MORPHOLOGY AND PHYSIOLOGY OF BUD DEVELOPMENT 
AND CONING IN PINUS CONTORTA DOUGL.

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ABSTRACT

Variations in the timing and rates of bud development and coning were recorded in *Pinus contorta* Doug. (lodgepole pine) growing both in the field and in temperature, and photoperiod controlled environments ± GA 4/7. Quantitative analysis of bud development was performed.

In Central Scotland, axillary bud (AB) initiation began in late May and cone differentiation began in early to mid-July for both male and female cones. Most female cones differentiated in July and August although a few developed in September. Male cones differentiated until late-September. The sequence of development in male and female buds was morphologically indistinguishable until cone differentiation began, whereupon 2ry cataphylls surrounding potential cones increased in size faster than similar 2ry cataphylls around potentially vegetative ABs.

More male cones differentiated at 20°C and in long days (LDs) than at 10°C or in short days (SDs) where female cone differentiation appeared to be stimulated. Photoperiod appeared to have a greater effect on the rate and sequence of bud development than either temperature or GA 4/7 application. In LDs, initiation and growth of buds was reasonably constant throughout the experimental period, while in SD, fertile 1ry cataphyll initiation stopped within a month of treatment being applied; fewer ABs were initiated per bud; the plastochrone ratio and area of a primordium at initiation increased; the mean radial distance decreased; ABs in sub-basal and sub-apical positions grew and differentiated faster than ABs in other positions; and buds became cylindrical in shape rather than conical.

GA 4/7 application did not appear to affect the overall sequence of differentiation in buds as male, female and vegetative buds all differentiated on treated trees. However, GA 4/7 did cause an increase in the rate of growth of buds and stimulated the production of bud parts. The implications of developmental differences are discussed in relation to possible mechanisms for the control of bud development and coning in lodgepole pine.
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LIST OF ABBREVIATIONS

AB  Axillary bud
ABA  Abscisic acid
API  Area of a primordium at initiation (p 36)
ABD  AB diameter (p 37)
BA  Bare area (p 36)
B.C.  British Columbia
C  Carbon
CCC  Chlormequat
CPD  Critical point drier
FAA  Formalin/acetic acid/70% ethanol (5:5:90 by volume)
d.f.  Degrees of freedom
FC  Forestry Commission
FU  Rust urn volume (p 35)
GA3  Gibberellic acid
GA 4/7  Mixture of gibberellins 4 and 7
ITE  Institute of Terrestrial Ecology
LD  Long day
l.s.d.  Least significant difference
MRD  Mean radial distance (p 36)
MSUD  Mean stem unit diameter (p 35)
MSUL  Mean stem unit length (p 35)
MSUV  Mean stem unit volume (p 35)
N  Nitrogen
P  Phosphorus
PR  Plastochrone ratio (p 36)
Q.C.I.  Queen Charlotte Island
RRGR  Relative radial growth rate (p 36)
SD  Short day
S.E.  Standard error
SEM  Scanning electron microscope
UD  Unit diameter (p 35)
UL  Unit length (p 34)
USA  United States of America
V.R.  Variance ratio
1ry  Primary
2ry  Secondary
*  Significant at p=0.05
**  Significant at p=0.01
***  Significant at p=0.001

All measurements are in scientific units and are shown using standard abbreviations.
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1. INTRODUCTION

1.1 Problems of increasing timber productivity using tree selection techniques

As the need for timber increases and huge areas of virgin forest in Canada and Scandinavia are harvested, the necessity for planting high quality, fast growing trees, productively, and in large quantities, increases. Greater productivity may be brought about through silvicultural methods, but these are becoming more expensive (Zimmermann 1972, Pharis 1975) as the costs of fertilizers, insecticides, fungicides and labour increase. The use of agrochemicals on the large scale necessary in forestry may also be potentially hazardous to the environment.

If genetically superior 'plus' trees could be used in afforestation, yields could be improved. Techniques have now been developed which allow large quantities of cuttings from plus trees to be propagated, thus allowing some improvement in stock to be achieved. Tissue culture techniques are also being developed which may allow even more rapid propagation of trees (Durzan 1979). But, trees may only be effectively used as a source of material for micropropagation when they are in a juvenile state. This makes the selection of plus trees hazardous, as the trees may not have developed all the characteristics necessary for accurate selection, and the population from which the plus trees are selected may not contain trees of the required quality to use as parent material. Therefore selection either cannot take place until the trees have reached a stage where they are not ideally suited to being propagated, or a potentially less accurate selection procedure based on only a few characteristics must be used.

One solution to the problem of tree selection is to breed plus trees with all the characteristics which will make them highly productive. But, many economically important conifers do not flower until they are ten to twenty years old (Glerych 1967, Lines 1977) and seed production is often sporadic or periodic, making crossing and bulking up of material within a reasonable time
difficult (Jackson & Sweet 1972, Pharis 1975, Longman 1978). Within most tree species a few individuals may be found which flower precociously, and they may be selected for breeding. However, in the long term, selection of early flowering individuals may create problems because the production of cones decreases the potential for vegetative growth on a tree, and early flowering may be an inherited trait (Morris, 1951, Fielding 1960, Tappeiner, 1969). Increasingly floriferous, less vegetatively productive trees would be bred.

Developing a means by which coning could be both monitored, and when necessary, controlled by techniques for inducing potentially vegetative plus trees to cone, is thus important to the advancement of forestry practice. For monitoring and control of bud development to be achieved, it is necessary to be able to recognise divergence of development as soon as possible and to understand the factors, internal and external, which affect and control bud development.

Therefore, during this investigation, morphological and physiological aspects of bud development and coning in lodgepole pine were studied with the aim of obtaining a greater understanding of the mode of action of the factors controlling bud and cone development in conifers, to provide a means for predicting the outcome of bud development in advance, and to allow this outcome to be modified.

1.2 Taxonomy and ecology of lodgepole pine

*Pinus contorta* Douglas was chosen for use in this study because it is a commercially important conifer in the Pinaceae. It is native to western north America where it has a wide range from lower California to Alaska and inland to the Rocky Mountains. There are three main varieties (sub species (Critchfield (1957)) *Pinus contorta* var. *contorta* found coastally; var. *latifolia* found inland on the rocky mountains and var. *murrayana* found in the Sierra Nevada. (For a description of the ranges of the varieties of *Pinus contorta* see Critchfield (1957)). Lodgepole pine exhibits an extremely large ecological amplitude with respect to climate over its geographical range with temperature, rainfall, wind, humidity, air mass movement and daylength quite variable.
It has a fairly constant requirement for light. Although it will survive in moderate shade, it grows best in full sunlight everywhere (Satterland 1975). Lodgepole pine has become increasingly valuable in Great Britain because it grows well on the poorest upland soils of the North and West, where it is used either as a pioneer species or as a shelter for more exacting species.

1.3 Bud development and coning in conifers, especially lodgepole pine

The anatomy and morphology of bud development and cone differentiation is not easy to study in conifers because of their arborescent, perennial growth habit, extended juvenile phases and because in Pinus all leaf, branch and cone initiation occurs within a well protected overwintering bud up to nine months before shoot extension; only a few buds on a tree initiate cones and coning buds are often either male or female.

The anatomical and cytohistological structure of conifer buds was first recorded by Doak in 1935 for Pinus species. In the last 30 years an ecological/physiological approach to the study of bud development has led to an understanding of the changes in structure in different seasons and different environments (Sacher 1954, Duff & Nolan 1958, Gifford & Mirov 1960, Hanawa 1966,67, Garret & Zahner 1973, Bolland & Sweet 1979). The work of Van Den Berg & Lanner (1971), Cannell & Willett (1975), Lanner & Van Den Berg (1975), Cannell (1976) and Cannell & Bowler (1978), specifically on Pinus contorta, has provided much information about vegetative bud development in this species.

The timing and mode of initiation of cones has been determined for a few species of conifers. The structure of a floral pine bud is described by Doak (1935), and Duff & Nolan (1958). Abnormalities in the reproductive system which lead to anomalous coning structures are described by Black (1961). The most comprehensive early study of the timing of cone development was carried out by Gifford & Mirov (1960), who determined the dates of cone initiation in Western USA for 15 species of Pinus. There are several general reviews on cone initiation (Puritch 1972, Owens 1980) and an up to date summary of floral initiation in forest trees (Owens & Blake 1985).
The timing of cone initiation in *Pinus contorta* var. *contorta* and var. *latifolia* growing in British Columbia was studied (Owens & Molder 1975) and variations in the timing between the two subspecies were identified. The anatomy and morphology of cone development in lodgepole pine, before and after pollination, is described by Owens & Molder (1984). As the timing of bud development and coning may vary in Scotland from that of trees growing in their natural range, it is essential to identify the timescale in Scotland so that floral induction or enhancement treatments may be applied when trees are most likely to be receptive to the treatment.

Although data relating to the timing of cone initiation and maturation are available, very little is known about the time-span of cone initiation and differentiation within a single tree in a year; the amount of variation in timing between clones and provenances, or the effects that environmental factors have on the timing of cone initiation. Even less is known about the rate of cone differentiation and the effects of genetic and environmental factors on this rate. As the factors affecting floral initiation may have no effect on differentiation of cones, and as there may be some overlap between the timing of initiation and subsequent differentiation of cones in a population of buds, it is essential to know how much variation occurs in the timing of cone initiation and in the sequence and rates of cone differentiation. This will allow a more accurate determination of the mode of action of environmental or cultural factors affecting bud and cone initiation and development.

In the Pinaceae, seed cones are normally produced on trees first, usually a few years before pollen cones (Wareing 1958). Wareing studied the distribution of male and female cones (and thus indirectly male and female buds) on *Pinus sylvestris* (Fig. 1.1) and showed that female cones were normally produced on vigorous branches towards the top of a tree and male cones on comparatively less vigorous shoots towards the base of a tree. This pattern is similar in other conifers (Balsalm fir (Powell 1977), longleaf pine (Varnell 1976)). Male cones on lodgepole pine shoots normally develop from axillary buds (ABs) situated towards the base of a bud while female
Figure 1.1 Diagram of the branches of a single 12-13 year old tree of *Pinus sylvestris* (Scots pine), showing the distribution of male and female cones. Modified from Wareing (1957). Data collected by K.A. Longman.

The distribution of male and female cones on lodgepole pine is very similar to that for Scots pine.
cones develop from a pseudo-whorl of ABs close to the apex of a bud. In lodgepole pine more than one pseudo-whorl of female cones may be initiated, with short-shoots and sometimes long-shoots developing between the two whorls. This type of growth is termed polycyclic.

1.4 Photosynthesis and movement of assimilates in conifers.

The developing buds of conifers are strong metabolic sinks (Dickmann & Koslowski 1970). In Scots pine most of the photosynthate for bud development comes initially from needles which expanded the year before (Dickmann & Koslowski 1968, 1970), while from mid July onwards the photosynthate for bud development is translocated from newly expanded needles. In conditions where trees are adequately irrigated and fertilized, export of photosynthate from newly expanded needles may begin in early July.

Over all locations and positions on a tree, shoot length is highly correlated with the length and diameter of the bud from which it is formed (Koslowski et al 1973) and bud size can be used as an index of height growth in shoots. There also may be significant correlations between the length of a shoot and the size of the bud which forms on it, with largest buds forming on terminal leader shoots and bud size decreasing with increasing distance from the tree-top to the branch the bud is on (Koslowski et al 1973). Except under severe environmental conditions the potential annual increase of shoot length of pines appears to be governed by the number of short-shoots initiated in unopened buds. The correlations between shoot sizes and bud development have been used as a basis for proposing that the amount of assimilate in a shoot may directly affect the development of the bud at the tip of that shoot, with larger shoots providing more assimilate so that larger buds can develop. The number of short-shoots initiated is partially determined by environment. However, defoliation experiments have shown that the number of needles on the shoot also affects bud development. When developing cones, which are the largest sinks for photoassimilate (Dickmann & Koslowski 1968, 1970) were removed from trees, bud survival increased (Ebell 1971), although
Increased carbohydrate content did not appear to directly affect the survival of reproductive buds (Ebell 1971).

1.5 Factors implicated in the control of bud development and coning in conifers, especially lodgepole pine

Many studies have been carried out to determine the effects of environment and/or applied treatments on bud development and coning in conifers, often with the aim of enhancing female cone production. The factors or applied induction treatments which have been examined can be separated into three groupings:

1. Environmental factors including effects of light quality and intensity, photoperiod, temperature, water availability and mineral nutrient availability;
2. Growth regulators especially gibberellins, auxins and cytokinins;
3. Silvicultural techniques which may be stress inducing like pruning, root pruning, layering, girdling and grafting.

These factors or treatments have been studied independently and in conjunction with each other. Although the direct effects of the treatments have been measured, eg. shoot, needle and bud extension rates, most of the measurements have been of the number of needles or cones developing subsequently, and shoot growth the following year. These studies have provided some understanding of the effect of each factor or treatment, but specific data relating to mode of action of factors or treatments on bud development and coning, the rate of response of the bud, the time at which buds are receptive to the factors and the variation in response in different buds are all unavailable.

1.5.1 Environmental factors

Few studies have compared the effects of environmental factors directly on short-shoot initiation and differentiation, although Duff & Nolan (1958), Koslowski (1963), and Marion et al (1968) have emphasised the importance of the previous season’s environment, during the period of bud formation, on production of short-shoot primordia for current-year needles.

Many investigators try to demonstrate relationships between environmental factors and floral initiation in
geographic regions, stands, and on individual trees, but, such relationships are frequently difficult to identify (Owens & Blake 1985). Although some correlation between weather conditions and the size of the cone crop are probable (Daubenmire 1960), Owens and Blake caution that "intercorrelations" among dependent and independent variables "may occur between, or among all factors affecting floral initiation" and as these intercorrelations may mask the causal relationships they "should be considered in all attempts to interpret the effects of environmental factors."

Light intensity

Light intensity appears to involved in controlling the number of cones initiated on a tree and the position of these cones. Most of the evidence for the effect of light intensity on flowering is indirect, comparisons having been made between trees growing on south and north facing slopes, or between open canopied or exposed trees compared with closed canopied trees or trees growing in a closed stand. In general, coning is more abundant on the south side of the crown of exposed trees or trees with open canopies or trees growing on a south facing slope (Smith & Stanley 1969, Kosinski & Giertch 1979, Simpson & Powell 1981) and shading the south side of a tree has been shown to reduce flowering more than shading the north side of a tree (Giertch & Krowlikowski 1978). Shading in general reduces flowering in fruit trees (Jackson & Sweet 1972). There is a tendency for more female cones to develop in buds located distant from a tree trunk when compared with buds located close to the trunk, and greater numbers of cones develop on tree tops and on the edges of trees than in the centre of the crown (Kosinski & Giertch 1979). This evidence suggests that high light intensity is beneficial for at least the development of female cones. Kosinski & Giertch (1982) studied the effects of increased light directly, by inserting optical fibres into buds of Pinus sulvestris and Picea abies. They managed to induce the formation of female strobiles. They believed that the increase in female cone development was related to an increase in the the proportion of far-red light absorbing phytochrome in the bud, as the effects of the bud scales which absorb more red than far-red light had been bypassed.
Photoperiod

Photoperiod has been shown to have a great effect on the vegetative development of conifers. In many tree species long days (LDs) hasten and prolong growth while short days (SDs) induce early dormancy (Vaartaja 1960). SDs led to early cessation of needle elongation, reduced shoot internode extension, and initiation of fewer internodes in buds (Wareing 1950a, b, Downs & Borthwick 1956). Longer needles developed in LDs. LDs also had a marked effect on height growth of shoots the year after treatment (Longman 1982). Breaking a 14 hour dark period into two 7-hour periods increased the height, dry weight and nitrogen absorption of jack pine seedlings, prevented the formation of adult foliage and reduced the formation of dormant buds (Giartitch & Farrer 1961). Although the effect of photoperiod on shoot and needle extension and subsequent bud development is well documented, no information seems to be available about the effects of photoperiod on the sequence or rate of bud development.

The link between photoperiod and coning in conifers is not as clear as in herbaceous plants, many of which have been shown to flower in response to LDs or SDs. Initial studies with Pinus showed no effects of photoperiod on coning (Mirov 1956, Wareing 1958), so it was regarded as being a day neutral genus, although Giertych (1967) proposed that male cones in Pinus were initiated before female cones and that male strobiles were analogous to LD plants and female strobiles to SD plants. In 1982, Longman showed that the number of female cones on lodgepole pine increased when plants were grown in SDs. As Durzan et al (1979) had shown that female cone production was inhibited in Picea glauca by interrupting the nights with 1 to 100 seconds of red light, there is now some evidence to suggest than cone initiation in the Pinaceae may be photoperiodically controlled.

In Thuja plicata full strobilus development occurred if the photoperiodic sequence of SD followed by LD is given after an initial period of more than 3 months in LD when induction takes place (Pharis et al 1969). An LD requirement was also demonstrated in GA3 induced pollen cones in Cupressus arizonica (Pharis et al 1970).
Differences in the natural time of reproductive bud differentiation in *Thuja plicata* led Owens and Pharis (1971) to suggest that it is not the absolute daylength which seems to be important, but whether it is increasing or decreasing. Owens and Blake (1985) suggest that "In all conifers where there is a demonstrated effect of photoperiod on bud induction or sex, there is a natural difference in time of male and female cone bud differentiation. In species without this difference, photoperiod may act indirectly by causing the end of shoot elongation which coincides, in conifers studied thus far, with reproductive bud differentiation (Greenwood 1980)." Although the evidence linking photoperiod to cone initiation and differentiation in conifers is sparse and in some cases contradictory, recent work suggests that increasing daylengths may be important for male cone development and decreasing daylengths for female cone development.

Temperature

Temperature has had a well-noted effect on coning (Daubenmire 1960, Matthews 1963, Puritch 1972, Owens & Blake 1985). High temperatures during the period when cones were differentiating promoted abundant cone production (Fober 1976). Daubenmire (1960) showed that in Ponderosa pine, over 7 years, there was a positive correlation between high temperatures in June-September and the number of cones initiated, while temperatures in April-May were not correlated with cone initiation. Fober (1976) working with *Pinus sylvestris* found that higher temperatures in May as well as July and August were important for increased cone crops. Philipson (1983) examined the role of heat and drought on cone development in Sitka spruce and concluded that although both treatments stimulated male cone development, drought appeared to be the most effective cultural treatment.

The mode of action of high temperatures stimulating cone formation may be by increasing growth rates, although photosynthesis, carbohydrate metabolism, water and nutrient uptake, transpiration and metabolism of growth substances may also be affected. There is a problem with determining a causal relationship between high temperature and increased coning because increases in temperature are normally correlated with reduced
moisture content in the atmosphere and increases in light intensity, both of which have also been implicated as possibly affecting cone initiation and development in conifers. It is impossible to identify which of the possible modes of action is most likely, because no data are available to relate temperature to coning in conditions where all other factors were known to be constant. However, the information gained from field studies suggests that high temperatures may promote the development of an increased number of cones when compared to lower temperatures.

Water availability

Water stress has a marked effect on vegetative bud development (Garrett & Zahner 1973). Buds of red pine subjected to drought during June-July were more than 30% smaller than unstressed buds at the end of the growing season. The number of short-shoots on a shoot was governed only by conditions of June-July of the initiation year, with drought during this period reducing the number of short-shoots developing by 30%. Shoot extension was also affected by conditions in June-July the year before extension occurred. As needle length appeared to be affected by the number of short-shoots on a shoot, when the water supply was deficient in the middle of the previous year, longer needles developed, compensating for the reduction in number.

Positive correlations have been shown between low rainfall during the period when reproductive buds are initiated, and subsequent cone production. Fober (1976) concluded from a correlation of meteorological factors and seed crops in *Pinus sylvestris* that more female buds were initiated during years when there was a sunny, dry spring (March and April) and a warm, sunny summer (July). There was no correlation between rainfall during the late summer when female cones were differentiating and number of female cones produced. Lowry (1966) and Eis (1973) found that high precipitation in the spring before female cones were initiated increased the number of cones developing on *Pseudotsuga menziesii*. Eis (1973) found that a higher proportion of cones were female rather than male if the weather in June was warm, dry and sunny. Lowry (1966) found no correlation between a dry summer and female cone differentiation.
The correlation between low rainfall alone at the time of cone differentiation and increased cone production is therefore not consistent, while experiments in which trees have been droughted have shown definite promotive effects on cone development. Cultural treatments which have been used to induce moisture stress include drought treatments, root pruning and controlled irrigation. Not only has withholding water stimulated coning in conifers (Philipson 1963, Owens & Blake 1985), watering from March to November in some pines, increased the number of male cones produced the next season (Owens & Blake 1985).

Mineral nutrient availability

Although few studies have specifically examined the mineral nutrients essential for bud development and coning, those which appear to be most important for stimulating flowering are N and P (Jackson & Sweet 1972). The effects of fertilizers on cone initiation have been reviewed by Matthews (1963), Puritch (1970) and Jackson & Sweet (1972) while Owens & Blake 1985 have summarised the results from 49 experiments carried out since 1951. After reviewing all the data on early fertilizer treatments, Matthews (1963) proposed that all other factors being equal, trees growing on fertile sites tended to produce more seed than than those on less fertile sites. Ross & Pharis (1985) believed that the specific role of fertilizers in bud development and coning was a matter of some controversy. According to Sweet & Hong (1978), the major role of N is to improve tree vigour, increasing crown size and thus the potential sites where cone buds may differentiate when conditions created by other inductive agents are favourable. However, Ebell & McMullen (1970) proposed that specific products of N metabolism, amino acids and arginine in particular, play a direct role in coning, and Ebell (1972) speculated that arginine may play a regulatory role in cone bud differentiation.

The results of fertilizer treatments have been very variable (Owens & Blake 1985). Application of nitrate N rather than ammonia N (Ebell & McMullan 1970, Ebell 1972a) during the period of vegetative bud break (Ebell 1972b) increased the number of female cones produced on Pseudotsuga menziesii; late summer application of
fertilizer to Pinus taeda increased female coning (Schmidtling 1974, 1975) while fertilizing with NH₄NO₃ doubled the numbers of female buds developing in Pinus elliottii (Barnes & Bengston 1968). Therefore, the timing of application of fertilizer, the type of fertilizer applied and the co-factors involved eg. watering or root pruning may all affect the response of trees, which may vary with species. In general, high soil fertility appears to favour the production of cones. There is evidence that many nutrient deficiencies eg calcium, magnesium, copper, may adversely affect flowering and seed production (Owens & Blake 1985).

Other Factors
Other factors which have been implicated in cone initiation include injury caused by late frosts (Ebell 1971), injury which causes girdling, defoliation (Giertych 1970), resin tapping and root damage (Owens & Blake 1985). The cone crops produced in response to these injuries have been referred to as a stress crop (Brondo 1970).

Conclusion
Although all the environmental factors listed can affect bud development and specifically coning, they do not always elicit a response from trees. This may be related to periodicity of flowering. The number of especially female cones developing on a tree at any one time may be internally regulated so that a balance is maintained between vegetative and reproductive growth. This may be the reason why after a good cone harvest, trees of certain species do not cone significantly in the following year or years. This coupled with the interactions and correlations between environmental variables and the long reproductive cycles in some conifers has led to no clear understanding of the effects of environment on bud and cone initiation and development, although high temperatures, high light intensity, low rainfall and high soil fertility all appear to favour coning.

1.5.2 Growth regulators
The relationship between growth regulators and bud development and coning has been studied using two
different approaches. The first has involved applying a
growth regulator to trees at varying times in the season
and recording the subsequent development of the trees
(Puritch 1972, Pharis & Kuo 1977, Ross & Pharis 1985,
Owens & Blake, 1985). Auxins, gibberellins, cytokinins
and abscisic acid have been applied, and although the
results have been inconclusive, in general the only group
of growth regulatory substances which has had significant
effect on the coning potential of trees is the
gibberellins. Gibberellic acid (GA3) is very effective in
stimulating cone production in the Cupressaceae and
Taxodiaceae, while GA 4/7 has been shown to stimulate
coning in the Pinaceae. Although GA3 is effective in
stimulating cone production when applied alone, GA 4/7 is
more effective when applied in conjunction with one or
more of the previously discussed environmental
treatments. For a comprehensive summary of the results of
most of the growth regulator treatments applied to forest
trees see Owens & Blake 1985.

The second approach to studying the role of growth
regulators in bud development and coning has involved
extracting endogenous growth regulators from floral and
vegetative buds of different ages at different times of
the year (Kopcewicz et al 1967, Kopcewicz 1968, 1969,
1979). Although few of the regulators extracted were
specifically identified, and different bioassays were
used to determine the active concentrations of the
regulators, these studies had the advantage over the
application techniques of being a measure of the type and
quantity of different regulators within the plant during
different stages of development and are thus a record of
the growth regulators most likely to be implicated in any
chemical control of development.

Gibberellins

Application of some of the gibberellins can be used to
manipulate flowering in some conifers. The growth
regulator GA3 has been very successful in increasing cone
production in the Cupressaceae and Taxodiaceae (Kato
1959, Hashizume 1959, Pharis et al 1965, Pharis & Morf
1968, Pharis 1976, Longman 1982), although it is believed
to inhibit flowering in woody angiosperms (Jackson &
Sweet 1972). There are few instances of increased coning
in response to application of GA3 in the Pinaceae although Chalupka (1981) obtained increased male cone initiation after treatment of *Picea abies*. Conifers in the Pinaceae do respond to less oxidised gibberellins, especially GAs 4, 7 and 9, by flowering earlier, and by producing greater numbers of cones (Pharis et al. 1976, Tompsett 1977, Greenwood 1982). The best cone induction has been obtained when the GA 4/7 mixture was applied with an adjunct treatment e.g. girdling (Ross 1975, 1978, Wheeler 1978), root puning (Ross et al. 1985), and heat or water stress (Philipson, 1983).

**Auxins**

Auxins may be important in flowering of herbaceous plants, but appear to play only a secondary role in the flowering of conifers (Puritch 1972). The transition from the vegetative to the reproductive stage of Scots pine development was correlated with a decrease in indole acetic acid levels (Galoch et al. 1979). The formation of female cones was correlated with higher levels of auxin than the formation of male cones (Hashizume 1969), although Galoch et al. (1979) found no correlation between levels of auxin and sex determination. In general when auxin has been used to induce coning, it has caused a reduction in the numbers of cones forming (Hashizume 1959, Bleymuller 1976).

Abscisic acid (ABA), cytokinins and chlorimequat (CCC).

Differentiation of female inflorescences was correlated with an increase in the level of an ABA like inhibitor, while differentiation of male inflorescences was associated with a decrease in the amount of this inhibitor (Galoch et al. 1979). When used in conjunction with gibberellins, ABA has had a stimulatory effect on cone initiation although it had no significant affect when applied alone (Bleymuller 1976, Chalupka 1979).

No correlation between sex determination and the levels of cytokinins has been observed (Galoch 1979), although when used with gibberellins, cytokinins induced increased levels of coning (Ross & Pharis 1984) as did CCC (Ross et al. 1983).

1.5.3 Cultural techniques

Girdling, banding and strangulation have been used
successfully to promote cone initiation in conifers (Hashizume 1970, Ebell 1971). These techniques were employed in an attempt to increase carbohydrate content in the crown of a tree by impeding downward movement. The success of the treatment has been related to a high carbon (C)/nitrogen (N) ratio which was believed to be favourable to flowering (Kramer & Koslowski 1960, Ebell 1971).

Root pruning or transplanting has been used as a means of inducing cone formation in conifers. It has not always been successful, possibly because of the time of treatment or because of environmental factors being unfavourable. The treatment may cause moisture stress, although any flower inhibiting substance produced by roots may also be diminished in amount (Philipson 1983, Ross et al 1985). Root pruning of Pseudotsuga menziesii in March reduced shoot elongation and also water potential during the period of reproductive bud development (June) (Webber et al 1985), reduced mitotic activity in terminal buds, delayed cone development for two to four weeks (Owans et al 1985) and induced the formation of increased numbers of cones (Ross et al 1985, Owens & Blake 1985).

Other techniques which have been used to stimulate coning include thinning, which increases the amount of light reaching the lower parts of trees, branch pruning or debudding (Wareing 1953), which may free lateral buds from apical dominance effects, grafting (Mirov 1951), and layering or shoot orientation (Jackson & Sweet 1972). These techniques can give very good results with some species while having little effect on others.

The literature related to the effects of environments or cultural practices on bud development and coning is large and somewhat contradictory, possibly because experiments have been carried out to manipulate bud development before studies to determine the duration of cone initiation and differentiation were carried out; treatments were applied at the wrong time, or other factors which may be involved in bud development were not controlled. The intercorrelations between environmental factors and the long cycles of reproductive development have also added to the confusion. It is possible to identify certain factors which affect bud development. High temperatures, high light intensity, low rainfall and
high soil fertility in the period before or during cone differentiation appears to favour the production of cones. If gibberellins are applied to trees especially in conjunction with a stress technique, enhanced coning may be induced.

1.6 Possible mechanisms for the control of bud development in conifers.

Although little direct evidence is available concerning the control of bud development in conifers, several hypotheses have been proposed. The first of these was proposed by Romberger & Gregory in 1974. Like Jackson & Sweet (1972), Romberger & Gregory rejected a flowering mechanism based on a single stimulus. They argued that woody perennials could not survive under the control of a single stimulus, and proposed that numerous biochemical/physiological systems had to be "permissive" if reproductive structures were to initiate and subsequently to develop. They suggested that flowering was a matter of "differential morphogenesis" which was determined by the microenvironment within the primordia and related to differences in size, shape, position, and competitive status of the primordia. They proposed that morphological differences arose as a result of different meristem activity of the primordia, affecting competition between these primordia and changing the supply-sink relations of metabolites and regulators. Once differences in the microenvironment occurred, cells within the primordia would respond by following different pathways of differentiation and form male or female cones.

Although Romberger & Gregory (1974) proposed that in Picea abies seedlings, primordia which developed into ovuliferous scales arose in the axils of primary foliar primordia in embryonic shoots that had initially taller internodes or higher rates of vertical growth, they had not tested their hypothesis nor had evidence relating to the cause of different sequences of differentiation in mature coning shoots.

