 1967) and by the amplification of rRNA cistrons (Jacqmand et al., 1981). Protein concentration also shows a sustained increase in parallel with RNA (Jacqmand et al., 1972; Miller, 1976).

The events of evocation are similar in plants which show the ability to flower at the seedling stage (e.g. Pharbitis and Chenopodium) and in those requiring a period of ageing before they flower (e.g. Xanthium) and therefore occur regardless of developmental age of the meristem at the time of induction.

NECESSITY OF THE EVENTS OF EVOCATION FOR SUBSEQUENT FLOWERING

The events of evocation follow as a result of induction, but changes in photoperiod produce a plethora of morphogenetic changes in plants in addition to flowering (Vince-Prue, 1975). Which of the events of evocation are important or necessary for commitment to flowering? Do some events occur as a result of changes of photoperiod, regardless of flowering? Strong evidence for the essentiality of particular events would be their constant occurrence in plants induced to flower, their inhibition thereby inhibiting flowering, and their promotion in a vegetative apex thereby inducing subsequent flower formation.

RNA Synthesis

Pharbitis nil can be induced to flower by a single dark period. The incorporation of $^{3}H$ uridine into RNA increased, immediately at the end of the critical dark period of 11 h, to twice that in the vegetative plants kept in LD (Gressel et al., 1978). Plants subject to the dark period but given three 5 min light breaks at 4, 8, and 12 h did not flower and did not show an increased rate of RNA synthesis. Particular care was taken during extraction to minimize the effects of the very active ribonuclease in these plants. Lack of such precautions may account for the different results of Stiles and Davies (1976). Although the tissue taken for these experiments was the whole plumule, the results probably represent what was happening in the apex because the increase in the rate of RNA synthesis occurs not only within the apical dome but also in the young leaves (Arzee et al., 1975). The very early increase in Pharbitis therefore occurs only when the apex goes on to flower.

In Sinapis the increase in the rate of RNA synthesis also
occurred only in plants which went on to flower, whether induced by a LD or by a displaced SD (Pryke and Bernier, 1978a). The first increase in the rate of RNA synthesis was also immediately at the end of the critical day. Treatment with cytokinin can mimic some aspects of the synchronised cell division but cannot induce flowering (Bernier et al., 1977) and does not result in an increased rate of RNA synthesis (Pryke and Bernier, 1978a).

In *Silene* plants induced with 7 LD, the RNA concentration in the apex increased by about 25% and remained higher than vegetative plants (Miller and Lyndon, 1977). In fractional induction treatments which resulted in flowering, the RNA concentration always increased and remained higher than in the SD vegetative controls, whereas in those which did not induce flowering, the RNA concentration increased only temporarily before falling again to the value in the controls. The conclusion was that a sustained RNA increase was a necessary precursor to flowering, although RNA increase alone is not sufficient for flowering in *Silene*. Application of GA3 to shoot apices resulted in a significant increase in RNA concentration in the apex but did not result in flowering or an increase in the growth rate of the apex unless inductive LD were given simultaneously (Miller and Lyndon, 1977).

The relationship between RNA content, growth rate and flowering was examined in *Silene* by comparing plants induced by LD at 13°C with non-induced plants in SD at 20°C (Miller and Lyndon, 1977). Both treatments resulted in almost the same concentration of RNA and the same growth rate at the apex, showing that whether or not the plants flowered did not depend on either the absolute RNA concentration or apical growth rate. However, the non-induced plants in SD at 13°C had a lower RNA concentration and a lower growth rate than those in LD at this temperature. These results are consistent with a higher growth rate and RNA content being necessary for flowering under strictly comparable inductive and non-inductive conditions. They are also consistent with an increased growth rate of the apex being associated with an increased RNA content.

In *Chenopodium* (SDP) an increase in the growth rate and the RNA content of the apex do not seem to be essential for evocation. During the first inductive dark period there is a decrease in RNA and DNA synthesis in the apex and apical growth is inhibited. The increase in RNA after induction is attributed to enhanced growth in the course of floral differentiation (Seidlova, 1980a,b; Seidlova and Sadlikova, 1983). An inhibition of growth before or during induction may actually promote flowering. When 6-azauridine, an inhibitor
of RNA synthesis, was applied (via the roots) for 1-2 days before induction, the RNA content of the apex decreased and the leaves each appeared about one day later than in the untreated controls, consistent with an inhibition of apical growth, but flowering was promoted (Seidlova and Krekule, 1973).

Increase in RNA content and increase in growth rate of the apex are general events of evocation, except perhaps in Chenopodium. Events in non-flowering apices stimulated into growth may closely resemble some of the changes at the beginning of evocation. When axillary buds of Cicer arrietinum were stimulated into vegetative growth by cytokinin there was a trebling of the mitotic index within an hour and an increased rate of synthesis of RNA within 1.5 h. An increase in growth rate was detectable after 2 h (Usciati et al., 1972). These observations show that an increase in growth rate, whether or not part of evocation, may be preceded by an increase in synthesis of RNA and by changes in mitotic index, indicating perturbations of the cell cycle. At least some of the increase in RNA and protein and some of the changes in mitotic frequency may be expected to be linked to increases in growth rate rather than being involved more directly in flowering.

Protein Synthesis

Obviously, different genes are expressed in the flower and in the vegetative shoot, but does this change in gene expression occur during evocation? Is evocation perhaps a general activation of the apex as Evans (1969, 1971) suggests, with major changes in gene expression occurring only during flower development? Certainly there is no overriding reason to invoke changes in gene expression for the early stages of flower initiation, which do not seem to differ qualitatively from the sort of changes which can be observed in changing phyllotaxis in vegetative apices (Lyndon, 1979b).

If evocation does involve the expression of new genes then this should be detectable as the synthesis of novel proteins during evocation. Early changes in gene expression could therefore be looked for either as new proteins or new mRNAs. In Sinapis apices there was an increase in synthesis of rRNA and sRNA during evocation but not of poly(A)-containing RNA (mRNA) (Pryke and Bernier, 1978a) although increases have been found in evoked buds of Pharbitis (Stiles and Davies, 1976). Qualitative changes, and especially small ones, would not, however, be detected by the methods used.

Apical meristems of Sinapis were labelled with $^{35}$S methionine and the complements of newly synthesized proteins in vegetative and evoked meristems (51-53 h after the beginning
of induction) were compared using the technique of isoelectric focussing and polyacrylamide gel electrophoresis (Lyndon et al., 1983). Most of the differences in the patterns of proteins synthesized were quantitative and most of the proteins were common to both evoked and vegetative meristems. However, there were some possible qualitative differences. In particular a few of the proteins synthesised in the evoked apex but not (or to a much lesser extent) in the vegetative apex occupied the same positions on the gels as proteins which are characteristic of floral buds, although in the evoked apex the flower primordia had not yet begun to be initiated. Whether such changes would be found in the earlier stages of evocation is not known.

Another approach to investigating changes in protein complement during evocation is to examine changes in amounts of antigenic proteins characteristic of vegetative or flowering apices. On evocation the apical meristem of *Stnaptis* shows the appearance of two antigens not present in the vegetative apex and the disappearance of one characteristic of the vegetative apex. Neither of the new proteins was specific to flowering however, since they could both be found in other parts of the vegetative plant (Pierard et al., 1977, 1980). When antisera were raised to the floral organs, two antigens were found specific to the stamens and one to the pistil, but none of them could be detected in the apical meristem; they were restricted to the almost mature stamens and pistil respectively and appeared to be proteins concerned with the male-female recognition system (Jacqmaud et al., 1984).

Changes in protein composition and enzyme activity have been shown to precede the morphological changes of flower initiation. The major problem is, what enzymes would be expected to change in activity in order to bring about the morphological changes? Changes in the shoot apex that can be predicted are a decrease in the activity of enzymes of photosynthesis and an increase for enzymes involved in pigment synthesis in the petals, and for synthesis of characteristic constituents of the stamens (e.g. sporopollenin) and the pistil. These changes could be fairly late in flower formation, just as the appearance of the antigens specific to stamens and pistil was late in the development of these organs. The evidence so far is consistent with qualitative changes in gene expression occurring late in floral realization rather than in evocation. Some of the earliest changes in evocation may involve synthesis of enzymes of respiratory metabolism but again these are likely to be quantitative rather than qualitative changes.
Synchronization of Cell Division

Conspicuous features of evocation are the characteristic increases in the mitotic index which have been observed in inflorescences of a whole range of different species (Bernier, 1971; Bernier et al., 1981). Experiments with *Sinapis* have shown that it has not been possible to dissociate flowering from the early increase in mitotic index (Bernier et al., 1974). No matter whether flowering was induced by a LD of 13 or more hours, by a displaced 18-h LD, or a displaced 18-h SD, there was an increase of mitotic index 16-28 h after the start of induction. In each case this was followed by a second mitotic peak about 36 h later, consistent with a synchronous cell cycle. When the plants were induced with a 16-day but were deprived of CO₂ for the last 8 h, the initial mitotic wave was delayed and so was flower initiation. In these experiments there was therefore a consistent temporal link between the rises in mitotic index, indicative of a synchronous cell cycle, and subsequent flowering.

A good test of the importance of synchrony would be specifically to inhibit it and subsequently examine the plants for evidence of effects on flower initiation. Recently, *Silene* plants were given 7 LD and then kept in darkness for 48 h before placing them in SD (Grose and Lyndon, unpublished results). The synchronous divisions were suppressed but the plants went on to flower, although flower initiation was delayed by approximately 48 h. The conclusion drawn was that synchronization itself was not an essential process for subsequent flower initiation but that it was a secondary effect of some stimulus required for an as yet unidentified aspect of flower initiation.

In *Sinapis*, the synchronized cell cycle occupies most of the period of evocation. In *Silene*, which has a much shorter cell cycle than *Sinapis* and a requirement for at least 4 cycles for induction, the synchronized cell cycles occur at the end of evocation, just before sepal initiation. In *Silene*, many events of evocation must already have occurred by the time the cells become synchronized. During synchronization the cells become more physiologically isolated from each other (Goodwin and Lyndon, 1983) and it could be that this is the important feature and still occurs even when the growth of the apex and synchronization are inhibited by darkness.

Early Changes in Cell Cycle

In *Silene*, early changes in the cell cycle occur during the first LD. Since even 3 LD are not inductive for most *Silene*
plants, are these early changes relevant to flowering? It has been shown that if *Silene* plants were given 6 LD but with the first 20 min of the low light intensity extension of each LD replaced by darkness, then flowering was inhibited (Taylor, 1975). These experiments have been repeated and confirmed (Ormrod and Francis, 1983). Exposure to this 20 min dark treatment for only the first 3 LD was still sufficient to inhibit flowering by 90%. The change in the cell cycle, measured as an increase in the proportion of cells in the $G_2$ phase of the cell cycle each evening at 20.00 in plants given 3 LD, was not found in the plants given 20 min darkness at the beginning of each extended light period. The suppression of the $G_2$ increase and the suppression of subsequent flowering are therefore correlated. The initial change in the cell cycle resulting from the first few minutes of the first LD may therefore be an important part of evocation in *Silene*. Whether this is because the changes in the cell cycle are essential or whether these are simply side effects of phytochrome changes which are achieving something else for evocation is not yet known. Light must also be required later in each LD, since the critical daylength for *Silene* is about 12 LD (Lyndon, 1984).

Another LDP, *Sinapis*, differs in that the first 20 min of the extended day are not critical because *Sinapis* will flower when given a displaced SD (Kinet, 1972). This necessarily means that instead of the normal LD there is darkness from the 8th to the 16th h of the inductive cycle.

**Which are the Essential Events of Evocation?**

The general occurrence of many events of evocation suggests that they are necessary for flowering although it has been possible to inhibit some of them without inhibition of flowering in some plants. However, the inability to separate events of evocation from subsequent flowering may not mean that they are essential, but only that the appropriate experimental treatment to divorce them from flowering has not yet been found. Alternatively, it is possible that an event may be obligatory in one species but not in another; for example, synchrony may be necessary for *Sinapis* but not for *Silene*, while events triggered by the first 20 min of light during each extended day seem essential in *Silene* but not in *Sinapis*.

Perhaps the early cell cycle changes in *Silene* accomplish the same as synchronization in *Sinapis*. If, for instance, it is an increase in growth rate which is essential it may be unimportant exactly how it is achieved.

Some events of evocation are clearly linked. The occurrence of a synchronous cell cycle will necessarily result in
Changes in mitotic index, rates of RNA synthesis, rates of protein synthesis, and nucleolar volume. The importance of some individual events may therefore be not in themselves but in some other aspect of the process of which they are an obligatory part. Other sets of events, perhaps like synchronization in *Silene*, may be by-products of the action of a stimulus whose principal effect is to alter an as yet unrecognized aspect of cellular functioning.

**THE SIGNIFICANCE OF EVOCATION**

**What does Evocation lead to?**

One way to try to understand the nature of evocation is to ask what it leads to and what type of changes would therefore have to occur in the shoot apex on flowering. For the early stages of flower initiation it is difficult to visualize what types of genes would have to be activated in order to change the form of the apex and the arrangement of the primordia on it. However, a consideration of these early morphological changes can perhaps give some clue to the type of cellular change which evocation may bring about.

On flowering the rate of initiation of primordia increases markedly, as exemplified by *Chrysanthemum* (Schwabe, 1959), *Triticum* (Kirby, 1974) and *Silene* (Lyndon, 1979a). This is despite a reduction in relative growth rate at this time which has also been shown for *Chrysanthemum* (Jeffcoat and Cockshull, 1972), *Triticum* (Williams, 1966) and *Silene* (Lyndon, 1979a). This is possible because the phyllotaxis also usually changes so that the plastochron ratio decreases. The implication is that the reduction of the size of the primordia at initiation, relative to the apical dome, which is a characteristic of lower initiation in *Silene* (Lyndon, 1978), is probably universal. In *Silene*, sepals are smaller than leaves at initiation, both relative to the apical dome and in absolute terms, and there is a further decrease in primordial size on initiation of the petals and stamens.

Probably then, one of the changes that occurs at flower initiation is in whatever controls the size of primordia at initiation relative to the apical dome. Substances which consistently affect the positioning and number of primordia at node and the fusion of primordia, are auxins and auxin antagonists (Soma, 1968; Schwabe, 1971; Meicenheimer, 1981). Gibberellic acid may speed up the rate of primordium initiation and alter phyllotaxis (Bernier et al., 1981) and it may do so by altering primordial or apical size since it resulted in a reduction in the plastochron ratio in *Xanthium* which mimicked the reduction occurring on floral induction.
(Maksymowych and Erickson, 1977). If, on flowering, there are relatively major changes in the rates of synthesis of growth substances in the shoot apex and young primordia, they might be difficult to duplicate by exogenous application and large reductions in the rate of synthesis would be almost impossible to mimic experimentally.

Does Evocation lead to Changes in Growth Substance Synthesis?

The initiation of shoots or roots from callus can be manipulated by altering the relative concentrations of growth substances in the media. If the formation of flowers is analogous then we would expect alterations in the relative rates of synthesis or availability to the apex of growth substances to be a feature of flower initiation. Such changes have been brought about in callus by altering the concentrations of auxin (Cheng, 1972) or cytokinin (Meins and Lutz, 1980) in the medium, causing the callus to habituate or to revert to dependence on an exogenous supply of the growth substance. If apical tissues were at all to resemble such callus, then changes in the rates of synthesis of one or more growth substance at evocation might be brought about by a short pulse of increased or decreased amounts coming from elsewhere in the plant. If evocation were to involve changes in the ability to synthesize some growth substances in the apical meristem, this might allow the expression of genetic information in a way not possible in the vegetative apex. Changes in gene expression during evocation could then be limited to a very few genes concerned with the regulation of growth substance metabolism and could be quantitative rather than qualitative.

The Nature of the Commitment to Flower

The ending of the juvenile stage in trees means that meristems have become competent to flower under the appropriate circumstances of environment and position on the plant although not every meristem does so. In the herbaceous plant Silene which once induced, continues to flower until it dies, each newly formed axillary bud bears two or more leaves below each flower, suggesting that each meristem is unable to flower until it has produced two leaves. Conversely, in induced Pharbitis, the apices of the lateral buds are not able to form flowers if they have more than two primordia, although at the same time other buds on the plant are forming flowers (King and Evans, 1969). The difference, therefore, between a vegetative and a flowering plant is that in the flowering plant the meristems now have the competence to form flowers although they may not necessarily do so.
This is also shown by experiments with cultures of epidermal thin layers from flowering tobacco plants. These can be made to develop to form roots, leaves or flowers according to the environment in which they are grown (Tran Thanh Van, 1980). Similarly, callus from flowering tobacco could be made to develop reproductive shoots or only vegetative ones, according to whether or not glucose was present in the medium (Chailakhyan et al., 1975) but callus from vegetative plants with or without glucose always produced only vegetative shoots. The competence of the parent plant to flower was transmitted through culture i.e. in the cellular state. This argues for a stable difference at the cellular level between the vegetative and the reproductive states. Stable differences have been shown in the ability of callus to habituate to growth substances in the culture media (Meins and Binns, 1979), but differences between callus from vegetative and flowering plants have not yet been looked for.

Is Evocation the Same in all Plants?

The transition to flowering is a 2-step process – commitment and realization. Clearly, to recognize a meristem as being evoked it is necessary for it to be subject to conditions which will allow the commitment to flower to be expressed. The action of a stimulus from the leaves could be at either step. Inhibitors can be used to prevent flowering and so identify the time of commitment. But in some plants the apex may already be committed and the effect of signals from the leaves would be to cause realization. There may also be plants in which specific stimuli are required for commitment and realization. Both steps could perhaps result from a single stimulus, or separate stimuli may be required, either simultaneously or sequentially. If signals from the leaves achieve different steps in different plants then the events in the apex leading to flowering would be expected to be different in plants requiring evocation from those requiring only realization.

Changes in Gene Expression

Even if changes in gene expression to bring about differentiation of the floral organs occur relatively late in flower development, the initial morphological changes might require early changes in the regulation of the expression of a few genes. This suggests a possible significance of the initial cell cycle events in *S. lutea* in which the proportion of cells in G2 is enhanced for 6 or more hours (Francis and Lyndon, 1978a; Francis, 1981a). It is known that chromatin undergoes
successive cycles of condensation and decondensation during normal cell cycle (Nagi, 1977). In Sinapis, the ratio of dispersed to condensed chromatin increased when the nuclei were in $G_2$ and reached maxima at or just before the peaks of mitosis (Havelange and Bernier, 1974; Havelange and Jeanny, 1984). At the first peak of RNA synthesis, 18 h after the start of induction (Pryke and Bernier, 1978a), there was a marked shift of RNA synthesis from the nucleolus to the chromatin (Bronchart et al., 1970). If this were to result in a change in the degree of transcription of a few key genes, then an increase in the proportion of cells in $G_2$ as one of the first events in evocation might result in a quantitative shift in gene expression. If these genes were concerned with the synthesis of regulatory metabolites then changes in apical development could conceivably result. If evocation is a change in cellular state (commitment) then there might be a critical event common to all evoked apices. The problem is to know what sort of cellular changes might be expected in order to bring about changes in metabolism to produce flowers.

ACKNOWLEDGEMENTS

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THE CONTROL OF THE CELL CYCLE IN RELATION TO FLORAL INDUCTION

D. Francis & R.F. Lyndon

INTRODUCTION
Following the arrival of the floral stimulus, a number of events occur in the shoot apex which commit it to flowering (Bernier, Kinet & Sachs, 1981) and are defined as evocation (Evans, 1971). An increase in the growth rate and changes in the mitotic index are two events which have been consistently found in evocation. Since cell size in apices seems to remain more or less constant, the changes in growth rate imply changes in the mean length of the cell cycle. Changes in mitotic index also indicate alterations in the component phases of the cell cycle. The questions posed here are therefore (1) how does the cell cycle in the shoot meristem change during floral evocation? (2) what are the regulating agents and how is the cell cycle regulated? and (3) what is the significance of these changes to flowering?

THE CELL CYCLE IN VEGETATIVE AND FLOWERING APICES
The vegetative apex of most higher plants typically has a zonate structure. The central zone at the summit of the meristem stains weakly with RNA- and protein-specific dyes, reflecting a lower concentration of RNA and protein relative to the peripheral zone on the flanks of the apex where leaves are initiated. As cells are displaced downwards, by growth, from the central to the peripheral zone their cell cycles shorten (Lyndon, 1973, 1976). In Chrysanthemum segetum and Rudbeckia bicolor G1 is shorter in the peripheral zone although in Rudbeckia G2 is also shortened but not to the same extent. In Pisum sativum, G1, S and G2 are all shortened in the peripheral zone although mitosis itself is of the same duration in both central and peripheral zones, as in the other two species (Table 1).

On flowering, an increase in the rate of growth and cell division typically occurs just before flower initiation in a range of unrelated species; this implies a shortening of the cell cycle (Lyndon & Francis, 1985).
Alterations in the cell cycle on flowering are also indicated by transient increases in the mitotic index which are some of the first events in shoot apices during evocation (Bernier, 1971). The first mitotic "burst" may occur very soon after the floral stimulus is presumed to reach the apex, and characteristically there is a second mitotic "burst" about 20-30 h later. An increase in labelling index (the percentage of cells labelled with DNA precursors) after the first mitotic peak suggests that cells may be progressing through a synchronous cell cycle from the first to the second mitosis. However, only for Sinapis alba and another LD plant Silene coeli-rosa, is there detailed evidence of synchronous divisions and synchronous cell cycles during evocation.