Tompsett (1978) put forward a proposal, based on Romberger and Gregory's hypothesis, that the relationship between apical meristem growth rate and bud development
may be a causal one. He based his proposal on the suggestion of Wareing (1958), that the vigour of a shoot may be related to the types of buds it bears, and the result that there is a slower frequency of bud scale formation in vegetative buds growing towards the base of a Sitka spruce tree than on male or female buds. Tompsett suggested a scale of growth rates on which bud types were specified in the following order of increasing early vigour: weak vegetative; male; intermediate vegetative; female; strong vegetative. The trend may be valid, but needs more evidence to prove because Tompsett compared bud vigour on different regions of the crown rather than between coning and vegetative buds on comparable shoots. After applying GA 4/7 to Sitka spruce trees growing in a polythene house, Tompsett & Fletcher (1973) suggested that increasing gibberellin concentrations above the optimum enhanced apical meristem growth rates, thereby converting potentially male and female buds into intermediate vegetative or strong vegetative types. Conversely, reducing the GA 4/7 concentration below the optimum may have reduced meristem growth rates and converted potentially male or female buds into weak or intermediate vegetative buds. Unfortunately, no record of growth rates after application of treatment was made, and recent evidence (Ross et al 1985) suggested that treatments which stimulated cone formation in Pseudotsuga menziesii (root pruning and root pruning + GA 4/7) inhibited apical mitotic activity and growth. Citing studies on P. menziesii and other conifers in the Pinaceae which show very little developmental differences between probable male cone, female cone and vegetative bud primordia until only shortly before the bud types become distinct, Owens et al (1985) believed that it was a gross oversimplification to suppose that the early growth rate of a bud, or distribution of mitotic activity within it, determined the bud’s subsequent pattern of differentiation.

The second hypothesis concerning the control of development in conifer buds, is based on the nutrient diversion hypothesis of Sachs (1977) and Sachs & Hackett (1977). The nutrient diversion hypothesis was based on the idea that "a morphogenetic event will occur if the tissues receive the substrates for growth" and that
"genetic information in some tissues is not expressed simply because nutrients for cell growth are diverted elsewhere by action of competing sinks or inadequate supply of photosynthetic tissues". Sachs and Hackett proposed that gibberellins would inhibit flower development in angiosperms because they stimulated subapical meristematic activity. In Pinus it is axillary meristems which differentiate into cones, therefore it is possible that applications of gibberellins and other cone inducing treatments may be stimulating the development of especially female cones, by diverting assimilates away from the terminal meristem to axillary meristems. Although the nutrient diversion hypothesis provides explanations for the action of many diverse types of treatments, there is no unequivocal evidence to support the belief that carbohydrates play a direct, or even supportive, role in flower initiation and early differentiation processes in woody plants. Jackson & Sweet (1972) and Ross & Pharis (1985) proposed alternative hypotheses for the effects of cone inducing treatments. Ross et al (1984) did find that application of both GA3 and GA 4/7 to terminal vegetative buds caused a significant reallocation of dry matter and 14C-photosynthate within the bud from the apical dome and from subtending structural tissues to potentially reproductive lateral long-shoot primordia. But as only GA 4/7 significantly promoted flowering, it was proposed that the effects of nutrient diversion on enhanced growth of lateral long-shoot primordia were secondary to "a direct morphogenetic effect of GA 4/7 on cone bud differentiation" (Ross & Pharis 1985).

Pharis (1976) proposed a third hypothesis for the control of flowering in conifers, based on the role of GA 4/7 in cone initiation. He suggested that vigorously growing conifers utilize endogenous GAs preferentially for vegetative growth, and it is only when environment or other factors restrict this growth that GAs are available for floral initiation. Pharis & Ross (1984, 1985), cite about 60 research papers detailing success of inducing cone initiation in conifers using GAs 4, 7 and 9. Ross & Pharis (1985) discuss the evidence for and against the hypothesis. In favour of the hypothesis they cite the results that levels of less polar GA-like substances in
Picea menziesii increased relative to more polar GAs following inductive treatments of water stress and nitrate treatments (Pharis 1977), and that the increase in endogenous GA 4/7-like substances was associated with a slowed conversion of GA4 to biologically inactive metabolites after highly inductive root pruning (Pharis & Ross 1985) and plastic tainting treatments (Chalupka et al 1982). Against the hypothesis is evidence that in cone induction treatments, growth regulators are applied and taken up in amounts far exceeding that required for reproductive bud development with the level of GA 4/7 taken up by shoots being 5000 times higher than in untreated shoots (McMullan 1980). McMullan (1980) and Dunberg & Oden (1983) proposed that induction resulted not from a direct morphogenetic effect but more from a stress effect. McMullan (1980) believed that even with dilution by the expanding shoot and metabolism of GAs, the concentration in differentiating buds would be likely to be considerably higher than normal. On the other hand, Ross & Pharis (1982) suggested that only a small proportion of applied GA 4/7 would be absorbed by the tree, and the differentiating primordia have access only to a small proportion of that. 95% of applied (3H)GA4 passes to adjacent stem and needle tissue (Pharis & Ross 1985) and the (3H)GA4 is rapidly metabolised to inactive forms (Wample et al 1975). The amount of GA 4/7 in differentiating buds has not yet been determined.

Conclusion

The mechanisms controlling bud development and coning in conifers are far from being understood with a variety of factors affecting the development of buds. There appears to be a role for nutrient diversion, possibly controlled by differential growth rates or the effects of redistribution of assimilates by non-polar gibberellins. How environmental variables or cultural techniques affect growth rates, endogenous gibberellin concentrations and nutrient diversion must be understood so that a definitive answer to the control of bud development can be obtained.
1.7 Approach and methods used to study bud development

As a means of studying bud development and coning in lodgepole pine, five aims were identified. These were:

1. Establishing the timecourse of development in buds, especially the timing of cone initiation and differentiation;

2. Identifying differences in the sequence of development in male and female buds;

3. Analysing quantitatively the effects of selected environmental variables and growth regulators on bud development and coning;

4. Identifying the correlations between the structure of a shoot and the structure of a bud which would provide some evidence of the intrinsic control of development in buds and allow the outcome of bud development to be predicted in advance;

5. Developing a means of identifying the stage and rate of development of buds without dissection.

The first part of the study, therefore, involved identifying the sequence of morphological changes occurring during bud differentiation and coning in lodgepole pine, especially the time at which male, female and vegetative buds become morphologically distinct. This was done using the SEM to examine buds of potentially different sexes during the growing season. The timecourse of bud development in different clones in different years was then determined so that variation in the timing of bud development could be identified and related to different factors, especially the effect of photoperiod on bud development and cone initiation.

A method of analysis was developed to monitor changes in the rate of growth and development of buds as a whole, and initiation, growth and development of individual bud parts. The results of the analysis were used to determine whether differentiation of various bud structures occurred in response to different rates of initiation or growth of buds. This technique was used to
examine the hypothesis of Romberger & Gregory that cone
development occurs in response to variations in the
sizes of primordia at initiation leading to different
rates of growth and thus to different outcomes of
development.

Three controlled environment experiments were carried
out during the project. The first involved studying the
effects of temperature on bud development. Changes in
the rates of growth and sequence of differentiation in
buds grown at three temperatures were analysed, and the
results related to the possible effects of temperature
on bud development and cone initiation. Experiments 2
and 3 examined the effects of SD and LD photoperiods on
bud development and coning, with the specific aim of
testing the hypothesis that male cones are initiated in
LDs and female cones in SDs. The relationship between
cessation of apical meristem growth and initiation of
female cones and long-shoots was examined to determine
if apical growth had to stop before female cones and
long-shoots could differentiate. The rate of growth of
buds at the time of application of LD and SD
photoperiods was examined to analyse the relationship
between growth rate, bud development and photoperiod and
to determine whether low growth rates may limit the
production of especially female cones.

In experiment 3, the effects of application of GA 4/7
on bud development and coning in LDs and SDs were
examined. The rate of growth of ABs vs the bud apex was
examined specifically to determine whether GA 4/7
increased the growth rate of ABs relative to the apex as
proposed by Sachs and Hackett (1977), which may then
provide some evidence for the mode of action of
gibberellins in coning in that they may be involved in
directing assimilate to or from different assimilate
sinks.

Finally, correlations were sought in lodgepole pine
shoots and buds between the structure and stages of
development, so that the stage and rate of development
within the bud could be accurately predicted without
dissection, and the outcome of the bud differentiation
sequence could be predicted in advance. A study of the
correlations between the effects of environmental
factors on shoot parameters and on bud parameters was
also made, to determine if the same factors affect the
shoot and bud in similar ways. Variation in response to treatment in different parts of the tree were analysed to identify differences which may be important in determining the zonation in the types of buds initiated on a tree. The internal control of bud development and coning in trees was examined by determining the response to treatment in buds on shoots of varying sizes on different parts of trees.
2. MATERIALS AND METHODS

2.1 Characteristics of the trees studied

The plants used were Pinus contorta Dougl. (lodgepole pine) grown from material originally collected as seed from sites on the west coast of the USA and Canada. Details of the age, sex, provenance, propagation conditions and subsequent history for each of the clones examined are given in Table 2.1. The location of the provenance for each clone is given in Fig. 2.1.

2.2 Field survey of bud development and coning

The trees selected for the field survey had to be very floriferous to ensure that buds sampled before cone initiation had taken place had a high probability of being potential coning buds and did not remain vegetative. By comparing the early stages of differentiation in potential male and female buds, differences in the sequence of development in buds of different sexes could be followed. The range of material collected allowed male and female cone development to be compared within trees and between trees, clones and provenances in one season and between seasons.

2.2.1 Plot Locations.

Trees were sampled from three sites, two on Bush estate, approximately six miles south of Edinburgh and one at Farfield, one mile east of Bush.

(1) Site 1 at ITE, Bush.

Trees were sampled from two plots at this site. 
Plot 1: Clones 8987, 8988 and 8989. Grid reference NT 245 638. The trees were growing in the nursery at ITE. They had been planted in 1971 as part of a provenance trial. Most of the other trees planted in 1971 have been removed, with the exception, mainly, of the trees at the edges of the plot, and replaced with small (height < 1m) cuttings.
Plot 2: Clones 8996 and 8997. Grid reference NT 245 638 These clones were growing in the experimental orchard at
Figure 2.1 Map of western USA and Canada showing the provenance locations for the trees used in this project. See Critchfield (1957) for a map of the geographical distribution of the four subspecies of lodgepole pine.

KEY
1. Skagway, Alaska
2. Cedarvale, Skeena River, B. C.
3. Shuswap Lake, B. C.
4. Mount Ida, B. C.
5. Sooke, B. C.
6. South Cooskai, Washington
7. Newport, Oregon
8. North Bends, Oregon
ITE. The trees were planted at approximately 2 m spacing with the clones arranged in alternate rows.

Table 2.1 Characteristics of the trees used. Part A includes information about the trees growing in the field which were sampled during the field survey. Part B includes details about the potted trees used in experiments 1-3.

### 2.1 A

<table>
<thead>
<tr>
<th>Clone (No. of trees)</th>
<th>Sex</th>
<th>Provenance</th>
<th>Obtained as</th>
<th>Seeds sown/ cuttings rooted</th>
<th>Subsequent history</th>
<th>Reason for selection for this research</th>
</tr>
</thead>
<tbody>
<tr>
<td>8987 (1)</td>
<td>Male</td>
<td>Newport, Oregon, USA</td>
<td>Seed</td>
<td>1970</td>
<td>Planted out in the ITE nursery in 1971</td>
<td>The three trees were of the same age, had been propagated in the same way and were growing in the same location. Each coned regularly, and together showed a wide range of sex expression.</td>
</tr>
<tr>
<td>8988 (1)</td>
<td>Female</td>
<td>Cedarvale, Skeena River, B.C.</td>
<td>Seed</td>
<td>1970</td>
<td>Planted out in the ITE nursery in 1971</td>
<td></td>
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<tr>
<td>8989 (1)</td>
<td>Male plus female</td>
<td>Canada</td>
<td>Seed</td>
<td>1970</td>
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<tr>
<td>8021 (16)</td>
<td>Female plus male</td>
<td>G.C.I. x Olympics, Canada</td>
<td>Cuttings from Broa Forest, England</td>
<td>1971</td>
<td>Planted out in the FC nursery in 1972</td>
<td>Both clones had a reliable coning pattern and a large amount of material was available. Clone 8021 regularly produced polycyclic shoots.</td>
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<tr>
<td>8981 (4)</td>
<td>Male</td>
<td>Shuswap Lake, B.C. Canada</td>
<td>Cuttings from Shin near Lairg Scotland</td>
<td>1974</td>
<td>Planted out in the FC nursery in 1976</td>
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<tr>
<td>8978 (4)</td>
<td>Male plus female</td>
<td>Skagway, Alaska, USA</td>
<td>Cuttings from Sela Muir Forest, Scotland</td>
<td>1972</td>
<td>Planted out at Farfield (ITE) in 1973</td>
<td>To increase the number and provenance range of the clones sampled.</td>
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<tr>
<td>8979 (2)</td>
<td>Male</td>
<td>North Bends, Oregon, USA</td>
<td></td>
<td>1972</td>
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<tr>
<td>8996 (12)</td>
<td>Female plus male</td>
<td>Sooke, Vancouver Island, Canada</td>
<td>Cuttings</td>
<td>1975</td>
<td>Planted out in the seed orchard at ITE in 1981</td>
<td>The two clones were used in experimental and results from the control trees were included in the field survey.</td>
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<tr>
<td>8997 (12)</td>
<td>Male plus female</td>
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<td>Cuttings</td>
<td>1975</td>
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* Queen Charlotte Island x Olympics hybrid
The experimental trees were surrounded completely on three sides and partly on the fourth side by tall lodgepole pine trees which had been left standing after a provenance trial.

(2) Site 2 in the FC’s experimental plantation at Bush.

In this site trees were sampled from two plots, both of which were within 400 m of the ITE site.

Plot 3 : Clone 8021. Grid reference NT 246 640
The sixteen trees of clone 8021, which were growing next to the parent trees of clone 8004 used in Experiment 3, were planted 2 m apart in two rows with a wide path between the rows.

Plot 4 : Clone 8981. Grid reference NT 247 639
The four trees of 8981 were growing in a row spaced 2 m apart surrounded by trees of different clones. The parent
trees of clone 0986, which was used in Experiment 3 were also growing in this plot.

(3) Site 3 at Farfield near Roslin.

Plot 5 : Clones 8978 and 8979. Grid reference NT 262 631

These two clones were planted as part of a provenance trial at Farfield, near Roslin, which is about one mile east of ITE. Trees near the edge of the closely spaced stand were sampled.

2.2.2 Sampling dates.

The dates in each year when buds were sampled for the field survey are given in Table 2.2. The sampling dates for buds which were primarily collected as part of an experiment are given in the relevant experimental details.

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<td>30 July</td>
<td>26 May</td>
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<td>5 Aug</td>
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<td>8 Oct</td>
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2.2.3 Sampling technique

Method One, used for clones 8987, 8988 and 8989.

Buds were selected from positions on the tree where male or female cones were likely to be initiated (for diagram of the normal distribution of male and female cones on Pinus see Figs 1.1 and 2.4). Buds were selected from branches which had already produced the sex of cone required for the sample, as typically once a branch has started to produce one type of cone, there is a higher
probability it will produce that type the following year than a branch which has not coned or one which produced the opposite sex of cone. After cone differentiation had taken place, buds could be selected more accurately because male and female coning buds have different shapes. Male buds are often oval to conical with a cuspidate apex while female buds are typically cylindrical/spathulate with an acute cuspidate to asterate apex (see Fig. 2.2).

Figure 2.2 Typical shapes of male and female buds of lodgepole pine after cones have begun differentiating.

Terminal buds were cut from the tree at their junction with the surrounding lateral buds, using a scalpel. The buds were placed in labelled McCartney bottles and the fixative formalin/acetic acid/70% ethanol (5:5:90 by
(FAA) (Johansen 1940) was added. The bottles were sealed with wax and stored until dissection.

Method two: used for clones 8021, 8981, 8978, 8989, 8996, 8997 and 8994 and 8996 growing in pots. This sampling method allowed correlations between stages of shoot and stages of bud development to be identified. First year shoots were removed from the tree just below the junction where first year basal lateral shoots were growing out (see Fig. 2.3).

Before the terminal bud was removed from the shoot, a record was made of the following shoot parameters: Number of basal lateral shoots; shoot basal diameter; number of needle pairs on shoot; number of cones of each sex on shoot; length of shoot covered by needles; length of shoot covered by male cones; lengths of five needles; number of lateral buds on shoot.

The terminal bud was then removed from the shoot, placed in an individually labelled bottle and FAA was added. The bud was stored in FAA until dissection.

2.3 Preliminary fixation and dissection of buds

Within 2 h of excision all the buds to be examined were fixed in FAA for a minimum of six weeks. This ensured adequate fixation and resulted in the dissolving away of much of the waxy resins from the bud scales so making dissection of the buds easier.

FAA was used routinely, even though it is not recognised as being a generally useful fixative for SEM analysis (Falk et al 1971, O'Brien and McCully 1983), because it was convenient, facilitated dissection, had been used successfully by Cannell (1976), and Cannell & Willett (1975) for fixing pine buds. An adequate SEM fixation image was produced.

Buds were dissected in 70% ethanol, using a Nachet binocular microscope with variable x10 to x40 magnification and fibre-optics light source. The undissected length and maximum diameter of each bud was measured using a glass micrometer ruler placed in the ethanol solution beside the bud.

The first stage of the dissection involved removal of all the 1ry cataphylls using fine forceps. The number of
Figure 2.3 Diagrammatic representation of a first year shoot of lodgepole pine with long-shoots, cones and buds attached.
Buds were removed from up to 5 positions on potted trees, depending on the size of the tree and its age. These positions are indicated below, along with the age of each of the sampled branches, and the age-range of needles and the types of cones they were likely to have initiated, if any. Lodgepole pine trees may be predominantly male, female or have male and female buds in about equal numbers. The two cone types are typically found on different parts of a tree, with female cones on vigorous 1st and 2nd order branches on the upper part of the tree and male cones on somewhat less vigorous particularly 2nd and 3rd order branches towards the base of the tree.
each type of 1ry cataphyll removed was noted.

The second stage involved the selection of a typical spiral parastichy of ABs from which all the sterile 2ry cataphylls surrounding the ABs were removed. The number of 2ry cataphylls removed and the type of AB from which they were removed was recorded. In some cases, before the 2ry cataphylls were removed, the length of the outermost 2ry cataphyll was measured.

The third stage of dissection was done only if female cone or long shoot ABs were present. The 2ry cataphylls surrounding each female cone or branch AB were removed after the length of the outermost 2ry cataphyll had been measured.

When all the relevant parts of the bud had been dissected, the length and diameter of the dissected bud was measured. For some buds the height of the visible AB meristems was also measured along with a rough measurement of the height and diameter of the bud apical dome. The bud was then either discarded or, if it was to be prepared for SEM analysis, was stored in absolute ethanol before undergoing further treatment.

2.4 Fixation of buds for SEM analysis

2.4.1 Preparing buds for critical point drying

Buds were washed sequentially in 80%:20%, 60%:40%, 40%:60%, 20%:80%, 0%:100% ethanol:acetone mixtures, with at least 30 minutes in each wash, then washed in three changes of absolute acetone with at least one of the washes being overnight. The buds were stored in airtight containers over silica gel until they were critical-point dried following normal critical-point drying techniques, except when, because of their large size, some buds were washed more often (4-5 times) and left soaking in CO₂ for longer (3-4 h) than prescribed in the instructions.

2.4.2 Mounting and gold coating of specimens

Specimens were mounted on single aluminium stubs using either Durafix or Araldite as the adhesive. All the small buds and most of the large buds were mounted upright. The very large buds were mounted sideways with the dissected spiral parastichy visible. All the areas of the specimen which were not of interest were painted over with
Silver-dag to improve the electrical conductivity of the bud and reduce charging in the SEM (Hall 1978). Buds were coated with a thin film of gold in an Emscope Sputter coater following the instructions described in the Emscope manual. Small buds were coated for three minutes with the apex of the bud closest to the gold source. Larger buds were often given a further two-minute coating. Buds were then stored in plastic specimen holders over silica gel until they were examined in the SEM. If there was more than a two day delay between coating and examination in the SEM, buds were recoated in the sputter coater for one-two minutes before being examined.

2.5 SEM Analysis of Buds

The buds were examined in a Cambridge series 250 SEM fitted with a single specimen holder to allow easier movement of the bud within the chamber. Specimens were normally viewed with the low voltage anode in the column and at a low voltage (5-10 kv) setting, although the settings varied depending on the size of the specimen.

Buds were examined quickly in the SEM, then photographs were taken. Because measurements were going to be taken from the micrographs, bud parts were photographed parallel to the plane of view of the camera.

For nearly all the buds examined in the SEM, micrographs of the whole bud viewed from the side and from the top were taken. This allowed measurements of bud length, bud diameter, apical dome height, and other parameters of the apical dome to be made. Undissected ABs were photographed in side view, so that the length of the outermost AB 2ry cataphyll and the width of the AB plus cataphylls could be measured. Dissected ABs were photographed from above so that the number of 2ry cataphylls surrounding them and the arrangement of these cataphylls could be determined. Developing male and female cones were photographed in side and top view so that their increase in size relative to other cones and the number of microsporophylls or bract scales they had initiated could be calculated. Photographs were taken using an Olympus 35 mm camera and Ilford FP film or a Rolex 120 mm camera and Kodak film. Negatives were developed and printed using standard techniques. In experiments 2 and 3 many drawings
were made directly from the enlarged images and measurements were made either directly from the enlarged image or from the drawing.

2.6 Measurements of tree, shoot and bud parts

Measurements were made using a mm rule, an eyepiece micrometer or calipers unless otherwise stated.

2.6.1 Tree and shoot parameters measured

1) **Tree height**: From soil level to the tip of the leader branch.

2) **Branch height**: Height from soil level to the base of the insertion of the branch into the main stem.

3) **Branch length**: Length of first order branch from its insertion point into the main stem of the tree to the tip of the first order terminal bud.

4) **Branch basal diameter**: The diameter of the first order branch, measured within 10 mm of the branch’s insertion point with the main stem.

5) **Shoot length**: Length of a first year shoot, from the proximal junction with the last formed first year lateral shoot to the base of the terminal bud, or, if no year 1 lateral shoot was present, from the scar of the first formed year 1 sterile 1ry cataphyll to the base of the terminal bud (see Fig. 2.3).

6) **Shoot basal diameter**: The diameter of a first year shoot, within 10 mm of the insertion point of the last formed first year lateral shoot, or, if no lateral shoot is present, within 10 mm of the scar left by the year 1 sterile 1ry cataphylls (see Fig. 2.3).

7) **Number of basal lateral shoots**: Number of lateral shoots growing out at the base of a shoot. Unless otherwise stated, the lateral shoots are 1st or 2nd order first year lateral shoots developing at the base of either a year 1 leader or 1st order terminal shoot.

8) **Number of needle pairs (short-shoots) on shoot**: Number of short-shoots present on 1 year’s growth of shoot. Normally a measure of the number of extending short-shoots on a first year shoot.

9) **Length of shoot covered by needles**: The length of shoot over which short-shoots are growing, measured from the
2.6.2 Bud parameters measured

On polycyclic buds, where more than one cycle of bud parts was present, the number of bud parts in each cycle was counted or measured separately. Cycle one was always the first-formed cycle consisting of basal sterile lry cataphylls and possible combinations of some or all of the following: undifferentiated; male; short-shoot; female and/or long-shoot ABs and apical sterile lry cataphylls. The second and any subsequent cycle consisted of combinations of more undifferentiated, short-shoot, female and/or long-shoot ABs, with another cycle of sterile lry cataphylls. On very rare occasions, male cone ABs were seen in the second cycle of ABs.

Where polycyclic buds had developed the measurements made on each cycle of bud parts are clearly and individually labelled.

A. Measurements made directly on bud parts.

1. **Undissected bud length**: Length of bud from the proximal junction with the last-formed lateral bud to the tip of the cataphyll covered bud apex (see Fig. 2.3).

2. **Undissected bud maximum diameter**: Diameter of bud measured across the widest part of the bud, usually at the base of the bud (see Fig. 2.3).

3. **Undissected bud diameter 5 mm from tip of bud**: Diameter of the bud measured close to the apex of the bud.

4. **Number of basal sterile lry cataphylls**: Number of sterile
1ry cataphylls between the last formed lateral bud and the first formed AB.

(5) **Number of fertile 1ry cataphylls**: Number of cataphylls subtending any type of AB between the last-formed basal and first-formed apical sterile cataphylls.

(6) **Number of short-shoots, male or female cones or long-shoots**: Number of ABs visibly differentiating into short-shoots, male or female cones or long-shoots.

(7) **Number of apical sterile 1ry cataphylls**: Number of sterile 1ry cataphylls above the last-formed fertile cataphyll. In single cycle buds these 1ry cataphylls surround and protect the apical dome. In polycyclic buds these cataphylls are formed between two cycles of fertile cataphylls.

In buds still initiating ABs these cataphylls are only temporarily sterile. After about 15 plastochrons ABs will begin to differentiate in their axils. Normally about five newly-initiated cataphylls were too small to be accurately counted or removed from the bud, but the final number was corrected for this.

(8) **Total number of cataphylls**: Total number of cataphylls initiated from the production of the last-formed lateral bud to the time of sampling. About five of the last-formed apical sterile cataphylls were normally missed from this count, but the final number was corrected for this.

(9) **Dissected bud length**: Length of the bud from the base of the scar left after removal of the first fertile 1ry cataphyll to the tip of the apical dome. Both dissected and undissected bud length and diameter were measured because measurements of dissected buds was thought likely to be a more sensitive measurement of response to treatment, while measurements on undissected buds were much easier to make. If the response to treatment affected both dissected and undissected buds in the same way, in future, all length and diameter measurements could be made on undissected buds. If the response was not the same, then the change in the rate and sequence of growth of buds cannot be determined from measurements of undissected bud length and diameter.

(10) **Dissected bud diameter**: Diameter of bud measured parallel to the long axis of the base of the scar left after removal of the first fertile 1ry cataphyll.

(11) **2ry cataphyll length**: Length of the outermost 2ry
cataphyll surrounding an AB. Measured from the base of the 2ry cataphyll, at the junction with the top of the scar left after removal of the subtending fertile 1ry cataphyll to the top of sterile 2ry cataphyll.

(12) 2ry cataphyll width: Maximum width of the AB plus all surrounding sterile 2ry cataphylls. This is a measure of the maximum width of the first two 2ry cataphylls formed around an AB.

(13) AB (male, female, short-shoot, long-shoot or undifferentiated) meristem height: Height of the AB apical meristem from the junction with the last-formed 2ry cataphyll to the tip of the dome of the apical meristem. In male and female ABs the apical dome included areas where microsporophylls and bract scales were being initiated.

(14) AB (male, female, short-shoot, long-shoot or undifferentiated) meristem diameter: Maximum diameter of the AB at the base of the apical meristem, above the junction with the last formed sterile 2ry cataphyll. On male, female and branch ABs, this measurement was taken below the area where microsporophylls, bract scales or fertile cataphylls respectively were being initiated.

(15) Apical dome height: Height of the bud apical dome from the basal junction with the last formed sterile 1ry cataphyll to the top of the apical dome. This and dome diameter (No 16) were only approximate measures because of the difficulty of measuring dome dimensions directly on the dissected bud.

(16) Apical dome diameter: Diameter of the bud apical dome measured across the basal junction with the last-formed sterile 1ry cataphyll.

(17) Bud diameter 1 mm below the apical dome: Measurement of the diameter of the bud a set distance from but close to the apex of the bud.

B. Bud measurements taken from electron micrographs.

(1) Dissected bud length, dissected bud diameter, 2ry cataphyll length and 2ry cataphyll width: These measurement were made as described in parts 1-4 of the last section.

(2) AB identification number: Each AB was numbered from 1 to n in order of appearance, with n equal to the total number of ABs initiated.

(3) Unit length (UL): The distance in length between ABs x and
\( y \) is the next AB on the spiral going from the base of the bud to the apex.

The length measurements were made on enlarged drawings or prints of the side view of a bud, with the spiral being used to calculate the unit length clearly in view. A line was drawn parallel to the base of the scar left after removal of the fertile lry cataphyll subtending AB \( x \), and a further line drawn parallel to the base of the scar left after removal of the fertile lry cataphyll subtending AB \( y \). The distance between the two parallel lines was then measured. Measurements of unit lengths were then made between other ABs arranged on the same spiral, or occasionally between ABs on different spirals.

(4) **Unit diameter (UD)**: The diameter of a bud measured level with the base of an AB eg. \( x \) or \( y \), and parallel to the base of the scar left after removal of the fertile lry cataphyll subtending the AB.

(5) **Mean stem unit length (MSUL)**: The mean length of a stem unit (basal lry cataphyll plus AB) in any one frustum, calculated by dividing the unit length of the frustum by the number of stem units in the frustum, where the number of stem units equals \( c \):

\[
MSUL = \frac{(UL_{xy})}{c}
\]

(6) **Mean stem unit diameter (MSUD)**: The mean diameter of a stem unit in the middle of a frustum, ie. the mean of the values for basal and apical frustum diameter:

\[
MSUD = \frac{(UD_{x} + UD_{y})}{2}
\]

(7) **Frustum volume (FU)**: The volume delimited by the diameters of the bud measured at \( x \) and \( y \) and with unit length from \( x \) to \( y \). The volume of this frustum was calculated as:

\[
FU = \frac{((UD_{x})^{2} + (UD_{y})^{2} + (UD_{x} \times UD_{y}))}{4} \times \frac{\pi}{3} \times UL_{xy}
\]

(8) **Mean stem unit volume (MSUV)**: The mean volume of a stem unit in the middle of a frustum, calculated from mean stem unit length and diameter:
Apical dome height: Height of the apical dome above the last formed 1ry cataphyll. Measured as the distance from a line parallel to the base of the last formed cataphyll to the tip of the apical dome.

Because the buds used were in general large with many stem units, and because the changes over the whole of the bud were required, this approximate measure of apical dome height was used rather than a more accurate measurement which could have been obtained by sectioning buds.

Minimum radial distance (MRD): The mean radial distance of the youngest primordium from the apical centre. For measuring method see section 2.6.2 C

Plastochrore ratio (PR): The ratio of the distance from the centre of the apical dome to the centres of primordia $x$ and $y$, where $x$ and $y$ are recently formed primordia and primordium $x$ was initiated one plastochrone before primordium $y$ (Richards 1951). For measuring method see section 2.6.2 C

Radial relative growth rate per plastochrone (RRGR): Calculated as the natural logarithm of the plastochrone ratio:

$$RRGR = \log_e PR$$

Rare area of apex: Calculated as the cross-sectional area with centre at the apical centre and radius equal to the minimum radial distance:

$$BA = (\text{MRD})^2 \times \pi$$

Area of a primordium at initiation (API): Area of a primordium at initiation measured relative to the area of the apex is calculated as being twice the natural log of the plastochrone ratio (Richards 1951):

$$API = 2\log_e PR$$

Area of apex without ABS: Cross-sectional area with centre at the apical centre and radius equal to the
minimum distance to the centre of the fertile 1ry cataphyll subtending the last formed AB.

(16) **AB diameter (ABD)**: The sterile 2ry cataphylls surrounding ABs are arranged in an irregular pattern caused by the AB being squashed by the core of the bud, by other ABs surrounding it and by the 1ry cataphylls which cover it. The pattern of 2ry cataphyll initiation approximates to opposite, decussate, with a mean divergence angle of 90° between leaf pairs. The diameter was measured at 90° to the last pair of 2ry cataphylls initiated so that it was always measured across the positions where the next 2ry cataphylls were likely to form. In some buds one of the 2ry cataphylls of a pair was initiated before the other so some of the diameter measurements were made between the area where the next primordium was expected to form to the area where the last primordium had formed. In these circumstances, the diameter was measured to the outside edge of the already formed primordium i.e. the measurement was made as if the primordium had not been formed. The measurement was made to edge of the bare area of the apex, where it adjoined with an already formed cataphyll.

(17) **Number of cataphylls surrounding an AB**: Number of sterile 2ry cataphylls surrounding the apical meristem of an AB.

(18) **AB plastochrone ratio**: The ratio of the distance from the centre of successively formed 2ry cataphyll primordia to the apical centre.

(19) **Cone length**: The length of a cone measured from the scar left after removal of the last formed 2ry cataphyll to the top of the apical dome.

(20) **Cone diameter**: Diameter of a cone measured across its base level with and parallel to the scar left after removal of the last-formed AB.

(21) **Number of microsporophylls on male cone**: The number of differentiating microsporophylls on the sides of a male cone, calculated by counting the number of microsporophylls visible on a micrograph of the side view of a male cone and doubling the number.

(22) **Number of bract scales on female cone**: The number of differentiating bract scales on the sides of a female cone, calculated as for microsporophylls (No. 21) or by counting the number of bract scales seen on a micrograph.
C. Technique used to identify the arrangement of primordia on the apical dome of a bud and calculate the dimensions of apical parts.

More than 90% of buds examined in the SEM had their primordia arranged in spiral parastichies which fitted into the normal Fibonacci series, with a primordium divergence angle of approximately 137°. Anomalous buds had either a 4:7 spiral arrangement with divergence angle of 99° or a bijugate arrangement where the parastichy numbers were twice that found in Fibonacci patterns and the divergence angle was 68°. The constancy of the arrangement of primordia on the apical surface was used as an aid to calculating the apical centre.

Firstly, the order of initiation of the last twelve primordia initiated was determined from photographs taken directly over the apical dome. Divergence angles of primordia around the approximate apical centre were calculated and using these calculations and a knowledge of the spiral arrangements of the primordia it was relatively easy to identify the primordial order. A grid of concentric circles on clear acetate sheeting was placed over each of the last 12 primordia in turn and the centre of each primordium was estimated by arranging the best fitting circle as accurately as possible inside the circumference of the primordia and equalising as much as possible the areas of the primordia outside the circle so that these areas were equally spread around the circumference of the circle. A pencil mark was then made through the hole in the centre of the acetate grid marking the estimated centre of the primordia. This was repeated for at least the last twelve primordia initiated by the bud.

A transparent mm grid was laid over the drawing or photograph of the apex of the bud and the cartesian coordinates of the centre of each of the primordia were determined. Using these coordinates a computer program 'Angles' was used to calculate the centre of a triangle using the cosine rule. The program (See appendix 1 for computer listing) uses an iterative technique based on the cosine rule to calculate the apical centre from each
possible set of three primordia which were initiated successively (see Erickson & Meicenheimer 1977). This gave 10 estimates for the apical centre from which a mean position of the centre was calculated. The distance of each of the primordia away from the centre was determined and 11 estimates of the plastochrone ratio were calculated and averaged to give the mean plastochrone ratio for each bud. The minimum radial distance was calculated as the distance from the last formed primordium to the apical centre. These two parameters were then used to calculate other parameters of the apical dome.

A similar technique was employed to calculate the cross-sectional area of an apex without ABs, the only difference being that the first coordinate to be read into the computer program was that for the centre of the last-formed fertile cataphyll subtending the last-formed AB. By calculating the radial distance from the apical centre to this fertile 1ry cataphyll, it was possible to determine the area of the apex over which only sterile 1ry cataphylls were initiated.

2.7 Techniques used to analyse results

The results were analysed using standard statistical techniques, with the aid of the computer package 'Genstat' (Alvey et al. 1982). The assumptions made and the mathematical techniques used in the analyses of variance, regression analyses and statistical modelling are given in version 4.4 of the Genstat reference manual.

In the multiple regression analyses (chapter 4), the statistical models were built using an iterative technique to order a set of variates, with the variate which minimised the residual sums of square of the regression most fitted first, and the variate which had least effect on the residual sums of squares fitted last. A summary analysis of variance table was then calculated for the ordered variates. The analyses were used to determine the relative importance of highly correlated tree and shoot variates plus other factors on the structure and stage of development of the bud.
2.8 Growth room and seed orchard experimental designs

2.8.1 Experiment 1: Effects on bud development and coning of temperature in LD applied from the end of shoot extension.

The experiment lasted for 99 days from 27th May to 2nd September 1983. Twelve potted trees of clone 8994 and eight of clone 8996 were used in each of three treatments. (For characteristics of the trees used see Table 2.1). The trees were used in 1981 to study the effects of GA 4/7 application on coning. They were repotted into 10-inch pots in spring 1983, just before the start of the experiment.

(1) Experimental conditions

Treatment 1 (room 1): Warm temperature regime.
Plants were placed in growth room 1 from day 1 to day 42 at a constant 20 ± 1°C. Photoperiod was 16 h light (0900-0100) and 8 h darkness (0100-0900) and photon flux density was 450 µmol m\(^{-2}\)s\(^{-1}\) ± 20 µmol m\(^{-2}\)s\(^{-1}\) measured at tree top height (trees were approximately 1.2 m tall). Light was supplied solely by fluorescent tubes.

Treatment 2 (room 2): Cool temperature regime.
Plants were placed in growth room 2 from day 1 to day 42 at 10 ± 1°C. The photoperiod and photon flux density were the same as in room 1.

Plants in treatments 1 and 2 were placed outdoors from day 42 to 99.

Treatment 3: Plants were kept outdoors during the whole of the experiment. Temperature, photoperiod and photon flux density all fluctuated daily and with season.

The plants in all three treatments were watered when required.

Note: On day 14, growth room 1 was closed down for 2 h. Temperature remained at approximately 20°C, but photon flux density was reduced by about 50%.

From approximately 1200 on 18th July to 2300 on 19th July 1983, temperature control in both rooms was lost. The
temperature rose to 30°C in room 1 and 25°C in room 2 during part of this time.

(2) Tree layout

Because of their large size and the lack of space available for moving them around in the growth rooms, the plants were arranged in a way which randomised the edging effects. Plants were given numbers using Tables of random numbers, then assigned to one of the treatments. Within each treatment trees were positioned in one of two blocks depending on clone. This was because the trees of clone 8996 were smaller and had fewer branches than those of clone 8994. The 20 trees in each treatment were arranged in these blocks throughout the experiment. When the plants were outdoors they were placed in a sheltered area between glasshouses, with the blocks for each treatment slightly apart.

(3) Sampling

A minimum of six buds of clone 8994 and four buds of clone 8996 per sample were removed, each bud from a different tree on day 1 (27/5/83) and on each of seven other collection dates at approximately 14-day intervals. Three trees of clone 8994 and two trees of clone 8996 were left unsampled throughout the experiment, to allow the effects of treatment to be examined on the fully developed shoots in 1984.

Buds were removed from trees at the junction between the lateral attachment point and the extending short shoots on the first year shoot (Fig. 2.3). Buds were removed from five positions on the tree (Fig. 2.4). The sampled buds were fixed, dissected and prepared for SEM analysis as described in sections 2.3-2.5.

Measurements of tree, shoot and bud parameters were made at the beginning of the experiment, on each collection date and after buds were dissected at the end of the experiment.

In May 1984, measurements were made, on extending shoots from both sampled and unsampled trees, and included number of trees with polycyclic shoots, number of male cones and number of female cones in cycle 1 and cycle 2.
2.8.2 Experiment 2: The effects of photoperiod on bud development and coning, from the start of the bud development cycle.

The experiment lasted for 77 days from 6th March to 23rd May 1984. Fourteen two-year old plants of clones 8021 and 8881 respectively were used in each of two treatments. (See Table 2.1 B for characteristics of the clones used). Material had been collected in 1982 and rooted on a mist bench. The trees were potted up in autumn 1982, overwintered in a cool glasshouse then placed in a plunge bed in spring 1983. In December 1983 the plants were placed in a cold room at 5°C in 18 h LD to prechill them and remove any dormancy effects. In January 1984, the plants were placed in an unheated growth room in 18 h days to encourage shoot extension to begin.

After one month, seven plants of each clone were moved to their allocated growth cabinets where the temperatures were 18°C during the 18 h day (0900-0300) and 13°C during the 6 h night (0300-0900), so that shoot extension would be speeded up.

(1) Experimental conditions

Treatment 1 (cabinets 3 and 6): LD environments.
Photoperiod was 18 h with 10 h (0900-1900) of light from fluorescent plus incandescent lamps; 9 h (1800-0900) of low photon flux density (approx. 1.8 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) from the incandescent lights only and 6 h darkness (0300-0900). Temperature was 18°C ± 0.5°C for 10 h (0900-1900) and 13°C ± 0.5°C for 14 h (1900-0300).

Treatment 2 (cabinets 4 and 5): SD environments.
Conditions were the same as treatment 1 except that the photoperiod was 10 h, with the low photon flux density incandescent lights on only between 0930 and 1830 and there was 14 h darkness (1930-0900).

The photon flux density in each cabinet was set as close as possible to 195 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) with fluorescent and incandescent lamps on. At the beginning of the experiment the average radiometer readings in each cabinet were, Cabinet 3: 206.6 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), Cabinet 4:
although the actual photon flux density in each cabinet was higher in the centre and lower at the edges of the growing area.

(2) Tree layout: Pairing plants

Measurements of tree height, terminal shoot length and bud length were made for each plant. For each clone separately, trees were ordered from highest to lowest and assigned a value for each character measured. The three values assigned to each plant were then totalled and the plants were ordered into largest and smallest plants on the basis of these totals. The plants were assigned numbers in order of size and arranged into seven groups of four similar plants. Plants from each clone were arranged in pairs by matching the numbers allocated to the plants during the sizing process. The most uniform four blocks of plants from each clone were assigned to the centre positions in each of the growth cabinets, one plant from each clone per cabinet. The plants in less uniform blocks were assigned to edge positions in separate cabinets. By having a similar layout of matched plants in each of the cabinets, with plants separated into edge and centre positions, any differences in conditions between cabinets or within a cabinet, especially differences in photon flux density between the centre and the edge of a cabinet could be identified.

(3) Sampling

On day 1 (6 March) and on each of 6 other collection dates, normally at 14 day intervals, a pair of plants with the same identity number was sampled from each cabinet, with all the pairs sampled being taken from the same position in each cabinet i.e. one block of four plants were sampled on each date. Plants from the centres of the cabinets were sampled on alternate sampling dates to plants from edge positions so that the more uniform sized plants were sampled at regular intervals throughout the experiment. Any differences in the response to treatment between centre and edge plants could thus be identified more easily.

After measurements had been made on the sampled trees,
all the viable buds (terminal buds developing on shoots where needles were or had just extended) on each tree were removed just below the junction between the shoot and the developing lateral buds.

Buds were fixed in FAA, then dissected in 70% ethanol, and bud parameters were measured. Leader buds were prepared for SEM analysis, where a more detailed study of development was made (for technique see sections 2.3-2.6). After trees had been sampled, they were replaced in their original positions in the growth cabinets but raised on platforms so that the shading effects on unsampled trees would remain as constant as possible.

Tree height, shoot length, bud length and needle length were measured on unsampled trees throughout the experiment. When trees were sampled, these and other shoot and bud parameters were measured.

2.8.3 Experiment 3: The effects on bud development and coning of photoperiod and treatment with GA 4/7 applied from the middle of the bud development cycle.

The experiment lasted for 85 days from 11th May to 3rd August 1984.

24 plants of clones 8004 and 8986 were used in each photoperiodic treatment. (For characteristics of the clones see Table 2.2.) The cuttings were propagated as in experiment 2. They were placed in a plunge bed in spring 1983 and remained there until 30th March 1984 when they were placed in a glasshouse to encourage shoot extension. At the beginning of May 1984, the plants were placed in growth rooms and the experiment was begun.

(1) Experimental conditions

The growth rooms used were the same as in experiment 1 (P 40) but modified by the addition of temporary incandescent light fittings mounted before the start of the experiment to increase the amount of far-red light available to the plants. The photon flux density in each room, measured at soil level, was maintained at 350 μmol m⁻² s⁻¹ by having all the incandescent lamps (27 μmol m⁻² s⁻¹) and most of the fluorescent tubes (323 μmol m⁻² s⁻¹) switched on and by switching in new fluorescent
tubes to compensate for the loss of light from existing tubes.

Treatment 1 (room 1) : LD environment. Temperature was a constant 15 ± 1°C. Photoperiod was 18 h with 10 h of light (0900-1300) supplied by fluorescent tubes and 9 h (1800-0300) supplied by incandescent lamps, and 6 h of darkness (0300-0900).

Treatment 2 (room 2) : SD environment. The temperature and photon flux density in room 2 were identical to room 1 throughout the experiment while the photoperiod was identical until day 29. From day 29 to day 85, photoperiod was 10 h, with 10 h light from fluorescent tubes (0900-1900), and 9 h from incandescent lamps (0930-1830), and 14 h of darkness (1900-0900).

(2) Application of GA 4/7
11 plants of each clone per room were injected with GA 4/7 and a further 11 plants per clone per room were injected with absolute ethanol as controls. 400 µg of gibberellin was applied in 10 µl absolute ethanol to a hole drilled in the stem of each treated tree, just below the junction between the stem and the lowest branches. The ethanol was allowed to evaporate, then the hole was sealed with the wound sealing compound Arborex. The control plants were injected with 10 µl of ethanol before the GA 4/7 was applied, to avoid contamination of the injection equipment.

(3) Tree layout : Pairing Plants
Plants were paired as in experiment 2, with the exception that measurements only of tree height and shoot length were used for clone 8986 because these trees were pre-selected for bud sizes between 16-21 mm in length. Within each clone, consecutively-numbered plants were arranged into twelve groups of four plants, alternate plants in each group being assigned to rooms 1 and 2. Odd-numbered interclone pairs were placed in exactly the same position in a block in room 1 as even-numbered pairs in room 2. The largest eight plants of each clone were assigned to block A, the middle sized eight to block B and the smallest eight to block C. In each room, one
member of each intraclone pair was randomly selected as control plant and the other as treatment plant. All interclone pairs received the same treatment. This allowed variations in response to treatment in plants of different sizes to be monitored.

(4) Sampling

Plants were sampled at 14-day intervals until transfer to SD on day 29, and were then sampled at 10 to 12-day intervals. On days 1 and 15, four plants were sampled, one from each clone per room. As all plants were in LD until day 29 and no GA 4/7 had been injected, replicate samples per clone had been harvested. On day 29, eight plants were sampled, two from each clone per room, very shortly after the treated plants had been injected with GA 4/7 and before conditions in room 2 were altered to provide SD, so again duplicate samples were harvested. From day 39 to day 79, two injected and two uninjected plants per clone per room were sampled at each collection. Paired trees from matching positions in each room were sampled at the same time. A randomly generated repeating pattern was used to select the plants to be harvested on each collection day so that plants from different blocks with a wide range of sizes would be sampled on each occasion.

Measurements of shoot length and bud length were made during the period of shoot extension on unsampled trees. Other shoot and bud parameters were measured after sampling.
Morphological aspects of bud development and coning were studied in lodgepole pine buds collected over four years from field sites in Central Scotland. The sequence of development in male, female and vegetative buds was examined in detail. The rate and timing of bud and cone development in male and female buds was studied to determine the variation in development on trees of predominately different sexes from different clones and years. Part of this study involved determining if cone development in the field occurred in response to changes in photoperiod with male cones developing in LD and female cones developing in SD (Giertych 1967, Longman 1982).

The size and rate of growth of buds in length and diameter was compared, to determine if any variations in these parameters in buds of different sexes and clones could be used to predict the stage of a bud's development; the likelihood of the bud developing cones; and the time at which cones would start differentiating.

The ABs which had the potential for differentiating into cones were studied, to identify any variations in their growth and development which may indicate why the ABs followed a particular sequence of development, and to allow differentiating cones to be recognised earlier. Correlations were made between sizes and rates of development of bud parts and types of cones initiated, to identify any relationships between growth of bud parts and coning potential.

### 3.1 A general description of bud development

From an analysis of over 1000 buds studied, bud development has been categorised into a preliminary development stage the year before the bud forms (year -1) and six development stages which occur in the year the bud is formed (year 0). Buds of lodgepole pine are determinate structures, where, except in polycyclic buds, all the parts of a shoot which extend and develop in the spring of year 1 are initiated in the buds the year before.
before extension occurs (year 0). Female cones normally differentiate from ABs close to the apex of the bud (Plate 1(a)), while male cones differentiate acropetally from the base of a bud (Plate 1(b)). Not all the bud parts differentiate in the year they are initiated; differentiation may take place in the spring of year 1, just before shoot extension occurs.

Here, the emphasis is on determining the sequence of events in buds during their early stages of development. The final stages of differentiation, which occur just before shoot extension, have already been studied by Owens and Molder (1975).

Bud development was categorised into six main stages to allow comparisons of rates of development to be made between buds which did not start differentiating at the same time or which differentiated different numbers of parts. This allowed variations in the duration and rate of differentiation for each stage of bud development in different sexes of buds on different sizes of shoots in different positions on trees to be identified so that factors which could cause this variation could be studied.

The start of each stage of bud development was marked by a new structure being initiated or differentiated on a bud as this was an easy and reliable quantitative method of deciding how well developed a bud was.

Preliminary Stage. Development of a new bud starts in the autumn of the year before the bud is visible on the tree, when the first sterile lry cataphylls which protect the apex of the overwintering bud are initiated. A variable number of sterile lry cataphylls, commonly 20-40, are initiated depending on the size, age and position of the shoot they are growing on.

As the apex of the bud enters dormancy so do the sterile lry cataphylls surrounding it. The first formed sterile lry cataphylls, although normally small in size, have already differentiated into recognisable bud scales. The sterile lry cataphylls initiated by the apex at the end of the bud growth period remain as small, undifferentiated cataphyll primordia until the spring of year 0.
Scanning electron micrographs of the apex of a female bud viewed from above and the base of a male bud viewed from the side

1 a. Scanning electron micrograph of the apex of a female coning bud of lodgepole pine with all 1ry and some 2ry cataphylls removed to show the arrangement of the female cones around the bud apical dome. The apices of the developing cones are larger in diameter than the apex of the main bud. For a fuller description of the arrangement of female cones around the apex of a bud see Fig. 3.4. and section 3.2.2.

1 b. Side view of base of a male bud of lodgepole pine with both 1ry and 2ry cataphylls removed to show male cones in different stages of development. Cones towards the base of the buds are more fully differentiated than those closer to the apex. For a full description of the different stages of male cone differentiation see Fig. 3.2 and section 3.2.1.
Stage 1. The first stage of development of a bud, in the spring of year 0, between April and May (Cannell & Willett 1975), is the increase in size of the apical meristem of the bud. This increase in size coincides with the increase in length and diameter of sterile cataphyll primordia initiated in the autumn of the year before and the initiation of new sterile 1ry cataphyll primordia (Stage 1 of Fig. 3.1). After cataphyll initiation has restarted, it is very difficult to identify in which year the cataphylls were initiated. For this reason, the total number of sterile 1ry cataphylls on a bud was used as the measure of activity of the apex during the autumn of year -1 and the spring of year 0 and not as a measure of the rate of development in year 0.

Stage 2. The second stage of development of a bud begins with the initiation of fertile 1ry cataphylls by the apex of the bud. These fertile 1ry cataphylls are identical at initiation to sterile 1ry cataphylls, so it is not until about 15 to 20 plastochrons later, when AB primordia can be seen differentiating in the axils of the fertile 1ry cataphylls that they can be identified. Because of the constancy by which approximately 15 to 20 cataphylls are initiated before ABs begin to differentiate, the beginning of stage 2 was timed from the beginning of AB initiation (Stage 2 of Fig. 3.1). AB initiation begins between mid May and late June depending on clone, provenance and season.

Stage 3. The third stage of bud development begins with the initiation of sterile 2ry cataphylls around the AB primordia. This stage begins three to four weeks after stage 2, when about 30 ABs have been initiated on a bud. The beginning of 2ry cataphyll initiation indicates the beginning of the visible stages of differentiation of ABs (Stage 3 of Fig. 3.1).

Bud development is similar in all buds until the end of stage 3, although the time at which development starts and the rate at which it proceeds appears to depend on a number of features related to the genetics and the age of the tree and the environment in which it is growing.

By the end of stage 3, ABs are beginning to differentiate and the sequence of bud development varies
Stage 1

Enterted apex

New sterile
try cataphyll

Stage 2

ABs forming
in axils of
try cataphyll

Stage 3

2ry cataphyll
initiated
around ABs

Stage 4

Short shoots
and/or scale
cones starting
to differentiate

Stage 5

Apex decreasing
in size

ABs surrounded
by 2ry cataphyll

Stage 6

Small apex
Female cones
and Long
shoots
differentiating

Approximate scale

500 μm

Figure 3.1 Diagrammatic representation of six stages of bud development in Lodgepole pine. For a full description of each stage see section 3.1.
depending on the sex of the bud, the number of branches it produces and the number of cycles of parts it initiates. The later stages of development of a vegetative bud will now be described, and, where appropriate, variations in this sequence in male and female buds are identified.

Stage 4. The fourth stage of development in vegetative buds is the completion of the initiation of sterile 2ry cataphylls around ABs. In some buds this is closely followed by ABs differentiating into short-shoots, each with two visible needle primordia (Stage 4 of Fig. 3.1). The timing of the start of this stage may vary with clone or year, but normally occurs from mid August to late September. In polycyclic buds needle differentiation may occur as early as mid July, while in small vegetative buds the ABs may not differentiate into short-shoots until the spring of year 1, even though all the sterile 2ry cataphylls have been initiated by the autumn of year 0.

In male buds, the first formed ABs differentiate into male cones instead of short-shoots. Normally male cones can be seen differentiating before needle primordia are recognisable on vegetative buds of the equivalent size. The number of sterile 2ry cataphylls surrounding the potential male cone AB varies between six and ten depending on the clone and the position of the AB with respect to the rest of the bud. Normally fewer sterile 2ry cataphylls surround a differentiating male cone than are found surrounding a short-shoot. The number of male cone to short-shoot ABs initiated on a shoot is not constant and may vary from 1 to 150 male cones on shoots with 1 to 300 short-shoots, depending on the size and position of the shoot on the tree, the environmental conditions and the clone.

For male buds, stage 4 begins when male cone differentiation begins, so the start of stage 4 would generally be the time when the most basal ABs on a bud either differentiate into male cones, short-shoots or have initiated all their sterile 2ry cataphylls and have become dormant. For male buds, stage 4B can be recognised when differentiation of new male cones ceases and the undifferentiated ABs in the region of developing male
cones either begin differentiating into short-shoots or initiate between six and ten sterile 2ry cataphylls and become dormant until the spring of year 1, when short-shoot differentiation takes place.

**Stage 5.** The beginning of stage 5 is marked by the cessation of fertile cataphyll initiation. It is very difficult to recognise when fertile 1ry cataphylls stop being initiated and sterile 1ry cataphylls are initiated instead, so the beginning of this stage is more accurately marked by the end of AB initiation, which can be recognised when all the ABs on a bud have at least one sterile 2ry cataphyll covering them (Stage 5 of Fig. 3.1).

**Stage 6.** Bud development stage 6 begins when the last-formed AB or buds become recognisable as potential long-shoots either because they have begun differentiating into long-shoots or because they have stopped differentiating while the other apical ABs are still increasing in size and initiating 2ry cataphylls (Stage 6 of Fig. 3.1). With the first type of development, the last-formed AB primordia initiate more than 15 sterile 2ry cataphylls, then initiate fertile 2ry cataphylls with small buds in their axils. Except on very vigorous shoots, it is unusual for more than twelve 2ry ABs to be formed as the long-shoot differentiates. The sterile 2ry cataphylls surrounding the long-shoot increase in length so that they are noticeably longer than cataphylls surrounding short-shoots. The outermost cataphylls surrounding long-shoots are narrower, the shape of the AB plus cataphylls is more pointed and the apical dome is smaller than is found on female cones, which are the only type of AB which differentiating long-shoots could be mistaken for.

The second type of long-shoot development occurs on smaller buds which have initiated fewer ABs. All the ABs except the last-formed one or two initiate six to twelve sterile 2ry cataphylls. The outermost sterile 2ry cataphylls reach a maximum length of at least 600 µm while only three or four sterile 2ry cataphylls > 400 µm long surround the last formed ABs. These ABs are always noticeably smaller and less developed than other ABs.
formed only one or two plastochrones before them. The small ABs can be recognised as potential long-shoots which have become dormant before differentiating.

In female buds, the beginning of stage 6 is marked by the differentiation of female cones. Female cone ABs are normally initiated immediately before the long-shoot ABs and between one and seven female cones may be initiated, although they may not all become fully differentiated. Usually there are one to three female cones on a single-cycle bud. The early stages of differentiation of female cone ABs vary slightly from the early stages of differentiation of long-shoots. As with long-shoot differentiation, ABs differentiating into female cones initiate many more sterile 2ry cataphylls. Unlike long-shoot differentiation, as these extra 2ry cataphylls are initiated, or even before, the apex of the AB increases in length and width from 100 μm to 200 μm and the outermost sterile 2ry cataphylls increase correspondingly in length and width to cover the enlarged AB apex.

In female buds the start of stage 6 is marked by the differentiation of female cones, with stage 6B being recognised as beginning when long-shoots start differentiating. As female cones or potential long-shoots become recognisable, the apex of the main bud decreases in diameter to approximately 400μm and sterile 1ry cataphyll initiation ceases. At this stage the bud normally becomes dormant until the spring of year 1 when the bud development sequence is repeated.

In polycyclic buds the initiation of sterile 1ry cataphylls lasts for only about 15 plastochrones and the apex does not decrease noticeably in size. After about 15 plastochrones, the apex begins to initiate a second cycle of fertile 1ry cataphylls and development stages 2 to 6 are repeated. Although buds with three and four cycles of parts may be found on lodgepole pine trees, never more than two cycles of bud parts were found on any buds during this project.

3.2 General description of cone development

Male and female cone differentiation has been
separated into six developmental stages using similar criteria to those used in identifying bud development stages. By having a timetable of developmental events and by studying the timescale over which these events occurred, variations in the rates at which cones differentiated could be examined and an attempt made to recognise the factors causing the variation.

3.2.1 Development of male cones.

Stage 1. The first stage of male cone development is morphologically indistinguishable from short-shoot development, with ABs producing about eight sheathing sterile 2ry cataphylls (Stage 1 of Figure 3.2; Plate 2(a)).

Stage 2. As the AB 2ry cataphylls increase in length from about 500 to 800 µm and the diameter of the apex of the AB increases from 100 to 200 µm, the apex of the AB becomes rounded in cross section and the apical dome makes up most of the AB length (Stage 2 of Fig. 3.2; Plate 2(b)). It is ABs at the base of the main bud which differentiate acropetally into male cones. This characterises the first stage at which male cones become morphologically different from other types of ABs, and it is from this stage that the start of male cone differentiation is measured.

Stage 3. When the AB is > 200 µm in diameter, microsporophylls are initiated acropetally from the base of the meristem. The microsporophyll primordia are smaller than 2ry cataphyll primordia and are arranged in a 5:8 phyllotaxis as opposed to the opposite decussate arrangement of 2ry cataphylls (Stage 3 of Fig. 3.2; Plate 2(c)).

Stage 4. As the diameter of the cone apex increases to about 300 µm, the rate of microsporophyll initiation also increases. By the end of stage 4 half the apex is covered by microsporophylls (Stage 4 of Figure 3.2; Plate 2(d)).

Stage 5. The microsporophyll primordia progressively cover more of the apical dome until the apex is
Figure 3.2 Diagrammatic representation of six stages of male cone differentiation. For a full description of each stage see section 3.2.

- Apex of bud
- 2ry cataphyll
- Enlarging apex
- Enlarged apex
- Microsporophyll
- Large apex
- More microsporophylls forming
- Reduced apex
- Numerous microsporophylls
- Apex covered by microsporophylls

Approximate Scale
500 μm
Plate 2

Scanning electron micrographs of six stages in the development of male cones

For a description of male cone development in lodgepole pine see section 3.2.2.

2 a. Undifferentiated AB with small apex and two 2ry cataphylls viewed from above.

2 b. Side view of an undifferentiated AB with two differentiated 2ry cataphylls and the 3rd cataphyll visible as a bulge on the side of the apical dome. The apex of the AB is more pointed—an indication that the AB may differentiate into a male cone.

2 c. Side view of a male cone. 2ry cataphylls have been removed to show the microsporophyll primordia beginning to differentiate acropetally from the base of the cone.

2 d. Microsporophylls have differentiated over more than half the male cone.

2 e. The microsporophyll primordia have covered all but the tip of the male cone.

2 f. Microsporophylls have completely enclosed the apex of the male cone. They are flat, with very divided edges, adpressed to the side of the male cone.
completely enclosed. The end of stage 5 is reached when the AB meristem is completely covered in microsporophylls (Stage 5 of Figure 3.2 Plate 2(e)).

Stage 6. The microsporophylls are initially smooth and raised, but as the cone develops, they become flatter with very divided edges, adpressed to the side of the cone. The cone increases in size, becomes more rounded in shape and darkens in colour from a pale cream to mid brown as it becomes more resinous. It then becomes dormant until the spring of the following year when the final stages of differentiation occur and pollen is produced (Stage 6 of Fig. 3.2; Plate 2(f)).

3.2.2 Development of female cones.

Stage 1. Female cones normally differentiate from the antepenultimate ABs. The early stages of their development are morphologically indistinguishable from other types of AB development (Stage 1 of Fig. 3.3; Plate 3(a)).

Stage 2. The female cone AB increases in size until its apex is noticeably larger than those of other types of ABs. As the first cone develops, up to six other female cones may start differentiating, while the apex of the terminal bud starts to initiate sterile 1ry cataphylls (Stage 2 of Fig. 3.3; Plate 3(b & c)). It is from this stage that female cones become morphologically distinguishable and the start of female cone differentiation is measured.

Stage 3. As each cone increases in size, up to 25 additional 2ry cataphylls are produced, tightly covering the apex of the cone (Stage 3 of Fig. 3.3; Plate 3(d)). A typical arrangement of female cones around the bud apex is shown in Fig. 3.4.