SYNCHRONISATION OF CELL DIVISION

Cell cycle in evocation in Sinapis

The mean length of the cell cycle in vegetative Sinapis plants is 288 h in the central zone and 157 h in the peripheral zone of the shoot apex (Bodson, 1975). It reduces to 35 h and 25 h respectively, when the first flower primordia are just being initiated. The shortening of the cell cycle is first observed as a wave of mitosis about 26 h after the beginning of induction (Fig. 1), without a prior wave of DNA synthesis, implying that cells already in G2 come into division, presumably because of a shortening of G2 (Kinet, Bernier & Bronchart, 1967; Bernier, Kinet & Bronchart, 1967). Cells accumulate in G1 (Jacqmand & Miksche, 1971), then there is a peak of

Table 1. Cell cycles in the central zone (CZ) and peripheral zone (PZ) of vegetative shoot apices

<table>
<thead>
<tr>
<th></th>
<th>Cell cycle phases (h)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Gl S G2 M C</td>
</tr>
<tr>
<td>CZ</td>
<td>115 10 7 3 135</td>
</tr>
<tr>
<td>PZ</td>
<td>32 8 8 3 51</td>
</tr>
<tr>
<td>Chrysanthemum segetum</td>
<td>Nougarede &amp; Rembur (1978)</td>
</tr>
<tr>
<td>CZ</td>
<td>38 13 17 1 69</td>
</tr>
<tr>
<td>PZ</td>
<td>15 8 4 1 28</td>
</tr>
<tr>
<td>Pisum sativum</td>
<td>Lyndon (1973)</td>
</tr>
<tr>
<td>CZ</td>
<td>&gt;40 19 14 &lt;1 &gt;&gt;40</td>
</tr>
<tr>
<td>PZ</td>
<td>9 12 9 &lt;1 30</td>
</tr>
<tr>
<td>Rudbeckia bicolor</td>
<td>Jacqmand (1970)</td>
</tr>
</tbody>
</table>
Fig. 1. Mitotic index (%), labelling index (%) and G2/G1 (>3C:<3C amounts of DNA) ratio in the peripheral zone of the shoot apex of 65-day old plants of *Sinapis alba* exposed to (a) LLD and 2SD (a) or (b) SD (b), (adapted from Bernier, Kinet & Bronchart, 1967; Jacqaud & Miksche, 1971).
labelling index indicating a wave of cells in the DNA synthetic (S)-phase of the cell cycle (Bernier et al., 1967). This is followed by a preponderance of cells in G2, and then the second mitotic peak about 34 h after the first. These events are consistent with a synchronous cell cycle during the interval between the mitotic peaks, corresponding with the values of 35 h for the mean cell cycle of the central zone in the flowering apex, and 25 h in the peripheral zone, measured independently from the accumulation of colchicine-metaphases (Bodson, 1975).

The maximum mitotic index measured in *Sinapis* was about 4% (in the second mitotic peak) whereas in a truly synchronous system one would expect a mitotic index approaching 100%. Since mitosis takes about one hour (Bodson, 1975) and since the first peak of mitotic index of just over 2% appears to be normally distributed over most of a cell cycle (Fig. 1), it would be equivalent to a mean mitotic index over the whole cell cycle of about 1.3%. If all cells were contributing to the mitotic peak the mean mitotic index should then be about 100/35 i.e. 2.9%. The observed mean value of 1.3% falls short of this and would be consistent with only about 45% of the cells of the shoot apex being involved in the first mitotic peak. The second mitotic peak reaches almost 4% and on similar assumptions could account for 100% of the cells of the apex. These considerations are consistent with the conclusion that only those cells that are in G2, when the floral stimulus arrives at the apex, contribute to the first mitotic peak (and perhaps to the synchronous cell cycle which follows) but that most or all of the cells are involved in the second mitotic peak. This suggests that the remaining 55% of cells not involved in the first mitosis are stimulated to synthesise DNA and progress into G2 and so are brought into the shorter (25-35 h) cell cycle. This interpretation is supported by the data showing that the growth fraction in the vegetative apex is about 34%, and increases to 61% after transition to flowering (Gonthier, Bernier & Jacqmand, 1984). A further implication would be that for faster cycling cells the control point regulating the length of the cell cycle is the passage from G2 to mitosis, and for slower cycling cells the entry into S-phase.

The relationship between the occurrence of successive peaks of mitosis and subsequent flowering has been examined in a series of experiments in *Sinapis* (Bernier, et al., 1974). In all the experiments attempted it was impossible to dissociate the flowering process from the occurrence of the first mitotic peak which therefore may be essential for evocation in *Sinapis*. However, the first mitotic peak could occur without necessarily being followed
by flowering, since plants given a 12 h LD, showed the first mitotic peak although not the second, and did not flower. In all plants which subsequently flowered there was a second mitotic peak and this occurred simultaneously with the initiation of flower buds. Synchronisation of the cell cycle may therefore be essential for flowering in *Sinapis*.

**Cell cycle in Silene**

Silene is a qualitative LD plant in which nine days, and 10-11 cell cycles elapse between the beginning of induction and the appearance of sepals (Miller & Lyndon, 1976). Measurements of the rates of growth of the apex suggested that there was a transient increase in the rate of cell division during the first LD and a more marked increase on about the eighth day after the start of induction (Table 2). Synchronisation of cell division was found to occur on day eight, just before the appearance of sepals on day nine. Approximately 20-50% of the cells became synchronised. Between 1500 h of day eight and 0100 h of day nine successive peaks were recorded for the mitotic index, proportion of cells in G1, DNA synthesis (labelling index), proportion of cells in G2, and mitotic index once more (Francis & Lyndon, 1979). The 10 h interval between successive mitotic peaks was identical to the length of the cell cycle measured for the cells of the apical dome by an

<table>
<thead>
<tr>
<th>Days after beginning of induction</th>
<th>0</th>
<th>1</th>
<th>2-7</th>
<th>8-9</th>
<th>10-13</th>
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</tr>
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<tbody>
<tr>
<td>Photoperiod</td>
<td>SD</td>
<td>LD</td>
<td></td>
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<tr>
<td>Cell cycle length (h)</td>
<td>20</td>
<td>13</td>
<td>20</td>
<td>10</td>
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<td>Approx. number of cell cycles</td>
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<td>1</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>......</td>
</tr>
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</table>

**Table 2. Changes in cell cycle length in the Silene apex**

Induction

Synchronisation

Flower development

Flower initiation
independent method (Miller & Lyndon, 1975). This suggests that in Silene, all the cells had cycles of a similar length but that, as in Sinapis, only a proportion of the cells were synchronised at the time of the first mitotic peak. In Silene, later work has shown that there are apparently two synchronous cell cycles, after which synchrony begins to disappear (Fig. 2).

In Silene, unlike Sinapis, synchrony of division and of cell cycles can be dissociated from flowering. When Silene plants were given 7 LD followed by 48 h darkness the mitotic index fell almost to zero, the apices temporarily stopped growing and synchrony did not occur (Fig. 2). When the plants were then placed in SD, growth was resumed (without synchronisation

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**Fig. 2.** Mitotic index (%) and G2/G1 ratio in the apical dome of 28-day old Silene plants exposed to 7 LD and 5 SD (days 8-12) (closed symbols—solid line) or exposed to 7 LD, 48 h darkness (days 8-9), and then 3 SD (open symbols—dotted line) (days 10-12). 50% of the 7 LD plants had 3 sepals by day 10 (\(\uparrow\)) whilst 50% of the dark treated plants had 3 sepals by day 12 (\(\downarrow\)). (Adapted from Grose & Lyndon, 1984).
of cell division) and flowers were initiated but with a delay of two days (Grose & Lyndon, 1984). The delay of flowering suggests that some growth had to take place subsequent to the end of induction for flowers to form, perhaps to allow the synthesis of molecules necessary for flowering but which are made only when the cells are actively going through the cell cycle.

The shorter cell cycle in Silene is detectable as a marked increase in cell number which has already occurred by the beginning of day eight (Miller & Lyndon, 1976) whereas the first synchronous cell cycle does not begin until late on day eight (Fig. 2) (Francis & Lyndon, 1979; Grose & Lyndon, 1984). This suggests that synchronisation may be imposed on a set of cells which are rapidly dividing.

Synchronisation in other species

In Xanthium (Jacqmand, Raju, Kinet & Bernier, 1976), Anagallis (Taillandier, 1978) and other plants, in the absence of information about the length of the cell cycle, it is impossible to be sure that successive mitotic peaks represent anything other than, at most, a partial synchronisation of small numbers of cells without a significant degree of synchrony. Until more information is forthcoming it may be incautious to assume that synchrony of cell division and the cell cycle is always characteristic of floral evocation except on a limited scale in each apex.

Evoked apices in which the cells become synchronised are unusual in that synchrony seems to be the result of, or superimposed on, cells which are growing faster and have shortened cell cycles. This unusual feature could perhaps be because only at evocation does the flow of substances reaching the apex change relatively suddenly, i.e. when components of the floral stimulus arrive.

Even if general synchronisation of cells throughout the apical meristem during evocation is the exception rather than the rule, the occurrence of mitotic peaks during evocation may be consistent with the occurrence of clusters of mitoses in particular regions of the apical dome. Such sub-populations of cells could possibly be concerned with the future initiation of floral organs, involving alterations in the polarity of growth which may relate to changes in the arrangement of primordia which occur at the beginning of flower morphogenesis (Lyndon, 1978).

CELL CYCLE DURING EARLY EVOCATION

In Sinapis the synchronous cell cycle occupies the whole of
evocation and extends up to the initiation of flower buds. Since the "point of no return" of commitment of the apex to flowering is about 44 h after the start of induction (Kinet, Bodson, Alvinia & Bernier, 1971) and just after the peak of DNA synthesis in the synchronous cell cycle, only the events of the cell cycle occurring before the peak of DNA synthesis can be concerned with the processes of evocation.

In Silene, in addition to an increase in growth rate on the eighth day after the start of induction, the growth rate was also faster during the first LD (Miller & Lyndon, 1976; Francis & Lyndon, 1978a), but this was not associated with synchronisation of cell division. In the plants exposed to 1 LD, G2 was longer than in the SD controls but G1 had become shortened from 10.0 to 3.8 h (Fig. 3) and so cells accumulated in G2 for about 12-14 h (J.C. Ormrod & D. Francis, unpublished data). The initial accumulation of cells in G2 could be detected as early as one hour after the beginning of the photoextension (Francis, 1981a). Since this consisted of 16 h low intensity light from tungsten bulbs and was preceded by 8 h high intensity light from fluorescent tubes and tungsten bulbs, there was a

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Fig. 3. Component phases of the cell cycle (h) in cells of the apical dome of 28-day old Silene plants exposed to (a) 1 LD or (b) exposed to 5 min far-red light at 1700h (FR) or (c) maintained in SD (adapted from Ormrod & Francis, 1985; J.C. Ormrod & D. Francis unpublished).
Francis & Lyndon: Cell cycle and floral induction

change in spectral quality which involved a decrease in the red/far red ratio (Smith, 1975; J.C. Ormrod & D. Francis, unpublished data). Silene was subsequently found to respond to a 5 min exposure to far-red light by an increase in the proportion of cells in G2 in the apex 3 h later. A short exposure to far-red light therefore effectively replaced the 16 h photoreversal in this respect (Francis, 1981b). Moreover, a similar exposure to red light or far-red followed by red light did not result in a G2 increase and the data were consistent with a low irradiance phytochrome response.

Interestingly, all of the various red and far-red exposures resulted in significant increases in the mitotic index relative to the untreated controls and so are not simple phytochrome effects in which far-red negates an effect of red light. Similar observations have been made on vegetative shoot meristems of etiolated Oryza sativa, in which the rate of cell division was stimulated by both red and far-red light (Rolinson & Vince-Prue, 1976). Since the mitotic index in the shoot meristem in Silene is proportional to the rate of cell division, increases in the mitotic index in response to both red and far-red light were consistent with a shortening of the cell cycle relative to the untreated control plants. However, in the two different treatments the shorter cell cycle would be achieved in different ways since far-red light resulted in an early accumulation of cells in G2 but red light did not (Francis, 1981b). Measurements of the duration of the cell cycle and its component phases in Silene have shown that both far-red, and red, light result in a shortened cell cycle, but with a marked shortening of G1 in response to far-red light (Fig. 3). In response to red, there is a dramatic shortening of G2, at least for a sub-population of cells within the apex (Ormrod & Francis, 1985). The shortened cell cycle brought about by far-red light reverts to a longer cell cycle comparable to, and at the same time as, that which occurs in untreated plants given 1 LD (Francis & Lyndon, 1978b). The effect of 1 LD on the cell cycle can therefore be ascribed to the effects of far-red light in the first 5 min of this LD.

The increase in the proportion of cells in G2 only 2 h after a 5 min exposure to far-red light poses the question of how this is achieved by the cells. There is evidence that S-phase of the cycle is shorter when the cell cycle is shortened during the first LD (Francis & Lyndon, 1978b; Ormrod & Francis, 1985). In 2 h the cells therefore must leave G1 faster than they enter it and rapidly traverse S-phase. This may be achieved by faster rates of DNA replication or activation of shorter replicons (see Francis, Kidd & Bennett, this volume). The reduction in the length of G1
could be either because the cells complete the necessary G1 events more rapidly in response to far-red light, or because on leaving mitosis the nuclei are already able to replicate their DNA, and simply do so almost immediately when far-red light has been received. There are examples from both animal and plant systems where G1 is bypassed or omitted completely from the cell cycle showing that G1 is not always a prerequisite for DNA replication (Clowes, 1967; Prescott, Liskay & Stancel, 1982).

Since one LD and the far-red treatment are both non-inductive in Silene (Miller & Lyndon, 1976; Francis, 1981a), what, if any, is the relationship between the rapid phytochrome-mediated accumulation of cells in G2 during the first LD (day 0) and the appearance of flower primordia 8 days and 9-10 cell cycles later? Similar accumulations of nuclei in G2 occur at 2000 h on the first 3 of 7 LD (i.e. days 0-2) but from days 3-6 these accumulations can no longer be detected in the apical dome. However, at 2000 h of days 7 and 8, a G2 increase reappeared in the apex even if the LD treatment was ended after 7 LD (J.C. Ormrod & D. Francis, unpublished data). These G2 increases occurred at exactly the time expected for cells synchronised after a normal induction of 7 LD (Francis & Lyndon, 1979), suggesting that the occurrence of synchrony of division on days 8 and 9 is linked to the effect of far-red light during the first 5 min of the photo-extension on each day of induction.

The importance for flowering, of the events triggered by far-red light in the first few minutes of the first 3 days of induction in Silene, is confirmed by experiments in which the plants were exposed to short periods of darkness during an otherwise inductive treatment. When Silene was given 7 LD but with a 20 min dark period at the beginning of the 16 h photoextension each day, flowering was suppressed (Taylor, 1975) or reduced to 8% of that of the controls in a normal 7 LD regime (J.C. Ormrod & D. Francis, unpublished data). Giving the dark period on only the first 3 of the 7 LD resulted in only 10% flowering and samples taken at 2000 h on each of these dark-interrupted LD showed that a G2 increase did not occur (J.C. Ormrod & D. Francis, unpublished data). Thus, the G2 increases occurring during 3 normal LD were correlated with subsequent flowering of 90-92% of the plants. These results are consistent with the hypothesis that in the apical dome of Silene during the first 3 of 7 LD, essential events are occurring which commit the vast majority of the plants to flower, and that these events are correlated with, and perhaps dependent upon, changes in the cell cycle.
Although a G2 increase was not detected during these dark-interrupted long days, the mitotic index in the apical dome increased significantly, consistent with a shortened cell cycle relative to the SD controls. A major difference between this shortened cell cycle and that occurring during 1 LD seems to be that whilst G2 was 34% of the LD cycle, it was only about 27% of the cell cycle in plants given the dark-interrupted LD (J.C. Ormrod & D. Francis, unpublished data). This suggests that in *Silene* it is the accumulation of nuclei in G2 which is correlated with flowering rather than the shortened cell cycle *per se*. Perhaps this protracted time spent in G2 (Fig. 3) is when a qualitative change in gene expression takes place in the apex of *Silene*.

In order to activate DNA synthesis, chromatin may have to undergo conformational changes (Bryant, 1982; Kelly & Trewavas, this volume), the most obvious being the dissociation of DNA from the histones and the subsequent separation of the strands of the DNA molecule. Changes in chromatin structure may therefore modulate the events of DNA replication (De Pamphilis & Wassarman, 1980). Changes in chromatin structure have indeed been found during evocation; in *Sinapis*, the amount of dispersed chromatin reached maxima just before the peaks of mitosis (Havelange & Bernier, 1974). An increase in the dispersion of the chromatin occurred first in G1 nuclei 30 h after the beginning of induction, at about the time of the first mitotic peak, and then later also in the G2 nuclei (Havelange & Jeanny, 1984). Phytochrome may therefore modulate reactions in the nucleus which result in a rapid decondensation and modification of chromatin. It would be of obvious interest to know whether in *Silene* there are also changes in the degree of condensation which result from exposure to far-red light and are associated with the changes in the cell cycle during the first few LD. However, it is still not at all clear what specific changes in gene expression would be expected during evocation (Lyndon & Francis, 1985). Changes in chromatin structure are likely, in any case to result in a general activation or deactivation of many genes (Kelly & Trewavas, this volume). If changes in phytochrome are also affecting ionic fluxes or changes in the concentrations of growth substances in the cells of the apex, then changes in the cell cycle could perhaps be indicators of other underlying changes in the cell rather than themselves being the important events of evocation.
EFFECTS OF PLANT GROWTH REGULATORS ON THE CELL CYCLE DURING EVOCATION

Plant growth regulators have a wide range of effects on flowering ranging from the promotion of flowering by gibberellins in some LD plants and some trees, to complete inhibition. When inhibition of flowering involves inhibition of growth of the shoot apex then naturally the cell cycle in the apex will be affected in some way. However, there are relatively few instances where plant growth regulators have been shown to affect the cell cycle in the shoot apex in a way that is not simply part of a general inhibition of growth. Axillary buds inhibited from growing by apical dominance are usually arrested in G1 (e.g. Cottignies, 1979) and to the extent that auxin or abscisic acid may be responsible for the arrest, these growth substances can be regarded as inhibiting the onset of DNA synthesis (see Dunham & Bryant, this volume). In apices which have the potential to respond to an inductive stimulus only the effects of gibberellic acid and cytokinin on the cell cycle in the shoot apex have been studied.

Gibberellic Acid

In *Rudbeckia* the application of gibberellic acid causes the plant to bolt and flower and also causes increases in the mitotic index and the labelling index in the central zone and subapical pith of the shoot apical meristem (Bernier, Bronchart & Jacqmard, 1964; Jacqmard, 1968). The increase in labelling index seemed to precede the increase in mitotic index by several hours suggesting that the primary action of GA$_3$ was to induce the release of nuclei from G1 to S. Also, because the peak of DNA synthesis was only about 2 h before the peak of mitosis in the central zone and subapical pith, GA$_3$ probably resulted in G2 shortening, thereby releasing nuclei from G2 into mitosis. The parallel effect of gibberellic acid on the cell cycle in the shoot apex, and on promoting flowering, are probably linked. Gibberellic acid increased the rate of cell division in the central zone (as indicated by the increase in mitotic index) which is characteristically activated on flowering in the Compositae, of which *Rudbeckia* is a member (Nougarède, 1967).

Cytokinins

In plants in which application of cytokinin can promote flowering nothing is known about the effects of cytokinin in the shoot apex. Conversely, in the plants in which the effects of cytokinin on the cell cycle in the
meristem have been studied, cytokinin is ineffective in promoting flowering. In *Sinapis*, benzylaminopurine or zeatin applied to vegetative apices resulted in an increase in the mitotic index 10 h later, but without a subsequent increase in DNA synthesis. Moreover, the cytokinin-treated plants did not flower (Bernier, Kinet, Jacqmard, Havelange & Bodson, 1977). Because the cytokinin mimicked the increase in mitotic index which occurred in induced plants, it was suggested that a cytokinin may be the component of the floral stimulus responsible for the initial synchronisation of cell division during evocation.

The effect of cytokinin in the shoot apex is clearly to promote the progression of cells from G2 to mitosis, as it does generally in other tissues and other organisms (Fosket, 1977). Whether cytokinin is part of the floral stimulus or whether it can merely substitute for a component of the floral stimulus is not clear from these experiments.

**CONTROL OF PROGRESSION THROUGH THE CELL CYCLE IN RELATION TO FLOWERING**

A characteristic feature of evocation is a faster rate of growth of the shoot meristem, often reflected in an increase in the mitotic index. Because in any particular species the duration of mitosis remains constant, the mitotic index is a measure of the rate of cell division and is inversely proportional to cell cycle duration. The implication is that increases in the mitotic index are accompanied by decreases in the lengths of the component phases of the cell cycle. However if it is an increase in growth rate of the apex just before flowering which is important, perhaps in order to allow a new pattern of primordial arrangement to be set up, then the change in the lengths of the phases of the cell cycle may be a general response to a speeding up of growth. There could perhaps be no specific control points in these cycles; they could be regulated by the general rate of metabolism. Similarly although the imposition of synchrony in the shoot meristem of *Silene* implies control of some of the cell cycles in order to get them in phase, these controls may be inessential for flowering (Grose & Lyndon, 1984; Fig. 2).