Stage 4. When the apical dome of the cone is about 400 μm in diameter and surrounded by about 30 cataphylls, bract scale primordia are initiated acropetally from the base of the cone (Stage 4 of Fig. 3.3; Plate 3(e)).
Figure 3.3 Diagrammatic representation of six stages of female cone development in lodgepole pine. For a complete description of each stage see section 3.2.

Stage 1
- Apex of bud
- 2rY cataphyll

Stage 2
- Enlarging apex
- More 2rY cataphylls

Stage 3
- Numerous cataphylls

Stage 4
- Bract scales forming

Stage 5
- Apex half-three-quarters covered by bract scales,
  Ovuliferous scales forming

Stage 6
- Apex covered by bract and ovuliferous scales
- Ovules forming

Apex of a female bud viewed from above and showing six stages of female cone development.

Scale
500 μm
Scanning electron micrographs of six stages in the development of female cones

For a description of female cone development in lodgepole pine see section 3.2.3.

3 a. An undifferentiated AB with four 2ry cataphylls removed to show the small apical dome (approx. 100 μm in diameter) surrounded by a further five 2ry cataphylls.

3 b. A female cone at the earliest morphologically recognisable stage in its development. Seven of the 2ry cataphylls surrounding the cone have been removed to show the enlarged apex (approx. 200 μm) surrounded by a further six 2ry cataphylls.

3 c. A female cone with 2ry cataphylls removed to show bract scales beginning to differentiate at the base of the cone. The apex of the cone has increased in size to approx. 350 μm.

3 d. A female cone viewed from above after 2ry cataphylls have been removed. The apex of the cone is more than 400 μm in diameter and bract scales have differentiated all around the base of the cone.

3 e. A female cone with 2ry cataphylls removed to show the bract scales differentiating acropetally from the base of the cone which is now more than 0.5 mm in length and diameter.

3 f. Side view of a female cone with 2ry cataphylls removed to show bract scales almost completely covering the apex of the cone.
The apex of the bud is surrounded by about 55 sterile sterile cataphylls. Eight ABs can be seen clearly. The ABs have been numbered from the last formed or newest to the oldest. ABs 1-3 are likely to differentiate into long-shoots (branches). ABs 4-7 are differentiating into female cones. AB 8 will differentiate into a short shoot (needle pair). The female cone apices are larger than the bud apex. The undifferentiated branch bud apices have larger diameters than the undifferentiated short shoot apex.
Stage S. During the autumn, bract scale initiation continues until between half and three quarters of the cone is covered (Stage 5 of Fig. 3.3; Plate 3(f)).

Stage E. Bract scale initiation appears to occur more slowly than microsporophyll initiation. When the outermost cataphylls surrounding the cone have reached a length of about 2 mm and the undissected cone has become almost circular in longitudinal section, the cone becomes dormant until the spring of the following year, by which time bract scales have completely covered the apex of the cone and ovuliferous scales are being initiated. By the end of May, ovules are well developed and pollination occurs (Stage 6 of Fig. 3.3).

3.3 Timing and rate of development of buds

In order to determine if photoperiod was involved in controlling bud development, the time at which buds began and finished differentiating and their rate of development was studied.

The rate and duration of bud development varied depending on the sex of the bud and the clone (Fig. 3.5). Female buds of clone 8988 began and finished developing earlier in the season than other buds, while male buds of clone 8987 began later and had a slower rate of development. Male and female buds on the same clone had similar rates of development initially, but from development stage 3, the rate of development of female buds was slightly faster and development was finished earlier than in male buds.

When the timescale of development in different seasons was examined, the temporal order for each sex and clone was similar to that for 1983. Bud development began earlier and was completed earlier, especially in female buds in 1982, and proceeded more slowly initially in 1983 for all clones (Fig. 3.6). The rate of development for each clone appeared reasonably constant over the four seasons.

An analysis of variance of regression of stage of development against collection date was carried out for data collected before the beginning of October in each
Figure 3.5 Mean stage of bud development plotted against collection date for buds of clones 8987, 8988 and 8989 (male and female buds separately). Mean values include data from buds collected between 1981-1984.
Figure 3.6 Mean stage of bud development plotted against collection date for buds collected between 1981-1984. Mean values include data from clones 8987 (male), 8988 (female) and 8989 (male and female) all combined.
year. The results suggested that although buds of different sexes and clones began developing at significantly different times in all four years, the rate of development for different clones in all years and different sexes in 1981 and 1983 did not vary. Male and female buds of clone 8989 appeared to develop at similar rates in all four years although they may have begun developing at slightly different times. The timing of bud development appeared to be more affected by sex of the bud and clone than the rate of development which remained very constant. When a summary analysis of variance was carried out (Table 3.7), the results obtained supported the individual analyses. The timing of the start of bud development varied with sex, clone and year. It is unlikely, with so much variation in timing that development is under strict photoperiodic control. The rate of bud development varied with season but was not significantly affected by the sex of the bud or the clone. It is unlikely that overall rates of bud development are linked with the initiation of a particular sex of cone.

3.4 Timing and rate of differentiation of cones

Cone differentiation in lodgepole pine occurred from early July to late September (Table 3.1), with female cones generally differentiated before male cones although male cone ABs were formed first and both sexes of cone differentiated at approximately the same time on the same tree. Neither male nor female cones differentiated in increasing daylengths.

3.4.1 Timing of cone differentiation.

The period over which female cones began differentiating was similar for all the clones examined in each season, but varied with season. This variation may have been related to differences in weather conditions in the spring or to periodicity of coning (Owens & Blake 1985). All the female cones on a clone began differentiating within three weeks with the exception of clone 8978 in 1983 where new cone differentiation began over a six week period.
Female cones on a single cycle bud were all initiated within a few days of each other, with a maximum of seven plastochrones between the initiation of the first and last ABs destined to be female cones. These ABs appeared to start differentiating into female cones within a few days of each other but the first formed AB was not always the one which started to differentiate first. All the female cones began differentiating in decreasing daylengths and, as they differentiated within a few days of initiation, this may indicate coning in response to decreasing photoperiods. Although a critical daylength has not been determined for cone initiation in lodgepole pine, the cones were not initiated in short daylengths as daylength in early July was >16 h and in early September was >12 h. The variation in the timing of cone differentiation suggests that some other factor may be involved in determining when female cones will differentiate, possibly with photoperiod also involved.

The time over which new male cones began differentiating varied more than for female cones. This may be because more male cones than female are normally initiated on a bud. In 1983 and 1984 most male buds appeared to be differentiating cones at some time in August. Male clones could be separated into two groups depending on the time at which they differentiated new cones. The first group consisted of all the clones which differentiated both male and female cones (clones 8989, 8996, 8997, and 8021). In this group, new male cones differentiated between mid July and late August in 1983 and between early July and mid August in 1984. The second group consisted of clones which are predominantly, if not exclusively, male coning (clones 8987, 8979 and 8981), in which new male cones began differentiating from mid August to late September in 1983, later than male cones on the first group of clones. The two late coning clones were from North Bends and Newport, Oregon, the most southerly provenances examined and the lateness in their coning may be related to provenance variation. Buds which initiated a large number of male cones, tended to take longer for all their cones to begin differentiating, especially if cone differentiation started quite late in the year.

Male cone differentiation on clones which produced male and female cones, occurred at approximately the same time.
as female cone differentiation, in decreasing daylengths. Therefore, photoperiod cannot be the sole factor determining the sex of cone to be differentiated unless development of cones is determined before the cones differentiate. Initiation of male cone ABs began in increasing daylengths, in early June on clone 8989 in 1983, but did not begin on clone 8987 before late June in any of the years studied. Therefore, male cone AB initiation does not appear to depend on increasing daylength. If the type of AB which will develop in the axil of a lry cataphyll is determined as the lry cataphyll is initiated, the sex of male cones may be determined in increasing daylengths and that of female cones in decreasing daylengths, but, there is no evidence for this predetermination.

In conclusion, the seasonal and clonal variation in timing, makes it unlikely that cone initiation and differentiation is controlled solely by photoperiod.

3.4.2 Rate of cone differentiation and growth

The rate at which male and female cones differentiated showed some variation between individual buds, clones and years. Three parameters were used to measure the rate of cone differentiation: the length of the outermost 2ry cataphylls covering the cone, the number of 2ry cataphylls covering the cone and the development stage of the cone. In male buds, the first formed AB which was differentiating into a male cone and the least differentiated male cone on each bud were measured. On each female bud, all the ABs which were differentiating into female cones were measured.

All three parameters of cone development correlated highly with each other for both male and female cones, although the correlation coefficients were larger for male cones (Table 3.2). Length of the cone meristem is highly correlated with 2ry cataphyll length (Fig. 3.7), and the other cone parameters measured (data not presented). The rate of growth and development of different parts of the AB during cone differentiation appears similar.

A. Male cones

The time-period over which initiation and growth took place varied between the first and last formed cones on a
Figure 3.7 Male cone meristem length plotted against length of the outermost 2ry cataphyll surrounding the cone meristem for clones 8978 and 8979 collected in 1983. Number of cones measured = 66. Correlation coefficient (r) = 0.900.
male bud, between clone and between season.

Table 3.1 The timing of the beginning of cone differentiation from 1981 to 1984 for all the the clones examined in the field survey.

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<tbody>
<tr>
<td>8987 (M)</td>
<td>MALE</td>
<td>Early August to September</td>
<td>Late July to September</td>
<td>Mid August to mid September</td>
<td>Early August to early September</td>
</tr>
<tr>
<td>8988 (F)</td>
<td>FEMALE</td>
<td>Mid to late July</td>
<td>Early to mid July</td>
<td>Early to mid August</td>
<td>Mid July</td>
</tr>
<tr>
<td>8989 (M&amp;F)</td>
<td>MALE</td>
<td>Mid July to early August</td>
<td>Early to mid July</td>
<td>Mid July to early August</td>
<td></td>
</tr>
<tr>
<td>8994 (M)</td>
<td>MALE</td>
<td>Mid to late July</td>
<td></td>
<td>Early to mid August</td>
<td></td>
</tr>
<tr>
<td>8996 (M&amp;F)</td>
<td>FEMALE</td>
<td></td>
<td>Early August</td>
<td>Late July to 4c*LL</td>
<td>Late July to mid August(2)</td>
</tr>
<tr>
<td>8997 (M&amp;F)</td>
<td>MALE</td>
<td></td>
<td></td>
<td>Early August(1)</td>
<td>July to early August(3)</td>
</tr>
<tr>
<td>8998 (M&amp;F)</td>
<td>MALE</td>
<td></td>
<td></td>
<td></td>
<td>Late July to late August</td>
</tr>
<tr>
<td>8999 (M)</td>
<td>FEMALE</td>
<td></td>
<td></td>
<td></td>
<td>Early August to mid September</td>
</tr>
<tr>
<td>8999 (M)</td>
<td>MALE</td>
<td></td>
<td></td>
<td>Mid August to late September</td>
<td></td>
</tr>
<tr>
<td>8999 (M)</td>
<td>MALE</td>
<td></td>
<td></td>
<td>Mid July to mid August</td>
<td></td>
</tr>
<tr>
<td>8999 (M)</td>
<td>MALE</td>
<td></td>
<td></td>
<td>Mid July to early August</td>
<td></td>
</tr>
<tr>
<td>8999 (M)</td>
<td>MALE</td>
<td></td>
<td></td>
<td>Mid August to September</td>
<td></td>
</tr>
</tbody>
</table>

(1) Female cones began being differentiated in early August but the time at which the differentiation of new cones stopped is not known.
(2) Female cones began differentiating at the top of the second cycle of bud parts in early October.
(3) No female buds were sampled.
The increase in 2ry cataphyll length on basal ABs can be separated into three phases (Fig. 3.8). In the first when the undifferentiated AB was initiating 2ry cataphylls (Stage 1 of male cone development), the increase in length was between 3 & 8 μm per day for each clone. In the second phase, as male cones started to differentiate, 2ry cataphylls increased in length by between 35-45 μm per day. In the third phase, growth of 2ry cataphylls slowed down as cones became completely covered in microsporophylls and microsporophyll initiation ceased.

Table 3.2 The correlation coefficients for number of cones per bud (Number), length of the outermost 2ry cataphylls surrounding a cone (Length), number of 2ry cataphylls surrounding a cone (Cats) and development stage of a cone (Dev) for first (1) and last (2) formed male cones of clones 8978 and 8979 (3.2A) and female cones of clone 8978 (3.2B). All coefficients significant at p<0.001.

### 3.2A Male cones

<table>
<thead>
<tr>
<th></th>
<th>Number 1</th>
<th>Length(1) 2</th>
<th>Cats(1) 3</th>
<th>Dev(1) 4</th>
<th>Length(2) 5</th>
<th>Cats(2) 6</th>
<th>Dev(2) 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1.00</td>
<td>0.80</td>
<td>0.60</td>
<td>0.62</td>
<td>0.72</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Length(1)</td>
<td>0.80</td>
<td>0.96</td>
<td>0.99</td>
<td>0.95</td>
<td>0.94</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Cats(1)</td>
<td>0.80</td>
<td>0.96</td>
<td>0.96</td>
<td>0.86</td>
<td>0.97</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Dev(1)</td>
<td>0.80</td>
<td>0.99</td>
<td>0.96</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Length(2)</td>
<td>0.62</td>
<td>0.95</td>
<td>0.88</td>
<td>0.94</td>
<td>0.89</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Cats(2)</td>
<td>0.72</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td>0.98</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Dev(2)</td>
<td>0.62</td>
<td>0.94</td>
<td>0.86</td>
<td>0.94</td>
<td>0.98</td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

### 3.2B Female cones

<table>
<thead>
<tr>
<th></th>
<th>Number 1</th>
<th>Length 2</th>
<th>Cats 3</th>
<th>Dev 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>0.72</td>
<td>0.74</td>
<td>0.74</td>
<td>0.56</td>
</tr>
<tr>
<td>Length</td>
<td>0.99</td>
<td>0.77</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td>0.74</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dev</td>
<td>0.74</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Although male cones on the clones studied showed the same pattern of changes in their growth rate, different clones did not initiate cones at the same time. Both
Figure 3.8  Mean length of outermost 2ry cataphylls surrounding a basal AB before and after the AB differentiated into a male cone in 1983. Mean and 95% confidence interval of 5 or 3 buds (clones 8987 and 8989 - last 3 collections) are shown. Empty symbols: in no buds were male cones differentiating; half-filled symbols: in some buds male cones were differentiating; filled symbols: all buds had male cones.
clones 8976 and 8987 were, in terms of size, between 20 and 30 days behind clone 8989 throughout most of the development season. Male cone development began earlier in 1982 than 1983 or 1984 (Fig. 3.10 A) although the rate of growth in all three years was constant. As cones reached a greater final length in 1982 than 1983, the size of the differentiated cone at the end of the season depended more on duration than rate of growth.

The results for number of cataphylls initiated (Fig. 3.9 A) and development stage reached (Fig. 3.9 B) in 1983 showed similar variations in timing for the different clones as cataphyll length. This was expected, because of the high correlations between the three parameters, although there was no obvious lag phase in the initiation of cataphylls to correspond with the slow initial development of ABs before cone differentiation began. The constant rate of production of 2ry cataphylls was almost identical over the initiation period in 1983 and 1984 (Figs 3.9 A & 3.10 B). Cataphyll initiation ceased in both clones in September unlike growth in length which was of variable duration. In 1982, cataphyll initiation began and finished earlier than in other years.

The time between initiation of male cone ABs (development stage 1) and differentiation of male cones (development stage 2-6) was similar at 40-45 days for clones 8976 and 8989 in 1983 (Fig. 3.9 B). The rate of cone differentiation was constant and similar in both clones (Fig. 3.9 B) and in 1983 and 1984 (Fig. 3.10 B).

The absolute growth rate in length was faster for the last formed male cones on a bud, compared to the first, over the first four to six weeks of growth of the cone (Fig. 3.11 A). The rate of initiation and growth of male cone 2ry cataphylls was similar (after an initial slow start for the first formed cones on some clones) (Fig. 3.11 B). The final number and length of cataphylls on the last formed cones was less than on the first formed cones from the same clone.

In summary, growth of basal male cones took place at a constant rate which was unaffected by clonal and seasonal variation in 1983 and 1984. The period of growth and final size of cones did vary between cones on the same bud, some seasons and some clones.
Figure 3.9 Mean number of 2ry cataphylls (3.9 A) and stage of development (3.9 B) of basal ABs before and after the ABs differentiated into male cones on clones 8987 and 8989 in 1983. Means of 5 buds (or 3 buds - last three collections).
Figure 3.10 Mean length (3.10 A) and number (3.10 B) of cataphylls surrounding the basal male cone AB and stage of development of that AB (3.10 C) before and after the initiation of male cones, plotted against collection date for buds of clone 8987 collected between 1982 and 1984.
Figure 3.11 Mean length (3.11 A) and number (3.11 B) of 2-ry cataphylls surrounding basal and apical ABs before and after they differentiated into male cones for clones 8978 and 8979 collected in 1983. Smooth lines indicate results from basal (b) ABs and dotted lines those from apical (a) ABs.
B. Female cones

The size and stage of development reached by a female cone varied with clone and season. Female cone differentiation began almost immediately after the female cone AB had been initiated. The newly formed 2ry cataphylls increased rapidly in length (Fig. 3.12 A). This was in contrast to the sequence in male cones, where the AB could be present on the bud for up to six weeks before cone differentiation began and where the growth rate of ABs destined to become male cones was initially slow. In 1982, the increase in cataphyll length slowed towards the end of the season and the cones became dormant while in 1983 the increase in 2ry cataphyll length did not slow (Fig. 3.12 A). Coning occurred relatively late in 1983 compared to other years and this may be part of the reason that cones continued developing. The number of cataphylls initiated on female cone ABs was more than twice the number found on male cone ABs (cf. Figs 3.12 B & 3.9 A). The 2ry cataphylls around female cones began growing at different times in different seasons (Fig. 3.12 A). The rate of 2ry cataphyll initiation was reasonably constant with time in 1983 and 1984 and initiation of cataphylls continued throughout the whole of the period studied, while in 1982, cataphyll initiation stopped at the same time as the outermost 2ry cataphylls stopped increasing in length. This link between cataphyll growth and initiation was not found with male cones where cataphylls continued increasing in length after initiation had ceased (Figs 3.10 A & B). Female cones took longer to differentiate fully than male cones as development stage 6 was not reached in the year the female cones were initiated. The final size and stage of development reached by female cones in a season may be linked to environmental variables, especially those stimulating early bud development. In 1982 cones differentiated earlier, grew faster and were larger when they stopped growing than cones initiated in 1983.

As with male cones, the rate of female cone differentiation appeared constant in at least two of the years studied, while the period of growth and final size reached did vary with season.

3.4.3 The significance of variation in the duration and rate of
Figure 3.12 Mean length (3.12 A) and number (3.12 B) of 2ry cataphylls surrounding female cone ABs and mean stage of development of female cones (3.12 C) before and after cone differentiation, plotted against collection date for buds of clone 8989 collected between 1982 and 1984.
male and female cone development in clones 8987, 8988 and 8989 from 1982 to 1984

The intercepts of the regression lines for male and female cones of different clones in different years varied significantly (Table 3.3). Therefore, the time at which cone development began varied significantly in different clones in each year and between years. As the variance ratio for the clone regression lines was only slightly significant for 2ry cataphyll length and not significant for either of the other two parameters, the rate of 2ry cataphyll initiation and rate of cone development were constant in each of the clones studied and there were only slight differences in rates of cone growth. The variance ratios for the regression lines for all except male cone 2ry cataphylls were highly significant, so the rate of growth and development of cones varied between years. Except for the stage of development of female cones, there was no significant difference in the intercept values for the regression lines of clone/year pairs, so cones began differentiating at approximately the same time each year. The rate of growth and development of cones was constant between clones in each year, but varied between years for both types of cone.

The time at which male and female cone ABs on the same clone (clone 8989) began differentiating varied significantly \((p > 0.001)\), probably because male cone 2ry cataphylls were initiated before female cone 2ry cataphylls. The rate of increase in 2ry cataphyll length also varied between sexes \((p > 0.05)\), possibly because of the lag phase in male cone growth. The variation in growth rate was not as significant as the variation in timing of growth. The difference in the timing of the start of cone AB growth on the same clone may be significant with regard to the type of cone which develops, but as male cone 2ry cataphylls on male buds and needle 2ry cataphylls on female buds were initiated at the same time, photoperiod or any other factor related to time cannot be determining the outcome of development of ABs initiated in the same part of the bud.

The size, number and stage of development of male and female cone AB parts were highly correlated with each other and their rates of growth and development varied little between clones, although rates varied significantly.
with season. The time at which male cone 2ry cataphylls began to increase in length and their rate of initiation varied significantly with that for female cones on the same tree.

Table 3.3 Summary analysis of variance of regression of three parameters of cone growth and development against collection date for male cones of clones 8997 and 8999 and female cones of 8988 and 8999. The data for number of cataphylls on male cones in 1982 (D.F.>1,108) and data collected after early October in each year was omitted.

<table>
<thead>
<tr>
<th>Variate or factor</th>
<th>D.F.</th>
<th>MALE CONES</th>
<th>D.F.</th>
<th>FEMALE CONES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length</td>
<td>Cataphyll Dev. Stage</td>
<td>Length</td>
</tr>
<tr>
<td>Day</td>
<td>1,156</td>
<td>641.0 ***</td>
<td>645.6 ***</td>
<td>415.2 ***</td>
</tr>
<tr>
<td>Clone</td>
<td>1,156</td>
<td>61.2 ***</td>
<td>93.8 ***</td>
<td>51.0 ***</td>
</tr>
<tr>
<td>Year</td>
<td>2,156</td>
<td>42.6 ***</td>
<td>1.9 n.s.</td>
<td>15.0 ***</td>
</tr>
<tr>
<td>Day/clone</td>
<td>1,156</td>
<td>5.3</td>
<td>3.3 n.s.</td>
<td>1.2 n.s.</td>
</tr>
<tr>
<td>Day/year</td>
<td>2,156</td>
<td>5.6</td>
<td>1.3 n.s.</td>
<td>10.8 ***</td>
</tr>
<tr>
<td>Clone/year</td>
<td>2,156</td>
<td>0.7 n.s.</td>
<td>0.1 n.s.</td>
<td>1.6 n.s.</td>
</tr>
<tr>
<td>Day/clone/year</td>
<td>2,156</td>
<td>0.0 n.s.</td>
<td>5.4</td>
<td>0.4 n.s.</td>
</tr>
</tbody>
</table>

This difference in timing may be involved in determining the type of cones differentiated although male cone ABs on predominantly male trees began growing at the same time as female cones and 2ry cataphylls around potential short-shoots on female buds began growing at the same time as potential male cones on male bud on the same tree.

3.5 Duration and rate of growth of buds

The rate of growth of buds and the time and rate of initiation and differentiation of bud parts were measured on different clones over four years. The aim was to try to distinguish differences in the rate or sequence of development of male and female buds more accurately, to identify any relationships between the development of vegetative bud parameters and cone development and to be able to predict if a bud was likely to develop cones, when this development would take place and what the rate of development was likely to be.
3.5.1 Growth of buds in length and diameter

Four parameters were used to measure the growth of the bud as a whole. These were undissected bud length and diameter and dissected bud length and diameter. The first two parameters were measured because any method developed to predict cone development from the size and shape of a bud will have to use measurements which are easily made. The second two parameters were used because they gave a more accurate measurement of the rates of growth of buds than measurements on undissected buds, where there was difficulty in accurately identifying the junction between bud parts initiated in year 0, and those initiated in year 1.

The undissected bud length of female buds (Fig. 3.13 A) was significantly larger than male buds, both on the same and on different clones, throughout most of the growing season. The rate of growth in undissected length of male buds of clone 8987 (Fig. 3.15 A) was large enough for the mean bud length for the clone to become larger than for female buds (Fig. 3.15 B & D) by mid September.

The changes in dissected bud lengths were similar to the changes in undissected length (Fig. 3.13 B), although mean bud length was only significantly different on two collection dates for the different sexes of bud from clone 8978.

Mean undissected bud diameter (Fig. 3.14 A) was almost constant in female buds, where very little growth was observed over the season, while male buds showed an increase in diameter over the whole period of study for clone 8978. Variation in the mean diameter values for clone 8979 obscured any relationship with time.

The dissected diameter growth rate was much larger in both male and female buds than the undissected diameter growth rate (cf. Figs 3.14 A & 3.14 B). Male buds had significantly smaller mean diameters than female buds initially, but had the potential for a faster diameter growth rate over a longer period, and were significantly wider than female buds of the same clone by the end of the season. There was variation in the diameter growth rate of male buds from different clones. The period and rate of growth of buds in length (3.15) and diameter (3.16) varied with season, although buds of the same clone and sex tended to have similar rates of growth in each season.
Figure 3.13 Mean undissected (3.13 A) and dissected (3.13 B) bud length plotted against collection date for buds of clones 8978 (male and female separately) and 8989 (male) collected in 1983. Means and 95% confidence limits of 5 buds are given.
Figure 3.14 Mean undissected (3.14 A) and dissected (3.14 B) bud diameter plotted against collection date for buds of clones 8978 (male and female separately) and 8979 (male) collected in 1983. Means and 95% confidence limits of 5 buds are given.
In conclusion, female buds were initially larger than male buds. Buds from male clones, although smaller initially, increased in length faster and could become longer than other buds although this did not always happen. The diameter growth rate of female buds was much slower than for male buds, and although female buds were wider initially, they tended to be narrower at the end of the season than male buds of the same clone.

Correlations between the sizes of the four bud parameters and stage of development were very significant (Table 3.4). Because of the similarity between the duration and rate of growth of the two length and two diameter parameters, dissected length and diameter only are presented in some analyses.

<table>
<thead>
<tr>
<th>Table 3.4 Correlation coefficients for undissected bud length and diameter and dissected bud length and diameter and stage of development for buds of clones 8987, 8988 and 8989 collected between 1981 and 1984. Degrees of freedom = 420. All results significant at p=0.001.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undissected length (ULen)</td>
</tr>
<tr>
<td>Dissected length (DLen)</td>
</tr>
<tr>
<td>Undissected diameter (UDiam)</td>
</tr>
<tr>
<td>Dissected diameter (DDiam)</td>
</tr>
<tr>
<td>Development stage (DS)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>ULen</td>
</tr>
</tbody>
</table>

When dissected bud length and diameter were compared over four years, variations in the time at which buds began to increase in length and diameter and in the rates at which they increased could be identified.

The time at which buds began to grow, their absolute growth rate in length and diameter and the time at which buds stopped growing in a season all varied between seasons (Table 3.5). In 1982, buds appear to have begun growing earlier or had a faster initial growth rate than in the other years studied, while in 1983 bud growth began later than in other years. Over the time-period studied in each year, the absolute growth rate in length and diameter was nearly always greater in male buds. As female
Figure 3.15 Mean dissected bud length plotted against collection date for buds of clones 8987 (3.15 A), 8988 (3.15 B) and 8989 (male 3.15 C, female 3.15 D) collected between 1981 and 1983.
Figure 3.16 Mean dissected bud diameter plotted against collection date for buds of clones 8987 (3.16 A), 8988 (3.16 B) and 8989 (male 3.16 C, female 3.16 D) collected between 1981 and 1983.
buds were initially larger and in nearly all cases reached 5 mm in length and 3 mm in diameter earlier than male buds, they must either have grown slowly over a much longer period early in the year, or the period during which female buds increased rapidly in size was before the time-period studied in this survey, where growth of female buds was slowing down.

Table 3.5 Results of fitting regression lines to dissected bud length and diameter for each clone and sex separately from 1981 to 1984. The absolute growth rate in length or diameter (μm·d⁻¹), percentage variation accounted for by the fitted line and the date at which mean dissected length = 5 mm and mean dissected diameter = 3 mm is given for clones 8987, 8988 and male and female buds of clone 8989.

<table>
<thead>
<tr>
<th>Year</th>
<th>Clone</th>
<th>Sex</th>
<th>Growth Rate</th>
<th>% Variation</th>
<th>Date</th>
<th>Mean Dissected Length</th>
<th>Mean Dissected Diameter</th>
</tr>
</thead>
<tbody>
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<td>1981</td>
<td>8987</td>
<td>Male</td>
<td>147</td>
<td>90.4</td>
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<td>89</td>
<td>36</td>
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<tr>
<td></td>
<td></td>
<td>Female</td>
<td>89</td>
<td>73.6</td>
<td>6 July</td>
<td>150</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>8988</td>
<td>Male</td>
<td>150</td>
<td>84.6</td>
<td>12 July</td>
<td>83</td>
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<td></td>
<td></td>
<td>Female</td>
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<td>79.8</td>
<td>30 July</td>
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</tr>
<tr>
<td></td>
<td>8989</td>
<td>Male</td>
<td>83</td>
<td>94.4</td>
<td>1 Aug.</td>
<td>83.5</td>
<td>83.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>79.4</td>
<td>41.6</td>
<td>17 June</td>
<td>27.1</td>
<td>27.1</td>
</tr>
<tr>
<td>1982</td>
<td>8987</td>
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<td>121</td>
<td>86.0</td>
<td>14 July (1)</td>
<td>101</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>16.9</td>
<td>74.6</td>
<td>17 June (1)</td>
<td>55.0</td>
<td>6</td>
</tr>
<tr>
<td>8988</td>
<td>Male</td>
<td>150</td>
<td>84.6</td>
<td>78.1</td>
<td>19 April</td>
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<td>17.5</td>
</tr>
<tr>
<td></td>
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<td>74.6</td>
<td>77.2</td>
<td>25 June (1)</td>
<td>42</td>
<td>19.2</td>
</tr>
<tr>
<td>8989</td>
<td>Male</td>
<td>83</td>
<td>94.4</td>
<td>87.7</td>
<td>26 June (1)</td>
<td>87.7</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>79.4</td>
<td>87.7</td>
<td>59.6</td>
<td>26 June (1)</td>
<td>59.6</td>
<td>19.2</td>
</tr>
<tr>
<td>1983</td>
<td>8987</td>
<td>Male</td>
<td>104</td>
<td>78.4</td>
<td>6 Aug.</td>
<td>82</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>88.3</td>
<td>88.9</td>
<td>20 July</td>
<td>75</td>
<td>18</td>
</tr>
<tr>
<td>8988</td>
<td>Male</td>
<td>81.7</td>
<td>88.9</td>
<td>80.1</td>
<td>29 July</td>
<td>87.7</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>88.9</td>
<td>80.1</td>
<td>87.7</td>
<td>16 July</td>
<td>51</td>
<td>14</td>
</tr>
<tr>
<td>8989</td>
<td>Male</td>
<td>87</td>
<td>90.1</td>
<td>50.7</td>
<td>26 Aug.</td>
<td>15 July</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>90.1</td>
<td>50.7</td>
<td>87.0</td>
<td>21 July</td>
<td>54.5</td>
<td>20</td>
</tr>
<tr>
<td>1984</td>
<td>8987</td>
<td>Male</td>
<td>138</td>
<td>60.1</td>
<td>2 Aug.</td>
<td>113</td>
<td>37</td>
</tr>
<tr>
<td>8988</td>
<td>Male</td>
<td>113</td>
<td>122</td>
<td>17</td>
<td>10 July</td>
<td>122</td>
<td>17</td>
</tr>
<tr>
<td>8989</td>
<td>Male</td>
<td>142</td>
<td>91.0</td>
<td>14 Aug.</td>
<td>16 July</td>
<td>122</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>122</td>
<td>90.1</td>
<td>87.0</td>
<td>23 July</td>
<td>54.5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>91.0</td>
<td>50.7</td>
<td>87.0</td>
<td>10 July</td>
<td>54.5</td>
<td>20</td>
</tr>
</tbody>
</table>

(1) Values were not calculated when the percentage variation accounted for was less than 25%

These results do indicate that bud development in female buds begins and ends earlier than in male buds. The higher growth rates of male buds suggests that the female cone initiation is not dependent on higher growth rates in bud length and diameter over the period studied. It is possible that high growth rates early in the season are of significance to female bud differentiation, with only those buds which have fast growth rates before July being able to differentiate female cones.
3.5.2 Significance of variation in the duration and rate of male and female bud growth

Analyses of variance of regression of dissected bud length and diameter against collection date were carried out to determine if there was any significant variation in the duration and rate of bud growth in male and female buds, on different clones between 1981 and 1984 (Tables 3.6 & 3.7). Because more than 50% of variance around the fitted line was accounted for, undissected bud length and diameter were included for analysis in the summary analysis of variance (Table 3.7).

To allow all the factors to be compared in a balanced analysis, modified day values were used. These were calculated by grouping together sampling dates from different years which occurred within six days of each other and calculating a mean value to represent the grouped values.

Values for bud length and diameter, although correlating highly with each other (Table 3.4), did not show the same pattern of variation with each of the factors studied. The absolute growth rate in dissected length was the same in male and female buds (Day/sex values) (Tables 3.6 A & 3.7) while the diameter growth rates were different. The absolute growth rate in undissected diameter (Day/year) varied least between different seasons (Table 3.7).

The large variation in the significant difference of the regression coefficients for each year may be related to differences in the time-period over which the buds appeared to be growing.

In general, the time at which buds began developing appeared to vary more between years than the absolute growth rate in length. The initial diameter measurements for male and female buds of clone 8989 showed more difference than the initial length measurements and the diameter growth rate varied more between the sexes than growth rate in length. As measurements of bud diameter are affected less by season and show more difference between male and female buds, diameter may be the best parameter to use to predict the sex of a bud before cones begin differentiating. The accuracy of the prediction would depend on having specific information about the clone being studied and the seasonal growth rate of the bud.
Undissected length and diameter may also be used to predict the sex of the bud but the prediction is unlikely to be as accurate.

Table 3.6 Variance ratios from analysis of variance of the regression of dissected bud length and diameter and stage of bud development with collection date. Using the regression coefficients to test for parallelism between regression lines for different sexes of bud from 1981 to 1984 for clone 8989 (3.6 A) and for different sexes and clones from 1981 to 1984 separately (3.6 B).

![Table 3.6 Variance ratios from analysis of variance of the regression of dissected bud length and diameter and stage of bud development with collection date. Using the regression coefficients to test for parallelism between regression lines for different sexes of bud from 1981 to 1984 for clone 8989 (3.6 A) and for different sexes and clones from 1981 to 1984 separately (3.6 B).](image-url)
3.5.3 The relative importance of bud size and time of year on the rate of bud development.

A multiple regression model was set up (section 2.7) to analyse the relationship between bud size and stage of bud development. The aim of the analysis was to determine if the size of a bud was more important to its rate of development than time of year. The stage of bud development reached was regressed against all four bud size parameters plus the collection date.

Table 3.7 Summary analysis of variance for four bud size parameters and one bud development parameter plotted as a factor of collection date. Using the regression coefficients to test for parallelism of regression lines for each clone, sex and year.

<table>
<thead>
<tr>
<th>Factor or variate</th>
<th>Undissected length</th>
<th>Dissected length</th>
<th>Undissected diameter</th>
<th>Dissected diameter</th>
<th>Stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>916.3 ***</td>
<td>2054.2 ***</td>
<td>426.8 ***</td>
<td>1335.1 ***</td>
<td>1025.2 ***</td>
</tr>
<tr>
<td>Year</td>
<td>71.5 ***</td>
<td>68.7 ***</td>
<td>59.0 ***</td>
<td>65.2 ***</td>
<td>29.7 ***</td>
</tr>
<tr>
<td>Clone</td>
<td>30.3 ***</td>
<td>33.3 ***</td>
<td>47.1 ***</td>
<td>78.4 ***</td>
<td>220.1 ***</td>
</tr>
<tr>
<td>Sex</td>
<td>63.7 ***</td>
<td>3.0 n.s.</td>
<td>4.8</td>
<td>31.4 ***</td>
<td>12.2 ***</td>
</tr>
<tr>
<td>Day/year</td>
<td>14.2 ***</td>
<td>16.9 ***</td>
<td>1.9 n.s.</td>
<td>8.8 ***</td>
<td>36.5 ***</td>
</tr>
<tr>
<td>Day/clone</td>
<td>30.1 ***</td>
<td>26.7 ***</td>
<td>36.4 ***</td>
<td>34.6 ***</td>
<td>0.4 n.s.</td>
</tr>
<tr>
<td>Day/sex</td>
<td>0.9 n.s.</td>
<td>1.5 n.s.</td>
<td>156.7 ***</td>
<td>173.5 ***</td>
<td>0.0 n.s.</td>
</tr>
</tbody>
</table>

An analysis of variance of the multiple regression model was calculated to determine the significance of each of the variables following the order they were assigned during the iterative technique. The factors clone, sex and season were also analysed (Table 3.8).

The results of the multiple regression analysis suggest that the stage of development reached by a bud is more strongly related to the size of the bud, than to the time of year. As factors affect the rate of growth of a bud they also affect the timescale of development. The criteria used to determine the six stages of bud development (Section 3.1) may be indirectly related to bud size, but no size parameter was used to delimit any of the developmental stages reached and buds of very different sizes have the potential to be at the same stage of development.
Table 3.8 Results of the multiple regression analysis for stage of bud development vs dissected and undissected bud length and diameter, collection date, clone, sex and year.

<table>
<thead>
<tr>
<th>Factor or variate</th>
<th>D.F.</th>
<th>Variance ratio</th>
<th>% variance accounted for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissected length</td>
<td>1,410</td>
<td>992.1 ***</td>
<td>58.2</td>
</tr>
<tr>
<td>Dissected diameter</td>
<td>1,410</td>
<td>30.3 ***</td>
<td>59.9</td>
</tr>
<tr>
<td>Collection date</td>
<td>1,410</td>
<td>15.7 ***</td>
<td>60.7</td>
</tr>
<tr>
<td>Undissected length</td>
<td>1,410</td>
<td>23.7 ***</td>
<td>62.0</td>
</tr>
<tr>
<td>Undissected diameter</td>
<td>1,410</td>
<td>3.5 n.s.</td>
<td>62.1</td>
</tr>
<tr>
<td>Year</td>
<td>3,410</td>
<td>5.0 **</td>
<td>62.8</td>
</tr>
<tr>
<td>Clone</td>
<td>2,410</td>
<td>102.6 ***</td>
<td>74.9</td>
</tr>
<tr>
<td>Sex</td>
<td>1,410</td>
<td>6.2 **</td>
<td>75.3</td>
</tr>
</tbody>
</table>

By developing an equation which includes a component for all the measured parameters of bud size, and time of year, it should be possible to predict the stage of development reached by any bud of a particular clone. Although the sex of the bud and the season have significant effects on the stage of development reached, their omission from the equation would not lead to a very significant decrease in accuracy of the prediction. Any prediction of the stage of bud development would only relate to the clone which was used to get bud size data. As male and female cone development begins at stage 4 and stage 5 of bud development respectively, the timing of the beginning of cone differentiation may be predicted.

As the timing of cone differentiation appears to be related more to the size of a bud and its growth rate than to collection date, this suggests that any role played by photoperiod in stimulating the initiation of cones must be a minor one. Factors which affect the time at which buds start developing and their rate of growth in the early part of the season are more important in determining the time at which cones will form. These factors also play a major part in determining the number of cones which develop per bud and their sex as more cones were initiated per bud in 1982 than in other years. There is also a correlation between the duration of growth and development of female buds and female cones, with cones growing and...
developing faster in years where bud development took place earlier and possibly at a faster rate (1982), and cones continuing to differentiate into the winter in years where bud development did not take place till later in the season (1983). Weather data could be used to identify possible reasons for the increased cone productivity of trees in 1982. Because no data was collected at the sites studied and because of the large positive correlations between temperature, light intensity and rainfall (Owens & Blake 1985) the accuracy of such an analysis is in doubt. The spring of 1982 was warmer and sunnier than that of 1983.

3.6 Differences in the sizes and rates of growth and development of bud parts on male and female buds.

By identifying any differences in the duration and rate of AB initiation and development it may be possible to determine what type of structure will differentiate from an AB before it is morphologically different from other ABs. Development of male and female cones was compared in section 3.2. However, as male and female cones differentiate from ABs in different parts of a bud, any variations in the timing or rate of development of these cone-initiating ABs may be due to differences in their position. To identify variations in the rate of AB growth and development which are related to a particular type of cone being differentiated, male cone development must be compared with short-shoot development and female cones with long-shoots. Therefore, by measuring the duration and rate of growth of ABs in different parts of a bud, it may be possible to determine why male cones are initiated at the base of a bud and female cones and branches near the top.

3.6.1 Axillary buds

The number of ABs initiated and rate of initiation was examined for 5 clones in 1983 (Fig. 3.17) to determine if there was any difference in the numbers ABs initiated on buds of different sexes, both from the same tree and from different trees with different coning potentials.

At the start of the study, potential female buds had a
Figure 3.17 Mean number of ABs per bud plotted against collection date for buds of clones 8978 (♂ & ♀), 8979 (♂), 8987 (♂) and 8988 (♀) (3.18 A) and male and female buds separately of clones 8978 and 8979 (3.18 B). All buds collected in 1983.
significantly greater number of ABs than male buds irrespective of clone (Fig. 3.17 A & B). During the early part of the season (until the end of July), AB initiation occurred at a similar constant rate of approximately 2 AB d\(^{-1}\) on nearly all the clones studied (Fig. 3.18 A). AB initiation rates correlated highly with increases in bud length (cf. Figs 3.13 & 3.15). The period over which ABs were initiated did not appear to be related to the coning potential of a bud as female buds of clones 8988 and 8989 stopped initiating ABs in late July and female buds of clone 8978 continued initiating ABs until mid-September (Fig. 3.17 B). The final number of ABs on buds from each clone showed a wide range depending on the positions the buds were sampled from, but in general, the sex of the bud did not affect the number of ABs initiated (Fig. 3.17 A). When male and female buds from the same tree were compared (Fig. 3.17 B), the total number of ABs initiated was substantially less on male buds than on female buds. Therefore, it is unlikely that the coning potential of a bud is directly related to the number of ABs it produces per se, but within a clone, there may be a relationship between number of ABs and coning potential.

The time at which AB initiation ceased varied considerably, but the variation was not related to the sex of the bud as female buds on clone 8989 stopped initiating ABs first, while female buds on clone 8978 stopped initiating ABs last. In summary, ABs started developing on female buds earlier than on male buds, but the rate of initiation of ABs and final number initiated appeared to be unaffected by the sex of the bud except when comparisons were made within the same clone.

When the number and size of 2ry cataphylls in different parts of buds were correlated with length and diameter of the bud as a whole (Table 3.9), about 50 percent of the variation in the cataphyll measurements could be accounted for by variations in length and diameter. Length was more highly correlated with the AB parameters than diameter. This suggests that longer and wider buds have ABs surrounded by more longer cataphylls. The length of the outermost 2ry cataphylls were highly correlated with the number of cataphylls (Table 3.9), with larger coefficients being obtained when cataphylls on the same part of a bud
were compared. Therefore, all ABs on a bud have similar rates of growth and development and these rates show parallel trends to the rate of growth in length and diameter of the bud as a whole.

Table 3.9 Correlation coefficients for number of 2ry cataphylls around the most basal (NBAB), most apical (NTAB) and last-formed short-shoot (NSS) AB; length of the outermost 2ry cataphylls around the last-formed short-shoot (LSS) ABs; dissected bud length and dissected bud diameter. All values significant at $p = 0.001$.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissected bud length</td>
<td>0.87</td>
<td>0.74</td>
<td>0.58</td>
<td>0.64</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Dissected bud diameter</td>
<td>0.87</td>
<td>0.70</td>
<td>0.49</td>
<td>0.57</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>NBAB</td>
<td>0.74</td>
<td>0.69</td>
<td>0.64</td>
<td>0.63</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>NTAB</td>
<td>0.58</td>
<td>0.49</td>
<td>0.64</td>
<td>0.93</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>NSS</td>
<td>0.64</td>
<td>0.53</td>
<td>0.64</td>
<td>0.95</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>LSS</td>
<td>0.64</td>
<td>0.57</td>
<td>0.63</td>
<td>0.93</td>
<td>0.97</td>
<td></td>
</tr>
</tbody>
</table>

3.6.2 Basal ABs

In 1983 2ry cataphyll initiation on the first-formed AB on each bud began between 15 and 20 days after the AB had been initiated (Fig. 3.5). 2ry cataphyll initiation began between late May and early July depending on clone (Fig. 3.18 A). 2ry cataphylls were initiated on basal ABs until mid September except on clone B978 where initiation was complete by the end of August. The period and rate of 2ry cataphyll initiation were only slightly different on male and female buds (Fig. 3.18 B). Fewer 2ry cataphylls were initiated on male buds. Therefore, the divergence of the developmental pathway leading to the production of male cones or short-shoots either did not involve the initiation of 2ry cataphylls or was very sensitive to any small difference in the rate or duration of 2ry cataphyll initiation.

The outermost 2ry cataphylls surrounding the most basal AB on female buds had slightly faster initial growth rates in length than those on male buds, although the rate of growth over the period when 2ry cataphylls were being initiated on buds was similar for the two sexes of bud (Fig. 3.18 C). As 2ry cataphyll initiation stopped, the increase in length of the 2ry cataphylls on
Figure 3.18 Mean number (3.18 A & B) and length (3.18 C) of 2ry cataphylls surrounding the basal AB on a bud plotted against collection date, for buds of different sexes and clones collected in 1983. M & F = male and female added together.
female buds also stopped, while on male buds the 2ry cataphylls increased rapidly in length as male cones began to differentiate. This increased growth rate marked the first recognisable stage of male cone differentiation. The period and rate of 2ry cataphyll initiation and growth varied only slightly between basal ABs on male and female buds on the same tree. The difference does not appear large enough to cause the differentiation of either short-shoots or male cones. 2ry cataphylls grew for a longer time and had a greater final length on male coning basal ABs on male buds than on short-shoot ABs on female buds, although the number of 2ry cataphylls initiated on male cones was less.

3.6.3 Apical ABs

The number of 2ry cataphylls surrounding the last-formed AB on each collection date varied between the clones studied (Fig. 3.19 A), although the values for clones 8978, 8988 and 8989 (group 1) (Section 3.4.1) were similar initially as were clones 8979 and 8987 (group 2). The time at which initiation began and the rate of initiation within each of the groups was also similar with group 1 initiating more cataphylls earlier and at a faster rate than group 2, the clones from southerly provenances.

When 2ry cataphyll initiation was compared between male and female buds on the same clone in 1982 and 1983, (Fig. 3.19 B & C), the 2ry cataphylls were initiated earlier on female buds. The rate of initiation was similar for the different sexes developing in 1983, but the rate of initiation slowed earlier on male buds and the final number of 2ry cataphylls surrounding the last-formed AB was less. Although more 2ry cataphylls were initiated around the apical AB on female buds in 1982 compared to 1983, there was little difference in the mean number surrounding male buds in the two years, suggesting that growing conditions may be limiting 2ry cataphyll development around apical ABs on female buds but not on male buds. As the last-formed AB was destined to become a long-shoot, differentiation of this structure on male buds either occurs in the spring of year 1 or the AB remains undeveloped. A study was carried out (Section 3.7) to find if there was a
Figure 3.19 Mean number of 2ry cataphylls surrounding the last-formed AB on a bud plotted against collection date for buds of different clones (3.19 A) and different sexes of the same clone (3.19 B & C). Buds collected in 1982 and 1983.
correlation between the production of male cones and the production of branches. In summary, the initial rate of 2ry cataphyll initiation around last-formed ABs was similar in male and female buds except those from southerly provenances, but the duration of growth was less on male than female buds from the same tree.

3.6.4 Last formed short-shoot ABs

The size and number of cataphylls initiated around the last potential short-shoot to be initiated was compared for male and female buds of clone 8989 (Fig. 3.20). Initiation (Fig. 3.20 B) and growth (Fig. 3.20 A) of 2ry cataphylls began on female buds before male buds. The rate of 2ry cataphyll growth in length may have been faster initially on female buds (Fig. 3.20 A) and the final length was greater. Both the period and rate of development of short-shoot ABs on male and female buds on the same clone varied markedly between the two years. The conclusion appears to be that the period of cataphyll initiation was earlier and the rate of cataphyll growth in length was significantly faster on female buds compared to male. The rate of 2ry cataphyll initiation in 1983 showed little difference between male and female buds. 2ry cataphyll initiation started earlier in 1982 than 1983. It is likely that conditions which stimulate early growth and development of buds (1982) may also stimulate fuller development of axillary structures within the bud, in some cases leading to the development of axillary structures which are not initiated at all or do not differentiate fully, in years of slower or late growth.

The analyses of rate and duration of AB initiation and development in different parts of buds showed that there were some variations in the differentiation of male and female ABs when compared to short-shoots and long-shoots respectively. The time at which 2ry cataphylls stopped growing in a season varied, with basal ABs around male cones on male buds growing for longer than those around short-shoots on female buds although more 2ry cataphylls were initiated around the short-shoots on female buds. ABs near the apex of female buds continued to initiate 2ry cataphylls which grew longer, after ABs on male buds had stopped growing for
Figure 3.20  Mean length (3.20 A) and number (3.20 B) of 2ty cataphylls surrounding the last formed short shoot AB on a bud plotted against collection date for male and female buds of clone 8989 collected in 1982 and 1983.
the season. Growth continued later in the basal part of a male bud and the apical part of a female bud, so after cones are initiated the rate and duration of growth in the part of the bud where the cones are forming varies. However, the timing and initial rate of initiation and growth of 2ry cataphyll around ABs in 1983 although varying between clones, did not vary significantly between male and female buds on the same clone. Therefore, although more ABs were initiated and their initiation began earlier on female buds when compared to male buds on the same clone, any differences in the rate or duration of AB development which may have been important to the outcome of AB differentiation, were not apparent until after cone initiation had taken place.

3.7 Structure of male and female coning shoots

Parameters of shoot size (Table 3.10) were correlated with the type and number of cones initiated on a shoot, to try to identify differences in the structure of male, female and vegetative shoots. Because the number of shoot parts which can develop is predetermined in a bud, an overview of the relationship between bud structure and cone initiation could be gained by examining shoot structure in relation to number of cones initiated.

Table 3.10 Correlation coefficients for number of cycles of shoot parts, shoot length, number of short-shoots, number of male and female cones and number of branches at the top of cycle 1. All values are significant at p=0.001 (d.f.=250) except the correlation between no. of male cones and no. of branches.

| No. of cycles | 1 | 0.75 | 0.81 | -0.30 | 0.63 | 0.39 |
| Shoot length  | 2 | 0.75 | 0.87 | -0.34 | 0.80 | 0.66 |
| No. of short-shoots | 3 | 0.81 | 0.87 | -0.56 | 0.80 | 0.38 |
| No. of male cones | 4 | -0.30 | -0.34 | -0.56 | -0.44 | -0.16 |
| No. of female cones | 5 | 0.63 | 0.80 | 0.80 | -0.44 | 0.44 |
| No. of cycle 1 lateral shoots | 6 | 0.39 | 0.66 | 0.38 | -0.16 | 0.44 |
| 1 2 3 4 5 6 |

There was a clear positive correlation between shoot length, number of short-shoots, number of cycles of bud parts and number of female cones on the shoot. The negative correlations between shoot parameters and number of male cones, although statistically significant were not
very high and no high correlations \((r > 0.7)\) were observed between number of cycle 1 lateral shoots and the other measured shoot parameters. Therefore, the correlations between all the parameters of shoot size and coning were higher and positive for female cones and lower and negative for male cones.

The number of male, female, male/female (shoots with both male and female cones attached) and vegetative shoots with 0-5 basal lateral shoots was calculated (Table 3.11) for shoots collected from different positions on trees in 1983. The number of male coning shoots with no basal lateral shoots accounted for more than half the number of male shoots collected. As the number of basal lateral shoots increased, the number of male coning shoots decreased. Although fewer vegetative shoots were sampled, they showed the same pattern of distribution as male coning shoots, while most of the female shoots had 2 or 3 basal lateral shoots attached. The greatest numbers of male/female shoots were collected on shoots with 1 or 2 basal lateral shoots. The distribution of male and vegetative shoots with number of basal lateral shoots on clone B981 was similar to that for clone 8021, except that as the number of lateral shoots increased from 3 to 6, increasing numbers of vegetative shoots were sampled. (No female shoots were sampled). In both clones, the fewer the number of basal lateral shoots, the greater the likelihood of male cones being present on the shoot and for clone 8021, female cones were more likely to be present on shoots with 2 or 3 basal lateral shoots and male/female cones on shoots with 1 or 2 basal lateral shoots. Vegetative shoots either developed on shoots with few or no lateral shoots or on shoots with many lateral shoots.

Table 3.11 Number of male, female, male/female and vegetative shoots of clone 8021, collected with 0 to 5 basal lateral shoots attached. Trees sampled in 1983.

<table>
<thead>
<tr>
<th>Sex of shoot</th>
<th>Number of basal lateral shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Male</td>
<td>65</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
</tr>
<tr>
<td>Vegetative</td>
<td>28</td>
</tr>
<tr>
<td>Male/female</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>98</td>
</tr>
</tbody>
</table>
Figure 3.21 Mean number of female and male cones per shoot plotted against number of basal lateral shoots for female (3.21 A) and male (3.21 B) shoots of clone 8021 collected in 1983.
For both female and male shoots, the number of cones per shoot increased with increasing numbers of basal lateral shoots (although the likelihood of shoots being male coning decreased) (Fig. 3.21 A & B).

The mean values for shoot length, number of short-shoots per shoot, number of cycles of shoot parts and number of basal lateral shoots on male, female, male/female and vegetative shoots were calculated (Table 3.12). Female shoots were longer than other shoot types, had more short-shoots and normally had two cycles of shoot parts. Male and vegetative shoots were similar in length, number of basal lateral shoots and number of cycles of bud parts, mean values of which were less than for female or male/female shoots. The number of short-shoots on vegetative shoots was greater than on male shoots. Bisexual shoots were intermediate in size between male and female shoots. Female cones only appeared to differentiate on shoots which had the potential to grow to greater than approximately 140 mm with a mean of more than 130 short-shoots. Male cone development appeared to be restricted to smaller shoots. Differentiation of male cones was correlated with a reduction in the number of short-shoots formed on a shoot.

Table 3.12 Mean values of shoot length, number of short-shoots per shoot, number of cycles of bud parts and number of basal lateral shoots on male, female, male/female and vegetative shoots of clone 8021 collected in 1983. Analyses of variance between the different sexes of shoot were carried out for the 4 shoot parameters and all gave variance ratios significant at p=0.001.

<table>
<thead>
<tr>
<th>Sex of shoot</th>
<th>Shoot length (mm)</th>
<th>No. of short-shoots</th>
<th>No. of cycles</th>
<th>No. of basal lateral shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>65.9</td>
<td>50.4</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Female</td>
<td>182.7</td>
<td>196.7</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Vegetative</td>
<td>73.6</td>
<td>92.3</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Male/female</td>
<td>103.1</td>
<td>84.8</td>
<td>1.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

There was an increase in mean shoot length (Fig. 3.22 A) and number of short-shoots per shoot (Fig. 3.22 B) in both male and female shoots as the number of basal lateral
Figure 3.22 Mean shoot length (3.22 A) and number of needles per shoot (3.22 B), on male and female shoots plotted against number of basal lateral shoots for clone 8021 collected in 1983. For number of shoots collected in each category see table 3.11.
shoots increased, although female shoots were always significantly longer with more short-shoots. The increase in length and number of short-shoots was linear for male shoots (except number of short-shoots on shoots with > 2 basal lateral shoots) and linear over most of the range for female shoots. The greater the number of basal lateral shoots differentiating, the longer the shoot and the greater the number of short-shoots and cones it had.

The number of short-shoots per cycle increased as the number of basal lateral buds increased in cycle 2 for both sexes of bud and until 3 basal lateral buds were differentiating in cycle 1 for male shoots (Fig. 3.23). On female shoots there was no increase in the number of short-shoots in cycle 1 as the number of basal lateral shoots increased (Fig. 3.23 A). The constant number of short-shoots in the first cycle may be related to all female coning buds being at the same stage of development when the initiation of sterile primary cataphylls was triggered, female cone differentiation began, and the formation of cycle 1 was completed.

The number of short-shoots initiated on a female coning shoot was examined to determine the range for number of short-shoots which were initiated before female cones developed (Fig. 3.24). Each cycle of bud parts was plotted individually. The frequency of female shoots as a function of the number of short-shoots produced before female cones developed shows a normal distribution both for the first and the second cycle of bud parts, with a mean value of about 110 short-shoots being initiated. The number of short-shoots in each cycle correlated with the number of cones initiated, with more short-shoots produced before 2 or 3 cones were initiated compared to 1 cone being produced (cycle 1).

When the number of short-shoots in cycle 2 on female shoots (including shoots which had only produced cones at the top of cycle 1) (Fig. 3.25) was examined, shoots which had initiated less than 80 short-shoots did not produce cones in cycle 2, while shoots which produced more than 130 short-shoots all produced female cones. Female cone initiation appeared to be inhibited until more than 80 short-shoots were initiated in a cycle of bud parts. Whether the limitation is related to something produced by the bud as a result of initiating short-shoots or to changes which occur in the bud during needle initiation is
Figure 3.23 Mean number of needles in cycles 1 and 2 plotted against number of basal lateral shoots for female (3.23 A) and male (3.23 B) shoots of clone 8021 collected in 1983.
Figure 3.24  Number of female shoots plotted against number of short shoots initiated before production of female cones in cycles 1 and 2, for shoots of clone 8021 collected in 1983.
Figure 3.25  Number of bycyclic shoots plotted against number of short shoots initiated in the second cycle, for female coning shoots of clone 8021. Shoots with female cones terminating the second cycle of shoot parts have been treated separately from shoots with no female cones in the second cycle.

Number of bycyclic shoots

No female cones in second cycle
Female cones in second cycle

Number of short shoots initiated in cycle 2
not known. It seems unlikely that the constancy of number of short-shoots initiated before female cones was related to all the buds growing at a similar rate and being at the same stage of development when cone initiation was triggered.

It was noted above, that fewer short-shoots were produced per bud on male buds. The mean number of short-shoots initiated on vegetative shoots with 1 or more basal lateral shoot was greater than on male coning shoots (Fig. 3.26 A), so the initiation of male cones not only reduced the potential number of short-shoots initiated on a shoot, but also the total number of stem units initiated on a vigorous shoot. There was no increase in the mean internode length as the number of basal lateral buds increased for male, female or vegetative shoots of clone 8021 (Fig. 3.26 B), but internode extension was greater on female and less on male shoots.

The structure of male and female coning shoots of clone 8021 varied significantly, female shoots being longer with more short-shoots and more basal lateral branches. The likelihood of male cones being present on a shoot decreased as the number of basal lateral shoots attached increased. Most female shoots had 2 or three basal lateral shoots attached. As the number of basal lateral shoots increased, shoot length, number of short-shoots, number of male and female cones and number of cycles of bud parts also increased, so the number of basal lateral shoots could be used as an accurate and easily measured predictor of the vigour of a shoot and of the number of cones on the shoot.

3.8 Summary of field survey results

The timing of the start of bud development in lodgepole pine varied with sex, clone and year, and this large amount of variation makes a strict photoperiodic control of development unlikely. The rate of bud development varied with season, but was not significantly affected by the sex of the bud or the clone. It appears unlikely that overall rates of bud development are linked with the initiation of a particular sex of cone. Rates of development at particular times in the year may be more important.

Cone differentiation in lodgepole pine occurred from
Figure 3.26  Mean number of stem units per shoot (3.26 A) and needle internode length (3.26 B) plotted against number of basal lateral shoots for shoots of clone 8021 collected in 1983.
early July to late September, with female cones generally differentiating before male cones although male cone ABs were formed first and both sexes of cone differentiated at approximately the same time on the same tree.

The time and rate at which male and female cone 2ry cataphylls on the same tree began to develop varied significantly. This difference may be related to the type of cones developing, although male cone 2ry cataphylls on predominantly male trees began growing at the same time as secondary cataphylls around female cones and potential short-shoots. If the type of bud which will differentiate in the axil of a fertile 1ry cataphyll is determined at the initiation of that cataphyll, male and female cones may be determined in different photoperiods. There was no difference in the development of the 2ry cataphylls around male and female cones, or in the initiation and earliest stages of differentiation of male and female cone ABs which would provide evidence for predetermination of cone development as the 1ry cataphyll is initiated.

Observations of antipenultimate ABs which have more, longer, secondary cataphylls and which appear to have begun differentiating into female cones before finally developing into short-shoots suggests that development of female cones is not determined when their subtending 1ry cataphylls are initiated.

The similarity between the timing and rate of development of the last-formed male cones and the first-formed short-shoots on a bud provides little information about why one structure develops into a male cone and one into a short-shoot.

Bud length and diameter varied significantly in response to collection date, clone and year, but dissected length and undissected diameter measurements were not significantly affected by the sex of the bud while undissected length and dissected diameter were. The absolute growth rate in length and diameter tended to be higher on male buds over the period studied each season, but female buds may grow faster in length early in the season. The time at which buds began developing appeared to vary more between years than the absolute growth rate in length, and the absolute growth rate in diameter varied least between different seasons. As measurements of bud diameter are affected less by season and show more
differences between male and female buds, it may be the best parameter to use to predict the sex of a bud, although the accuracy of the prediction would depend on having specific information about the clone being studied and the seasonal growth rate of the bud.