In both *Silene* and *Sinapis*, synchronisation of cell division is at its most complete immediately before floral morphogenesis. By this time, an entire sequence of events has already taken place to commit the apex to make a flower. The most probable interpretation seems to be that the arrival of substances at the apex (perhaps cytokinins or gibberellins), which are
necessary for flowering to occur, also have as a side effect the control of the passage of cells from one phase of the cell cycle to another, so that synchrony results fortuitously, at least in *Silene*.

In *Silene*, the changes that occur in the component phases of the cell cycle during the first 3 of 7 LD are not so easily reconciled to a simple requirement for faster rates of growth since it is not the shorter cell cycle which is correlated with subsequent flowering, but the accumulation of cells in G2. Thus, nuclei accumulate in G2 in response to LD not merely as a consequence of a shorter cell cycle but because of different controls on the passage of cells from one phase of the cell cycle to another. The tentative hypothesis is that G2 has a functional role during early evocation in *Silene*. Caution is necessary, however, since it has not yet been possible to show that accumulation or arrest of cells in a particular cell cycle phase leads to a particular developmental event. For example, cells differentiate equally well from G1 or G2 (e.g. Shininger, 1979).

A remarkable aspect of this cell cycle response during early evocation in *Silene* is the speed with which cells must leave G1 and traverse S-phase so that they can accumulate in G2 whilst G2 nuclei are rapidly entering mitosis. It will be of fundamental interest to elucidate the mechanisms operating during this shortened S-phase which enable rapid DNA replication. Recently, it has been found that nuclei of root-tips of *Pisum sativum* which permanently arrest in G2 do so because of incomplete DNA ligation (Van't Hooft, 1980 and this volume). These permanently arrested G2 nuclei were characterised by DNA with a molecular weight equivalent to replicon size rather than genome size. Thus, the data suggested that these nuclei were arrested in G2 with the vast majority of replicons non-ligated. Clearly, G2 accumulation in an exponentially growing population of cells in a shoot meristem cannot compare with a group of cells in the root which have ceased to grow. However, the suggestion is that ligation of replicons in actively growing cells may be a G2-dependent event.

In *Silene*, S-phase may have to shorten to less than 1 h for cells to rapidly accumulate in G2. This may in turn mean that ligation of replicons for these cells exclusively occurs in G2. Thus, for those cells speeded through S-phase, the recovery time for replicon ligation could result in a protracted G2, before these cells divide. Clearly not all cells would be affected in this manner, particularly those which are in late G2 which would be stimulated to divide in response to photo-extension, or a short exposure to far-red light.
Changes in the cell cycle in the apical dome of Silene at the start of floral induction may well be a reflection of other, as yet unidentified, events which are important for floral growth. Nevertheless the rapid accumulation of cells in G2 is at least a useful marker of the competence of the Silene apex to make a flower.

The availability of data for cell cycle behaviour in only three species, Sinapis, Silene and Rudbeckia, clearly highlights a requirement for much more information on a wider range of species. Only then will we be able to discern the relationship between changes in the cell cycle and the transition from vegetative to floral growth. If cell cycle changes do prove to be markers of other important events, then they could be extremely useful in pinpointing when critical changes which result in floral growth are likely to occur in the shoot apex.

ACKNOWLEDGEMENTS

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REFERENCES


INTRODUCTION

Silene is a genus in the Caryophyllaceae, subfamily Dianthoideae. The limits of the genus have long been uncertain. Melandrium is now included in Silene, and Viscaria has been abandoned and sunk in Lychnis. Confusion has also been sown by the current horticultural use of Viscaria to name some species of Silene and the tendency to give varieties specific epithets.

Silene is, in general, a genus of LD plants, most having a qualitative requirement. However, in S. cucubalis GA can replace the LD requirement, and S. oitites flowers best with vernalization followed by LD, the vernalization being replaceable by GA. S. alba requires either LD or vernalization. S.italic a, S. dioica, and S. nutans are apparently DN but require vernalization, with the possibility of low temperature being replaceable by GA in S. nutans (Table 1).

SILENE COELI-ROSA

En. Rose of Heaven

BOTANICAL INFORMATION AND NAMES

S. coeli-rosa (L.) Godron is an annual which is native to the Iberian peninsula and southwest Europe (see chapter Melandrium in Volume III). A red-flowered strain has been grown as a garden ornamental under the name of Viscaria cardinalis. A mixture of pink- and blue-flowered strains has been grown as V. occulata. V. candida is a white-flowered strain of this species.

FLOWER AND INFLORESCENCE STRUCTURE

The inflorescence is a monochasial cyme. The first flower is initiated and opens at the terminal apex of the shoot. The growth of the inflorescence is carried on by the outgrowth of axillary buds. The vegetative plant has a strictly opposite and decussate leaf arrangement with unequal buds at each node, and normally only the larger of the two develops, at least in the upper part of the plant. On flowering, this pattern of growth tends to continue so that the main inflorescence is therefore formed by the outgrowth of the bud at the node immediately below the terminal flower. This bud produces one pair of leaves (sometimes two or more pairs), and then a flower. The bud below this second-order flower itself grows out and repeats the process, and so on. Buds on the main stem at lower nodes also grow out in this same way, but often both buds at the lower nodes will grow out, although each tends to produce only a monochasial cyme. In prolonged LD all branches flower, but when plants are exposed to a limited number of LD the buds which grow out from the main stem at the lower nodes do not invariably flower.

The flowers have 5 sepals, initiated singly in sequence, but soon becoming fused at their bases. There are 5 petals, 10 stamens (5 antepetalous, 5 antepetalous), and 5 carpels (antesepalous) fused to form a free central ovary with septae at the very base.
Apical Morphology at Transition to Flowering

The changes at the apex as a result of induction have been described from scanning electron micrographs and from conventional sections. The first signs of flowering, which occurs at the terminal meristem, are an enlargement of the apical dome and the precocious development of axillary buds near the apex. This is soon followed by a change in phyllotaxis from opposite and decussate to helical, with the primordia of the floral organs initiated singly in sequence. The helix which describes the order of initiation of the floral organs is always opposite in direction to the helix of the axillary buds at the last few nodes below the terminal flower. Enlargement of the apex does not seem to be obligatory for flowering, since in plants starved of nutrients there is no measurable increase on flowering.

Anomalies of Structure

Variation in the numbers of floral parts normally occurs only in a very small proportion of plants, except at high or low temperatures when the proportion of plants having uncharacteristic numbers of floral organs, i.e., showing meristic variation, increases. On one occasion a very small proportion of plants growing out-of-doors formed structures which were not true flowers yet could develop ovaries, but not petals or stamens. The reason for the occurrence of these anomalous, proliferous flowers is not clear but it is thought to be an unusual combination of weather conditions allied to fungal infection of the plants.

RESPONSE TO PHOTOPERIOD

Photoperiod and Light Quality

S. coeli-rosa is a LDP. Plants have been kept in 8-hr SD for up to 9 months but flower initiation has never been found under these conditions. Several LD are required to induce flowering, usually at least 3 LD for most plants and 6 or 7 LD for 100% flowering, although flowering can sometimes be induced by only 2 LD.

When given seven 24-hr cycles of photoperiods with 8 hr light (fluorescent 90 to 100 W/m² + tungsten 15 W/m²) extended by various periods of tungsten light only (2 W/m²), the

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Table 1

REQUIREMENTS FOR INDUCTION OF SILENE SPP.

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<th>Species</th>
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<th>Ref.</th>
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</table>
Table 2
THE CRITICAL PHOTOPERIOD FOR FLOWERING OF S. COELI-ROSA

<table>
<thead>
<tr>
<th>Total length of photoperiod (hr)</th>
<th>% Flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
</tr>
</tbody>
</table>

*Note:* Plants 4 weeks after sowing were given 7 24-hr cycles of 8 hr light (fluorescent = tungsten) extended with low intensity tungsten light to give the total photoperiod shown. The plants were then transferred to short (8 hr) days and scored for flowering several weeks later.

critical photoperiod is about 16 to 17 hr (Table 2). However, this plant is sensitive to the R/FR composition and if given 7 cycles in which both tungsten (15 W/m²) and fluorescent (90 to 100 W/m²) lights are on continuously (i.e., 168 hr light), flowering is not induced. A relatively low R/FR ratio is therefore presumably required for induction. The critical daylength may also be expected to vary according to the spectral composition of the light during induction. Light intensity is also important, plants being induced less effectively when the light intensity was low during the high-intensity part of the photoperiod.

The spectral composition of the light immediately after the period of high-intensity light is important for cell division in the apex (Figure 1). When 5 min of either R or FR is given at this time the cell cycle is shortened. The effects of R and FR differ, however. With R light it is the G2 phase of the cell cycle which is shortened most, and with FR light it is G1. The significance for flowering of such effects of R and FR light on the cell cycle in the apex is unknown.

Light Breaks
When given a threshold photoperiod of 14 hr, flowering can be promoted by a LB of 2 hr in the following night. It was more effective in inducing most plants if given soon after the main light period. If given 5 hr after the end of the main light period, the number of flowers produced was increased, but since the leaf number below the terminal flower was not affected this suggested that it was the rate of flower development which was hastened rather than the time of flower initiation.

Interaction with Temperature
Over the range 13 to 27°C induction is more effective as the temperature is lowered, so that fewer LD are required to achieve a similar degree of induction (Figure 2).

Mineral Nutrition
Plants starved of nutrients by growing them from seed on sand and providing them only with distilled water always remain small and virtually unbranched, but if kept in LD they eventually flower, usually producing a single, small flower but occasionally two flowers.
Effects on Flower Development

When plants are maintained in inductive LD the time to anthesis is shortened and this is presumably, at least in part, because of the stimulation of growth which this treatment brings.
about in the early stages of flower initiation. Sepal primordia became visible 2 days earlier in continuous light than in 18-hr LD although the leaf number below the flower was unchanged, suggesting that in continuous light the time of initiation of the flower was not altered but its development was hastened.8

Effects on Stem Elongation

In the vegetative plant the internodes are short so that the plant is compact although not a rosette. Internode length is a function of daylength so that those internodes which are growing at the time of treatment elongate more in LD than in SD. This is so whether or not the LD treatment results in floral induction. The internodes also respond to applied GA by elongating and this is an effect which seems independent of induction.11

JUVENILE PERIOD AND EFFECTS OF PLANT AGE

There may be a juvenile period of 1 to 2 weeks. When plants were given seven 18-hr LD, none were induced to flower when this treatment started 1 week after sowing, but 21 and 100% flowered when LD were begun at the beginning of the second and third weeks, respectively. When 1-week-old plants were given 14 LD, 39% flowered. This shows that the plants are able to respond to induction when less than 2 weeks old but that induction must be longer when given to very young plants.8

FRACTIONAL INDUCTION

SD interpolated between LD delay or prevent flowering, unless the plant has received sufficient consecutive LD to commit it to flower, after which SD affect only the plant form, in restricting internode elongation and slowing the rate of flower development. The effects of interpolated SD are therefore evident only when LD are given in groups of not more than 3 at a time. When 4 SD are inserted between two sets of 3 LD flowering is not inhibited, whereas it is when 6 SD are used.11 Plants given 5 LD with 1 SD inserted between each of the LD did not flower,12 although in continuous alternating LD and SD the plants eventually flowered.13

EFFECTS OF DARKNESS

When 6 LD were each separated by 24 hr of darkness (instead of a SD), flowering was prevented. However if the periods of darkness were short, plants could be induced even if the total number of hours of light each day was only 12. When plants were given alternating periods of 4 hr light/4 hr darkness, then nine 24-hr cycles of this treatment gave almost complete induction.8

Periods of up to 144 hr of continuous darkness before induction have no effect on the subsequent induction although prolonged exposure to darkness kills many of the plants.8 When the plants have already been induced, exposure of up to 240 hr continuous darkness does not prevent flowering. The survivors, when returned to SD, eventually flower.14 If 48 hr darkness is given immediately after 7 LD then the synchronization of cell division which is normally found in the apex15 does not occur although the plants go on to flower, with a delay of approximately 48 hr in the time of flower initiation.14

A 20-min period of darkness at 1700 hr (immediately after the high-intensity light period) inhibited flowering in plants given 7 LD, but when the dark period was given at 0100 hr (i.e., in the middle of the period of low-intensity inductive light) there was no inhibition.8
EFFECTS OF GROWTH REGULATORS

So far it has not proved possible to induce flowering by exogenous application of growth regulators. The only effects found have been an acceleration of the appearance of the macroscopic flower bud when GA was applied during induction. IAA reduces flower number but does not alter the time of appearance of the buds: xanthoxin, B9 and ABA delay bud development: ABA also reduces flower number. The effects are, in general, obtained when the substances are applied during induction. GA is necessary for the development of the excised flower in culture and for the production of pollen.

SILENE DIOICA AND S. PENDULA — SEX EXPRESSION

The cytological basis for sex expression in S. dioica (= Melandrium rubrum) has been comprehensively summarized. Attempts to modify sex expression in this species by environmental means have not been successful. Chemical modification of sex expression has, however, been possible. Application of estrogens resulted in an increase in femaleness of the flowers and application of testosterone an increase in maleness. Inhibition of stamen growth, and an enlargement of the gynoecium in female plants, was observed when 'substantial doses' of NAA were applied.

Sex expression is also modified by infection with the smut fungus Ustilago violacea. The most marked effects are on the female flowers, in which stamen development is stimulated but calyx and ovary size are reduced. It has been suggested that the presence of the fungus results in a reduction of auxin levels in the neighborhood of the developing primordia of the floral organs.

In S. pendula, the plants can be rendered male-sterile and gynomonoecious by exposure to LD given continuously for 90 days after sowing. The stamens fail to develop properly, growth of the petals is inhibited, and growth of the gynoecium is promoted. A similar result is obtained when plants which are transferred to SD 40 days after sowing and then transferred back to LD, are treated with NAA. Plants similarly treated, but not given auxin, developed normal hermaphrodite flowers.

REFERENCES

The Growth of the Shoot Apical Meristem during Flower Initiation

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Abstract. The vegetative apex grows in an indeterminate, iterative mode. When flowers are formed it changes to determinate, sequential growth. The essential change in growth at the transition to flowering seems to be the decrease in primordium size at initiation, relative to the size of the apex. In the formation of the flower itself there may be a further requirement, that primordia undergo a decrease in absolute size at initiation. Where measured, this decrease is paralleled by a decrease in the size of the stem frustum at initiation. While in the case of the stem frustum this decrease in size has a predictable consequence, the reduced size of internodes in the mature flower, it is not clear how the reduction in size of the primordium is related to the subsequent development of flower parts. Reduction in primordium size seems to be fundamental to the changes in primordium arrangement and divergence angle that typically occur on flower formation, but not sufficient to cause the subsequent differentiation of floral parts. This presumably occurs as a consequence of the interactions between the developing primordia and substances present in the flowering apex.

Recent models of flowering show that a realistic simulation of apical growth on the transition to flowering can be made using the equations of catastrophe theory. To account for the growth and development of a flower meristem models must also account for changes in divergence angle and the new developmental pathways followed by the floral organs.

The data on the role of growth substances in the transition to flowering at the apex are at present insufficient. What is needed particularly is information on the identity of the putative inhibitor of primordium initiation that may operate within the apex, and its role in the growth changes that occur during and after the floral transition.

The events which occur in the shoot apex which commit it to flowering are termed evocation (Evans 1969). These events are followed by changes in the growth of the apex so that instead of leaves a flower or inflorescence is formed. We will consider the nature and significance of these changes in the growth of the apex, and examine possible models which may help to explain their role and lead to further investigations and understanding.

GROWTH CHANGES AT THE APEX LEADING TO AND DURING FLOWER INITIATION

The changes which usually occur at the apex as a result of evocation and before or at flower initiation are listed in Table 1. These changes are the beginning of a sequence which culminates in the formation of successive
whorls of floral organs. The apex therefore changes from an indeterminate, iterative mode of growth in the vegetative plant, in which the events of leaf initiation are repeated again and again, to a sequential mode of growth in the flower (although there may be periods of iteration, e.g. during the initiation of successive petals, stamens and carpels). When considering the significance of changes occurring at the transition between these 2 modes of growth, it is important to distinguish between those meristems that go on to form an inflorescence, and those that produce a terminal flower. The inflorescence meristem continues to produce leaves, or reduced leaves, with flowers as lateral structures, so that the growth changes are concerned with modifying the pattern in which leafy parts are produced by the apex. Changes more directly related to flower part production might be expected in the lateral meristems which these parts subtend. Only in the terminal flower meristem are the growth changes directly concerned with switching the apex to a new, sequential mode of development, in which the successive floral organs develop quite differently from leaves. We will therefore try to determine which growth changes are essential to flower or inflorescence initiation and, in addition, which changes are associated particularly with flower (as opposed to inflorescence) initiation.

Growth Rate

Although in most plants the rate of growth and cell division in the apical meristem increases just before or at flower initiation (LYNDON and FRANCIS 1984), in neither Ranunculus (a flower meristem) nor Epilobium (an inflorescence meristem) could a change in relative growth rate be demonstrated during the transition to flowering (MEICENHEIMER 1979, 1982). An increase in growth rate may therefore not always be necessary for flower initiation. Since the changes in the growth pattern at the apex that occur on flowering can be brought about in different ways even within the same species (LYNDON 1977), the increase in growth rate may facilitate these changes without always being essential. Alternatively, it may be the result of the arrival at the apex of stimuli which have other effects that are more essential for flowering.

Apex Enlargement

Enlargement of the apical dome results from an increasing proportion of tissue being allocated to the apical dome rather than to the primordium of each plastochron (LYNDON 1977), and is not necessarily a function of the relative growth rate of the apical cells. Thus an increase in the volume of
the apical dome may be accompanied either by an increase in the rate of growth and cell division, as in Sinapis (Bernier et al. 1967, Bodson 1975), or by no apparent change in growth rate, as in Xanthium (Erickson and Mieicnenheimer 1977, Maksymowych and Erickson 1977), Epilobium (Mieicnenheimer 1982), and Linum (Williams 1975). In Nicotiana the growth rate actually decreased (Williams 1975). A constant growth rate (or even a decrease) and a constant plastochron are quite consistent with an increasing apical dome size (Lyndon 1977).

Although in most plants the apical dome does enlarge on flowering (e.g. Chrysanthemum, Horridge and Cockshull 1979), in Perilla (Nougarède et al. 1964) and Humulus (Thomas and Schwabe 1970) the apical dome decreases in size. The formation of the flower may also be accompanied by an enlargement of the apical dome of the flower meristem, as in Silene (Lyndon 1977), although nutrient-starved plants flower with meristems only about half the normal size (Lyndon, unpublished data). Also, in Impatiens the flower apex remains the same size (despite an increase in growth rate) (Battey and Lyndon 1984). The models which have been developed for Chrysanthemum, incorporating the idea that enlargement of the inflorescence apex to a critical size causes flowering (Charles-Edwards et al. 1979, Thornley and Cockshull 1980) are therefore not applicable generally. Lyndon (1977) suggested that it is the change in the size of the apical dome relative to the size of the primordia on initiation which is important, rather than the absolute change in apical dome size.

**Primordium Arrangement and Size of Primordia at Initiation**

The arrangement of primordia at the shoot apex, or phyllotaxis, is uniquely defined by the plastochron ratio and the divergence angle between successive leaves (Richards 1951). The plastochron ratio is the relative radial distances of successive primordia from the apical centre (Richards 1948), and is a measure of the growth of the apex between the initiation of successive primordia. Where \( r \) is the plastochron ratio, \( \log_e r \) is the radial relative growth rate of the apex per plastochron and \( 2 \log_e r \) represents the area of a primordium at initiation relative to the area of the apical dome (Richards 1951). If the dimensions of the apical dome are known then the absolute area of the apical surface associated with a primordium on initiation can be estimated. To understand how the changes in phyllotaxis are brought about at the transition to flowering, it is therefore necessary to know how the size of the primordium at initiation (relative to the size of the apical dome), and the divergence angle, alter at this time.

In plants which make an inflorescence the leaf arrangement changes to a higher order of phyllotaxis on flowering. The plastochron ratio tends to decrease, so that the area of a primordium at initiation relative to the area of the apical dome also tends to decrease. This happens in Chrysanthemum (Evington 1955), Xanthium (Erickson and Mieicnenheimer 1977), Nicotiana and Linum (Williams 1975), in which phyllotaxis is spiral and the divergence angle remains unchanged; in Triticum, in which phyllotaxis is distichous (Williams 1975); and in Epilobium, in which the phyllotaxis changes from bijugate to spiral (Mieicnenheimer 1982). Calculations from the published data suggest that in Epilobium and Xanthium the absolute area of the primordium at initiation is constant or increases during the transition to
flowering (Table 2). The reduction in the area of primordia at initiation relative to the apical dome therefore appears to result from an increase in the size of the apical dome rather than a decrease in the size of the primordia. *Xanthium* plants treated with gibberellic acid, which did not cause flowering, showed similar changes in primordium and apical dome size, and hence in phyllotaxis (Table 2). WILLIAMS (1975) notes that in *Linum* the primordia "have very similar areas at initiation and they arise on the flanks of an apical dome which is gradually increasing in size". For *Nicotiana* and *Triticum* the data are insufficient to allow us to deduce whether the reduction in plastochron ratio takes place because of an increase in apical dome size alone, but this is strongly suggested by WILLIAMS' (1975) diagrams for

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**Table 2**

Area of apex and of primordia at initiation, calculated from published data.

<table>
<thead>
<tr>
<th>Area of apex</th>
<th>Area of primordium relative to apex</th>
<th>Primordial area on apical surface</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross sectional area of apex</td>
<td>$[10^2 \mu m^2]$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced</td>
<td>5</td>
<td>1.31</td>
<td>7</td>
</tr>
<tr>
<td>Vegetative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>132</td>
<td>1.30</td>
<td>171</td>
</tr>
<tr>
<td>GA3-treated</td>
<td>24</td>
<td>0.95</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.48</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>13</td>
<td>0.39</td>
<td>5</td>
</tr>
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<td>Chrysanthemum</td>
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</tr>
<tr>
<td>Vegetative</td>
<td>16</td>
<td>1.59</td>
<td>25</td>
</tr>
<tr>
<td>Flowering</td>
<td>116</td>
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<td>13</td>
</tr>
<tr>
<td>FLOWER MERISTEMS</td>
<td></td>
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<td>Silene</td>
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</tr>
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<td>11</td>
<td>0.71</td>
<td>7.5</td>
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<td>18</td>
<td>0.16</td>
<td>2.8</td>
</tr>
<tr>
<td>Stamens</td>
<td>12</td>
<td>0.11</td>
<td>1.4</td>
</tr>
<tr>
<td>Impatiens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>11</td>
<td>0.41</td>
<td>4.7</td>
</tr>
<tr>
<td>Petals</td>
<td>6</td>
<td>0.26</td>
<td>1.7</td>
</tr>
<tr>
<td>Reverted</td>
<td>8</td>
<td>0.20</td>
<td>1.6</td>
</tr>
<tr>
<td>vegetative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranunculus</td>
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</tr>
<tr>
<td>Vegetative</td>
<td>16</td>
<td>1.42</td>
<td>23.2</td>
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<tr>
<td>Sepals</td>
<td>23</td>
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<td>7.8</td>
</tr>
<tr>
<td>Stamens</td>
<td>54</td>
<td>0.04</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Corrected for apical angle.
** Values for a single leaf of a pair.
There are, however, 3 exceptions to this general pattern. In *Chrysanthemum* the apex enlarges but the absolute primordium size at initiation, after enlarging, then decreases on flowering (Evinston 1955) (Table 2). We do not know whether primordium size and phyllotaxis change in *Perilla* and *Humulus* on inflorescence formation, but because the apex decreases in size (Nougarède et al. 1964, Thomas and Schwabe 1970), it seems probable that the absolute size of the primordia at initiation also decreases.

The conclusion, based on the evidence so far available, is therefore that the change in phyllotaxis in an inflorescence apex at the transition to flowering often takes place because of an increase in the size of the apical dome so that only the relative size of a primordium at initiation is decreased. In some inflorescence meristems, however, there may be a decrease in the absolute size of the primordia at initiation.

There is usually a correlation between a particular phyllotaxis and the vegetative or the floral state. An example is the pineapple, in which the phyllotaxis changes from a 5/13 to an 8/21 arrangement on floret initiation, and then back to a 5/13 arrangement on reversion to leaf initiation when the crown is formed (Kerns et al. 1936). On the other hand, in *Xanthium* application of gibberellic acid does not result in flowering but brings about changes in phyllotaxis similar to those observed on flowering (Maksymowycz and Erickson 1977). The evidence is, therefore, that changes in primordium size and phyllotaxis typical of the floral transition may not necessarily result in flowering, although they appear to be essential prerequisites, since flowering is not observed in their absence.

In the flower the floral parts are often in pentamerous or hexameric arrangement whereas the leaves on the vegetative apex are not. There is, therefore, usually an obvious change in primordium arrangement and divergence angle on formation of the flower. In *Ranunculus*, for example, even though all the other floral primordia are in spiral arrangement, the petals are initiated as a whorl (Meicenheimer 1979). In the flowers in which measurements have been made — *Silene* (Lyndon 1978), *Ranunculus* (Meicenheimer 1979), and *Impatiens* (Battley and Lyndon 1984) — the primordia of the floral parts are smaller at initiation not only relative to the apical dome, but also in absolute size (Table 2). The change in primordium arrangement that occurs when the flower is formed in these species therefore results from a reduction in the absolute size of the primordia at initiation. It may be that this decrease in absolute primordium size is necessary so that, in addition, primordia can develop as floral organs rather than leaves.

Normally the changes in primordium size and arrangement in the flower meristem are correlated with the development of the primordia in the sequence: sepals, petals, stamens and carpels. In *Impatiens*, however, on reversion leaves are initiated although the apex is still producing primordia which are the same size at initiation as petals (Table 2). The phyllotaxis also remains pseudo-whorled or whorled, as in the flower, rather than returning immediately to the 3:5 arrangement characteristic of the vegetative meristem (Battley and Lyndon 1984). This evidence suggests that the control of the pathways of organ development is an aspect of floral growth which

Nicotiana; and in *Triticum* no evidence was found for a consistent change in phytomer size at initiation during the transition to reproductive growth (Griffiths 1981).
Table 3
Sizes of primordia and stem frusta at initiation relative to the generating tissue

<table>
<thead>
<tr>
<th></th>
<th>Primordium area ([2 \log e r])</th>
<th>Stem frustum length ([\log e r])</th>
<th>Ratio (\frac{2 \log e r}{\log e v})</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epilobium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>0.367*</td>
<td>0.373*</td>
<td>0.98</td>
<td>MEICENHEIMER 1982</td>
</tr>
<tr>
<td>Floral</td>
<td>0.230</td>
<td>0.250</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td><strong>Ranunculus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>0.711</td>
<td>0.630</td>
<td>1.13</td>
<td>MEICENHEIMER 1979</td>
</tr>
<tr>
<td>Sepals</td>
<td>0.230</td>
<td>0.197</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>Stamens</td>
<td>0.025</td>
<td>0.020</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td><strong>Impatiens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>0.41</td>
<td>0.35</td>
<td>1.17</td>
<td>BATTEY and LYNDON 1984</td>
</tr>
<tr>
<td>Petals</td>
<td>0.26</td>
<td>0.17</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>Reverted vegetative</td>
<td>0.20</td>
<td>0.22</td>
<td>0.91</td>
<td></td>
</tr>
</tbody>
</table>

*Values for a single leaf of a pair.

depends on different factors from those maintaining a change in the growth pattern of the meristem.

**Stem Frustum Size at Initiation**

The reduction in size at initiation relative to the apical dome may not be restricted to the primordium alone. The typical flower is characterised by a lack of internodes. This may be either because internodes are not initiated or, if they are, they do not elongate. The initial size of the stem frustum, which gives rise to the mature node + internode, has been estimated at the transition to flowering in *Impatiens* (BATTEY and LYNDON 1984). In this case the length of the frustum at initiation (relative to the generating tissue) decreases during flower initiation, in parallel with the decrease in the area of the primordium at initiation. Similar changes can be demonstrated in *Ranunculus* (a flower) and *Epilobium* (an inflorescence) (MEICENHEIMER 1979, 1982) (Table 3). This might suggest that there is a common mechanism in both flower and inflorescence meristems which controls the size of the primordium and frustum, relative to the apical dome. Changes in the absolute size of frusta at initiation might be expected to be correlated with the changes in length of successive internodes which are often characteristic of the transition to flowering.

**The Rate of Primordium Initiation**

The rate of primordium initiation commonly increases, and so the plastochron decreases, before or during the transition to both inflorescence and flower initiation. In the species for which estimates of the size of the primordia at initiation are also available (*Silene, Impatiens, Epilobium* and *Ranunculus*) the shortening of the plastochron appears to coincide with the reduction in
size of the primordia at initiation relative to the apical dome (Lyndon 1977, Meicenheimer 1979, 1982, Battey and Lyndon 1984). This is consistent with the rate of primordium initiation being a resultant of the growth rate of the apex and the size of the primordia at initiation. There seems to be no clear evidence that the plastochron itself is a determinant of apical growth. However, in the final stages of flowering, when the apical dome is used up by primordia being initiated closer to, and finally at, the summit, the relationship between primordium size and apical dome size breaks down and there is no longer any inhibition to primordia forming on the apical dome. Under these circumstances, since primordia are usually initiated in a predictable sequence, the rate of primordium initiation is presumably controlled solely by the rate at which apical cells attain competence to form primordia. However, some of the S.E.M. photographs that have been published of Helianthus inflorescence meristems (Marc and Palmer 1981) suggest that existing floret primordia may actually be promoting the initiation of new floret primordia.

MODELS OF FLOWERING

In the flower meristem there seems to be a reduction in absolute primordium size at initiation, suggesting that this change may be an essential prerequisite for the development of floral organs. There is, apparently, no such stringent requirement in the inflorescence apex, in which only a relative decrease in primordium size is typical. However, it is also clear that these changes are usually linked to many of the other changes characteristic of flowering (Table 1). Given this complexity, a useful approach is to incorporate the changes in growth rate, plastochron, primordium size, and apical size, that are known to occur during and after the floral transition, into a model of flowering. Testable predictions, based on the model, can then be made about the effects of specific experimental treatments on flower initiation and development. Such an approach has been followed for Chrysanthemum (Charles-Edwards et al. 1979, Thornley and Cockshull 1980). In their first model the required change in apical growth pattern on flowering was produced by introducing a competition factor, so that bract and floret primordia competed with the apical dome for assimilates, whereas the leaf primordia did not (Charles-Edwards et al. 1979). The model accounted for the change in growth pattern when the apex reached a critical size (Horridge and Cockshull 1979). As a result of introducing the competition factor the apex was eventually used up in the formation of floret primordia, and changes in primordium size at initiation, apex growth rate, and apical size similar to those found in other species took place. However, the question of why the attainment of a critical apical size should cause initiation of the first bract, with different metabolic properties from leaves, was left open.

In the second model (Thornley and Cockshull 1980), the equations of catastrophe theory were used to simulate the switch from vegetative to reproductive growth in the Chrysanthemum apex. The attainment of a critical apical size caused the apex to switch suddenly from one stable state (vegetative growth) to another (floral growth). Associated changes that were predicted were a decrease in primordium size, a shortening of the plastochron, and an immediate increase in apical growth rate. Whether all these changes, which are typical of flowering, actually occur as predicted in the Chrysanthemum...
apex is not yet fully known. This model is, however, stimulating in proposing that a sustained pattern of growth can nevertheless bring about an abrupt change of state once the system reaches a critical point. This is analogous to the proposal that an apex will eventually inevitably form a flower if it is left long enough. Induction would then be viewed as causing a change in one of the components of growth which would cause the apex to make the transition from the vegetative to the floral state sooner than in non-inductive conditions.

A basic assumption of both these models and of models for phyllotaxis is that the newly initiated primordia are sources of a morphogen which inhibits the initiation of new primordia until its concentration has fallen to a threshold value (Thornley 1975, Veen and Lindenmeyer 1977). We need to know whether such a substance exists, and if so what it is, since a change in primordium arrangement, as occurs in the flower, is presumably brought about by a change in its amount and distribution within the apex. Lyndon (1978) concluded, from a study of the primordium arrangement in Silene flowers, that an inhibitor produced by the primordia could also be determining the size of the primordia at initiation; this is a conclusion consistent with the model of Veen and Lindenmeyer (1977).

The models so far discussed are concerned with the change in the pattern of growth at the apex at the transition from the vegetative to the floral state, but not with the changes which occur in the formation of the flowers themselves. In the formation of the flower, as opposed to changes in the growth of the inflorescence meristem, several features must be accounted for. These are:

1) Primordia and stem frusta at initiation become smaller in absolute terms;
2) Changes in divergence angle to approximately 144° or 120° often occur and imply a sudden change in the relative effectiveness of successive primordia as inhibitors of the initiation of new primordia (Thornley 1975);
3) Primordia eventually occupy the summit of the meristem.

Changes (1) and (2) imply that a step-down in primordium size at initiation, and in postulated inhibitor production, occurs at flower initiation; (3) suggests that synthesis of an inhibitor by cells of the apical summit (as in the model of Veen and Lindenmeyer 1977) may decrease on flowering. If the inhibitor from the summit also inhibits the rate of cell growth then its decrease on flowering would allow an increased growth rate of the summit cells, which is what is typically observed (Lyndon and Francis 1984). The production of a substance by the summit of the apex which regulates the sizes of primordia at initiation is suggested by the observations of Snow and Snow (1952, 1955, 1959) that the size of primordia at initiation was reduced when the summit of the apical dome was weakened by being pricked.

We propose the following model to account for these changes when a flower is formed. Substance A is produced by primordia from the moment of their initiation, in an amount proportional to their size at initiation. It inhibits the initiation of new primordia above a threshold concentration and corresponds to the inhibitor proposed by Veen and Lindenmeyer (1977) and Thornley and Cockshull (1980). Substance B is produced by the summit of the apex. Its concentration determines the number of cells required for the
initiation of a new primordium. If the concentration of B is reduced at the
transition to flowering, smaller primordia will be initiated in the flower.
This, in turn, will reduce the amount of A produced by each primordium,
giving a sudden change in the relative effectiveness of successive primordia
as inhibitors of the initiation of new primordia.

It would be of interest to know whether known metabolites or growth
substances can produce the predicted effects on apical growth, and whether
known substances could be shown to be present in the apex and to change
in amount on flower initiation in the ways predicted by the models.

THE POSSIBLE ROLE OF GROWTH SUBSTANCES IN APICAL GROWTH
CHANGES ON FLOWERING

Auxins, and auxin analogues and antagonists, produce phyllotactic
changes when applied to apices (Tropaeolum, Ball 1944, Lupinus and
Epilobium, Snow and Snow 1937, Varnell and Vasil 1978, Meichenheimer
1981, Phaseolus, Soma 1968) or via the stem (Chrysanthemum, Schwabe
1971). Gibberellins have also been shown to affect phyllotaxis in Xanthium
(Maksymowycz and Erickson 1977) and Dipsacus (Cutter 1964). The
changes in leaf arrangement induced by gibberellic acid in Xanthium, and
by the auxin inhibitors NPA and CPIP in Epilobium (Meichenheimer 1981),
were very similar to those which precede flowering.

Evidence that young primordia produce auxin is that (1) young expanding
leaves are the main sources of auxin in the shoot (Goodwin 1978), (2) the
excision of young primordia prevents the induction of vascular tissues nor-
mally associated with them, but the primordia can be replaced (at least
partly) by auxin (Sachs 1977, Bruck and Paolillo 1984), (3) cultured
apical domes require for growth and leaf initiation a supply of auxin and
cytokinlin, but apical domes with 2 primordia do not (Shabde and Murashige
1977).

Auxins and related substances have been shown to alter divergence angle
and they may also be involved in regulating the size of primordia (Meichenheimer
1981). Treatment with NPA (an inhibitor of auxin transport) gave
larger primordia and with CPIP (an auxin antagonist) gave smaller pri-
mordia. A requirement of the models discussed previously is that inhibitor
production should be induced at sites of minimal inhibitor concentration.
If auxin were the inhibitor, this would imply that auxin synthesis is induced
by low auxin concentrations. This can happen in tissue culture showing
habituation (Cheng 1972).

These data are all consistent with the possibility that auxin might be the
inhibitor of primordium initiation. However, Shabde and Murashige (1977)
found that an auxin: cytokinin ratio of 1 : 3 allowed leaf initiation in cultured
apes, but a 3 : 1 ratio inhibited it. This suggests an interaction between
auxin and cytokinin in controlling primordium initiation. A gradient in the
ability of cultured explants of Nicotiana to habituate for cytokinin synthesis
parallels a gradient in their ability to form floral buds (Meins et al. 1980,
TURGEON 1982, TRAN THANH VAN 1973). Therefore, although the effects of
auxin on primordium initiation and arrangement suggest that it may play
a regulatory role within the apex, there are data which indicate that other
factors may be involved. More information is required on the distribution
of endogenous growth substances within the shoot apex before, during, and after the floral transition, before we can satisfactorily assess their importance in flowering.

REFERENCES


SHOOT APICAL MERISTEM DURING FLOWER INITIATION

The Effects of Vernalization on the Growth of the Wheat Shoot Apex

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ABSTRACT
The effect of vernalization on the growth of the wheat shoot apex was examined by comparing three genetic lines of Chinese Spring (CS) wheat having strong [CS (Hope SD)], medium (CS Euploid), or no [CS (Hope 5A)] vernalization requirement. The mean volume of the apical dome increased gradually in all lines, and then the apical dome enlarged rapidly as its relative growth rate (RGR) increased prior to double ridge formation. Phytomer volume at initiation remained constant, so that the ratio of phytomer to apical dome at primordium initiation decreased in successive plastochrons. In CS Euploid and in unvernalized CS (Hope 5D), the RGR of the apical dome tended to decrease at least until initiation of the collar primordium. The rate of primordium initiation at double ridge formation increased in proportion to the RGR of the apex at that time; i.e. it increased greatly in CS (Hope 5A) and vernalized CS (Hope 5D), less so in CS Euploid, but no increase was observed in unvernalized CS (Hope 5D). The time of formation of double ridges seemed to be independent of the growth rate or size of the apical dome. The number of tillers present at ear emergence was inversely proportional to vernalization requirement and was reduced by vernalization. Vernalization resulted in a decrease in the RGR of the newly-initiated leaf primordia in relation to the RGR of the apical dome and the axial part of the phytomer. Transfer of plants from long to short days at various times during growth showed that vernalization increased the number of labile primordia which could develop into either leaf, collar or spikelet. Vernalization therefore seems to alter the ability of the apex to respond to subsequent photoperiod rather than to affect its growth directly.

Key words: Triticum aestivum, wheat, chromosome substitution lines, shoot apex growth, vernalization.

INTRODUCTION
Vernalization is the promotion of flowering by previous exposure of a plant to low temperatures in the range 0 to 15 °C (Purvis, 1961). The shoot apex is the site of perception of the chilling stimulus (Curtis and Chang, 1930; Choroboczek, 1934; Purvis, 1940; Schwabe, 1954) and also the site of response, since it forms the flowers. If growth of a vernalized apex differs from that of an unvernalized apex then it might be possible to detect the effects of vernalization in the apex, before flowering, by measuring apical growth rates and changes in the partitioning of the apical tissues between phytomer and apical dome in successive plastochrons.

A convenient plant to use is bread wheat, as genetic lines have been developed which range in vernalization requirement from none to a strong requirement (Law, 1972; Law, Worland and Giorgi, 1976). In Chinese Spring (CS) wheat, CS (Hope 5A) and CS (Hope 5D) are single chromosome substitution lines in which either chromosome 5A or 5D, respectively, from the variety Hope has replaced its homologue in the variety Chinese Spring. These wheat lines permit comparisons of plants with a range of differing vernalization requirements due to allelic variation at single gene loci, Vrn1 on chromosome
5A and Vrn3 on chromosome 5D. Thus, any quantitative differences in the growth of the apices may be related to their quantitatively different responses to the vernalizing stimulus. The present work used three such lines: CS (Hope 5A) with no vernalization requirement and having the genetic construction \( Vrn1 \ Vrn3 \), CS (Hope 5D) with a strong requirement, \( vrn1 \ vrn3 \), and CS Euploid with an intermediate requirement, \( vrn1 \ Vrn3 \) (Law, 1972).

This work aimed to show whether the growth of the shoot apex while still producing leaves, and before flowering, was changed by vernalization in a way that could be correlated with the time taken from sowing to the appearance of double ridges. The experimental method has been to grow vernalized and unvernalized plants of these lines and, from sowing until initiation of double ridges, to measure the rates of initiation of primordia and the growth rates of the shoot apical dome and phytomers. The way in which the apex was partitioned at the formation of each new primordium was also followed by measuring the size of each phytomer at initiation in relation to the size of the apical dome from which it was formed. Experiments were also done to test the effect of vernalization on the number of primordia (labile primordia) whose developmental fate could be subsequently affected by the growing conditions.

**MATERIALS AND METHODS**

The three lines of wheat (Triticum aestivum L.) used in this study were CS Euploid and the substitution lines, CS (Hope 5A) and CS (Hope 5D). Dr C. N. Law of the Plant Breeding Institute, Cambridge, provided the seeds.

Grains were germinated in the dark on wet filter paper in Petri dishes at 22° for 24 h and then at 4° for 2 to 3 d. They were returned to 22° for a further 24 h before being planted in pots. This germination procedure ensured that any residual dormancy in the grains was broken and that even germination occurred.

Vernalization was done by prolonging the treatment at 4° for six weeks in sealed Petri dishes. The roots were then approx. 5 cm long and coleoptiles 2-4 cm long. Unvernalized seedlings had roots 2-3 cm and coleoptiles 1-2 cm long.

Seedlings were then transferred to sand, which was watered every other day with half-strength Hoagland's solution, and every day once the plants were 30 d old.