The time at which buds start developing and their rate of growth in the early part of the season may be important in determining the time at which cones will form and the number which will form per bud, as the timing of cone differentiation appears to be related more to the size of the bud than to collection date. This suggests that although photoperiodic stimulus may be involved in flowering in lodgepole pine, there is no photoperiodic switch which caused buds to start coning at the same time each year.

More ABs were initiated and their initiation began earlier on female buds when compared to male. The timing and initial rate of initiation of 2ry cataphylls around ABs varied for different clones but not always for different sexes of bud from the same tree, although basal ABs on female buds tended to initiate cataphylls either slightly earlier or slightly faster than ABs on male buds. The rate of growth of 2ry cataphylls around the last-formed short-shoots tended to be greater on female buds. Growth continued later in the basal part of a male bud and the apical part of a female bud, so after cones are initiated, the rate and duration of growth in the part of the bud where the cones are forming varied. Male cones appear to be limited to shorter shoots with fewer short-shoots while female cones do not differentiate at the top of a cycle of shoot parts until at least 80 and more normally 110 short-shoots have been initiated on the shoot. The size of a shoot appears to be the most important factor in determining the outcome of cone initiation. As bud and shoot size parameters are highly correlated, the size of a year 1 shoot may be very important in determining the size of the developing year 0 bud and the type and number of cones differentiating on it.
EXPERIMENT 1: EFFECTS OF TEMPERATURE IN LDS. APPLIED FROM THE END OF SHOOT EXTENSION, ON BUD DEVELOPMENT AND CONING

Needle and bud growth and development were compared at 10°C or 20°C constant temperatures in 16 h LD. As growth rooms were available only for 42 days, treatments were applied for this time, then plants were moved outside and grown under natural environmental conditions. Bud development was monitored until day 99. To allow comparisons between bud development in the controlled environments and in the field, some of the trees were kept outside during the whole of the experiment.

Needle length was measured to see how temperature affected the duration and rate of growth of an already determined structure. Undissected bud length and diameter and number and types of cataphylls and ABs were measured to identify any changes in rates and/or sequence of development which were attributable to temperature. The number of differentiating lateral buds was counted to determine if temperature affected the number of growing points on a tree.

The effects of the temperature treatments could have been modified by subsequent conditions, so in the spring of the following year the number of polycyclic branches and shoots and the number of male cone groups and female cones on each tree were counted.

Because the trees used in the experiment lacked uniformity, measurements of tree and shoot parameters were made to allow an analysis of the effects of variation in tree size, sampling position, shoot length, clone and pre-treatment on needle and bud growth and development.

4.1 Shoot and bud size parameters

There was a linear increase in needle length with time (Fig. 4.1 A & B), until about day 69 when needle extension slowed down or stopped. In clone 8996 at 20°C, extension continued only until day 57. The effect of treatment on rate of increase in needle length was significant, with needle extension rates greater at 20°C than 10°C or outside. There was also a significant effect on final needle length, with needles from the 20°C treatment longer than those from the 10°C treatment. Needle extension
Figure 4.1 Mean needle length plotted against days from start of experiment for needles of clones 8994 (4.1 A) and 8996 (4.1 B), grown in three treatments. Each value is the mean of 30 (8994) or 20 (8996) needles. The arrow marks the end of treatment.
stopped after the end of treatment so the effect of treatment on the duration of needle extension cannot be determined. As needle extension stopped at similar times in both treatments and clones, it seems likely that all the trees responded to a similar stimulus, possibly a photoperiod or temperature stimulus which caused a cessation of growth. The rate of growth of needles is markedly affected by temperature with higher temperatures giving faster growth rates.

Increases in undissected bud length (Fig. 4.2) and diameter (Fig. 4.3) and number of 1ry cataphylls per bud (Fig. 4.4) appeared linear with time, with clone 8994 having smaller mean values than clone 8996. Although not significant initially, this clonal difference was significant by day 95. Treatment had little effect on bud length (Fig. 4.2) and basal diameter (Fig. 4.3) on either clone except on days 15 & 29 (during the period when treatment was actually operative) when mean diameter was larger at 20°C. The rate of cataphyll initiation was generally initially greater at 20°C and less at 10°C (Fig. 4.4 A & B). As there was a high correlation between bud length and number of cataphylls, the increase in cataphyll initiation rate at 20°C was expected to be paralleled by an increase in length. As this did not occur, the rate of initiation of cataphylls was more responsive to the effects of treatment than growth rate in length, which may be controlled by factors other than temperature. It is possible that the inherent variation in the size of the buds sampled may have masked a temperature response. This explanation was tested in section 4.6.

Varying temperature had little effect on the rate or duration of growth in length of buds. The rate of growth in diameter and rate of cataphyll initiation was greater in the 20°C treatment during at least part of the time the trees were in the growth room.

4.2 Number and rate of initiation of ABs
The number of ABs in cycle 1 was plotted against collection date for each treatment and clone separately (Fig. 4.5 A & B). The number of ABs per bud was greater in the 20°C treatment than in the other two treatments, until day 42, by which time AB initiation had stopped. In the 20°C treatment the rate of initiation of ABs on clone 8994
Figure 4.2 Mean undissected bud length plotted against days from start of experiment for buds of clones 8994 (4.2 A) and 8996 (4.2 B), grown in three treatments. Each value is the mean of 6 (8994) or 4 (8996) buds. Arrows mark end of treatment.
Figure 4.3 Mean undissected bud diameter plotted against days from start of experiment for buds of clones 8994 (4.3 A) and 8996 (4.3 B), grown in three treatments. Each value is the mean of 6 (8994) or 4 (8996) results. Arrows mark end of treatment.
Figure 4.4 Mean number of iron cataphylls per bud plotted against days from start of experiment for buds of clones 8994 (4.4 A) and 8996 (4.4 B), grown in three treatments. Each value is the mean of 6 (8994) or 4 (8996) results. Arrow marks end of treatment.
was initially significantly greater (2.6 AB/day over the first 28 days) than in either of the other two treatments (1.6 AB/day), while the rate of initiation was faster only until day 14 for clone 8996. AB initiation in the 20°C treatment was completed earlier (day 42) than in the other treatments where initiation continued throughout the period studied except clone 8994 outside where initiation stopped by day 68. The rates of initiation in the 10°C and outside treatments were similar initially, but because some of the buds in the 10°C treatment were polycyclic, the mean number and rate of initiation of the ABs was less in the cold treatment than outside. The main effects of the treatments were that 20°C temperatures increased AB initiation rates when compared to the other treatments, and stimulated the production of polycyclic buds (Fig. 4.6). 10°C temperatures stimulated some polycyclic bud formation. As the rate of growth in the 10°C treatment and outside were similar between days 1 and 42 (Fig. 4.5), there does not appear to be a link between growth rate and polycyclicity.

4.3 Sequence of bud development

The sequence of bud development was markedly affected by temperature (Fig. 4.6), with all the buds from the 20°C and half from the 10°C treatments becoming polycyclic while none of the buds growing outside were polycyclic. The time spent by 20°C and 10°C treated buds initiating fertile cataphylls in cycle 1 (stages 2-4 of bud development) also varied.

In the 20°C treatment, all the buds were initiating fertile cataphylls by day 15 and had stopped by day 57, with 9 out of 10 buds initiating sterile cataphylls by day 42. On day 68, a few buds had started initiating a second cycle of fertile cataphylls and by day 85 three-quarters of the buds studied were polycyclic.

In the 20°C treatment, the length of time over which fertile cataphylls in cycle 1 were initiated was much less than at 10°C. At 10°C, AB initiation started before day 15 with one exception, and continued until at least day 57, with two buds still initiating cycle 1 fertile cataphylls on day 99. 5 out of 12 of the buds sampled on the last two collection dates were polycyclic, which was significantly fewer than in the 20°C treatment, although initiation of
Figure 4.5 Mean number of ABs in cycle 1 per bud plotted against days from start of experiment for buds of clones 8994 (3.5 A) and 8996 (3.5 B), grown in three treatments. Each value is the mean of 6 (8994) or 4 (8996) results. Arrows mark end of treatment.
Figure 4.6 The types of 1ry cataphylls being initiated by buds on days from the start of the experiment. 6 buds of clone 8994 and 4 buds of clone 8996 were examined from each of three treatments on each collection date.

KEY

- **Basal sterile 1ry cataphyll**
- **Fertile 1ry cataphyll in cycle 1**
- **Sterile 1ry cataphyll at top of cycle 1, on potential polycyclic bud**
- **Sterile 1ry cataphyll at top of cycle 1, on potential monocyclic bud**
- **Fertile 1ry cataphyll in cycle 2**
- **Missing values**

20°C Treatment | 10°C Treatment | Outside
the second cycle of fertile cataphylls began at the same
time in both treatments.

Buds in the outside treatment showed a similar sequence
of development to those at 10°C until day 69, when fertile
cataphyll initiation began to stop. The sterile cataphylls
initiated subsequently were all terminating a years' growth, as the apical meristem of the bud became dormant.
No polycyclic buds were formed in the outside treatment.
Although the type of cataphylls initiated varied, the
total number of cataphylls initiated in each treatment did not (Fig. 4.4).

4.4 Number of differentiating lateral buds

The number of differentiating lateral buds (Table 4.1)
showed no obvious relationship to treatment or clone. The
number of differentiating lateral buds appeared to be related to
the length and diameter of the terminal bud
with more on shoots with large terminal buds.

Table 4.1 Mean number of differentiating lateral buds on
clones 8994 and 8996 from three treatments on each
collection date. Each value is the mean for 6 (clone 8994)
or 4 (clone 8996) shoots.

<table>
<thead>
<tr>
<th>Collection day</th>
<th>Clone 8994</th>
<th>Clone 8996</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hot</td>
<td>Cold</td>
</tr>
<tr>
<td>1</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>15</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>29</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>42</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>57</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>69</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>85</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>99</td>
<td>1.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

4.5 Relationship between the sequence of development of buds
in year 0 and the structure of the shoots which extended
in year 1

When extending shoots were examined in 1984, the
outcome of the different sequences of bud differentiation
in the three treatments were seen. Figs 4.7 & 4.8 show the
number of trees, sampled and unsampled, in each treatment
with particular shoot characteristics. Trees grown at 20°C
and 10°C had many polycyclic shoots and some polycyclic
Figure 4.7 Effect of temperature treatments on numbers of trees with particular shoot characteristics, for trees sampled in 1983. 9 trees of clone 8994 and 6 trees of clone 8996 per treatment were sampled. Out = outside.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>20°C</th>
<th>10°C</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Polycyclic shoots</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. Polycyclic branches</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>C. Male cones</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D. Female cones</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>E. Subterminal female cones</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F. Polycyclic female cones</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 4.8 Effect of temperature treatments on numbers of trees with particular shoot characteristics, for trees unsampled in 1983. 3 trees of clone 8994 and 2 trees of clone 8996 per treatment were sampled. Out = outside.
branches while the trees grown continually outside had no polycyclic shoots or branches. The number of trees with fully differentiated female cones was greater after the 10°C treatment than any other.

There were some obvious differences between sampled and unsampled trees. No unsampled trees from the 10°C treatment had male cones or subterminal female cones. No sampled trees from the 20°C treatment had subterminal female cones. Thus removal of some buds for analysis may have affected the differentiation of buds left on the trees.

Treatment affected the number of growing points on a tree, but only if buds had been removed from the tree; the removal of buds had a greater effect on number of shoots than treatment. The total number of shoots on sampled trees (approx. 51 per tree after 20°C treatment; 36 per tree after 10°C treatment and 32 per tree after outside treatment) (Fig. 4.9 A) increased relative to those on unsampled trees (approx. 11 per tree after all three treatments). The total number of polycyclic shoots were also affected by sampling (cf. Figs 4.9 B & 4.10 B). There were slightly more shoots on unsampled trees from the 10°C treatment than the 20°C treatment. Sampled trees from the 20°C treatment had more than double the number of polycyclic shoots than those from the 10°C treatment. The number of polycyclic long-shoots increased on sampled shoots from the 20°C treatment (Fig. 4.9 C) compared to unsampled shoots (Fig. 4.10 C). Sampling markedly affected the number of male cone clusters on shoots from the 10°C treatment (cf. Figs 4.9 D & 4.10 D), increasing the number from 0 on unsampled shoots to approx. 7% of all sampled shoots. The number of shoots with female cones was greater on shoots from the 10°C treatment irrespective of sampling (cf. Figs 4.9 E & 4.10 E), although sampling decreased the percentage of shoots with cones from 48% on unsampled trees to 6% on sampled trees. The figures for sampled trees may be low because coning buds were sampled in 1983.

The effect of 20°C treatment was to increase the number of polycyclic branches and male cone clusters and to decrease the number of female cones differentiating. Increased temperatures in conjunction with bud pruning (sampling buds), increased the number of polycyclic shoots and long-shoots and decreased the number of male cone
Figure 4.9 Effect of temperature treatments on numbers of shoots with particular characteristics on trees sampled in 1983. 9 trees of clone 8996 and 4 trees of clone 8996 per tree were measured. Out = outside.

A. Total number of shoots

B. 2 cycles of needles (polydendric)

C. Polycyclic Long-shoots

D. Male cone clusters

E. Female cones
Figure 4.10 Effect of temperature treatments on numbers of shoots with particular shoot characteristics on trees unsampled in 1983. 3 trees of clone 8994 and 2 trees of clone 8996 per treatment were sampled. Out = outside.

A. Total number of shoots

<table>
<thead>
<tr>
<th>Treatment</th>
<th>20°C</th>
<th>10°C</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. 2 cycles of needles (poly cyclic)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>20°C</th>
<th>10°C</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C. Poly cyclic long shoots

<table>
<thead>
<tr>
<th>Treatment</th>
<th>20°C</th>
<th>10°C</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D. Male cone clusters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>20°C</th>
<th>10°C</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E. Female cones

<table>
<thead>
<tr>
<th>Treatment</th>
<th>20°C</th>
<th>10°C</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
clusters relative to shoots given the 10°C treatment.

4.6 Analyses of variance of five variates against the factors collection date, clone and treatment.

Analyses of variance were calculated for bud length, basal diameter, number of cataphylls, needle length and number of differentiating lateral buds separately against day, treatment, clone, and their interactions (Table 4.2). Only day was always a significant factor determining the size or number of the variates. Clone had a significant effect on all except differentiating lateral buds. Treatment only affected number of cataphylls and needle length. None of the interactions had significant effects on any of the variates except needle length where all three factors plus all their interactions were highly significant. Therefore the conclusion is that both needles and buds were increasing in size during the experiment, but only the rate of growth of the needles and the rate of initiation of cataphylls were affected by the treatment applied.

Table 4.2 Variance ratios from analyses of variance of five variates against day, treatment, clone and their interactions

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Bud Length</th>
<th>Bud Diameter</th>
<th>Number of Cataphylls</th>
<th>Needle Length</th>
<th>Number of lateral buds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>82.28 ***</td>
<td>42.90 ***</td>
<td>47.90 ***</td>
<td>578.53 ***</td>
<td>4.06 ***</td>
</tr>
<tr>
<td>Treatment</td>
<td>1.94 n.s.</td>
<td>2.13 n.s.</td>
<td>6.74 **</td>
<td>492.70 ***</td>
<td>1.17 n.s.</td>
</tr>
<tr>
<td>Clone</td>
<td>9.20 **</td>
<td>47.60 ***</td>
<td>23.31 ***</td>
<td>32.17 ***</td>
<td>0.03 n.s.</td>
</tr>
<tr>
<td>Day/treatment</td>
<td>0.64 n.s.</td>
<td>1.16 n.s.</td>
<td>0.90 n.s.</td>
<td>21.04 ***</td>
<td>0.75 n.s.</td>
</tr>
<tr>
<td>Day/clone</td>
<td>0.92 n.s.</td>
<td>1.10 n.s.</td>
<td>0.41 n.s.</td>
<td>3.76 ***</td>
<td>0.49 n.s.</td>
</tr>
<tr>
<td>Treatment/clone</td>
<td>0.04 n.s.</td>
<td>0.22 n.s.</td>
<td>0.13 n.s.</td>
<td>22.86 ***</td>
<td>0.59 n.s.</td>
</tr>
<tr>
<td>Day/treatment/clone</td>
<td>0.46 n.s.</td>
<td>0.47 n.s.</td>
<td>0.14 n.s.</td>
<td>3.78 ***</td>
<td>0.50 n.s.</td>
</tr>
</tbody>
</table>

Because the rate of initiation of cataphylls is a measure of the rate of growth of the apex of a bud, it was chosen as a likely parameter which would provide insight into the relative importance of different environmental variables controlling bud development. Number of cataphylls was also the only variate directly related to bud development which had been affected by treatment. For
these reasons it was chosen for further analysis.

Regression analysis was done to see if the rate of cataphyll initiation was constant with time throughout the period of study or if it varied, especially when sterile try cataphylls were initiated. The variance ratios obtained from analyses of variance of each treatment and clone separately were significant (Table 4.3) although the percentage variance accounted for by fitting the regression lines was greater for the outside treatment than the 10°C treatment and much less for the 20°C treatment. (See Fig. 4.4 for graphs of number of cataphylls against collection date.) Therefore, although initiation would appear to be linear with time, the rate of initiation in the outside was more constant than in the 20°C and 10°C treatments.

Table 4.3 Variance ratios and percentage variance accounted for from analysis of variance of regression of number of cataphylls against time.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variance ratio 8994</th>
<th>Variance ratio 8996</th>
<th>% variance accounted for 8994</th>
<th>% variance accounted for 8996</th>
<th>Number of samples 8994</th>
<th>Number of samples 8996</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 20°C treatment</td>
<td>51.20 ***</td>
<td>21.08 ***</td>
<td>52.2</td>
<td>40.9</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>2. 10°C treatment</td>
<td>65.50 ***</td>
<td>67.71 ***</td>
<td>65.0</td>
<td>68.3</td>
<td>46</td>
<td>31</td>
</tr>
<tr>
<td>3. Outside</td>
<td>107.53 ***</td>
<td>78.21 ***</td>
<td>70.3</td>
<td>71.4</td>
<td>45</td>
<td>31</td>
</tr>
</tbody>
</table>

When a test for parallelism of treatment was carried out, to determine if the growth rate varied in the 3 treatments, the intercept values for the treatment lines were significantly different although the slopes were not significantly different (Table 4.4). The same result was obtained when clones were analysed. No significant interaction between treatment and clone lines was detected. The conclusion is, therefore, that the overall rate of initiation for either clone in the three treatments did not vary significantly. Because of the initial increase in cataphyll initiation rate in the 20°C treatment, a significant difference in intercept value was obtained.
Table 4.4 Variance ratios form analyses of variance of the regression of number of cataphylls with time. Testing for parallelism of treatment and clone lines.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Variance ratio</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>354.86 ***</td>
<td>Slope of overall regression line sig. different from 0</td>
</tr>
<tr>
<td>Treatment</td>
<td>7.49 ***</td>
<td>Intercepts of treatment lines sig. different</td>
</tr>
<tr>
<td>Clone</td>
<td>26.08 ***</td>
<td>Intercepts of clone lines sig. different</td>
</tr>
<tr>
<td>Treatment/clone</td>
<td>0.10 n.s.</td>
<td>Intercepts of treatment/clone lines non-sig. different</td>
</tr>
<tr>
<td>Day/treatment</td>
<td>0.82 n.s.</td>
<td>Slopes of treatment lines non-sig. different</td>
</tr>
<tr>
<td>Day/clone</td>
<td>1.96 n.s.</td>
<td>Slopes of clone lines non-sig. different</td>
</tr>
<tr>
<td>Day/treatment/clone</td>
<td>0.16 n.s.</td>
<td>Slopes of treatment/clone lines non-sig. different</td>
</tr>
</tbody>
</table>

There was no difference in the rate of initiation of sterile cataphylls between the two cycles of fertile cataphylls in polycyclic buds when compared with the initiation of fertile cataphylls.

4.7 Fitting stepwise regression models to the five variates.

The relative importance of temperature in controlling bud development was determined by comparing its effects on the growth of a range of shoot and bud parameters with that of other environmental and structural factors and variables. The parameters modelled were bud length, basal diameter, number of cataphylls, needle length and number of differentiating lateral buds. The models were built using an established stepwise multiple regression technique. (See section 2.7 for method). The factors examined were day, treatment, clone, pre-treatment, position of bud on tree and sex of the shoot the bud came from. The variables included were shoot length, number of short-shoots on shoot, tree height, number of basal lateral shoots and total number of lateral buds. The percentage variance accounted for at each step of the analysis and the final variance ratios were calculated (Table 4.5) and from these a diagram showing the significant interactions between the modelled variates and the factors and variables affecting them was designed.
Table 4.5 Variance ratios (V.R.) from analyses of variance of the regression of up to five factors (position, treatment, day, clone and pretreatment) and six variables (number of lateral buds, tree height, number of needles, shoot length, number of lateral shoots and sex of bud) against needle length, bud basal diameter, number of cataphylls per bud, bud length and number of differentiating lateral buds. The method used to select factors and variables for modelling is given in section 2.7. The order (0) in which factors and variables were selected for the analyses are given.

<table>
<thead>
<tr>
<th>Factor or variable</th>
<th>Bud length</th>
<th>Basal diameter</th>
<th>No. of cataphylls</th>
<th>Needle length</th>
<th>No. of differentiating lateral buds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>6</td>
<td>1.0 n.s.</td>
<td>3</td>
<td>13.0 ***</td>
<td>6</td>
</tr>
<tr>
<td>Treatment</td>
<td>8</td>
<td>0.5 n.s.</td>
<td>7</td>
<td>3.4</td>
<td>1</td>
</tr>
<tr>
<td>Day</td>
<td>1</td>
<td>92.8 ***</td>
<td>1</td>
<td>41.6 ***</td>
<td>1</td>
</tr>
<tr>
<td>Clone</td>
<td>3</td>
<td>39.5 ***</td>
<td>2</td>
<td>84.7 ***</td>
<td>3</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>4</td>
<td>2.7 $</td>
<td>4</td>
<td>3.8 ***</td>
<td>6</td>
</tr>
<tr>
<td>No. lat. buds</td>
<td>7</td>
<td>1.8 n.s.</td>
<td>6</td>
<td>46.9 ***</td>
<td>8</td>
</tr>
<tr>
<td>Tree height</td>
<td>5</td>
<td>8.2 **</td>
<td>6</td>
<td>46.9 ***</td>
<td>8</td>
</tr>
<tr>
<td>No. needles</td>
<td>5</td>
<td>46.9 ***</td>
<td>2</td>
<td>172.8 ***</td>
<td>1</td>
</tr>
<tr>
<td>Shoot length</td>
<td>2</td>
<td>86.2 ***</td>
<td>5</td>
<td>14.7 ***</td>
<td>5</td>
</tr>
<tr>
<td>No. lat. shoots</td>
<td>9</td>
<td>1.7 n.s.</td>
<td>1</td>
<td>1.7 n.s.</td>
<td>2</td>
</tr>
<tr>
<td>Sex</td>
<td>4</td>
<td>2.2 n.s.</td>
<td>3</td>
<td>1.7 n.s.</td>
<td>9</td>
</tr>
</tbody>
</table>
The models of bud length, diameter and number of cataphylls accounted for more than 70% of variation in the size of these parameters (Table 4.5, Fig. 4.11). Day was the most important factor because it accounted for the greatest amount of variation. Clone was second most important, which emphasises the between-clone differences in the values for these parameters. The most important variable was either shoot length or needle number, both of which were measures of shoot size. The size to size (shoot length to bud length and diameter) and number to number (number of needles to number of cataphylls) correlations were higher than the correlations between non-similar types of measurements. As there was a high correlation between shoot length and needle number, once one of these variates was built into the model; fitting the other did not account for very much more variance.

Tree height - an indicator of the overall size of the tree, and position - a measure of the age of the branch and its distance from the top of the tree, were selected for all three statistical models (Table 4.5, Fig. 4.11) although they were not always significant. The lack of significance was related to the type of multiple regression model fitted where, because other variables which were correlated with tree height and shoot position (shoot length and needle number), had already been built into the model, most of the variance which could have been accounted for by tree height and position had already been accounted for.

The pre-treatment applied in 1861 affected bud length and number of cataphylls but not basal diameter (Table 4.5, Fig. 4.11). The application of gibberellin in 1881 had stimulated the production of longer buds and greater numbers of needles (Longman, personal communication) and these quantitative changes in development have had long lasting effects on the parameters affected. Pre-treatment affected only the two bud parameters which were highly correlated with shoot length and needle number (bud length and number of cataphylls). Pre-treatment did not affect the other variates, which are correlated less with shoot length and needle number.

Sex of the shoot was significant only in the model for
Figure 4.11 The significant interactions between modelled variates and the factors and variables affecting them.
Factors and variables fitted in the regression models which did not have significant variance ratios have been excluded from the diagram. Two levels of significance have been recognised: $P > 0.001$ and $P > 0.05 < 0.001$. 

<table>
<thead>
<tr>
<th>Factors affecting model</th>
<th>Variables affecting model</th>
<th>Variables being modelled</th>
<th>Variables being treated as a factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>No. of differentiating lateral buds</td>
<td>Pre-Treatment</td>
<td>Bud length</td>
</tr>
<tr>
<td></td>
<td>No. of cataphyllae</td>
<td>Clone</td>
<td>Day</td>
</tr>
<tr>
<td></td>
<td>Basal diameter</td>
<td>Treatment</td>
<td>Position</td>
</tr>
<tr>
<td></td>
<td>Needle length</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of laterals</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shoot length</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of needles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tree height</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of lateral buds</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
basal diameter (Table 4.5, Fig. 4.11). The results of the field survey indicated that bud diameter was more affected by the sex of the developing bud than other bud parameters. As the sex of the shoot and the sex of the bud are correlated, the diameter of the developing bud would also be likely to be correlated with the sex of the shoot.

The temperature treatment had a significant effect only on number of cataphylls (Table 4.5, Fig. 4.11). This last result was similar to that obtained from the analysis of variance (Table 4.2), although the significance of treatment was reduced after modelling, suggesting that if uniform plant material had been used, the effect of temperature may have been reduced. The length and diameter of the buds appeared to be related more to the size of the shoot on which they were growing and the clone than other factors, and were to some extent predetermined and invariable in the experimental conditions applied. If the treatment had been applied earlier, or for a longer period, it may have had more effect. The number of cataphylls initiated was more responsive to treatment and this may have been related to the change in the sequence of differentiation (see section 4.3) caused by the temperature treatment.

Treatment, day and sex were the three important factors determining mean needle length (Table 4.5, Fig. 4.11), with tree height and shoot length the variables involved. Needles on longer shoots began extending earlier and were slightly longer than on short-shoots while needles on male coning shoots began extending later but were significantly longer than on vegetative shoots by day 42. Field survey results indicated that short-shoot ABs were larger and more fully formed in the autumn on female buds than male. Needle extension may begin later on male coning branches because the ABs were not fully differentiated at the beginning of the season. The significance of the variance ratios obtained from the model (Fig. 4.11) differed from those obtained from analysis of variance (Table 4.2), with clone not significant in determining mean needle length. The clonal variation may now be accounted for by variation in the size of the trees used and their different coning potential. The significance of clone effects on bud
length and number of differentiating lateral buds was also changed after modelling, possibly because clonal variances could now be classified as differences in shoot structure. The multiple regression model for needle length illustrated that the growth of shoot structures on lodgepole pine may be significantly affected by temperature.

The model for differentiating lateral buds was not as good as the other models because only 54.7% of variation was accounted for (Table 4.5). Only needle number was highly significant (p = 0.001). Therefore the size of the shoot the lateral buds were on had more effect on the number of lateral buds which differentiated than any other factor or variable.

After fitting the significant factors and variables in the model for number of cataphylls, the mean adjusted values for number of cataphylls against time for the three treatments were calculated and a graph drawn (Fig. 4.12). This graph shows the effects accounted for by treatment after variances caused by other significant factors and variables were removed. There was a problem with the analysis because some of the values for shoot length and needle number, needed to calculate the adjusted values for days 1 and 15 were missing. The results for these days have been ignored. In treatment 1 (20°C), the number of cataphylls was greater until day 69, when the number of cataphylls on outside trees became greater. The number of cataphylls on buds grown at 10°C was consistently lower than the other treatments.

Correlation coefficients were calculated for all the variables fitted into the multiple regression models plus the five variates modelled. The coefficients were used to form a diagrammatic representation of the major correlations between bud and first year shoot variables (Fig. 4.13). The number and size of bud parts were highly correlated, as were shoot length and needle number. With the exception of the number of differentiating lateral buds and bud length, all the variates which were modelled were correlated with each other at a level greater than r = 0.5. Mean needle length was only significantly correlated with bud size variables. By using the equation which describes the relationship between the sizes of shoot and bud parts it is possible to predict the size a
Figure 4.12 Predicted values from modelling of number of cataphylls plotted against days from start of experiment. The effects of 3 treatments on number of cataphylls is shown, after the values for number of cataphylls were adjusted to remove variances caused by other significant factors and variables.
Diagrammatic representation of the correlations between bud variables and year 1 shoot variables. Correlations with $r > 0.707$, $r > 0.5 < 0.707$ and $r > 0.313 < 0.5$ are shown. Year 1 shoot variables have been categorised into those which are a measure of the available assimilate and those which are competing assimilate sinks.
bud is likely to grow to. Because of the significant difference in growth form between different clones of lodgepole pine, accurate predictions may be restricted to the clones used to obtain the original data.

4.8 Summary of results for experiment 1

Temperature affected the rate of needle extension more than any other parameter measured, with needles growing faster at 20°C than in the other treatments. The initial rate of AB and cataphyll initiation and diameter growth were also faster at 20°C. Bud length and number of differentiating lateral buds were not affected significantly by the temperature treatments. The sequence of development in buds varied in the different treatments. At 20°C, nearly all the buds initiated were polycyclic, at 10°C half the buds were polycyclic and no polycyclic buds were initiated in the outside treatment. Female cone initiation was stimulated by the 10°C treatment. Male cone initiation was markedly affected by 10°C treatment and pruning.

When the effects of tree and shoot parameters, and clone and pre-treatment were analysed, most of the variance in bud length and diameter, number of cataphylls and number of differentiating lateral buds could be accounted for. Either shoot length or needle number was the most important variable affecting the size or number of the modelled bud parameters. The effects of day and clone was also significant. To affect bud growth significantly using temperature, either more uniform material would have to be used or the treatment applied earlier and for a longer time.
Photoperiod affected the timing of bud development, the number of ABs which differentiated on a bud and may also have affected female cone differentiation. Needles were longer and more ABs were initiated on trees grown in LDs. None of the ABs initiated in LDs had differentiated by the end of the experimental period. Both long-shoots and female cones differentiated on buds grown in SDs. Male cones did not differentiate on any of the buds examined. Because of the small size and juvenility of the trees used in this experiment, only a few female cones differentiated. For this reason, the effects of photoperiod were studied, primarily, on vegetative shoot and bud parts.