Plants were grown in controlled environment rooms in 24 h cycles of 18 h light 6 h dark (long days). Phillips 65–80 W warm white fluorescent tubes gave irradiance at sand level of 150 W m⁻². Humidity was maintained at about 60–65 per cent, and the temperature was 18 ± 2 °C throughout. Unless stated otherwise, plants were grown throughout in long days (LD). In experiments to alter the developmental fate of some primordia, plants were grown in LD from sowing until after double ridge formation and at 2–7 d intervals samples each of three plants were transferred from LD to short days (SD) of 8 h light and 16 h darkness to complete their development. Some plants were transferred to SD on sowing. The final leaf number was measured when the ear was visibly differentiated. The number of primordia already initiated at the time of transfer from LD to SD was measured from longisections of apices of plants kept continuously in LD.

Plants with leaves and roots removed were fixed in ethanol/acetic acid (3/1, v/v), dehydrated in ethanol and transferred through xylene and embedded in Paraplast. Longitudinal 10 μm sections in the plane of the leaf insertions were Feulgen-stained (Francis and Lyndon, 1978). The total number of leaf primordia per plant was the sum of those in the median section plus any trimmed off beforehand. Primordia were also counted on plants dissected and examined under a dissecting microscope. A double ridge was counted as a single primordium.

Measurements of apical dome, phytomer and primordial volumes were made on the
The delineation of the apical dome, phytomer and primordium in a median longitudinal section of a wheat shoot apex.

Fig. 1. The delineation of the apical dome, phytomer and primordium in a median longitudinal section of a wheat shoot apex.

median longitudinal section for each apex (Fig. 1). The parts of the apex were delineated as described by Kirby (1974). The apical dome is defined as that part of the apex which lies above the horizontal line drawn from the axil of the youngest primordium, the axil being defined as the point of inflexion of the curve along the flank of the apical dome. Limits of phytomers are defined by the lines joining the axil to the inflexion at the base of the opposite, younger primordium. The primordium is defined by the approximately vertical line joining the ends of the lines limiting successive phytomers. For each component the volume of the median longitudinal section was a function of the total volume as measured in a sample of apices by summing the volume of every serial section comprising that apex (Miller and Lyndon, 1976). The correlation coefficient, \( r \), for total volume and volume of the median section was 0.959 \((n = 35)\) for the apical dome and 0.872 \((n = 25)\) for the phytomer. The maximum and minimum volumes of the apical dome at the initiation of the \( n \)th primordium (Fig. 4) were calculated as follows. A comparison of apical volumes showed that there was some overlap between the volume of the apical dome alone with \( n-1 \) primordia and the volume of the apical dome + phytomer with \( n \) primordia. These therefore represented respectively apices which were just about to initiate the \( n \)th primordium and those which had just done so. The mean of these values which overlapped was therefore taken as the mean maximum volume of the apex just before initiation of the \( n \)th primordium. The mean minimum volume of the apical dome at the beginning of plastochron \( n \) (just after the initiation of primordium \( n \)) was taken as the mean for the apical domes alone of the apices + phytomer used for the previous calculation. If there was little overlap, the three highest values of the volume of the apical dome with \( n-1 \) primordia, and the 3 lowest values of the apical dome + phytomer with \( n \) primordia were averaged. For some of the later plastochrons in CS (Hope 5A) and vernalized CS (Hope 5D) (where there were few apices with the same number of primordia) the total number of apices with a given number of primordia was less than six, so only the two largest and two smallest apices were used to obtain maximum and minimum values.

To measure the rate of cell division using colchicine the plants were first removed
carefully from their pots, and the roots were washed free of sand. Plants were then placed in 0.05 per cent (w/v) colchicine solution up to the level of the shoot apex, with the leaves out of the liquid. The solution contained 1 per cent (v/v) dimethyl sulphoxide to aid penetration of the colchicine. Plants sampled at intervals were then fixed in ethanol/acetic acid. Each apical dome was stained with lactopropene orcein and a squash preparation made (Dyer, 1963). Samples were taken over about 24 h, but the rate of division was calculated only over the period when the rate of accumulation of colchicine metaphases was maximal; from 5-6 to 11-13 h after colchicine treatment began. In all samples anaphases and telophases were absent during this period.

| TABLE 1. Growth and time (d) of ear emergence in Chinese Spring (CS) Euploid and substitution lines of CS wheat, when unvernalized (UnV) and vernalized (V) |
|-----------------|----------------|----------------|
|                 | CS (Hope 5A)  | CS Euploid     | CS (Hope 5D)   |
| Planting to ear emergence | 49.6±0.3 | 52.4±0.5 | 53.8±1.5 | 53±0.3 | 105±0.6 | 52±0.5 |
| Planting to initiation of the primordium which becomes the collar | 9 | — | 17 | — | 20 | 8 |
| Initiation of collar to double ridges | 8 | — | 16 | — | 44 | 9 |
| Planting to double ridges | 17 | — | 33 | — | 64 | 17 |
| Double ridges to ear emergence | 33 | — | 51 | — | 41 | 35 |
| Total number of leaves per plant | 7±0 | 7±0 | 10±0 | 7.5±0.2 | 12.7±0.2 | 7±0 |

Means (± s.e.) of 15–20 plants from two to three experiments. Other values from Fig. 2.

RESULTS

The effect of vernalization was to reduce the time to ear emergence in CS Euploid and CS (Hope 5D), but not in CS (Hope 5A), which behaved as a spring variety (Table 1). Vernalization of CS (Hope 5D) and CS Euploid reduced the time for ear emergence to the same as for CS (Hope 5A). Thus, vernalization hastened ear emergence principally by causing earlier formation of double ridges with consequently fewer leaves per plant. The duration of the period from double ridges to ear emergence was not obviously related to vernalization requirement, as Flood and Halloran (1984) have also shown.

The rate of initiation of primordia at the apex was very similar in all lines until collar initiation (Fig. 2). At about the time when the primordium destined to become the collar was initiated there appeared to be an increase in the rate of primordium initiation in many, but not all, experiments. A much more marked increase in the rate of initiation of primordia occurred when double ridges became apparent except in unvernalized CS (Hope 5D) in which there was no such increase. The rate of initiation of primordia at the time of double ridge appearance (Fig. 2) was inversely related to vernalization requirement and to the interval between planting and double ridge formation (Table 1).

Double ridge formation was preceded by a rapid increase in the volume of the apical dome, mainly attributable to increase in its height (Fig. 3). Because the volume of the apical dome depends on the growth made each plastochron and also the proportion of the apical dome which is cut off as phytomer at the end of the plastochron when the plastochron is initiated (Lyndon, 1977), the basis for the increase in height may be found by following the growth of the apical dome from plastochron to plastochron (Fig. 4). The relative growth rate (RGR) of the apical dome, redefined each plastochron as that part of the apex distal to the youngest primordium, is given by the slopes of the
inclined lines between minimum and maximum dome volumes (Fig. 4). A new primordium is initiated at each vertical line, which represents the difference between the maximal size of the apical dome at the end of a plastochron and the minimal size at the beginning of the next plastochron, and hence the size of the phytomer (primordium plus axial tissue) which is formed. The (geometrical) mean size of the apical dome is given by the midpoint of each inclined line. The mean size of the apical dome tended to increase faster with time in CS (Hope 5A), and probably in CS (Hope 5D)-vernalized, but to increase at a constant rate or slower in unvernalized CS (Hope 5D) and CS Euploid. There was also a tendency for the RGR to increase with time in CS (Hope 5A) (and probably CS (Hope 5D)-vernalized) but to decrease with time in CS Euploid and (more clearly) in unvernalized CS (Hope 5D). The proportion of the apical dome at the end of a

FIG. 2. Rate of initiation of primordia in CS Euploid and in substitution lines of Chinese Spring wheat. c, primordium subsequently forming the collar; dr, appearance of double ridges. Vertical bars indicate 1 s.e. Different symbols indicate different experiments. A, CS (Hope 5A); B, CS (Hope 5D)-vernalized; C, CS Euploid; D, CS (Hope 5D).
plastochron which was cut off as phytomer at the beginning of the next plastochron (the actual height of the vertical lines in Fig. 4, which represents the change in log volume) tended to diminish with time in all lines because the apical dome gradually increased in volume but the absolute size of the phytomer remained approximately constant and was similar in all lines; (mean volume of median section of phytomer in $10^3 \mu m^3$ was $41.6 \pm 1.8$ s.e.; range 24–64; $n = 29$). The apical dome therefore increased in height in CS (Hope 5A) and CS (Hope 5D)-vernalized because of the increase in RGR rather than a reduction in absolute size of the phytomer at initiation.

Measurements of the growth rate of apices of the two early flowering lines [CS (Hope 5A) and CS (Hope 5D)-vernalized] by the use of colchicine showed that the rate of cell division was approximately twice as fast at about double ridge formation than it was just after planting (Table 2). For plants with six leaves + primordia the calculated cell doubling times were 1.7 d for CS (Hope 5A) and 2.8 d for CS (Hope 5D)-vernalized. These compare with values of 2 d for CS (Hope 5A) and 1.6 d for CS (Hope 5D)-vernalized derived from the RGRs for plastochron 6 which in turn were obtained from measurements of the changes in apical dome size (Fig. 4).

Vernalization reduced the growth rate of the leaf primordium in relation to the rest of the phytomer or the apical dome (Table 3). When the RGR of a particular primordium and either its corresponding phytomer or the apical dome are compared, the ratio of RGR primordium/phytomer or primordium/apical dome was in general lower in the
TABLE 2. Rate of cell division, and cell doubling times, as measured by the rate of accumulation of colchicine-metaphases in the shoot apical meristem of CS (Hope 5A) and CS (Hope 5D)-vernalized lines of wheat

<table>
<thead>
<tr>
<th>Number of leaves plus primordia present when measurements made</th>
<th>Rate of cell division (% cells h⁻¹)*</th>
<th>Cell doubling time (d)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (Hope 5A)</td>
<td>6</td>
<td>1·70</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3·99</td>
</tr>
<tr>
<td>CS (Hope 5D)-vernalized</td>
<td>6</td>
<td>1·03</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1·86</td>
</tr>
</tbody>
</table>

* Mean rate of increase h⁻¹ of % metaphases.
† Cell doubling time = \( \frac{100 \log_2}{\text{Rate of cell division}} \); (see Lyndon, 1970).

Collar was the 8th primordium; double ridges were formed when 14 [CS (Hope 5A)] or 15 [CS (Hope 5D)-vernalized] primordia were present.

CS (Hope 5D)-vernalized than in the CS (Hope 5D), especially after collar initiation (primordium 8). The only exception was primordium 7.

Vernalization also reduced the mean number of tillers per plant at ear emergence from 5·3±0·3 in CS (Hope 5D), to 4·5±0·5 in CS Euploid and to 2·6±0·5 in CS (Hope 5A) and 2·4±0·1 in CS (Hope 5D)-vernalized.
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**Table 3. Effect of vernalization on the relative growth rate of the primordium in relation to the relative growth rates of the apical dome and the whole phytomer in Chinese Spring (Hope 5D) Wheat**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Primordium number</th>
<th>RGR d⁻¹ of primordium (1)</th>
<th>RGR d⁻¹ of apical dome (2)</th>
<th>Ratio (1)/(2)</th>
<th>RGR d⁻¹ of phytomer (3)</th>
<th>Ratio (1)/(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (Hope 5D)-vernalized</td>
<td>6</td>
<td>0.55</td>
<td>0.44</td>
<td>1.25</td>
<td>0.13</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.63</td>
<td>0.23</td>
<td>2.74</td>
<td>0.27</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.51</td>
<td>0.50</td>
<td>1.02</td>
<td>0.13</td>
<td>3.92</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.37</td>
<td>0.38</td>
<td>0.97</td>
<td>0.12</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.57</td>
<td>0.56</td>
<td>1.02</td>
<td>0.14</td>
<td>4.07</td>
</tr>
<tr>
<td>CS (Hope 5D)</td>
<td>6</td>
<td>0.77</td>
<td>0.50</td>
<td>1.54</td>
<td>0.16</td>
<td>4.81</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.62</td>
<td>0.45</td>
<td>1.38</td>
<td>0.14</td>
<td>4.43</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.63</td>
<td>0.40</td>
<td>1.58</td>
<td>0.13</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.45</td>
<td>0.36</td>
<td>1.25</td>
<td>0.05</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.78</td>
<td>0.23</td>
<td>3.39</td>
<td>0.06</td>
<td>13.00</td>
</tr>
</tbody>
</table>

The relative growth rates of the whole phytomer and the primordium (phytomer minus axial tissue) were obtained as the regression coefficients of log, phytomer or primordium volume as a function of time for several days after initiation.

**Table 4. Summarized main results of experiments with Chinese Spring Wheat Euploid and substitution lines to measure the effect of transfer of plants to short days (SD) after growth in long days (LD)**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Minimum leaf number</th>
<th>Maximum leaf number</th>
<th>Number of labile primordia</th>
<th>First primordium always forming a spikelet irrespective of treatment</th>
<th>Number of primordia already initiated at latest time of transfer to SD which could alter fate of labile primordia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (Hope 5A)</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>CS (Hope 5D)-vernalized</td>
<td>8*</td>
<td>13</td>
<td>5</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>CS Euploid</td>
<td>9</td>
<td>12</td>
<td>3</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>CS (Hope 5D)</td>
<td>12</td>
<td>14</td>
<td>2</td>
<td>16</td>
<td>26</td>
</tr>
</tbody>
</table>

* In earlier experiments with a different batch of grain the minimum leaf number was 7 (see Fig. 1).

The effect of vernalization on the developmental fate of labile primordia which can develop into either leaf, collar or spikelet (Gott, Gregory and Purvis, 1955) was investigated by experiments in which plants were grown in either LD or SD continuously from sowing, or were transferred from LD to SD after an initial period of growth in LD. The final leaf number varied according to the treatment, sometimes in a complex fashion. The main results are summarized in Table 4. The number of primordia which could develop into either leaf (or collar) or spikelet was two in CS (Hope 5A) and CS (Hope 5D), and three in CS Euploid, but in CS (Hope 5D)-vernalized there were five labile primordia. Vernalization had therefore increased the number of labile primordia. If CS (Hope 5D)-vernalized plants were grown in SD from sowing, the leaf number (13) was almost restored to that (14) of the unvernalized CS (Hope 5D) plants.
DISCUSSION

Before the appearance of double ridges, the only change found at the apex as a result of vernalization was a reduction in the growth rate of the primordium relative to that of the axial tissues and apical dome (cauline tissue), so that the growth of the young leaf primordium tended to be less vigorous in relation to the growth of the cauline tissues. Although similar to the effect of vernalization in shortening the leaves of rye (Purvis and Hatcher, 1959), in rye those leaves were already present when the seeds were vernalized whereas in the wheat the measurements were only of those leaves initiated after vernalization and sowing. The decreased growth rate of the primordium relative to the axis after vernalization may be related to the earlier transition to flowering in a way comparable to the reduction in the LD requirement in *Silene* plants when the growth rates of the primordium and phytomer were lower relative to the growth rate of the apical dome (Lyndon, 1977). Although the apex may become predisposed to make the

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Apical RGR d⁻¹</th>
<th>Apical dome height (μm)</th>
<th>Proportion of apical dome cut off as phytomer</th>
<th>Rate of initiation of primordia at the appearance of double ridges (primordia d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (Hope 5A)</td>
<td>0.39</td>
<td>68</td>
<td>0.46</td>
<td>2.1</td>
</tr>
<tr>
<td>CS (Hope 5D)-vernalized</td>
<td>0.50</td>
<td>67</td>
<td>0.51</td>
<td>3.3</td>
</tr>
<tr>
<td>CS Euploid</td>
<td>0.24</td>
<td>73</td>
<td>0.43</td>
<td>0.9</td>
</tr>
<tr>
<td>CS (Hope 5D)</td>
<td>0.20</td>
<td>95</td>
<td>0.32</td>
<td>0.3</td>
</tr>
</tbody>
</table>

transition to flowering earlier by becoming more sensitive to the inductive treatment (in the case of wheat LD), it is unclear what directly regulates the time of the actual transition. If the shift in the emphasis of growth from primordium to axial tissue is an essential part of vernalization, then it would be expected to be changed or reversed by devernalizing treatments.

The only difference between lines which could be related to the eventual rate of primordium initiation at double ridges was the RGR at collar initiation (Table 5). Since the RGR continued to fall with time in CS Euploid and CS (Hope 5D), then it might be expected that the sooner the transition to double ridges was made the higher would be the RGR of the apex at double ridge stage. This interpretation implies that the critical event would be the transition to double ridges, and that the rate of primordial initiation depends on the RGR of the apex at that time. If so, the effect of vernalization would be to bring forward the time of the appearance of double ridges. The rate of primordial initiation would simply be related to the time that this occurred. Similarly, change in all parameters in a comparable way in all lines would imply that the earlier the collar and double ridges were formed then the higher the RGR, the smaller the apical dome, and the greater the proportion of the apex cut off as phytomer at the time of initiation of the primordium ultimately forming the collar (Table 5). The implication is also that there is no critical value for apical size associated with collar initiation, which is, in any case, not a fixed point. It was possible to anticipate the appearance of double ridges only
from the increase in height of the apical dome immediately beforehand. It was not possible to predict the time of the appearance of double ridges from any other aspect of apical growth that was examined, and this included the rate of initiation of primordia, the RGR of the apex, and the size of the primordia and phytomers formed relative to the apical dome.

The marked increase in the rate of primordium initiation at the appearance of double ridges occurred at about the same time as the apical dome reached its maximum height in CS (Hope 5A) and CS (Hope 5D)-vernalized (Figs 2 and 3). The increase in the rate of primordial initiation was, therefore, preceded by the increase in height of the apical dome. The increase in the rate of initiation of primordia could have been associated with a further increase in RGR or may have been because of continued primordium initiation made possible by a decrease in the size of the apical dome, so that successive primordia would be formed progressively nearer the summit of the apex. The latter would account for the halt in the increase, or the decrease, in height of the apical dome also noted by Kirby (1974) and Cottrell, Dale and Jeffcoat (1981). In CS (Hope 5D) the eventual increase in height of the apical dome (Fig. 3), but without an increase in the rate of primordial initiation before double ridge formation (Fig. 2), suggests that they are not necessarily linked events.

One effect of vernalization was to increase the number of labile primordia. Thus, in CS (Hope 5D)-vernalized, primordia 10 to 13 could form spikelets in LD, whereas in CS (Hope 5D)-vernalized in SD or in the unvernalized CS (Hope 5D) in LD or in SD they could form only leaves or the collar. It was shown for rye that the effect of LD is because of a substance migrating from the leaves since a single leaf in LD hastened the flowering of vernalized plants (Gott et al., 1955). Vernalization in wheat, as in rye, therefore allows certain primordia to form spikelets in response to LD and so can be regarded as making the apex sensitive to substances produced in the leaves in LD. Double ridges were eventually formed even in SD, but then it took the same time after planting, and the same number of leaves were formed, whether or not the apex was vernalized (Griffiths, 1981) showing that LD are necessary for vernalization to result in earlier flowering.

There are clearly two stages in the progress to flowering in CS (Hope 5D) wheat, since for vernalization to be effective it must be followed by LD, and without vernalization CS (Hope 5D) produces the same number of leaves in LD as in SD and so LD are not effective alone either (Table 1). The effect of vernalization can be interpreted as producing a change in the state of the cells of the shoot apex which parallels the phase change which occurs during the transition from juvenile to adult in woody plants (Wareing, 1959). The cells thus become responsive (or sensitive, or competent to react) to floral stimuli, produced in the leaves in LD, which hasten flowering.

In all lines the fate of at least some of the primordia could be altered until double ridge formation occurred, but thereafter transfer from LD to SD was no longer able to alter the final leaf number; the conclusion is that vernalization increases the number of labile primordia and their developmental fate (and the site of the collar) is not fixed until double ridge formation, which is always later than the initiation of the last labile primordium.

The substitution lines, CS (Hope 5A) and CS (Hope 5D), differ from CS Euploid by having different alleles at the \textit{Vrn1} locus on chromosome 5A and the \textit{Vrn3} locus on chromosome 5D. The genetical constitution of CS Euploid is \textit{vrm1 Vrm3}, CS (Hope 5A) is \textit{Vrm1 Vrm3} and CS (Hope 5D) is \textit{vrm1 vrm3} – all the other vernalization genes are the same (Law et al., 1976). CS (Hope 5A) therefore carries alleles for reducing vernalization requirement at both loci, CS Euploid one and CS (Hope 5D) none – both the alleles in this line increasing vernalization requirement. Comparisons between CS (Hope 5A) and CS Euploid indicate the effect of allelic substitutions at the \textit{Vrn1} locus, and comparisons between CS (Hope 5D) and CS Euploid, the effects of substitutions at the \textit{Vrn3} locus.
Both the \textit{Vrn1} and \textit{Vrn3} loci are located at similar sites on each of the homoeologous chromosomes 5A and 5D. They are almost certainly genes which are functionally identical. The results of the present experiments support this by showing that the same developmental stages are affected when the variants at \textit{Vrn1} and \textit{Vrn3} are compared. It would be of interest to study another vernalization gene, e.g. \textit{Vrn5} (Law and Scarth, 1984) which is genetically different from \textit{Vrn1} and \textit{Vrn3} to see whether this influences a different stage of development.