Growth and development of needles and buds was studied in LDs and SDs from the start of the bud development cycle, to see if photoperiod affected either the rate of growth of needles and buds, or the rate of initiation or sequence of differentiation of ABs during the phase of rapid needle and bud development. If photoperiod was shown to affect cone differentiation, it could then be used as a tool to manipulate coning. Needle, shoot and bud lengths were measured to determine if the time taken to respond to treatment in different tree structures, and the type of response obtained from these structures, was similar. Bud parameters were measured to determine whether the sequence and/or rate of development of buds, and of ABs in different positions on buds, was affected by treatment. For experimental method see section 2.8.2.

Note: On day 43, buds were sampled from the smallest trees included in the experiment. The measured values for shoots and buds on these trees were all significantly lower than for other trees in the experiment. To minimise the effects of variation in tree height, covariance analyses were carried out. The last sample was taken on day 79 instead of day 85 because trees growing in the SD regime were showing signs of stress with needles turning yellow and beginning to rot. Measurements from these trees were also
smaller than expected.

5.1 Shoot parameters.

Terminal shoots had almost finished elongating by the start of the experiment, and elongation was complete before the photoperiodic treatment was applied (Fig. 5.1). Shoot extension ceased as the new bud became visible. Although the final shoot length reached by terminal shoots of clone 8921 was greater than for clone 8981, treatment appeared to have little effect on shoot length. Both the longest and the shortest terminal shoots were found on trees grown in SDs. Final shoot length appeared to be related more to initial shoot length, or at least to shoot length at the time the photoperiodic treatment was applied, than to the treatment itself.

When needle extension was monitored on the terminal shoots of the same unsampled trees throughout the experimental period, the rate of increase in needle length in LDs and SDs was similar until day 36, when extension ceased in SDs (Fig. 5.2). When needle length was measured on sampled trees on each collection date (Fig. 5.3), extension appeared to continue for longer in SDs (until day 57 or 71), and the rate of extension was apparently faster in LDs than in SDs from day 43 to day 71. Thus, the sudden cessation of needle growth on a tree in SDs was obscured by the variation in needle length between trees. There were no obvious special circumstances on day 36 which appeared to have triggered cessation of needle extension in SDs. It is possible that a response to photoperiodic treatment, identified as a cessation of needle extension, cannot occur until needles had reached a minimum length, which is variable depending on environmental conditions. It is also possible that there is a lag time of at least a month before needles respond to SDs by ceasing to elongate.

The rate of growth in length of needles over the whole shoot (ANY) was very similar to the rate of growth of comparable needles within 5 mm of the bud (TIP) (Table 5.1), although needles growing closer to the bud began growing later than other needles. Needles on clone 8021 were larger than needles on clone 8981 at the start of the experiment, but growth rate of needles in the two clones
Figure 5.1  Extension of terminal shoots of clones 8021 and 8981 in SD and LD. Each line represents the growth of a single shoot.
Figure 5.2  Effect of photoperiod on extension of needles on terminal shoots of clones 8021 and 8981 in LDs and SDs. Each value is the mean of ten needles from two shoots.
was very similar (cf. Figs 5.3 A & B). Therefore, in general, the rate of needle extension does not appear to be affected by photoperiodic treatment.

Table 5.1 Length of needles at start of experiment, and growth rate in length calculated by fitting regression lines to measurements made on shoots in 4 positions on clones 8021 and 8981 between days 1 and 71. Length measurements made on needles growing within 5 mm of the tip of the shoot (TIP) have been analysed separately from those made on needles growing in any position on the shoot (ANY). The % variation accounted for by fitting the regression lines is given. Data for both clones have been combined.

<table>
<thead>
<tr>
<th>Shoot position</th>
<th>Needle position</th>
<th>Length at start of experiment (mm)</th>
<th>Growth rate (mm day⁻¹)</th>
<th>% variation accounted for</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ANY</td>
<td>11.50</td>
<td>0.50</td>
<td>55.1</td>
</tr>
<tr>
<td>0</td>
<td>TIP</td>
<td>3.97</td>
<td>0.47</td>
<td>58.0</td>
</tr>
<tr>
<td>1</td>
<td>ANY</td>
<td>4.78</td>
<td>0.39</td>
<td>62.0</td>
</tr>
<tr>
<td>1</td>
<td>TIP</td>
<td>2.09</td>
<td>0.37</td>
<td>52.8</td>
</tr>
<tr>
<td>2</td>
<td>ANY</td>
<td>5.15</td>
<td>0.36</td>
<td>66.3</td>
</tr>
<tr>
<td>2</td>
<td>TIP</td>
<td>2.45</td>
<td>0.35</td>
<td>62.2</td>
</tr>
<tr>
<td>3</td>
<td>ANY</td>
<td>5.13</td>
<td>0.33</td>
<td>67.3</td>
</tr>
<tr>
<td>3</td>
<td>TIP</td>
<td>3.98</td>
<td>0.26</td>
<td>61.8</td>
</tr>
</tbody>
</table>

Needles on leader shoots (position 0, Fig. 2.4) had a considerably faster growth rate than needles growing on shoots in positions 1-3 (Table 5.1). They were also nearly always significantly longer at the start of the experiment, especially when ANY needles were examined. The initial sizes of needles on shoots generally decreased as the position numbers of the shoots increased, and although the rate of growth in length was not markedly affected by position, there was a consistent decline in growth rate with position for comparable needles. Needles began extending earliest on the shoots closest to the top of a tree, and in positions away from the tip of the shoot.

In conclusion, needle extension ceased in SDs by day 36, although inter-tree variation in needle length partially obscured the response when destructive sampling was done. Needle growth in LDs did not slow down before day 71. Photoperiod did not appear to affect the rate of needle extension, but markedly affected final length, with...
Figure 5.3  Effect of photoperiod on extension of needles on clones 8021 (5.3 A) and 8981 (5.3 B) grown in LDs and SDs. Each value is the mean of 20 needles from 2 terminal shoots. Error bars indicate L.S.D. at p<0.05.
needles becoming longer in LD than in SD. All needles on shoots of both clones responded in the same way. SD photoperiods appeared to take at least a month before affecting needle elongation. This would suggest that either needles were not responsive to the treatment until they had reached a minimum length, or the response of needles growing in SDs takes a long time to manifest itself.

5.2 Bud size parameters.

Measurements of undissected length (Fig. 5.4) and diameter (Fig. 5.5) showed that until day 43 there was little difference between treatments, but after day 43, buds growing in SDs were longer and wider and had a higher growth rate than those growing in LDs.

The mean values of length and diameter in each treatment, adjusted to take into account the covariate tree height, (Figs 5.4 B & 5.5 B), showed a more constant increase in length and diameter with time, and the mean values for day 43 were larger than the non-adjusted means. Therefore, variations in tree height affected the sizes of the buds with taller trees having longer buds than smaller trees. To identify more clearly changes in bud development related to photoperiod, covariance analyses were carried out on most of the data. Buds did not respond to photoperiod by varying in length and diameter until more than a month after treatment had started. From days 43-71, buds grew faster and were longer and wider in SD than in LD.

The response to the photoperiodic treatment was more significant on undissected length than undissected diameter, and interactions between photoperiod and clone and photoperiod and day were also more significant for length measurements.

When dissected length (Fig. 5.6 A) and diameter (Fig. 5.6 B) were examined, the effect of treatment on length was only significant when the data were adjusted for covariates, while the effect on diameter was not consistent or significant. These results were confirmed by the covariance analysis (Table 5.2). Therefore, although there is a high correlation between the undissected length and diameter and the dissected length and diameter of a
Figure 5.4 Effect of photoperiod on undissected bud length extension for buds grown in SDs and LDs. Values unadjusted (5.4 A) and adjusted (5.4 B) for the covariate tree height are given. 4 buds per sample. Vertical bars indicate ± S.E.

5.4 A Values not adjusted for covariate

5.4 B Values adjusted for covariate

Days from start of SD or LD treatment

Days from start of SD or LD treatment
Figure 5.5 Effect of photoperiod on undissected bud diameter extension. Values unadjusted (5.5 A) and adjusted (5.5 B) for the covariate tree height are given. 4 buds per sample (from clones 8021 and 8431). Vertical bars indicate ± S.E.
bud, the response to photoperiodic treatment in the different parameters is not the same. The response to treatment within a bud may not be accurately predicted by measuring the rate of growth and development of the undissected bud.

Table 5.2 Results of analyses of covariance for undissected (U) and dissected (D) bud length and diameter; number of Iry cataphylls (Iry cats), ABs and apical sterile Iry cataphylls (ASC); and PR, RRGR and MRD against collection date, clone and photoperiodic treatment (PP) plus all interactions except day/clone/PP which had no significant effect on any of the variables. The effects of treatment were analysed taking into account the different growth cabinets used in the experiment. The covariate used was tree-height as measured at the start of the experiment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PP</th>
<th>Clone</th>
<th>Day</th>
<th>PP/clone</th>
<th>PP/day</th>
<th>Clone/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>U length</td>
<td>6853.0 ** 27.4 ***</td>
<td>76.3 ***</td>
<td>13.3 **</td>
<td>1.7 n.s.</td>
<td>4.4 **</td>
<td></td>
</tr>
<tr>
<td>U diameter</td>
<td>392.7 * 14.8 ***</td>
<td>17.9 ***</td>
<td>6.0 *</td>
<td>2.3 n.s.</td>
<td>2.0 n.s.</td>
<td></td>
</tr>
<tr>
<td>D length</td>
<td>1231.0 * 2.6 n.s.</td>
<td>47.7 ***</td>
<td>2.9 n.s.</td>
<td>1.0 n.s.</td>
<td>3.9 **</td>
<td></td>
</tr>
<tr>
<td>D diameter</td>
<td>130.7 n.s. 1.6 n.s.</td>
<td>30.6 ***</td>
<td>0.6 n.s.</td>
<td>0.7 n.s.</td>
<td>0.8 n.s.</td>
<td></td>
</tr>
<tr>
<td>Iry cats.</td>
<td>0.1 n.s. 6.8 *</td>
<td>21.0 ***</td>
<td>3.4 n.s.</td>
<td>1.8 n.s.</td>
<td>1.1 n.s.</td>
<td></td>
</tr>
<tr>
<td>ABs</td>
<td>&gt;405300 *** 3.5 n.s.</td>
<td>35.1 ***</td>
<td>1.7 n.s.</td>
<td>3.5 *</td>
<td>4.7 **</td>
<td></td>
</tr>
<tr>
<td>ASC</td>
<td>29.6 n.s. 1.4 n.s.</td>
<td>11.2 ***</td>
<td>5.6 *</td>
<td>2.2 n.s.</td>
<td>5.1 *</td>
<td></td>
</tr>
<tr>
<td>PR &amp; RRGR</td>
<td>2972.5 * 4.5 *</td>
<td>1.7 n.s.</td>
<td>0.1 n.s.</td>
<td>1.7 n.s.</td>
<td>1.5 n.s.</td>
<td></td>
</tr>
<tr>
<td>MRD</td>
<td>12.3 n.s. 8.5 **</td>
<td>4.1 **</td>
<td>4.1 n.s.</td>
<td>1.6 n.s.</td>
<td>1.4 n.s.</td>
<td></td>
</tr>
</tbody>
</table>

The difference in the effect of photoperiodic treatments on undissected and dissected bud length and diameter, may be related to a greater response to treatment from Iry cataphylls than other bud structures. If Iry cataphylls responded in the same way as other leaves (needles), they will have stopped extending significantly earlier in SDs than in LDs. This may be the
Figure 5.6 Effect of photoperiod on undissected bud length (5.6 A) and diameter (5.6 B) extension. Mean values have been adjusted for the covariate tree-height.

4 buds per sample (from clones 8021 and 8981).
response which is measured when undissected bud length and diameter are measured, with longer lry cataphylls making longer buds in LDs. Because dissected buds are much smaller, the effects of photoperiod may be much more difficult to measure, and may be obscured by natural variation in the sizes of buds. It is also possible that the developing bud (dissected bud) responds differently to photoperiodic treatment when compared to already differentiated lry cataphylls (undissected bud).

Buds took about one month to respond to photoperiod. After this time growth in undissected and dissected length and undissected diameter was greater in SD than LD. There was no significant effect of treatment on dissected diameter. The initial size of the trees affected bud length and diameter noticeably. In general, buds grew faster in SDs, reached a greater length and diameter by day 71 and contained ABs which were differentiating. Buds grown in LDs had more ABs, none of which were differentiated.

5.3 Number of lry cataphylls and ABs and development in buds.

SDs caused the cessation of AB initiation in buds to occur sooner. On clone 8021, the rate of AB initiation was initially faster in SDs (Fig. 5.7 A), but there was no significant difference in the initiation rates in clone 8981 (Fig. 5.7 E). By days 29 (clone 8021) and 43 (clone 8981), AB initiation had stopped in SDs, while it continued in LDs for a further 4 weeks in each clone. These results were confirmed by an analysis of covariance (Table 5.2). The main effect of photoperiod was for SDs to cause the earlier cessation of AB initiation, while in LDs AB initiation continued throughout most of the experimental period.

The rate of initiation of fertile and sterile lry cataphylls was compared to determine whether the rate of initiation varied between the two types of lry cataphyll, and if this variation could be causing the cessation of AB initiation in the axils of lry cataphylls as sterile lry cataphylls were maintained on a bud. The mean number of lry cataphylls on buds in LDs and SDs (Fig. 5.8 A) was
Figure 5.7  Effect of photoperiod on initiation of fertile 1ry cataphylls on clones 8021 (5.7 A) and clone 8981 (5.7 B). Mean values adjusted for the covariate tree-height. Each value is the mean of 2 samples.
not significantly different until day 79, when initiation in SDs appeared to have stopped. There was a slight difference in the response of the 2 clones (Table 5.2). Only collection date was important in determining the total number of 1ry cataphylls on a bud (Table 5.2). SD photoperiods maintained all newly initiated 1ry cataphylls as sterile cataphylls around the bud apex after day 43 as could be determined from the cessation of AB initiation in SD (Fig. 5.7 A & B), and because in LDs, the number of sterile 1ry cataphylls around the apex of the buds did not vary significantly with time after day 15 (Fig. 5.8 B), while in SDs, the number increased four-fold between days 43 and 71.

There was no significant change in the number of 1ry cataphylls in SDs when compared to LDs between days 43 and 71 (Fig. 5.8 A) and the rate of initiation did not vary significantly (Table 5.2). Therefore, the rate of initiation of sterile 1ry cataphylls in SDs was not significantly different from the rate of initiation of fertile 1ry cataphylls in LDs.

The 1ry cataphyll initiation rate per day on buds growing in LDs was reasonably constant throughout the experiment (Fig. 5.8 A), while initiation rates on buds growing in SDs were fast until day 29, then initiation slowed to about a quarter of the previous rate between days 29 and 43 and remained low. The decrease in initiation rate occurred just before buds growing in SDs began initiating sterile 1ry cataphylls, therefore 1ry cataphylls destined to remain sterile may be initiated at a slower rate than previously initiated 1ry cataphylls which became fertile; the difference is not significant (Table 5.2). The absolute rate of 1ry cataphyll initiation did not seem to affect the outcome of 1ry cataphyll development. There may have been a reduction in the rate of 1ry cataphyll initiation as sterile 1ry cataphylls were maintained, but this (non-significant) change in rate did not occur until sterile 1ry cataphylls were being initiated. SDs induced the cessation of AB initiation, which normally precedes apical dormancy of the bud, without significantly affecting the rate of 1ry cataphyll initiation when compared to LDs. In SDs, the rate of initiation of fertile 1ry cataphylls did appear to be slightly faster than the rate of sterile 1ry
Figure 5.8 Effect of photoperiod on initiation of 1ry cataphylls (5.8 A) and on initiation of sterile 1ry cataphylls around the bud apex (5.8 B). Values have been adjusted for the covariate tree-height. 4 buds per sample (from clones 8021 and 8981).
cataphyll initiation. Growth in bud length and diameter were stimulated in SDs. In LDs, AB initiation continued and bud length and diameter growth rates did not increase.

5.4 Growth of the bud apex.

The size, and rate of growth of apices of terminal buds were measured to identify how photoperiod affected the size and rate of growth of the apical dome and to determine the response time to treatment. The plastochrone ratio (PR), which is a measurement of the rate of radial increase in the size of the apical dome in a plastochrone, was used to determine the effect of treatment on the rate of growth of the apical dome between the initiation of successive primordia. Initially there was a non-significant decrease in the PR (days 1-15) in both treatments (Fig. 5.9 A). From day 15, the PR in SDs increased at a constant rate, while in LDs the PR showed very little significant change with time. The mean values for PR on each collection date did not vary significantly between treatments until day 57. The results of the analysis of covariance of PR (Table 5.2), showed that the effect of treatment was only significant at p<0.05. The general trend was that in LDs the PR showed little significant variation while in SDs the PR increased from day 15. Therefore, the absolute radial growth rate of the apex per plastochrone was increasing in SDs while remaining reasonably constant in LDs.

Variations in bud sizes affected the minimum radial distance (distance from the centre of the last-formed primordium on a bud to the centre of the apical dome of the bud) (MRD) to the extent that no significant treatment effects could be identified (Fig. 5.9 B, Table 5.2). There may have been an increase in MRD during the period when fertile 1ry cataphylls were initiated in both treatments, and a decrease in MRD as sterile 1ry cataphylls were initiated, in SDs. Therefore, the apex of the bud may increase in size as fertile 1ry cataphylls are initiated, irrespective of the treatment applied, and then decrease in size as sterile 1ry cataphylls are initiated.

Buds grown in LDs showed no significant change with
Figure 5.9 Effect of photoperiod on the plastochrone ratio (PR) (5.9 A) and minimum radial distance (MRD) (5.9 B). Values adjusted for the covariate tree-height. 4 buds per sample (from clones 8021 and 8981).
time for either relative radial growth rate (RRGR) per plastochrone or area of a primordium at initiation (API) (Fig. 5.10 A), while there was an increase in RRGR and API per plastochrone in SDs. Therefore, in SDs, the RRGR of the apex per plastochrone and the size of a primordium as it was initiated increased, with the increase clearly statistically significant (p = 0.05) four weeks after the beginning of the SD treatment. Analysis of covariance showed that the effect of treatment on RRGR was significant at p = 0.01.

The RRGR of the apex per day was calculated by fitting straight lines to the data for number of 1ry cataphylls per bud between days 1 and 29 and then between days 29 and 79 (Fig. 5.8 A). (Two lines were fitted because the plastochrone appeared to be increasing in SDs from day 29.) The average length of a plastochrone was then calculated for buds grown in each treatment during both time-periods. The RRGR per day (Fig. 5.10 B), although decreasing with time, did not vary with treatment. This suggests that although the effect of treatment on 1ry cataphyll initiation was non-significant (Table 5.2), the plastochrone was increasing in SDs as can be seen by the reduction in 1ry cataphyll initiation rates (Fig. 5.8 A). The effects of SD photoperiods on the bud apex was to increase the plastochrone, increase the API and reduce the MRD.

The buds which had initiated female cones (only found in the SD treatment), were initiating sterile 1ry cataphylls when sampled. Both buds had small apical domes and the RRGR and API were large. Because only 2 buds had female cones and no buds had male cones, it was impossible to determine if the RRGR, MRD or API varied as cones were initiated. It was also impossible to determine if female cone differentiation had occurred as an indirect effect of SDs on the growth of other bud parts, which in turn had a stimulatory effect on the development of cones. It is possible that the cessation of AE initiation while the RRGR per day was higher than would normally be expected (approx. 0.06/day in SDs on day 29 and 0.04/day in LDs on day 71; Fig. 5.10 B) could have indirectly stimulated cone initiation.

Although the results showed a lot of variation, there was evidence that in LDs growth of the apex was constant.
Figure 5.10 Effect of photoperiod on relative radial growth rate (RRGR) per plastochrone and the area of a primordium at initiation (API) (5.10 A) and RRGR per day (5.10 B). Values adjusted for the covariate tree-height. 4 buds per sample (from clones 8021 & 8981).
while in SDs 1ry cataphyll initiation rate slowed slightly as sterile 1ry cataphylls were initiated. The PR and API increased, while MRD decreased. As more of the apex of the bud was used up in the initiation of sterile 1ry cataphylls, the apical dome decreased to the size seen in dormant buds.

5.5 Length and diameter of stem units

The length and diameter of stem units were measured to determine whether the size or rate of stem unit extension was affected by treatment and if this effect was differential on stem units in different parts of buds. The aim was to discover if the type of growth which resulted from the application of different photoperiods matched the different sequences of growth identified in male, female and vegetative buds sampled in the field study.

Stem unit length decreased as the distance from the base of a bud increased, and this decrease was approximately linear (Fig. 5.11). Therefore the absolute rate of stem unit extension per plastochrone was similar over the whole bud. The rate of extension per plastochrone was, by day 57, significantly higher in SDs than in LDs (Fig. 5.12 A). In LDs, the MSUL growth rate did not vary significantly after day 15, although the rate increased until day 43. The length of stem units close to the apex of buds did not vary with treatment but the length of stem units close to the base of buds were, after day 43, longer in SDs than in LDs (Fig. 5.11). SDs did not have a measurable effect on the length of stem units at initiation but the rate of extension of these stem units was faster than in LDs (Fig. 5.12 A).

Unlike growth in bud length, after day 29, the rate of growth in diameter in different parts of a bud growing in SDs was not always linear (Fig. 5.13). In SDs, the diameter of a bud was generally largest in the sub-basal part of a bud, about 15-40 ABs from the base, with the diameter perhaps decreasing slightly towards the base and for the most distal 50 or so units, decreasing linearly towards the apex. In LDs the diameter of a bud decreased in an approximately linear fashion from base to apex (Fig. 5.13). Although the maximum dissected bud
diameter (Fig. 5.6 B) did not vary significantly with treatment, when two paired plants, one from each treatment, were compared on each collection date (Fig 5.13), after day 27, the terminal buds on plants growing in SDs were wider over their whole length than on plants growing in LDs. By day 57, the diameter over most of the bottom half of a bud was the same, and buds were cylindrical, as opposed to the conical shape of buds growing in LDs (cf Plate 4a & 4b). After day 57, the rate of increase in diameter was the same in all the stem units at the base of the bud with the measurement across each node approximately 3.2 mm. The maximum diameter of the buds grown in SDs and sampled on day 79 was no larger than the diameter of buds on day 71. This was probably because the buds sampled on day 79, were from trees showing signs of stress, and the buds were smaller than may have been expected (Fig. 5.13).

The effect of photoperiod on the diameter growth of buds was firstly for SD to stimulate an increased growth rate per plastochrone compared to LDs; secondly for SD to stimulate growth in diameter preferentially in the sub-basal part of a bud until the diameter was greater than 3.2 mm when growth per plastochrone became constant in this area compared to the top half of the bud where the absolute growth rate was linear per plastochrone and the relative growth rate increased as distance from the apex of the bud decreased, similar to growth over the whole length of buds in LDs; and thirdly for SD to stimulate the production of cylindrical buds in SDs as opposed to conical buds in LDs. Cylindrical buds examined in the field survey were normally either female coning or vegetative, while conical buds were often male coning.

This would suggest that although very few cones differentiated during this experiment, the sequence of bud differentiation seen in SDs was similar to that which occurs when female cones develop and that seen in LDs was similar to what happens when male cones are produced.

5.6 Rates of growth of ABs in different parts of the bud

As male cones are initiated in ABs at the base of a bud and female cones and long-shoots on ABs close to the tip of a bud, treatments which differentially effect the
Figure 5.11 Mean stem unit length (MSUL) in relation to position on the stem from the base (B; unit 0) to the tip (T; unit 70) of the stem for buds of clone 8981 grown in LDs (■) and SDs (●). One terminal bud from each treatment was measured on each day.
Scanning electron micrographs of buds of clone 8981 grown in LDs (4 a) or SDs (4 b).

Side views of buds of clone 8981 grown in LDs (4 a) or SDs (4 b) and sampled on day 79. All 1ry cataphylls and some 2ry cataphylls have been removed to show developing ABs. The bud grown in LDs (4 a) has numerous small ABs and is conical in shape. The bud grown in SDs (4 b) although similar in length (approx 6 mm) and basal diameter (approx 3 mm) has many fewer, larger, and more differentiated ABs; a wider apical diameter; and a cylindrical shape. For more details of the development of buds in LDs and SDs see section 5.3.
Figure 5.12 Effect of photoperiod on mean stem unit length (MSUL) growth rate (5.12 A) and AB growth rate (measured as the rate of growth in length of the outermost 2ry cataphylls surrounding ABs) (5.12 B). Values adjusted for the covariate tree-height. 4 buds per sample.
Figure 5.13 Stem unit diameter in relation to position on the stem from the base (B, unit 0) to the tip (T, unit 70) of the stem, for buds of clone 8981 grown in LDs (■) and SDs (○). One terminal bud from each treatment was measured on each day.
timing or rate of growth and development of ABs in different parts of a bud, may affect the outcome of AB differentiation, by stimulating or inhibiting the formation of male or female cones or branches. The size of ABs in different parts of buds was studied to determine whether photoperiod affected AB size and rate of growth, and if it did, to determine whether the effects were constant in all the ABs on a bud, and if not, whether variations could possibly be affecting the outcome of AB development. The length of the outermost cataphylls surrounding an AB was used to estimate AB size and growth rate, as this was a measurement which could be made easily and accurately, and which correlated highly with the number of cataphylls initiated and the size of the axillary meristem (Section 3.4.2).

After day 28, the lengths of the outermost 2ry cataphylls surrounding ABs grown in SDs were all longer than those surrounding the corresponding ABs on buds grown in LDs, irrespective of the position of the AB on the bud (Fig. 5.14 A & B). The effects of treatment on AB growth per plastochrone (as estimated from measurements of the rate of increase in the length of the outermost 2ry cataphylls surrounding ABs) was initially similar to the effect of treatment on stem unit diameter, with the size of ABs decreasing linearly from base to apex in LDs; sub-basal ABs being larger than basal or apical ABs in SD and the rate of growth of ABs faster in all except the most apical ABs in SDs compared to LDs. After AB initiation ceased in SDs (before day 43), all the ABs on buds in SDs were larger than comparable ABs in LDs (see 5.14 A & B for data relating to basal and apical ABs).

The AB growth rate per plastochrone (measured as the rate of growth in length of the outermost 2ry cataphylls per plastochrone) (Fig 5.12 B) was approximately linear with time in SDs and by day 43 was significantly faster than in LDs. In LDs, the rate of growth in cataphyll length did not vary significantly with time after day 43. Thus photoperiod had a marked effect on the growth of ABs, as in LDs growth in length of the 2ry cataphylls surrounding ABs was constant in all the ABs on a bud over nearly all of the period studied, while in SDs, the growth rate in length was not constant over the whole bud but was faster in the sub-basal ABs and rate of growth increased with
Figure 5.14 Effect of photoperiod on the length of the outermost 2ry cataphylls surrounding basal (5.14 A) and apical (5.14 B) ABs. Vertical bars indicate ± S.E. 4 buds per sample (from clones 8021 and 8981).
time over the whole of the experimental period.

Photoperiod markedly affected the timing of AB differentiation, and also affected female cone differentiation. Male cones did not differentiate in any of the buds examined in this experiment. No ABs differentiated on buds grown in LDs, while both short-shoots and female cones differentiated on buds grown in SDs. By day 71, short-shoots were recognisable on terminal buds of clone 8021, and by day 79 nearly all the ABs on terminal buds had differentiated into short-shoots. On clone 8918 short-shoots did not differentiate until day 79, while female cones were recognisable by day 71. Only the largest two trees of clone 8918 grown in SDs had female cones. On day 79, potential long-shoots could be recognised on both clones grown in SDs. Therefore, in LDs, ABs did not differentiate while in SDs short-shoots, female cones and long-shoots all developed. Differentiation of female cones occurred only on the largest trees used in the experiment. Therefore, although SDs may promote female cone differentiation, other factors must be involved in determining whether coning will occur.

5.7 Summary of results from experiment 2

In SDs needle extension was slower than in LDs and extension in SDs stopped after about six weeks of treatment, while in LDs it continued throughout the experimental period. In general, the rate of growth of buds and the rate of initiation and growth of ABs on buds, continued at an approximately constant rate throughout the experimental period in LDs, with the exception of AB initiation which stopped by day 71. In LDs the size and RRGR of the apex was also constant and no ABs differentiated into shoots or cones. In SDs, however, the apex of the bud stopped initiating fertile cataphylls 29-43 days after treatment was applied, although the rate of cataphyll initiation was not significantly affected. The PR and API increased and the MRD of the apex decreased. SD treatment may have affected the apex first, by stimulating an increase in PR after day 14, but because of the variation in apical sizes between buds, the difference in PR was not significant.
until day 57. By day 43, initiation of ABs had stopped, the rate of growth in stem unit length and diameter was faster than in LDs, and the 2ry cataphylls surrounding ABs were significantly longer when compared to ABs in LDs. ABs on buds growing in SDs had differentiated into female cones and short and long-shoots by the end of the experiment. Therefore, the effect of short photoperiods appeared to be to induce the apex of the bud to become dormant, while the rate of growth of the stem units and the rate of differentiation and growth of ABs was stimulated, with ABs in sub-basal and, when female cones differentiated, in subapical positions, growing fastest. Because only two of the buds growing in SDs had female cones, more than short photoperiods are required before female cones will differentiate. As the trees with female cones were the largest of clone 89B1 used in the SD treatment, coning may have been inhibited because of a lack of 'vigour' in the smaller trees of clone 89B1.
6. EXPERIMENT 3: EFFECTS OF PHOTOPERIOD AND TREATMENT WITH GA 4/7, APPLIED FROM THE MIDDLE OF THE BUD DEVELOPMENT CYCLE, ON BUD DEVELOPMENT AND CONING

6.1 Introduction

Trees grown in LDs and SDs from the middle of the bud development cycle were examined to determine whether bud development was affected by photoperiodic treatments applied after bud development had begun. GA 4/7 was applied to trees in both photoperiods on day 1, to determine whether GA 4/7 affected bud development, and if so, whether the response to GA 4/7 application was mediated by photoperiod. Growth rates in length and diameter were compared with those obtained when photoperiodic treatments were applied from the beginning of the bud development cycle (experiment 2) to determine whether the type and rate of response to photoperiod was independent of application time. The rate of growth and development of bud parts was also examined to identify any effects of treatment which may be correlated with the type and number of bud parts, especially cones, which were initiated on buds.

As there was some evidence that the onset of bud dormancy and initiation of female cones was triggered by SDs, bud growth and development was monitored in LDs before application of LD and SD treatments (day -20 to day 1), to allow the sequence of events which occurred after SDs were applied to be monitored, and the response time of the buds to changes in the photoperiod to be determined. (For experimental details see section 2.8.3.) All the results presented relate to the terminal buds on trees.