\textbf{ACKNOWLEDGEMENT}

This work was made possible by the award of a SERC/CASE award to F.E.W.G.

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Apical Growth and Modification of the Development of Primordia During Re-flowering of Reverted Plants of *Impatiens balsamina* L.

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**ABSTRACT**

*Impatiens balsamina* L. was induced to flower by exposure to 5 short days and then made to revert to vegetative growth by return to long days. After 9 long days reverted plants were induced to re-flower by returning them to short days. Petal initiation began immediately and seven primordia already present developed into petals instead of into predominantly leaf-like organs. However, the arrangement of primordia at the shoot apex, their rate of initiation and size at initiation remained unchanged from the reverted apex, as did apical growth rate and the length of stem frusta at initiation. The more rapid flowering of the reverted plants than of plants when first induced, and the lack of change in apical growth pattern, imply that the reverted apices remain partially evoked, and that the apical growth pattern and phyllotaxis typical of the flower, and already present in the reverted plants, facilitate the transition to flower formation.

Key words: *Impatiens balsamina*, flower reversion, partial evocation, shoot meristem, determination, leaf development.

**INTRODUCTION**

Flowering in the short day (SD) plant *Impatiens balsamina* is unusual because the terminal flower meristem reverts to leaf initiation if the plants are returned to long days (LD) during flower formation (Krishnamoorthy and Nanda, 1968; Debraux and Simon, 1969). Such reversion does not involve reversal of the increase in apical growth rate and change in phyllotaxis that accompany flowering in *I. balsamina* (Battey and Lyndon, 1984). The reverted apex therefore differs from both the original vegetative apex and the flowering apex, because it has a phyllotaxis characteristic of the flowering apex, but produces leaves.

The shoot apex in the reverted plants is therefore a vegetative meristem that has a growth pattern like a flowering meristem. If reverted plants are made to re-flower, by transfer to SD, and there is a further increase in apical growth rate and a change in phyllotaxis, the implication would be that these changes are a necessary precursor of flower formation. In this case we might expect the response of the reverted plants to SD to be very similar to that of plants when originally induced. On the other hand, if it is not the changes themselves but rather the type of growth pattern at the apex which is necessary for flowering, then we might expect there to be little or no change in growth pattern in apices of reverted plants on re-flowering. The plants might also be expected to respond to SD more rapidly than when they were induced originally.

Plants which had been induced to flower by SD and then made to revert by transfer to LD were therefore made to re-flower by return to SD. The changes in apical growth rates and in the arrangement, size and rate of initiation of primordia were compared

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during re-flowering of these reverted plants and during flowering of vegetative plants when first induced. It will be shown that not only did the reverted plants respond to SD more rapidly than did vegetative plants when first induced, but primordia already present on the reverted meristem could be made to switch their developmental pathway and grow as petals, instead of as leaf-like organs.

MATERIALS AND METHODS

Plants were grown as described by Battey and Lyndon (1984), except that only plants with red flowers were used because they gave the most uniform reversion response (Battey, 1985). Plants were raised from seed in non-inductive LD. On the 7th day after sowing (designated day 0, the beginning of the experimental treatments) plants were induced by 5SD and then made to revert by transfer back to LD (Battey and Lyndon, 1984). Reverting plants were made to re-flower by transfer back to SD after 9LD.

Morphological characteristics were scored when the flowers were fully opened and the stamens clearly visible. The effect of the different photoperiodic treatments (5SD+9LD+SD or 5SD+LD) on flower form was quantified by measuring the percentage of the surface of the mature parts of the flower, or reverted flower, occupied by petal pigment. The plant parts were removed at their points of attachment to the stem, and their total areas and the areas over which coloured (petal) pigment occurred, were traced and measured using a Reichert-Jung ‘Videoplan’ digitizer.

Measurements of rates of apical growth and primordium initiation, of sizes of primordia and frusta at initiation, and of phyllotaxis, were made as described by Battey and Lyndon (1984), except that the plastochron ratio was measured on photographs of the first transverse section to graze the apical surface of each plant, rather than on scanning electron micrographs of the shoot apex. The centre of the apex was fixed by equalizing the radial distances of the primordia of the youngest whorl or pseudo-whorl as far as possible, rather than by the method described previously (Battey and Lyndon, 1984). This was because of possible shifts in the relative positions of older primordia as they were displaced away from the apex.

RESULTS

Initiation of primordia

After 5SD + 9LD (day 14) reverted plants had initiated about 26 leaves plus primordia (Fig. 1A) and were on the point of initiating normal leaves (Fig. 2). On re-transfer to SD (5SD + 9LD + SD) the rate of initiation of primordia (2.1 ± 0.3 d⁻¹) remained the same as in reverted plants subsequently maintained in LD (5SD + LD: 2.4 ± 0.3 d⁻¹) (Fig. 1A). However, all primordia initiated after re-transfer to SD developed as petals (defined as organs lacking a spur and having > 50 per cent of their surface red-pigmented – see Battey and Lyndon, 1984). Seven primordia already present at the time of re-transfer to SD on day 14 also developed as petals, having more than 50 per cent of their surface red-pigmented (Fig. 2). Primordia 18 and 19 formed organs intermediate between leaves and petals but with significantly more petal pigment than in the reverted plants. Primordia 20–26, which would have developed as intermediate organs, predominantly green and leaf-like and with < 20 per cent of their surface red-pigmented (Fig. 2), became predominantly petaloid in pigmentation and morphology. The petals formed as a result of the 5SD + 9LD + SD treatment were not preceded by the formation of bracts (organs < 50 mm long when mature and lacking a petiole and/or having a spur), which are formed when plants are induced to flower by transfer to SD after being grown only in LD (Battey and Lyndon, 1984). The plants given 5SD + 9LD + SD went on to
Fig. 1. Change in mean number of primordia (± s.e., two to seven plants per sample) in (A) reverted (5SD+LD) (□), and re-flowering plants (5SD+9LD+SD) (▼), between days 14 and 26 after selection for developmental uniformity on day 0; and in (B) vegetative (LD) (△), and flowering plants (SD) (●), between days 29 and 42 after selection for developmental uniformity on day 0 (plants to be induced to flower were transferred to SD on day 29). Full lines are those given by regression equations.

Fig. 2. Mean percentage area with petal pigment in mature parts of reverted (5SD+LD) (R4) (□) and re-flowering plants (5SD+9LD+SD) (▼). *, 5SD+9LD+SD significantly different from 5SD+LD at $P = 0.05$. Combined data from six experiments. Vertical arrow indicates the mean number of leaves plus primordia on return to SD after 5SD+9LD (see also Fig. 1A).
make subsequently normal flowers, initiating the first stamen at part $46.5 \pm 1.6$ and the first carpel at part $69.5 \pm 2.0$.

The lack of formation of bracts and the switch of primordia already present to petal development as a result of the $5SD + 9LD + SD$ treatment could have been not because they were reverted plants but because the SD treatment was being given to necessarily older plants than those maintained in SD from the start of the experiment. To test the effect of the age of plants on their response to SD, plants which had been grown only in LD were transferred to SD on day 29. At this time plants in LD had initiated about 36 leaves plus primordia (Fig. 1B), somewhat more than were present on reverted plants at the time of their re-transfer to SD on day 14 (Fig. 1A). LD plants on day 29 could therefore be regarded as of roughly comparable plastochron age but greater chronological age than reverted plants on day 14. When such plants were transferred to SD, the rate of initiation of primordia increased significantly ($P < 0.05$) from $0.6 \pm 0.3$ primordia d$^{-1}$ in LD, to $2.4 \pm 0.4$ primordia d$^{-1}$ ($29LD + SD$) (Fig. 1B), at about the time that petal initiation began. This is comparable with the increase from $0.9$ primordia d$^{-1}$ to $3.8$ primordia d$^{-1}$ in plants transferred to SD on day 0 (Battey and Lyndon, 1984). In the plants given $29LD + SD$, the first leaf without an axillary structure was part $31.9 \pm 2.0$ and the first bract was part $34.4 \pm 1.1$. Since there were about 36 leaves plus primordia present on day 29 at transfer to SD, these primordia already present at the time of transfer had their development modified by the transfer to SD. This did not occur when plants were transferred to SD on day 0 (Battey and Lyndon, 1984). In plants given $29LD + SD$, the first part with petal pigment was part $37.6 \pm 1.5$ and the first petal was part $39.5 \pm 1.3$. These parts were initiated about one and three plastochrons, respectively, after transfer of the plants to SD (Fig. 1B). In plants transferred to SD on day 0 the first part with petal pigment, and the first petal, were initiated four and six plastochrons, respectively, after transfer (Battey and Lyndon, 1984). Therefore, although the changes in morphology, and the sequence in which they occurred during the transition from leaf to petal initiation were the same in plants transferred to SD on day 0 and in those transferred on day 29, in the older plants the transition was accomplished much sooner after the beginning of induction by SD and affected some of the primordia already present on the meristem. However it is clear that although the rapid response of the plants given $5SD + 9LD + SD$ may have been due in part to their age at the time of the second transfer to SD, this does not account for the major differences in responsiveness of the apex. These differences must therefore result from the previous flower induction and reversion of the apex.

Vegetative controls maintained in LD showed spiral phyllotaxis with a divergence angle of $138.0 \pm 0.8^\circ$. After transfer to SD, whether on day 0 (Battey and Lyndon, 1984) or day 29, the phyllotaxis changed to whorled or pseudo-whorled so that by the mid-petal stage the divergence angle between the young petal primordia could no longer be determined. Reverted plants had already achieved a whorled or pseudo-whorled phyllotaxis which was still persisting when they were transferred back to SD ($5SD + 9LD + SD$), and continued into the flower. Re-flowering of reverted plants was therefore not associated with a change of primordium arrangement.

**Changes in apical growth**

The apical meristems of re-flowering plants initiating petals or stamens ($5SD + 9LD + SD$) were very similar to those of reverting plants initiating leaves ($5SD + LD$) (Table 1). The distance of the youngest primordium from the apical centre was similar in both, and so was the distance of the first primordium below the apical surface from the apical centre. The area of the bare apical surface and the area of the annulus bearing primordia on the apical surface was therefore also similar in both
TABLE 1. Area of the apical surface and areas of primordia on initiation in reverted (5 SD + LD), re-flowering (5 SD + 9 LD + SD), vegetative (LD), and flowering (transfer to SD on day 29) plants (all data obtained from serial transverse sections of the shoot apex)

<table>
<thead>
<tr>
<th>Type of apex</th>
<th>Stage of development (total no. of leaves + primordia)</th>
<th>No. of plants per sample</th>
<th>No. of the youngest primordium (µm)a</th>
<th>Mean distance from the apical centre of the first primordium below the apical surface</th>
<th>Area of annulus bearing primordia on apical surface ((10^3 \times \mu m^2)) ± s.e.</th>
<th>No. of primordia at initiation relative to area of apical surface ((2 \log_e r))</th>
<th>Absolute area of primordium at initiation (apical area (2 \log_e r)) ((10^3 \times \mu m^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverted</td>
<td>Leaf (30-46)</td>
<td>6</td>
<td>65.7</td>
<td>Treatment: 5 SD + LD</td>
<td>26.4 ± 3.1</td>
<td>6</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Leaf (47-69)</td>
<td>4</td>
<td>60.5</td>
<td></td>
<td>20.5 ± 6.4</td>
<td>5</td>
<td>0.20</td>
</tr>
<tr>
<td>Re-flowering</td>
<td>Petal (30-46)</td>
<td>8</td>
<td>64.9</td>
<td>Treatment: 5 SD + 9 LD + SD</td>
<td>33.8 ± 4.6</td>
<td>6</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Stamen (47-69)</td>
<td>4</td>
<td>73.3</td>
<td></td>
<td>31.1 ± 2.9</td>
<td>6</td>
<td>0.18</td>
</tr>
<tr>
<td>Vegetative</td>
<td>Leaf (36-44)</td>
<td>7</td>
<td>63.1</td>
<td>Treatment: LD</td>
<td>22.3 ± 1.8</td>
<td>3</td>
<td>0.33b</td>
</tr>
<tr>
<td>Flowering</td>
<td>Bract (35-39)</td>
<td>3</td>
<td>61.9</td>
<td>Treatment: SD (transferred from LD on day 29)</td>
<td>32.1 ± 2.4</td>
<td>3</td>
<td>0.42b</td>
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<tr>
<td></td>
<td>Early petal (40-49)</td>
<td>5</td>
<td>77.0b</td>
<td></td>
<td>37.5 ± 5.0</td>
<td>5</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Mid petal (50-59)</td>
<td>3</td>
<td>68.8</td>
<td></td>
<td>38.9 ± 4.7</td>
<td>9</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Geometric means. Analyses of variance on columns 4-6 and 8: 5 SD + LD and 5 SD + 9 LD + SD treatments - differences between stages not significant. LD and SD treatments - b, significantly different from other stages at \(P < 0.05\).
treatments. Since there were five to six primordia on the apical surface in both treatments the sizes of primordia would be expected to be similar. This was shown by the similar areas of primordia at initiation relative to the apical surface area, as measured by the plastochron ratio. The absolute area of the primordia at initiation was also similar in both treatments (Table 1). The length of the stem frustum at initiation also remained unchanged on re-flowering of reverted plants (Table 2). The area and vertical relative growth rates of the apex were either comparable or at least not more in the re-flowering plants than in the reverted plants (Table 3).

In plants flowering with SD begun on day 0, there was a reduction in primordium size at initiation when petals and stamens were initiated, compared to vegetative plants in LD (Battey and Lyndon, 1984). If the lack of reduction of primordium size in the re-flowering plants were a function of their age at the time of re-flowering then it would be expected that plants of a comparable plastochron age which had been maintained in LD and were then given SD would also show no reduction in the area of primordia when petals and stamens were initiated. Plants which were vegetative and had been kept in LD were therefore transferred to SD on day 29. In these plants although there was an increase in the area of the annulus bearing the primordia, the number of primordia on the apical surface also increased from three to nine. The area of the primordia at initiation therefore decreased, in both relative and absolute terms, when petals were initiated (Table 1). The area and vertical relative growth rates were both higher in the plants given SD on day 29 than in the vegetative plants forming leaves in LD (Table 3). The lack of change in the apical growth pattern, when reverted plants were made to re-flower, is therefore a function not of the age of the plants but of their previous exposure to SD. Re-flowering of reverted apices and the formation of petals and stamens instead of leaves occurs without the changes in primordium arrangement and apical growth rate, or the reduction of primordium area at initiation, that are found on initial induction of flowering by SD.

**DISCUSSION**

When reverted plants of *I. balsamina* were returned to SD after 5SD + 9LD, there were no changes (of those measured) in apical growth and phyllotaxis. Therefore, flowering of the reverted apex appears to involve only a change in the type of organ produced, floral parts being formed instead of leaves. This contrasts with the marked alterations in phyllotaxis and growth pattern that accompany the transition of the vegetative (LD) apex to flowering on transfer to SD, whether this transfer occurs on day 0 (Battey and Lyndon, 1984), or day 29. It also contrasts with the marked increase in the rate of primordium initiation, the decrease in primordium size at initiation, and the change in phyllotaxis, that accompany flowering in both *Silene coeli-rosa* and *Ranunculus repens* (Lyndon, 1978, 1979a; Meicenheimer, 1979). The implication is that these changes do not need to occur in *I. balsamina* to allow the switch from leaf to petal production. However, it may be that the attainment of a new mode of apical growth is a prerequisite for flowering, and that reverted *I. balsamina* plants have already achieved the necessary changes as a result of their 5SD treatment. The more rapid re-flowering of reverted plants is consistent with the idea that the pattern of growth typical of the floral apex facilitates the transition to flower part production. This suggests that the transition to flowering at the apex of *I. balsamina* may take place in two stages. First the growth pattern at the apex changes, and then, facilitated by this, primordia differentiate as floral organs instead of as leaves. A similar conclusion is suggested by the anomalous flowers formed by *Silene* (Lyndon, 1979b). In these flowers the phyllotaxis was modified from decussate to spiral, and aberrant leaves were produced which were intermediate in size at initiation (relative to the apical dome) between leaves and sepals. Eventually carpels
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of apex</th>
<th>Stage of development (total no. of leaves + primordia)</th>
<th>No. of plants per sample</th>
<th>(1) Mean distance of the first primordium below the apical surface (length of the tissue generating the frustum) (µm)(^a)</th>
<th>(2) Length of the frustum at initiation relative to the length of the tissue generating the frustum (log(_e)v) (± s.e.)</th>
<th>Absolute length of the frustum at initiation (1) × (2) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSD+LD Reverted</td>
<td>Reverted</td>
<td>Leaf (30-46)</td>
<td>6</td>
<td>10.1</td>
<td>0.17 ± 0.02</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaf (47-69)</td>
<td>4</td>
<td>5.4</td>
<td>0.19 ± 0.02</td>
<td>1.0</td>
</tr>
<tr>
<td>SSD+9LD+SD Re-flowering</td>
<td>Petal (30-46)</td>
<td>5</td>
<td>7.5</td>
<td>0.17 ± 0.02</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stamen (47-69)</td>
<td>3</td>
<td>12.6</td>
<td>0.16 ± 0.01</td>
<td>2.0</td>
</tr>
</tbody>
</table>

\(^a\) Geometric means presented; analysis of variance on log transformed data not significant.

---

**Table 3. Area and vertical relative growth rates (d\(^{-1}\)) of the apical surface and frusta of reverted (5SD + LD), re-flowering (5SD + 9LD + SD), vegetative (LD) and flowering (SD) (transfer to SD on day 29) plants**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of apex</th>
<th>Stage of development (total no. of leaves + primordia)</th>
<th>Area relative growth rate per day (2 log_e v × rate of primordium initiation)</th>
<th>Vertical relative growth rate per day (log_e v × rate of primordium initiation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5SD + LD</td>
<td>Reverted</td>
<td>Leaf (30-46)</td>
<td>0.43</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaf (47-69)</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td>5SD + 9LD + SD</td>
<td>Re-flowering</td>
<td>Petal (30-46)</td>
<td>0.48</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stamen (47-69)</td>
<td>0.38</td>
<td>0.34</td>
</tr>
<tr>
<td>LD</td>
<td>Vegetative</td>
<td>Leaf (36-44)</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>SD</td>
<td>Flowering</td>
<td>Early petal (40-49)</td>
<td>0.55</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid petal (50-59)</td>
<td>0.36</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\* No estimate of log_e v is available for this stage.
were produced in some cases. One interpretation of this is that the growth pattern changes sufficiently to allow aberrant leaves, and sometimes carpels, to form, but that the change is not sufficient to facilitate complete, normal, flower formation.

In reverted *I. balsamina* plants returned to SD, not only was the transition to flowering accomplished more rapidly than in plants transferred to SD for the first time, but there was also a retrospective effect of SD on primordia already present at the time of re-transfer. Primordia up to nine plastochrons old showed an increase in the percentage of their surface occupied by petal pigment at maturity. This indicates that these primordia were not completely committed until about 10 plastochrons old; this was not observed in the primordia already present on vegetative plants transferred to SD for the first time. It suggests that exposure to SD may sensitize not only the apical meristem but also the young primordia it initiates after return to LD, to further SD.

The more rapid response of the reverted apex of *I. balsamina* on re-flowering than on flowering on first transfer to SD, and the altered sensitivity of primordia already present to SD, both suggest that reversion does not involve de-evocation of the apex. If evocation is defined as the processes which lead to flowering (Evans, 1969), then some of these processes have apparently been completed during the first SD; transfer to LD, although causing leaf production to resume, does not reverse these processes. This reversal might perhaps eventually have occurred when the normal vegetative growth pattern was resumed about six weeks after the beginning of the experiments (Battey, 1985). The separation, on reversion and re-flowering of *I. balsamina*, of the metabolic changes associated with flower part initiation, from those controlling primordium arrangement and apical growth, may also occur in *Xanthium pennsylvanicum*. In this species gibberellic acid treatment caused the phyllotaxis to change from a 2:3 to a 3:5 contact parastichy arrangement (Maksymowych and Erickson, 1977) as occurs at the transition to flowering (Erickson and Meicenheimer, 1977), but without inducing flowering. It is not known whether *X. pennsylvanicum* plants treated with gibberellic acid respond more rapidly to inductive conditions than untreated plants. This would be expected if, as appears to be the case in reverted *I. balsamina*, the attainment of the growth pattern characteristic of the flower facilitates the transition to flower part initiation.

If only some of the processes of evocation have been completed in the reverted *I. balsamina* apex, then it could be described as partially evoked. Partial evocation has also been described in *Sinapis alba*, in which high intensity light during a single SD caused some of the events characteristic of evocation in this species, but did not result in flowering (Havelange and Bernier, 1983). However, in *I. balsamina* the more rapid re-flowering on return to SD than on first exposure to SD could have resulted from changes that occurred in the leaves, as well as in the apex. If this were so, then the plant would also be partially induced. Further experiments are needed to resolve the question of whether the leaves are partially induced after the 5SD treatment.

The results presented here indicate that commitment to flowering may not be an all-or-none process. In *I. balsamina* it seems that commitment is a gradual or step-wise process, with the changes in growth pattern occurring before the changes that bring about flower part formation. The late commitment of primordia to either leaf or petal development may be a result of this gradual commitment of the meristem to flower formation.

ACKNOWLEDGEMENT

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Synchronization of Cell Division During Flower Initiation in Third-Order Buds of Silene

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Accepted: 28 July 1986

ABSTRACT
Plants of Silene coeli-rosa were induced to flower with seven long days and then returned to non-inductive short days. Third-order buds were formed more than three weeks after the beginning of induction and third-order flowers were initiated about one week later. Comparison of the mitotic index with the ratio of cells in the G2 and G1 phases of the cell cycle for each third-order apex provided evidence for synchronization of cell division just before flower initiation. It is suggested that this results from changes of competence of the apical cells to react to their internal environment rather than because of the arrival of a floral stimulus at the shoot apex.

Key words: Silene, cell division synchrony, flowering, evocation, mitotic index, cell cycle, competence.

INTRODUCTION
In plants induced to flower, successive peaks of mitotic index occur during evocation, and these have been interpreted as indicating some synchrony of cell division in the shoot apex (Bernier, 1971; Lyndon and Francis, 1984). More detailed observations have shown that in Sinapis and Silene, and also probably in Xanthium, the apical cells undergo one or more synchronous cell cycles during evocation or just before flower initiation (Bernier, Kinet and Bronchart, 1967; Francis and Lyndon, 1979, 1985; Jacqmard et al., 1976). Synchrony in Silene can be suppressed without suppressing flowering (Grose and Lyndon, 1984) and is therefore characteristic of normal flowering rather than essential to it. It may therefore occur in response to the arrival at the apex of some stimulus formed during induction and which has some essential effect in causing flower initiation, but at the same time promotes synchronization.

If synchrony were, in this way, an incidental effect of induction and evocation, it might be expected to occur in the apex only at this one time, before formation of the first flower. In this case the formation of much later flowers, after the plants have been returned to short days which are non-inductive but allow flowering to continue, should not be associated with synchronization of the apical cells, since there would be no reason to expect a new flowering stimulus arriving at the apex from the leaves.

Accordingly, the aim of this work was to examine the initiation of flower buds in Silene which are formed long after induction and evocation, and after the plants have been returned to a non-inductive environment, to see whether or not synchronization of cell division could be detected at the apex. The third-order buds were chosen for study because they are not yet formed when the plants are induced by seven long days. Only the second-order bud is present as an undifferentiated bud just below the developing first-order flower when it is initiating floral organs (Lyndon, 1978a), and the third-order bud has not been formed at all. The third-order flower is initiated well after the first-order flower has begun development and about one month after the beginning of induction, and therefore almost three weeks after evocation must have been completed. The exact time of initiation of the third-order flower buds, and the length of the cell cycle in them, was therefore measured and evidence was obtained that synchronization of cell division was indeed found in the third-order buds.

MATERIALS AND METHODS
Plants of Silene coeli-rosa (L.) Godron were grown from seed in short days (SD), after 28 d selected.
for developmental uniformity, subjected to 7 long days (LD) and then returned to SD (Miller and Lyndon, 1976). The day of selection and the beginning of the 7 LD was designated day 0. Squash preparations of Feulgen-stained shoot apical meristems were prepared, and the mitotic index (MI) and G2/G1 ratio (ratio of number of cells with > 3C amount of DNA to number of cells with < 3C amount of DNA) were measured as described and detailed previously (Francis and Lyndon, 1978). For measurements of MI, all the cells of an apical squash were examined. Only metaphases, anaphases and telophases were counted as mitotic figures, because prophases were not always distinguishable from some interphase nuclei. For G2/G1 ratios usually the first measurable 150 cells encountered on a slide were recorded. For some apices fewer cells, but never less than 50, were measured.

The stage of development of an apex was recorded after dissection and observation under a dissection microscope. Volumes of longitudinal median sections were measured from camera lucida tracings and were proportional to total volumes (Miller and Lyndon, 1976).

RESULTS

Formation of third-order buds and their rate of growth

The inflorescence is a monochasial cyme. The first-order flower is formed by transformation of the terminal meristem of the shoot. Immediately below the first-order flower an axillary bud grows out, producing one (sometimes two or very rarely three) pair(s) of leaves before forming the second-order flower. This process is repeated by a bud immediately below the second-order flower to produce the third-order flower and so on as the inflorescence grows.

The first-order flower is initiated in 50 per cent of the plants by day 10-5, i.e. the 3rd sepal of the first-order flower has been initiated, allowing it to be unequivocally identified as a flower (Lyndon, 1979 and unpublished). At this time the second-order bud is just an axillary bulge to one side of the young first-order flower (Plate 1B, C in Lyndon, 1978a). The third-order bud is not formed until day 20-0 (Fig. 1), when just over 50 per cent of the second-order buds have initiated flowers (data not shown). The leaf pair on the third-order bud is initiated by day 25-5 (Fig. 1) and the flower by day 32-5.

The time between the initiation of the first leaf pair (day 25-5) and the subsequent pair of primordia on the third-order bud (day 30-5), whether they are another leaf pair (23 per cent of the plants) or the sepals (77 per cent of the plants), is 5-0 d (Fig. 1). The increase in volume of the apical dome above the first pair of primordia when just newly initiated (day 25-5) to that of the whole meristem above this same pair when sepals have just been initiated (day 30-5; Fig. 1) corresponded to a volume doubling time of 45 h (Table I). The increase in volume of the whole 3rd-order bud between the initiation of the first leaf pair and the second pair of primordia corresponded to a

<table>
<thead>
<tr>
<th>Days after beginning of induction</th>
<th>Percentage of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>28</td>
<td>80</td>
</tr>
<tr>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>36</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>44</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1. Initiation and growth of third-order buds of Silene. As indicated by the arrows, 50 per cent of plants have (a) a third-order bud (---) by day 20-0, (b) one leaf pair (—○—) by day 25-5, (c) two pairs of primordia (leaf pair, sepals, or two leaf pairs (—▼—)) by day 30-5, (d) three sepals, i.e. unequivocally a flower (—△—) by day 32-5.


**Table 1. Growth of apex of third-order buds during the plastochron before the initiation of the sepals**

<table>
<thead>
<tr>
<th></th>
<th>Vol. ((10^4 \mu m^3)) of longitudinal median section</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apex above first leaf pair</td>
</tr>
<tr>
<td>Apices just initiated one leaf pair</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>Apices just initiated next pair of primordia (sepals in 77 per cent of plants) (5-0 d later)</td>
<td>1.71 ± 0.18</td>
</tr>
<tr>
<td>Increase in log volume (a)</td>
<td>2.05</td>
</tr>
<tr>
<td>No. of doublings (\log_2 ) per 5-0 d</td>
<td>2.96</td>
</tr>
<tr>
<td>Doubling time (h)</td>
<td>40.5</td>
</tr>
</tbody>
</table>

Each value mean of six apices, ± 1 s.e.

doubling time of 52 h. From these two estimates it is therefore reasonable to assume a volume doubling time of 40–50 h and, assuming all the cells to be meristematic, a cell cycle time of 40–50 h.

**The cell cycle in third-order buds**

The simplest way to look for synchrony in third-order buds was to examine samples of buds on successive days and see if there were fluctuations in mitotic index (MI), as would be expected if there were synchrony and a cell cycle of about 2 d, or whether MI was more or less constant, which would be expected if the cells in the buds were asynchronous.

The MI varied considerably between apices, even on the same day, so that although there seemed to be variation in MI there was no evidence for synchrony (Fig. 2). However, apices were found on days 27 and 30, and one apex on day 29, in which there were no mitotic figures whatsoever. This was after careful examination of all the cells in the squash for each of these seven apices, a total of more than 18000 cells altogether. Although the data in Fig. 2 therefore do not provide evidence for synchrony or asynchrony in division, there is a suggestion that there could perhaps be some synchrony in individual apices during the plastochron between the formation of the leaf pair on the third-order bud on day 25-5 and the formation of sepals on day 30-5. G2/G1 ratios were also measured, since if there were synchrony between, as well as within, apices this ratio should have varied with time. Like the MI, the variation on each day was too great to be sure that there was a real fluctuation (data not shown).

Since this evidence was unsatisfactory, perhaps because of non-synchronous development of different apices, another approach was tried. Clearly if there were synchronization of the cell cycle within each apex in the plastochron before the formation of the sepals of the third-order bud, but different plants were only approximately or not at all synchronous with respect to each other, synchrony would not be demonstrated by taking replicated samples at intervals. However, certain predictions can be made about the relationship of G2/G1 ratios to MI, which should allow the detection of apices within each of which cell division is synchronous even though they may not be synchronous with each other.

In an asynchronously dividing population of cells the MI, the G2/G1 ratio, and the relationship
between them should remain constant with time. However, if there is complete or almost complete synchrony of cell division and synchrony of the cell cycle within individual apices the MI and G2/G1 ratio should change as follows. When cells are in G1 the MI will be zero and G2/G1 will also be zero (A on Fig. 3). As the cells enter the DNA-synthesis phase of the cell cycle (S) the G2/G1 ratio should reach unity (B). As the cells emerge into G2 the G2/G1 ratio will rise to > 1, but when all cells reach G2 the G2/G1 ratio will become infinity (C). MI should remain zero throughout interphase. Then as the cells enter mitosis MI will rise. But as MI rises, some cells will progress through into G1 and so the G2/G1 ratio will fall and become measurable once more. Since at mitosis every cell leaving G2 gives rise to two cells in G1, when mitosis is maximal the G2/G1 ratio should reach 0.5, since at this point there are twice as many G1 cells as G2 cells (D in Fig. 3). Then as all the cells pass through mitosis and into G1, the MI will fall to zero at the same time as the G2/G1 ratio also falls to zero (D to A in Fig. 3). Although the progression along the graph from A, B, C, D to A represents the traverse through the cell cycle, time itself is not represented on this graph. Thus, if cell division and the cell cycle were synchronous within each apex of a population of sample, even though each apex were not synchronous with any other apex, the values for MI and G2/G1 ratio measured simultaneously in each apex should nevertheless fall on this graph, according to where the cells are in the cell cycle. If, on the other hand, the cells within each apex are asynchronous then MI and G2/G1 ratio should both tend to be constant and the values for all apices should cluster around a point, corresponding to mean MI and mean G2/G1 ratio.

Measurements were made for each of the 44 apices represented in Fig. 2. MI and G2/G1 ratio both being measured from the same squash preparation. The results are shown in Fig. 4, and correspond reasonably to the distribution as in Fig. 3 which would be expected for apices in which synchronization occurs. Note particularly that the highest MIs correspond to a G2/G1 ratio of about 0.5, as predicted from Fig. 3. Figure 4 differs from Fig. 3 in showing no values of a G2/G1 ratio of > 0.3 which correspond to MI of zero, nor of infinity, but this would be consistent with a relatively long G1, where the G2/G1 ratio is < 0.5, and a relatively short G2, and incomplete synchrony. The data of Fig. 4 agree much more closely with the distribution expected of apices showing synchrony than of apices with completely asynchronous cell cycles, when the values would cluster around the point representing the mean MI of 0.75 and mean G2/G1 ratio of 0.51. The lack of values with MI < 0.65 in combination with G2/G1 ratios between 0.35 and 0.95 again corresponds better with Fig. 3 than with a random clustering. The maximum MI of 2.25 per cent corresponds to about 4.5 per cent if prophase had been included, and is therefore about half that
observed during synchronization in first-order buds (Francis and Lyndon, 1979; Grose and Lyndon, 1984).

The conclusion to be drawn, therefore, is that loose synchronization of cell division and of the cell cycle does occur in third-order buds in the plastochron before the formation of sepals, but it tends to occur independently in each apex, i.e. the apices are not synchronous with each other.

An observation of cytological interest is that in those apices in which MI was zero the structure of the nuclei was particularly granular, with distinct masses of heterochromatin, similar to the appearance described for articulating nuclei in G1 or G2 (Barlow, 1977).

**DISCUSSION**

The occurrence of loose synchronization of cell division and of the cell cycle in third-order buds poses the problem of what causes it. It is unlikely to be a specific stimulus produced as a result of induction since the third-order flowers form 32 d after the beginning of induction (Fig. 1) and 25 d after the plants have been returned to non-inductive SD. Also the formation of flowers on other axillary branches, and successive flowers on the same part of the inflorescence, is not synchronous with the formation of the third-order flowers. Synchrony in the third-order buds could perhaps result from some stimulus coming from the pair of leaves on the third-order branch immediately below the third-order flower. If so, this would imply that the young developing leaves become secondarily induced as they develop and then begin producing a stimulus which results in synchronization of the cells in the third-order bud. The logical conclusion would then be that synchronization in the first-order bud is also produced by a stimulus from the leaf pair below it, and since these (the 9th pair) are formed only on the 6th day of induction (Miller and Lyndon, 1976) these presumably too could have been secondarily induced. Moreover, if synchronization is the result of a stimulus from the most recently formed pair of leaves it is not a direct event or effect of evocation, or else evocation occurs each time a pair of young leaves is formed at the apex of second-, third-, etc., order buds. The simplest conclusion is that synchronization is not an event of evocation but occurs as part of the process of the initiation of each flower in *Silene*.

A reduction of the size of the primordia at initiation presumably occurs each time a flower is formed, because of the distinctive change in primordial arrangement which accompanies flower formation (Lyndon and Battey, 1985). It also occurs in the third-order flowers (R. F. Lyndon, unpublished data). Since it occurs in many or all flowers, including those plants which require no special environmental stimulus to flower, it is presumably the result of a change in competence of the apex so that it can grow in a floral rather than a vegetative mode. If the occurrence of synchrony in the meristem just before the initiation of the sepals is also regarded as a result of change in competence of the cells there is no need to postulate a specific stimulus for these events. It is only necessary to assume that the apical cells undergo some change which allows them, just before the flower is formed, to react in a different way to floral stimuli already present in the plant so that the cells become synchronized.

The commitment to flower brought about by evocation is a change in competence of the cells. The change in competence to flower is at the cellular level, since it can be transmitted through tissue culture (Chailakhyan *et al.*, 1975). Competent cells can, however, only form flowers when they are exposed to the right environment (Cousson and Tran Thanh Van, 1981). Similarly, the synchronization of cell division and of the cell cycle in the apex of *Silene* is expressed only when the cells are exposed to the appropriate environmental conditions (Grose and Lyndon, 1984). The reduction in the size of the primordia at initiation is progressive as the flower develops (Lyndon, 1978b) and occurs after synchronization of cell division (Grose and Lyndon, 1984). If synchronization and reduction in primordial size both result from a change in the competence of the cells we must conclude either that competence for different stages in development is achieved at different times or that the internal environment changes and thus allows the expression of different aspects of competence. This latter would be consistent with the hypothesis of differentiation-dependent development (Sachs, 1978), which implies that the first steps in differentiation modify the system in such a way that the pathway of development is subsequently affected. If the primary effect of the floral stimulus is to induce a change in competence of the apical cells only the first event of evocation can be unambiguously attributed to the direct effect of the floral stimulus. Although subsequent evocational events might be the result of the arrival of more floral stimulus, or new components of it, at the apex, it seems equally plausible that they may result from changes at the cellular level in the apex which have altered its competence to react to
existing metabolites and ultimately to form flowers.

ACKNOWLEDGEMENTS
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(ed. J. Atherton)
Butterworths, London.
1987
INITIATION AND GROWTH OF INTERNODES AND STEM AND FLOWER FRUSTA IN *SILENE COELI-ROSA*

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**Introduction**

The formation of a flower is first recognizable as a change in the arrangement of the primordia at the shoot apex, accompanied by a decrease in the size of the primordia at initiation, and a lack of internodes. There is a decrease in the length, at initiation, of the stem frusta that give rise to the node plus internode (Lyndon and Battey, 1985). The final lengths of internodes may therefore be related, at least in part, to the sizes of the stem frusta at initiation. The formation of flowers, in which internodes are suppressed, is very often accompanied by a simultaneous increase in the growth of internodes below the flower, suggesting that either potential internodal cells in the flower are not produced, or they may be formed at frustum initiation but do not grow and multiply.

The problem of the flower, in terms of frustum formation and growth, is therefore: why do internodes not form? Are the necessary cells not formed at frustum initiation? If not, then the primordium plus frustum of the flower is a different morphological entity from that in the vegetative plant. Or are the frusta similar at initiation but develop differently? These questions can be answered only by finding out what happens at the earliest stages of frustum initiation and growth. Most previous work on internode growth has been concerned with the growth of internodes already several mm long at the start of measurements (Garrison, 1973; Enright and Cumbie, 1973; Maksymowycz, Maksymowycz and Orkwiszewski, 1985). The very earliest stages of the growth of stem frusta have, however, been followed in some plants. The frusta grew in breadth rather than length for several plastochrons after initiation in *Lupinus* and *Chrysanthemum* (Sunderland and Brown, 1956; Berg and Cutter, 1969), but in *Arropyron* older frusta showed an initial lag in growth both in breadth and in length (Smith and Rogan, 1975). Frustum growth has not been followed beyond the apical meristem and not in flowering plants. It is at these earliest stages of frustum growth, when the internode is only a fraction of a mm long, that cell division predominates and when gibberellic acid has a major effect (Sachs, Bretz and Lang, 1959). It is presumably at these earliest stages that the developmental pathways of vegetative and floral frusta diverge.

In order to understand why internodes do not form in the flower one can pose the converse question: why do internodes form in the vegetative shoot? By comparing frustum initiation and growth in the vegetative shoot and in the flower it should be possible to pinpoint the differences in growth pattern and so gain some insight into
the type of cellular changes that are associated with the transition to flowering and why internodes are therefore suppressed in the flower.

The intention here is to make such a comparison, of the growth of frusta from initiation to the later stages of development, and to compare the sizes at initiation, the rates of growth, the response to long-days, and the distribution of cell division and elongation in frusta in vegetative and flowering plants. Frustum growth was followed in *Silene coeli-rosa*, which is an obligate long-day plant, which has opposite decussate leaves, and in which the terminal apex transforms into the first flower.

**Experimental**

Plants of *Silene coeli-rosa* L. Godron were grown from sowing in SD (short-days) and when 28 or 30 days old, they were selected for developmental uniformity according to the length of the third leaf pair in the ways previously described (Miller and Lyndon, 1976). SD consisted of 8 h light/16 h dark. LD (long-days) were the same but with low level illumination from incandescent lights during the 16 h, otherwise dark, period. Temperature was 20 °C throughout. The day of selection was designated experimental day 0.

For sectioning, shoots stripped of their oldest leaves were fixed in 3:1 ethanol:acetic acid, then dehydrated in an ethanol series, transferred by stages to xylene and embedded in Paraplast. Serial longitudinal sections 10 µm thick, in the plane of the odd-numbered leaf pairs, were stained usually with haematoxylin and orange G or fast green.

Frustum length was measured as the distance between the axils of successive leaf pairs, the frustum for a particular leaf pair consisting of the node of that leaf pair and the internode below it. Frustum breadth was the width of the stem at the leaf axils at the base of the frustum.

Measurements for Figures 22.2 and 22.3 and Table 22.5 were made on plants defoliated to expose the frusta. The lengths of the frusta were measured with a ruler if they were more than about 5 mm long, otherwise they were measured under a dissecting microscope with an ocular micrometer. All other measurements were made on sections. The outlines of the sections were traced using a camera lucida, and measurements were made on the scale drawings so obtained. For counting the numbers of cells in the frusta, lines were drawn down files of cells while they were viewed with the camera lucida and the number of cells passed in traversing the length of the frustum was recorded. This was repeated for six files per frustum per apex. Only pith cells were measured.

**GROWTH OF STEM FRUSTA**

The growth of stem frusta is best exemplified by comparing successive frusta along the stem. Since the growth pattern is the same for plants with 9 and with 12 pairs of leaves (Figure 22.1) then the increase in size of successive frusta also represents the temporal sequence of development. Each plastochron is about 4 days (Lyndon, 1977), and so Figure 22.1 represents about 20 days of development. Since frusta are necessarily exactly one plastochron apart this gives a more accurate representation of their growth than by sampling different plants at different times. The breadth of the frustum increases exponentially from initiation, which is taken to be when the frustum can be recognized as the axis associated with the youngest pair of leaf
Figure 22.1  Growth in length and breadth of successive stem frusta in *Silene* plants in SD. Each set of points represents the means from three plants.

Figure 22.2  Frustum elongation in SD in *Silene*. The points represent means from 6 different plants each day. Frustum numbers shown on the graph.
Initiation, Growth of Internodes and Stem and Flower Frusta in Silene coeli-rosa primordia. Length of the frustum increases very little for the first two plastochrons, but then elongation begins and is exponential.

The pattern of growth of successive frusta is similar (Figure 22.2) but with a tendency for the relative elongation rate to fall with successive frusta. Growth may not be truly exponential over the whole of the growing period; the apparent S-shaped curve for frustum 4 was also found in a duplicate experiment, which gave very similar overall results.

On transfer to LD the rate of frustum and internode elongation is promoted in all frusta (Figure 22.3). The relative elongation rate in LD clearly increases to a maximum and then decreases; the period of exponential growth is restricted to the

![Graph of frustum elongation in Silene in LD and in plants given 7 LD and returned to SD. The points represent means from six different plants each day.](https://example.com/graph.png)
Figure 22.4 Effect of LD on growth in length and breadth of frustum 8 in *Silene*, LD (--- ○ ---); SD (--- ● ---). Each point is the mean from three plants.

first half of the growing period. The effect of LD on the elongation of frusta depends on their stage of development at the time the LD treatment was begun. Frustum 1, which was almost at mature length in SD on day 0 (see Figure 22.1) was affected least, but even this frustum increased to about 7 mm instead of the 4–5 mm achieved normally in SD. The effect of LD is greater on successively younger frusta and becomes maximal in frustum 5 (Figure 22.3). Later frusta attained similar final lengths to frustum 5 when the plants were continued in LD until flowering. The implication is that only frusta less than about 100 μm in length at the beginning of LD treatment were able to respond maximally. This corresponds to frusta at the very beginning of their elongation phase (see Figure 22.1), when they are not much longer than at initiation. This conclusion is supported by data from older plants that were kept until day 20 in SD (i.e. 48 SD from sowing) and then transferred to LD. The maximal response after 21 LD was shown by frustum 8, which was the youngest frustum more than 100 μm long when the LD treatment was begun. The duration of frustum growth may be reduced slightly by LD (compare frusta 4 and 5 in Figures 22.2 and 22.3). The response of the frusta to LD, i.e. the increase in log final length in LD over log final length in SD, seems to be proportional to the fraction of the duration of frustum elongation left to that frustum at the onset of LD.

When plants were given 7 LD—a fully inductive treatment (Miller and Lyndon, 1976)—and then returned to SD the rate of frustum elongation reverted to that of
The effect of LD on frustum growth was almost entirely on elongation. The effect on all frusta was similar to that on frustum 8 (Figure 22.4) shown for illustration. The LD perhaps reduce the initial period of little extension from about 8 to about 4 days (cf. Figure 22.2 and 22.3); frusta 8 and 9) but the main effect is on increasing the rate of elongation in the main elongation phase. The breadth of all frusta was unaffected by LD except perhaps for a transient increase in growth rate which was often observed when the frusta began to elongate more rapidly in LD (Figure 22.4).

The growth of frusta in SD, LD and 7 LD in a duplicate experiment was similar to that reported here. The main conclusion is that frustum final length depends on the effects on elongation which operate on very young frusta when they are still only 100 μm long.

---

**Figure 22.5** Effect of LD (—○—) on elongation of 9th stem, sepal, and petal/stamen frusta in *Silene*. Controls given 7 LD and returned on day 7 to SD (—●—). Each point is the mean from three plants.
GROWTH OF FLOWER FRUSTA

Associated with the sepal frustum in *Silene* are the five sepal primordia, and with the petal/stamen frustum five petal and 10 stamen primordia. It was not feasible to try to distinguish petal from stamen frusta as the axils of the petals are above the bases of the outer whorl of stamens.

The potential for elongation of flower frusta can be measured by comparing their growth in plants maintained in LD and in plants which have been induced with 7 LD and then returned to SD, in which initiation and growth of leaf pair and frustum 9 and the flower occur while the plants are in SD (Figure 22.5). The effect of LD on the sepal and petal/stamen frusta is to increase their growth rate to a similar extent to that of the frustum associated with the last leaf pair. These frusta clearly have the initial potential to elongate, but the petal/stamen frustum does not continue to do so, since internodes are not formed between the individual petals and stamens. As in the stem frusta there was little effect of LD on the increase in breadth of the sepal and petal/stamen frusta, which was slightly slower than in the stem frusta. The sepal frustum appeared to grow in length from inception and did not show an initial period of increase in breadth alone (Figure 22.5). The sepal frustum at inception is longer than other frusta (Table 22.1). This is reflected in its final length at anthesis since the pedicel (to which it gives rise) is almost invariably longer than the last internode. On the other hand the petal/stamen frustum, although it is comparable in length at initiation to a stem frustum, does not elongate markedly. The length of the frusta at maturity is therefore not determined only by their length at initiation.

The length of frustum per primordium (at initiation) does however decrease on flowering from 31.5 μm for stem frusta (two primordia per frustum) to 22.8 μm for the sepal frustum (five sepals) and to 5.1 μm for the petal/stamen frustum (15 primordia; five petals, 10 stamens).

Table 22.1 LENGTHS OF YOUNGEST FRUSTA IN *Silene*, BEFORE THE NEXT PRIMORDIUM OR WHORL WAS APPARENT

<table>
<thead>
<tr>
<th>Frustum</th>
<th>Length (μm)</th>
<th>No. of apices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem*</td>
<td>63 ± 1</td>
<td>(50)</td>
</tr>
<tr>
<td>Sepal</td>
<td>114 ± 8</td>
<td>(9)</td>
</tr>
<tr>
<td>Petal/stamen</td>
<td>77 ± 3</td>
<td>(13)</td>
</tr>
</tbody>
</table>

* Frusta 7–13 inclusive. The values for different stem frusta were not significantly different.

CELL GROWTH IN FRUSTA

Differences in the growth of frusta can be found at the cellular level. In stem frusta two distinct regions are found. At the upper, nodal end of the frustum, at and just below the level of the axils of the leaves, the cells are apparently randomly packed and are not in files; below this is a region that gives rise to the internode in which the pith cells are in clear files which have frequent transverse walls. The youngest frustum associated with newly initiated leaf primordia consists of eight layers of cells which are not distinguishable from each other. However, in the second youngest frustum the lower four layers of cells can be seen to be beginning to enlarge (Table 22.2). This continues during the next plastochron and only in the third plastochron after initiation is transverse cell division observed, and cell number per frustum length
Table 22.2  GROWTH OF FRUSTUM PITH CELLS IN VEGETATIVE *Silene* PLANTS WITH 8 PAIRS OF LEAVES + PRIMORDIA IN SD

<table>
<thead>
<tr>
<th>Frustum</th>
<th>Frustum length (µm)</th>
<th>Total no. of cells per frustum length</th>
<th>No. cells in:</th>
<th>Cell length (µm cell⁻¹)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper</td>
<td>Lower</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>57</td>
<td>7.7</td>
<td>7.7</td>
<td>7.4</td>
<td>All cells similar</td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>8.3</td>
<td>4.0</td>
<td>4.3</td>
<td>7.4</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>7.9</td>
<td>4.2</td>
<td>3.7</td>
<td>9.6</td>
</tr>
<tr>
<td>5</td>
<td>157</td>
<td>15.1</td>
<td>4.4</td>
<td>10.7</td>
<td>12.0</td>
</tr>
<tr>
<td>4</td>
<td>348</td>
<td>33.0</td>
<td>6.3</td>
<td>26.7</td>
<td>10.9</td>
</tr>
<tr>
<td>3</td>
<td>868</td>
<td>53.3</td>
<td>7.8</td>
<td>46.5</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Each value is a mean from three apices.
increases. This corresponds with the onset of the frustum elongation phase 2 plastochrons after initiation (see Figure 22.1).

Increase in cell number and hence occurrence of transverse division to give files of cells, occurs at first only in the lower part of the frustum (Frustum 5; Table 22.2). Although cell number in the upper part of the frustum also begins to increase (Frustum 4, Table 22.2), the internode, and hence nearly all the increase in length of the frustum, derives from the elongation and cell divisions in the files of cells formed from the four cell layers in the lower half of the original frustum. The internode therefore originates from the lower half of the frustum and the node from the upper half; the two halves can be seen to be growing differently from the first plastochron after initiation and before transverse cell division begins. Cell length in the elongating frustum remains similar both in its upper and in its lower parts, although elongation is almost entirely restricted to the lower half. This implies that cell division keeps pace with cell elongation so that mean cell length remains similar in upper and lower parts of the frustum (Table 22.2).

In the growth of the petal/stamen frustum no such distinction could be made at any stage between cells in the upper and lower part of the frustum. The cells did not obviously form files but retained a packed, random appearance like those in the upper (nodal) part of the stem frusta. Cell extension and cell division appeared to be equally spread throughout the frustum except in older frusta in which the cells towards the base of the frustum tended to be larger (Table 22.3). In the petal/stamen frustum there was therefore no visible differentiation of cells specialized to divide transversely and to elongate. The number of cell layers in the youngest petal/stamen frustum at initiation is about 13, or about one cell layer per primordium (Table 22.3).

### Table 22.3 GROWTH OF PITH CELLS IN THE PETAL/STAMEN FRUSTUM OF Silene

<table>
<thead>
<tr>
<th>Frustum length (µm)</th>
<th>No. of apices</th>
<th>Total no. of cells per frustum length</th>
<th>Cell length (µm cell⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper half</td>
</tr>
<tr>
<td>80-90</td>
<td>(2)</td>
<td>13.2</td>
<td>6.3</td>
</tr>
<tr>
<td>115-120</td>
<td>(2)</td>
<td>16.2</td>
<td>7.3</td>
</tr>
<tr>
<td>135</td>
<td>(2)</td>
<td>16.2</td>
<td>8.4</td>
</tr>
<tr>
<td>204</td>
<td>(1)</td>
<td>22.2</td>
<td>8.1</td>
</tr>
<tr>
<td>324</td>
<td>(1)</td>
<td>29.8</td>
<td>8.4</td>
</tr>
<tr>
<td>420</td>
<td>(2)</td>
<td>25.5</td>
<td>12.2</td>
</tr>
</tbody>
</table>

As the sepal frustum grows, immediately after initiation (Table 22.4), the cell number does not increase but the cells elongate to about twice their original length. This occurs to the same extent throughout the frustum although the cells in the lower half are longer than those in the upper half. From initiation of the frustum the pith cells are in files which derive from the files of cells formed in the apical dome during its enlargement after the initiation of the last pair of leaf primordia and before the initiation of the sepals. When the sepal frustum has elongated to more than 500 µm, transverse cell divisions accompany extension but these occur equally in both halves of the frustum. Like the petal/stamen frustum, there is no distinction into cells forming the node and cells forming the internode. However, in the petal/stamen frustum all cells are apparently potential nodal cells but in the sepal frustum they are all potential internodal cells. When transverse cell division becomes frequent in the sepal frustum the cells in the upper half become slightly longer than the cells in the
Table 22.4 GROWTH OF PITH CELLS IN THE SEPAL FRUSTUM OF Silene

<table>
<thead>
<tr>
<th>Frustum length (µm)</th>
<th>No. of apices</th>
<th>Total no. of cells per frustum length</th>
<th>Cell length (µm cell⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper half</td>
</tr>
<tr>
<td>120–150</td>
<td>(4)</td>
<td>12.8</td>
<td>8.6</td>
</tr>
<tr>
<td>180–240</td>
<td>(5)</td>
<td>10.9</td>
<td>17.7</td>
</tr>
<tr>
<td>310</td>
<td>(1)</td>
<td>13.6</td>
<td>20.5</td>
</tr>
<tr>
<td>640–670</td>
<td>(2)</td>
<td>27.7</td>
<td>27.8</td>
</tr>
<tr>
<td>930</td>
<td>(1)</td>
<td>38.3</td>
<td>27.7</td>
</tr>
</tbody>
</table>

lower half (Table 22.4). The pith of the sepal frustum becomes very regular in appearance in longitudinal section and consists of cells elongating and dividing transversely. Cell length remains more or less constant, implying that cell division keeps pace with cell extension.

GROWTH OF THE SEPAL FRUSTUM

As the flower begins to develop the sepal frustum develops in a way unlike any other frustum. This can be followed by seeing how the vascular structure of the sepal frustum changes during its growth (Figure 22.6). The course of the procambium is at first like that in other frusta (1 and 2, Figure 22.6). But as the frustum grows the points

Figure 22.6 Successive stages (1–7) in the development of the sepal vascular traces from initiation (1) to anthesis (7) in Silene.
at which the procambium turns downwards into the stem become moved upwards
above the axils of the sepals (4, 5, Figure 22.6). This is apparently because of the
growth of the inner tissues but not the outer tissues at the sepal insertions, so that the
inner part of the sepal frustum, including the pith and the vascular tissues, grows up
above the sepal axils. The true petal/stamen frustum is those tissues above the
uppermost points of the sepal vascular traces in the axis. The slight bulging of the
outer tissues just above the sepal axils (5, Figure 22.6) is characteristic, and is
consistent with an expansion of inner tissues above a constraining ring of outer tissue
at the sepal axils. The internode which thus forms between the sepals and the petals
(7, Figure 22.6), and which at anthesis is about 2 mm long, is therefore almost entirely
derived from the sepal frustum growing up beyond the axils of the sepals. Thus there
is no true internode between sepals and petals in Silene.

Discussion

The reason that internodes are suppressed in the flower seems to be because the cells
that give rise to the internode by forming files are not formed in the petal/stamen
frustum. The successive frusta constitute a sequence starting with the vegetative stem
frusta, which form nodal cells and internodal cells. In the sepal frustum nodal cells are
not evident and the whole frustum is essentially internodal but the cell files have a
lower frequency of the transverse divisions typical of the stem frusta. In the petal/
stamen frustum all cells appear to be nodal in type and cells giving rise to files are not
initiated.

The transition to flowering may therefore consist of three steps:
1. The change in primordium arrangement.
2. The suppression of internodes because the cells generating them are not formed.
3. The differentiation of the floral organs.

The suppression of the internodes seems to occur after the change in primordial
arrangement because, in Silene, an internode is formed by the sepal frustum even
though the primordial arrangement has changed from that in the vegetative plant.

A stem frustum clearly gives rise to the node, which shows little elongation, and the
internode which is the main site of stem elongation. Some recent evidence suggests
that the node and internode may originate from distinct cell layers when the frustum
is formed. In Sambucus there are tannin-containing coenocytic cells which extend the
length of each internode (Zobel, 1985). These coenocytes originate in a single layer of
cells at the base of the apical dome. The suggestion is that these coenocytes are
therefore indicators of the cell layer which gives rise to the internode. The single cell
layer between successive coenocytes at the base of the apex gives rise to the nodal part
of each frustum.

In Silene there is a maximum of four cell layers from which the internode originates
(see Table 22.2). Although Silene does not contain coenocytic cells as in Sambucus,
it does of course contain sieve elements which can be seen readily in sections stained by
the periodic acid–Schiff method. Not infrequently only two sieve elements can be seen
to extend through the internodal portion of a young frustum and another two
through the nodal portion, suggesting that the frustum in Silene may originate from a
total of only four cell layers at the base of the apical dome, and the internode also only
from two cell layers, or one layer per leaf for the internode and one for the node. In
the petal/stamen frustum of Silene there are about 13 cell layers at initiation (see
Initiation, Growth of Internodes and Stem and Flower Frusta in Silene coeli-rosa

Table 22.3) and 15 associated primordia (5 petals; 10 stamens), equivalent to about 0.9 cell layers per primordium. Such a reduction to one cell layer associated with each primordium would at least be consistent with the hypothesis that one of the cell layers—that giving rise to the internode—is not formed in the flower. The hypothesis proposed here is therefore that the initiation of a leaf primordium also results in the designation of a minimum of two layers of cells as frustum, one layer giving rise to the node and one layer to the internode, and that in the flower the internodal initials are not formed and that there is only one layer of frustum initials associated with each primordium.

It is characteristic of flowers that primordium area at initiation and frustum length at initiation are both reduced compared with the vegetative apex (Lyndon and Battey, 1985). When proliferous ‘flowers’ were formed in Silene (Lyndon, 1979) the primordia were less than half the area at initiation of leaf primordia and the length of each frustum was half the length of a frustum associated with one leaf primordium in the normal vegetative plant. Judging by the congested nature of the proliferous ‘flowers’ the frustum layers missing were those giving rise to the internodal initials. In Ambrosia, when the phyllotaxis changed from decussate to spiral the length of the internode between successive single leaves was approximately two-thirds that of the internode between successive leaf pairs (Soma and Kuriyama, 1970). All of these observations are consistent with the hypothesis that the frustum and primordium are initiated together and that there is some sort of proportionality between the size of a primordium at initiation and the length of the frustum associated with that primordium. Translated into cellular terms this could perhaps mean that there is a specific number of cell layers initiated as frustum for each primordium formed.

At inception of stem frusta there is no internode present and the leaf bases extend down into the leaf axils. Only after about 2 plastochrons when the internode elongates (see Table 22.2) do the adjacent cells of the leaf base also elongate and divide to form the outer tissues of the internode. The sepal frustum is unusual in that the primordia are initiated towards the distal end so that the internode is present from inception. The cell files that give rise to the pedicel pith are formed directly from the cell files in the enlarged apical dome and do not arise subsequently by division as do the cell files in the pith of the stem frusta. The petal/stamen frustum resembles a stem frustum in that the primordium bases extend down into the organ axils, but differs in that extension of the bases of the primordia to contribute to internodes does not occur and so the bases of the petals and stamens remain in close contact in the flower.

The curious growth of the sepal frustum in which the inner tissues of pith and vascular strands extend upwards past the sepal axils, with compensatory growth of the cortical cells at the sepal node and subsequent extension of the cortical and epidermal cells just above the sepal axils, eventually gives rise to an internode about 2 mm long in the mature flower. The way this internode is formed very strongly suggests that the driving force for elongation of this internode comes from the internal cells, especially the pith. Indeed the visual impression from sections is that the driving force for elongation of all the internodes could come from the elongation of the files of pith cells (Sachs, 1882). Supporting evidence is that in Silene armeria the pith rib-meristem cells in the apex are the ones most activated by gibberellic acid, which promotes stem elongation (Besnard-Wibaut, Noin and Zeevaart, 1983).

The rate of elongation of internodes in Silene is promoted in LD (Figure 22.3) but reverts to a lower rate when the plants are transferred back to SD. Internodes growing or not yet initiated at the time of transfer back to SD do not attain the length of internodes of plants maintained in LD (Figure 22.3). However, when such plants returned to SD approach anthesis the distal internodes begin to extend and at
Table 22.5  INTERNODE LENGTHS AT OR NEAR ANTHESIS IN Silene INDUCED BY 7LD

<table>
<thead>
<tr>
<th>Frustum</th>
<th>Internode length (mm)</th>
<th>Frustum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepal</td>
<td>21.3</td>
<td>14.0</td>
</tr>
<tr>
<td>10</td>
<td>15.0</td>
<td>9.7</td>
</tr>
<tr>
<td>9</td>
<td>10.0</td>
<td>6.7</td>
</tr>
<tr>
<td>8</td>
<td>8.4</td>
<td>5.0</td>
</tr>
<tr>
<td>7</td>
<td>6.3</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>6.6</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
<td>9.0</td>
</tr>
<tr>
<td>4</td>
<td>12.7</td>
<td>13.7</td>
</tr>
<tr>
<td>3</td>
<td>18.0</td>
<td>17.7</td>
</tr>
<tr>
<td>2</td>
<td>8.9</td>
<td>8.3</td>
</tr>
<tr>
<td>1</td>
<td>7.7</td>
<td>8.7</td>
</tr>
</tbody>
</table>

Mean of 7  Mean of 3

anthesis there is a gradient of basipetally decreasing internode length in the pedicel and the last three stem frusta (Table 22.5). Plants with an extra leaf pair below the flower also show the same pattern with three longer internodes below the pedicel, showing that this late elongation is related to the position of the flower. This suggests that as the plant reaches anthesis the developing flower stimulates the elongation of the terminal frusta, perhaps by the production of gibberellins by the stamens (Murakami, 1975).

The petal/stamen frustum, although it enlarges as the flower develops, does not increase in longitudinal cell number much beyond 30 or so cells per file (see Table 22.3). Restriction of growth of this frustum coincides with the development of the flower nodal vascular structure at the base of this frustum, where the traces from the petals, stamens and carpels all join with the vascular strands from the sepals. In Silene all vascular strands in the flower join at the same level, unlike at the leaf insertions where there is a typical leaf gap and the leaf trace joins with the stem vascular bundles one node or so below the node of insertion. Sachs (1969) has shown that whether or not new vascular traces join with older ones can depend on the relative flux of auxin through the new and old strands. A new strand will fuse with an older one if the ratio of auxin in the new to that in the old strand is high. If the fusion of vascular bundles in Silene is governed in this way, the fusion of all the flower traces with the strands from the sepals at the base of the petal/stamen frustum would imply that sepals, unlike leaves, are poor sources of auxin. We do not know whether the reduction of auxin production by new primordia may be one of the events of the transition to flowering. Changes in auxin production by primordia, or in auxin transport, may well be involved in the change in primordial arrangement and the formation of whorls of organs that occurs on flower initiation. Changes in primordial size and fusion of primordia, as occurs in floral whorls, can be induced experimentally by chemicals which are antiauxins or inhibitors of auxin transport (Meicenheimer, 1981).

The development of vascular tissues may also be more rapid after the transition to flowering than in the vegetative shoot, as shown by careful measurements of the rate of vascular differentiation in Perilla (Jacobs and Raghavan, 1962). This may also be indicative of changes in the availability of metabolites and growth substances at the shoot apex associated with the transition to flowering and early flower development. Premature differentiation of vascular tissues may also have a role to play in the control of development on flowering.
Initiation, Growth of Internodes and Stem and Flower Frusta in Silene coeli-rosa

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BESNARD-WIBAULT, C., NOIN, M. and ZEEVAART, J. (1983). Mitotic activities and levels of nuclear DNA in the apical meristem of Silene armeria (strain S1.2) following application of gibberellin A₃. Plant and Cell Physiology, 24, 1269–1279