6.2 Bud size parameters

Both photoperiod and GA 4/7 affected the rate of growth and final size of buds, with longer, wider buds developing in LDs compared to SDs and in GA 4/7-treated buds compared to untreated buds (Fig. 6.1 A & B). Application of GA 4/7 to buds grown in SDs appeared to countermand the effects of SD treatment alone, so that by
Figure 6.1  Effect of photoperiod and GA 4/7 on bud extension in length (6.1 A) and diameter (6.1 B). Each value is the mean of 4 samples except for day 1 when 8 samples were measured.
the end of the experiment, buds treated with GA 4/7 and
grown in SDs were as long (Fig. 6.1 A) and broad (Fig.
6.1 E) as buds grown in LDs without GA 4/7.

Buds which had been injected with GA 4/7 and grown in
LDs showed significant response to treatment before other
buds (day 35), and were longer and broader than other
buds (Fig. 6.1 A & E). The effect on bud length and
diameter of photoperiod alone, was not significant until
day 56, although the rate of growth in length (Fig. 6.1
A) increased throughout nearly all of the period studied
in control buds in LDs, while the rate of growth began
decreasing within 24 days of application in control buds
in SDs. The effect of SDs appeared to be to reduce the
growth rate of buds, while that of GA 4/7 was to increase
the growth rate of buds. Application of GA 4/7 to buds in
SD appeared to increase growth rates in length to that
seen in LD control buds. Therefore, both photoperiod and
GA 4/7 had significant effects on the size and rate of
growth of buds in length and diameter, although buds may
respond faster to applications of GA 4/7 than to changes
in photoperiod.

As the rate of growth in length and diameter was
greater in buds grown in SDs when photoperiodic treatment
was applied at the start of the bud development cycle
(experiment 2), the timing of application of
photoperiodic treatments may be important in determining
the strength of response which will be obtained.

6.3 Growth of the bud apex

The size, and rate of growth of apices of terminal
buds were measured to determine if, how, and when, both
photoperiod and GA 4/7 affected the size and rate of
growth of the apex of buds. As in experiment 2, the
plastochorone ratio (PR), minimum radial distance (MRD),
relative radial growth rate (RRGR) per plastochrone, area
of a primordium at initiation (API) and size of the bare
area of the apex were calculated from electron
micrographs of bud apices.

The effect of SDs and LDs on PR was significant by day
24 (Fig. 6.2 A), with the PR increasing approximately
linearly in SDs until day 46, while the PR did not
increase significantly in LDs in GA 4/7-treated plants.
Figure 6.2 Effect of photoperiod and GA 4/7 on plastochrone ratio (PR) (6.2 A) and minimum radial distance (MRD) (6.2 B). Each value is the mean of 4 samples except for day 1 when 8 samples were measured.
and only increased significantly in LD control plants on the last collection date. There was no significant effect of GA 4/7 application in either SDs or LDs until the last collection date, when the PR was greater in control buds than in GA 4/7-treated buds, grown in LDs. Thus, the radial expansion of the apex of buds grown in SDs increased per plastochrone, while the effect of LDs on radial expansion of the apex was minimal. Application of GA 4/7 had little effect in SDs or LDs. The response to different photoperiodic treatments, in terms of changes in the PR, was similar to that obtained when LD and SD treatments were applied to buds from the beginning of the bud development cycle (Fig. 5.9 A).

The effect of photoperiod on the MRD (Fig. 6.2 B) was similar to that obtained in experiment 2, with the MRD decreasing in SDs when compared to LDs; this decrease being significant by day 24, when GA 4/7-treated and control buds were compared independently in the different photoperiods. There was no significant decrease with time of the MRD in GA 4/7-treated buds grown in LDs, while the MRD in control buds grown in LDs did decrease slowly with time during the experiment. On certain collection dates (days 35 and 56) there was a significant effect of GA 4/7 application on the MRD of buds grown in LDs. GA 4/7 did not have a significant effect on the MRD on buds grown in SDs. Therefore, in SDs the MRD decreased at an initially faster rate to a shorter length than that seen in LDs, while the effect of GA 4/7, which was only significant in LDs, was to maintain the MRD at a longer length than was measured in buds not treated with GA 4/7. The response to GA 4/7 may be dependent on the buds growing in LD photoperiods.

The RRGR per plastochrone and API (Fig. 6.3 A), were affected by treatment in a similar way to the PR (Fig 6.2 A). An increase in the RRGR and API was stimulated in SDs when compared to LDs, while application with GA 4/7 had little significant effect in either LDs or SDs (Figs. 6.3 A & B).

The size of the bare area of an apex (Fig. 6.3 B) responded in a similar way to treatment as the MRD (Fig 6.2 B). The size of the bare area in SDs with or without GA 4/7 decreased at approximately the same rate as before treatment was applied. In GA 4/7-treated trees grown in
Figure 6.3 Effect of photoperiod and GA 4/7 application on relative radial growth rate (RRGR) per plastochrone and area of a primordium at initiation (API) (6.3A), and bare area of apex (6.3 B). Each value is the mean of 4 samples except for day 1 when 8 samples were measured.

6.3 A
S.E.D. ± 0.53 x 10^-2 (RRGR) (Last 5 collections only)

6.3 B
S.E.D. ± 1.52 x 10^-4
(Last 5 collections)
LDs there was no significant change in the size of the bare area of the apex until day 46, while in general, the apices of control trees decreased in size at a slightly slower rate than apices grown in SDs.

The effect of photoperiod on apical development was observed (and sometimes significant) within 2 weeks of application of treatment. In SDs, the PR, RRGR per plastochrone and API all increased, while the MRD and bare area of the apex decreased. In LDs there was little significant effect of photoperiod on apical parameters until the end of the experiment when the change in each parameter was similar to that seen approximately three weeks earlier in buds grown in SDs. The effects of application of GA 4/7 on apical development were minimal, with only the MRD and bare area of the apex increasing to any significant extent after GA 4/7 was applied.

Because the exact time of cone initiation was not identified with accuracy, it was impossible to determine whether changes in the size and rate of growth of the apex in SDs occurred before or after female cones were initiated. The PR, RRGR per plastochrone and API all increased in SDs before sterile 1ry cataphylls were initiated in increased numbers around the apex of the bud (Fig. 6.3) and before female cones could be recognised on buds (Table 6.1). Therefore, changes in the size and rate of growth of apices of buds, which occurred in response to SDs, which were applied earlier than they would normally occur under natural conditions, may be important in stimulating the initiation of female cones before buds become dormant.

6.4 Number of 1ry cataphylls and ABs and development of bud parts

SDs caused very rapid cessation of AB initiation (Fig. 6.4 A), with buds grown in SDs with GA 4/7 applied, stopping the initiation of ABs to any significant extent within 12 days of application of treatment. Application of GA 4/7 to buds grown in LDs also slowed the rate of initiation of ABs initially, but this reduction appeared temporary, as the rate of AB initiation increased after day 24. In LDs, AB initiation occurred in control buds at the same constant rate as before treatment was applied,
until day 24 when that rate became more variable before slowing by day 46. Possibly, because of the variation in the number of ABs on buds, the response to treatments in terms of numbers of ABs initiated on each day, was not always significant. The variance ratio from an analysis of variance of the experiment from day 1 was only significant for photoperiod. Therefore, although both photoperiod and application of GA 4/7 affected the initiation of ABs, the effect of photoperiod only was significant.

6.4.1 Male and female cone development

The number of cones differentiating in each treatment was compared to determine whether the outcome of AB development had been affected by photoperiodic treatment and/or application of GA 4/7. Cone development had begun by day 12, and by day 46 the outcome of bud development appeared to be determined in all the terminal buds sampled. Varying the photoperiod affected the number of male and female buds differentiating (Table 6.1), with a greater number of male buds differentiating in LDs than SDs and female buds developing only in SDs. Applications of GA 4/7 increased the number of coning buds relative to the control buds growing in the same photoperiod. GA 4/7 application did not appear to control the sex of the cones differentiating on a bud. Sex determination seemed to be under some sort of photoperiodic control, as more male buds differentiated in LDs and female buds in SDs.

When the total number of male cones differentiating per bud in each of the treatments was calculated (Fig. 6.4 B), significantly more cones differentiated on buds in LDs, although a few male cones differentiated in SDs. GA 4/7 application also increased the number of male cones differentiating irrespective of photoperiod, although the effect of GA 4/7 application was much less (non-significant) in SDs than LDs.

There was a significant interaction (p<0.01) between photoperiod and GA 4/7 application when the experiment was analysed using the analysis of variance technique, so GA 4/7 application to buds may affect firstly, the likelihood of buds initiating cones and secondly, the number of cones which differentiate. The sex of the cones
Figure 6.4  Effect of photoperiod and GA 4/7 application on initiation of ABs (6.4 A) and differentiation of male cones per bud (6.4 B). Each value is the mean of 4 samples, except for day 1 when 8 samples were measured.

6.4 A
S.E.D. = ±17.0
(Last 5 collections only)

6.4 B
S.E.D. = ±8.2
(Last 5 collections only)

Days from beginning of LD or SD treatment

Days from beginning of LD or SD treatment
which differentiate appears to be dependent on the photoperiod the plants are growing in. Because few female cones developed, it was impossible to analyse the difference in number of female cones per bud statistically, but female cones developed only on buds grown in SDs (approximately 1.0–1.5 cones per bud depending on collection date).

Table 6.1 Number of male, female and vegetative buds sampled on each collection date from the beginning of the LD or SD treatment. All the buds sampled before day 1 were vegetative.

<table>
<thead>
<tr>
<th>DAYS FROM START OF TREATMENT</th>
<th>SEX OF BUD</th>
<th>LD CONTROL</th>
<th>LD + 6A 4/7</th>
<th>SD CONTROL</th>
<th>SD + 6A 4/7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MALE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VEGETATIVE</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>MALE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VEGETATIVE</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>MALE</td>
<td>2 (37,47)</td>
<td>1 (31)</td>
<td>1 (27)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>VEGETATIVE</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>24</td>
<td>MALE</td>
<td>1 (36)</td>
<td>2 (45,49)</td>
<td>1 (2)</td>
<td>1 (14)</td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>VEGETATIVE</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>35</td>
<td>MALE</td>
<td>0</td>
<td>4 (42,50,68,69)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VEGETATIVE</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>46</td>
<td>MALE</td>
<td>1 (59)</td>
<td>4 (20,40,55,61)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEMALE</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VEGETATIVE</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Figures in brackets are the numbers of male cones differentiating in male buds

In experiment 2, where no GA 4/7 was applied to buds, the number of buds which initiated cones was small, similar to the result obtained in control plants in experiment 3, but the effect of photoperiod in controlling the type of cone which was initiated was
similar. The use of photoperiod and GA 4/7 together may provide a tool for controlling the outcome of cone development in experiments, with photoperiod determining the type of cones initiated, and application of GA 4/7 soon after the bud begins to develop, increasing the number of cones which develop.

6.4.2 Number and rate of initiation of 1ry cataphylls

Variations in the rate of response of buds to photoperiodic treatment were examined by comparing the rate of initiation of sterile and fertile 1ry cataphylls (Fig. 6.5 A). The aim was to ascertain whether the rate of initiation of 1ry cataphylls could be determining the type of 1ry cataphylls which were initiated. AB initiation in buds grown in SDs ceased by day 35, and the apices of buds was entering the dormancy phase, identified by an increase in the number of sterile 1ry cataphylls surrounding the apical domes of buds (Fig. 6.5 A). In LDs, there was little evidence of AB initiation ceasing, as the number of sterile 1ry cataphylls did not increase beyond the approximately 20 new 1ry cataphylls found around the apices of all buds. Therefore, in SDs, fertile 1ry cataphyll initiation ceased soon after application of treatment, but initiation did not cease in LDs.

GA 4/7 did not affect the number of sterile 1ry cataphylls around the apices of buds growing in LDs, but in SDs, more sterile 1ry cataphylls were initiated on GA 4/7-treated buds. The mean number of all 1ry cataphylls on a bud (fertile plus sterile) (Fig. 6.5 B) was affected by both photoperiod and GA 4/7 application, but the response to both photoperiod and application of GA 4/7 was erratic. In general, the rate of initiation of cataphylls was slower in SDs than in LDs and 1ry cataphyll initiation was initially slower in LDs after application of GA 4/7 when compared to control buds but increased after day 24. When the variance ratios from the analysis of variance of number of 1ry cataphylls were calculated, only photoperiod had a significant effect on the total number of 1ry cataphylls on a bud.

The rate of 1ry cataphyll initiation which could be
Figure 6.5 Effect of photoperiod and GA 4/7 on initiation of apical sterile 1ry cataphylls (6.5 A) and initiation of 1ry cataphylls in general (6.5 B). Each value is the mean of 4 samples, except day 1 when 8 samples were measured.
calculated from the mean numbers of 1ry cataphylls on buds (Fig. 6.5) was very variable, because buds of different sizes were sampled on each collection date. It was therefore difficult to determine whether the sterile 1ry cataphylls initiated in SDs were produced at a slower rate than the fertile 1ry cataphylls initiated in LDs. As fertile 1ry cataphyll initiation ceased very shortly after application of SDs, and fewer 1ry cataphylls were initiated in SDs, the results support the hypothesis that sterile 1ry cataphylls were initiated at a slower rate than fertile 1ry cataphylls. In experiment 2, the rate of initiation of sterile 1ry cataphylls did not differ significantly from that of fertile cataphylls, but there was evidence that sterile 1ry cataphylls were initiated at a slightly slower rate.

From these results, it was impossible to determine whether the rate of initiation of 1ry cataphylls was involved in controlling firstly the type of 1ry cataphyll which developed and secondly for fertile 1ry cataphylls, the type of AB which differentiated in the axes of the cataphylls.

6.5 Rates of growth of ABs in different parts of the bud

The rate of growth of ABs has been proposed as one of the factors which determines the outcome of AB development. More basal ABs differentiated into male cones in LDs, and female cones differentiated only from sub-apical ABs on GA 4/7-treated buds in SDs (with the exception of 1 M/F bud which differentiated on a SD control bud). To determine whether photoperiod or GA 4/7 affected the size, and duration and rate of growth of ABs and thus affected the outcome of AB development, the size of ABs at the base and near the apex of buds, measured as an increase in the length of 2ry cataphylls surrounding the most basal and apical ABs on buds (Table 6.2), was calculated.

The length of basal ABs increased faster on GA 4/7-treated and control buds growing in LDs than on comparable buds in SDs. In SDs, apical ABs increased in length faster than apical ABs in LDs and on plants not injected with GA 4/7, the rate of increase in length of 2ry cataphylls around apical ABs was greater than round
basal ABs. Application of GA 4/7 significantly increased the rate of growth of 2ry cataphylls around basal ABs, while it marginally reduced the rate of growth of apical ABs, when compared to control plants grown in the same photoperiod. The rate of growth of 2ry cataphylls around basal ABs on GA 4/7-treated plants grown in SDs was increased to the level seen on control plants grown in LDs. Therefore, the GA 4/7 may be having a stimulatory effect on basal AB growth similar to that of LDs. In general, application of GA 4/7 resulted in faster growth rates of basal ABs, and may have reduced the growth rates of ABs near the apices of buds. In SDs, the rate of growth of apical ABs was faster, while in LDs basal ABs grew faster. Therefore the rate of growth in length and the final length of the outermost 2ry cataphylls surrounding ABs was affected both by the photoperiod in which buds were grown, and by the application of GA 4/7.

Table 6.2 Mean lengths of the 2ry cataphylls surrounding the first (Basal) and last (Apical) formed ABs on buds grown in SDs and LDs ± GA 4/7. Values given are means of 4 buds except day 1 when 8 buds were measured.

<table>
<thead>
<tr>
<th>Days from start of LD/SD treatment</th>
<th>Basal LD - GA 4/7</th>
<th>Apical Basal LD + GA 4/7</th>
<th>Apical SD - GA 4/7</th>
<th>Apical SD + GA 4/7</th>
</tr>
</thead>
<tbody>
<tr>
<td>-27</td>
<td>108</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-14</td>
<td>249</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>365</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>600</td>
<td>101</td>
<td>590</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>860</td>
<td>96</td>
<td>968</td>
<td>92</td>
</tr>
<tr>
<td>24</td>
<td>1040</td>
<td>198</td>
<td>1527</td>
<td>94</td>
</tr>
<tr>
<td>35</td>
<td>753</td>
<td>100</td>
<td>1716</td>
<td>92</td>
</tr>
<tr>
<td>46</td>
<td>1046</td>
<td>204</td>
<td>2010</td>
<td>108</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td></td>
<td>850</td>
<td>710</td>
</tr>
</tbody>
</table>

As the growth of basal ABs (the ABs which differentiate into male cones) was preferentially stimulated compared to apical ABs in LDs and the growth of apical ABs (some of which may differentiate into female cones) was preferentially stimulated in SDs, one way in which photoperiod may be affecting the outcome of sex determination in buds is by affecting the rate of growth of ABs in different parts of buds. As the most noticeable effect of GA 4/7 was to increase the size and...
rate of growth of basal ABs, and as GA 4/7 also increased the number of male cones initiating, it is possible to hypothesise that GA 4/7 increased the rate of growth of ABs beyond a threshold necessary for cone initiation to take place and therefore more cones were initiated than when GA 4/7 was not applied.

The rate of development of ABs in different parts of buds, measured by the rate of initiation of sterile 2ry cataphylls around ABs (Fig. 6.6), also appears to be under photoperiodic control, but the response to treatment was not identical to that for growth in length of the outermost 2ry cataphylls. Buds initiated more 2ry cataphylls around both basal and apical ABs in SDs than in LDs, irrespective of whether GA 4/7 was applied (Fig. 6.6). The response of basal ABs to treatment was less significant in LDs, possibly because fewer 2ry cataphylls were initiated and 2ry cataphyll initiation was completed earlier around male cones when compared to vegetative ABs. Thus, the effects of treatment on initiation of 2ry cataphylls and on the rate of growth of these cataphylls were different.

The rate of initiation of 2ry cataphylls around apical ABs did correlate highly (r=0.91) with the rate of growth of the 2ry cataphylls (cf. Table 6.2 and Fig. 6.6 B); the 2ry cataphylls being initiated faster and growing longer in SDs than LDs. The correlation between number and length of 2ry cataphylls around basal ABs (r=0.60) was much lower than for apical ABs. The 2ry cataphylls around basal ABs grew longer in LDs although fewer 2ry cataphylls were initiated and initiation finished earlier than in SDs. These results suggest that the rate and duration of 2ry cataphyll initiation and growth of outermost 2ry cataphylls may be affected by photoperiodic and GA 4/7 treatments, but the response to the treatments varied depending on the parameter being measured.

When the rates of growth of ABs in experiment 2 (Fig. 5.14) were compared with growth rates measured in experiment 3 (Table 6.2), the response to photoperiod was not the same in both experiments. Although the same response to photoperiod was observed in apical ABs, the basal ABs growing in SDs grew faster when treatment was
Figure 6.6 Effect of photoperiod and GA 4/7 application on initiation of 2ry cataphylls around the basal (6.6 A) and apical (last-formed) (6.6 B) AB on a bud. Each value is the mean of 4 samples, except for day 1 when 8 samples were measured.
applied from the start of the bud development cycle (experiment 2) than when applied from the middle of the bud development cycle, where basal ABs grew faster in LDs (experiment 3). Also, male cones did not differentiate on buds in experiment 2 and they did on control buds in experiment 3. Therefore, the rate of growth of ABs may be significantly affected by the time the treatment was applied. This variation in response to treatment needs to be examined more closely, to determine whether the response is dependent on the clones being used, on the natural coning potential of the ABs or is truly related to the time of application of treatment.

6.6 Length and diameter of stem units

The length and diameter of stem units were measured to determine whether the size or rate of stem unit extension was affected by either photoperiodic treatment or application of GA 4/7; if the treatments affected parts of buds differentially; if the treatments interacted with each other; and if the response to photoperiod was the same when applied in the middle of the bud development cycle as when applied at the start of the development cycle. Any variations in response to treatment could then be compared with the types of buds differentiating, to determine whether differences in the size or rates of growth of stem units could in any way be responsible for variations in the outcome of AB development.

Buds with male cones developing on them had much larger basal stem unit lengths than vegetative buds irrespective of the treatment applied (Fig. 6.7). The effects of photoperiod on the mean length of stem units and stem unit extension rate were not very significant. When comparisons were made between buds grown in LDs and SDs, after day 12, the mean lengths of stem units appeared to be related more to the sex of the bud being examined than to the photoperiod the bud was growing in, irrespective of whether GA 4/7 had been applied (Fig. 6.7). When comparisons were made between buds containing male cones (Fig 6.7 F, G and H), the absolute values for stem unit length varied, but the mean lengths of stem units decreased from the base of the bud (or close to the base of the bud) to the apex in a similar way.
irrespective of the photoperiod the buds were growing in.

Differences in the timing of application of the photoperiodic treatment may have affected the response to treatment. Variations in the rate of growth in length of stem units, observed in buds grown in SDs from the start of the bud development cycle (experiment 2: Fig. 5.11) were not observed when treatment was applied from the middle of the bud development cycle (experiment 3).

Application of GA 4/7 appeared to have a greater effect than photoperiod on mean stem unit length (Fig. 6.7), with, from day 35, the basal stem units on buds treated with GA 4/7 being longer than on untreated buds. Although the lengths of stem units were markedly affected by the types of buds differentiating, it is impossible from this experiment to determine whether either photoperiod or application of GA 4/7 caused any change in the growth in length of stem units, which was causal in determining the type of buds which developed. As more male cones developed on buds treated with GA 4/7, and as these buds had longer basal stem units than untreated buds, the increased number of cones initiated may be related to an increase in the growth in length of basal stem units.

Unlike experiment 2, where buds grown in SDs became wider at the base and cylindrical in shape compared to the conical buds grown in LDs (Fig. 5.13; Plate 4), the stem unit diameters of buds grown in SDs (Fig. 6.8) did not have a discernibly different pattern of growth from buds grown in LDs. GA 4/7 did not appear to have significantly affected the diameter of stem units in any position on buds. If there was any effect of GA 4/7 application, it was to slightly increase the diameter of stem units towards the base of buds sampled on days 35 and 46 (cf. Figs 6.8 E & F and G & H). Like the results for stem unit length, variations in diameters of stem units were most marked between male coning and vegetative buds. The differences in diameter were reasonably constant between male and vegetative buds over most of the length of the buds' sampled (cf. Fig. 6.8 C & D). The length and diameters of stem units appear to be correlated as buds with larger stem unit diameters also tended to have longer stem unit lengths. None of the buds represented in Fig. 5.8 were cylindrical in shape,
Figure 6.7  Mean stem unit length (MSUL) in relation to position on the stem from the base (position 0) to the tip of the stem for buds of clone 8996 grown in LDs and SDs ± GA 4/7. One terminal bud from each treatment was measured on each day. (m=male, f=female, v=vegetative)
possibly because so many of the buds sampled after day 24 had male cones differentiating on them. Thus, although buds grown in SDs in experiment 2 were ultimately cylindrical while those grown in LDs were conical, the sex of a developing bud must be more important in determining the shape of the bud than the photoperiod the bud is growing in and differences in bud shape may only be obtained when the photoperiodic treatment is applied earlier in the bud development cycle or when buds with the same type of cones or no cones are compared.

6.7 Summary of results from experiment 3

Both photoperiod and application of GA 4/7 affected the size and rate of growth of buds and of bud parts. Photoperiod, when applied from the middle of the bud development cycle, affected the rate and duration of bud development; the final size of the bud; the number, final size and stage of development of bud parts and the type of cones differentiating. Application of GA 4/7 appeared to increase the length and diameter of buds in general and of basal ABs on buds in particular. The number of male cones and to a lesser (non-significant) extent female cones differentiating was also increased by application of GA 4/7.

There was some evidence that the initiation of male and female cones may be mediated by photoperiod, with LDs stimulating preferential growth of basal ABs on buds and also stimulating more male cones to differentiate from these ABs while in SDs apical ABs grew larger and more female cones developed. The length of basal ABs are enhanced still further, irrespective of photoperiod, when GA 4/7 was applied to trees, and coning was also enhanced by application of GA 4/7. Although many more male cones developed on buds grown in LDs and treated with GA 4/7, buds grown in LDs without GA 4/7 also had male cones. A few buds grown in SD contained male cones, but more female buds differentiated in SDs. GA 4/7 appeared to have little effect on the type of part which differentiated.

The apices of buds responded to photoperiod in a similar way to that noted in experiment 2, with LDs having little effect on apical parameters while SDs
Figure 6.8  Mean stem unit diameter (MSUD) in relation to position on the stem from the base (position 0) to the tip of the stem for buds of clone 8996 grown in LDs and SDs ± GA 4/7. One terminal bud from each treatment was measured on each day. (m=male; f=female; v=vegetative)
caused an increase in the PR, RRGR per plastochrone, and API, and a decrease in the MRD and bare area of the apex. GA 4/7 affected apical growth by increasing the MRD of the apex, and possibly by increasing the time taken by apices growing in SDs to enter dormancy. GA 4/7 did not appear to have any effect on the outcome of primordial initiation at the apex of the bud.

Although the response to photoperiod was often similar to that observed when treatment was applied from the start of the bud development cycle (experiment 2), there were differences in the strength of response. Possibly because both male and female cones differentiated in this experiment, the significant shape changes observed in experiment 2 (cylindrical buds developing in SDs and conical buds developing in LDs) were not as obvious in experiment 3.

The time taken for buds to respond to treatment varied from 12 days to 35 days, depending on the bud parameter being measured and the treatment applied. The response to treatment was observed first as a change in the size and rate of growth of the apex when SDs were applied. As the PR, RRGR per plastochrone and API increased, AB initiation ceased in SDs. Although the sequence of changes which occurred as the apices of buds initiated female cones and then become dormant could be clearly identified, the timing of events was not precise enough for causal events to be identified.

The results of this experiment suggest that to experimentally control bud development, especially the differentiation of cones of a particular sex, both GA 4/7 and LDs (male cones) or SDs (female cones) should be applied during the early stages of development of buds, either before or during the phase of rapid AB initiation.
7. DISCUSSION

7.1 Introduction

Three hypotheses have been proposed for the control of bud development and coning in conifers. When expressed in terms of bud development in lodgepole pine, they are:

(i) Variations in size, shape, position or competitive status of ABs lead to differences in the meristematic activity and thus differential morpogenesis (Romberger and Gregory 1974);

(ii) Differentiation of some types of axillary structures is restricted because the meristematic tissues do not receive the necessary substrates for growth (Sachs & Hackett 1977). As the amount of available substrate increases, the following order of AB differentiation (based on Tompsett 1977) may be proposed: weak vegetative; male; intermediate vegetative; female; strong vegetative;

(iii) GA 4/7 has a direct morphogenetic effect on cone development, but this effect is not apparent until vegetative growth is restricted by the environment or other variables because conifers utilise endogenous GAs preferentially for vegetative bud development (Pharis 1976).

These hypotheses, along with other possible explanations, will be compared with the results obtained in this study in an attempt to determine how bud development and coning is modified and controlled in lodgepole pine.

7.2 Timing of bud development and coning- the relative importance of external factors

There is a great deal of variation in the timing of bud development and coning in lodgepole pine growing in Scotland (Table 3.1). This variation may be related to the sex of the bud, the clone and/or the year sampled. The provenances from which trees were originally
collected may also affect the timing of cone initiation and differentiation, with trees from southerly provenances producing cones later than trees from more northerly latitudes.

There is greater variation in the timing of male cone differentiation, possibly because as more male cones than female differentiate per bud, more time is needed for cone differentiation to be completed.

Seasonal variations in the timing of cone differentiation appear to be related to the conditions affecting the early growth and development of the bud. However, the complex interrelationships between environmental factors like temperature, water availability and light intensity make it difficult from field survey results to identify the specific cause or causes of early cone differentiation in any one year. Any attempt to correlate the timing of cone differentiation with weather data may be of marginal benefit because the effects of even a single variable on tree physiology are only poorly understood (Owens & Blake 1985), and because the effects appear to vary depending on the structure of the tree itself.

Little information exists regarding the effects of temperature on the timing of bud development and coning. Neither the field survey nor experiment 1 designed to identify the effects of varying temperatures on bud development and coning, provided clear evidence of a link between temperature and the timing of cone differentiation, although 20°C temperatures did stimulate the formation of sterile 1ry cataphylls followed by temporary apical dormancy, faster, and in more buds than 10°C temperature regimes. If growth rates can limit the types of buds developing on trees, then increases in temperature which may cause increases in these growth rates above a threshold, earlier in the season, may be important to the the timing of bud development and coning.

Results from the field survey suggested that either photoperiod played only a minor role in determining the timing of cone differentiation and the type of cone developing, since male and female cones both differentiated at the same time, in decreasing daylengths (Section 3.4.1), or, different parts of a tree respond
differently to photoperiodic stimuli as male and female cones on the same clone differentiated in the same photoperiod. When the effects of LDs and SDs on the timing of cone development were studied in controlled environments (experiments 2 & 3), the results were inconclusive. Although cone initiation was not consistently stimulated in either LDs or SDs, significant variations in the sequence of development of buds, including the cessation of AB initiation, were observed within a month of applying SDs, while in LDs, AB initiation continued. It therefore appears likely that the timing of bud development and cone initiation is affected by photoperiod, but the response may be modified by other factors like growth rate to allow different parts of a tree to respond independently to the same photoperiod. SDs may promote female cone development, possibly at the same time as apical dormancy is triggered. If bud growth rates are slow, dormancy may occur without female cone differentiation taking place.

The size and/or position of shoots appears to affect the time at which buds begin to develop in a season, with buds on first or second order branches towards the outside or top of a tree beginning to differentiate before buds on lower order branches nearer the base of the tree. The timing of cone initiation may also be affected by the size and/or position of shoots, although there was no clear evidence of this from the field survey results. Variations in timing may be related to the micro-environment around large branches preferentially stimulating the development of buds, or to the competitive status of these branches being more favourable for early bud development than smaller branches as there may be more stored assimilate available for growth.

There is some evidence that buds which develop earlier in a season may be more likely to initiate female cones (clones 8978, 8990 and 8989; Table 3.1). The mechanism whereby early bud differentiation may increase the likelihood of female cone initiation has not been elucidated, but various hypotheses may be proposed.

One possible explanation is that buds must reach a 'critical size', or initiate a critical number of
primordia, before female cone initiation can occur. Horridge & Cockshull (1979) proposed that in Chrysanthemum, flower bud initiation and leaf initiation were closely linked so that under controlled conditions flower bud initiation constantly occurred after the same number of leaves had been initiated. This result is similar to that obtained in the field survey where female cones did not differentiate on a bud until at least 80 short-shoots were produced (Fig. 3.24). Cockshull (1976) suggested that the response to LD in Chrysanthemum was similar to the 'autonomous determination' of flower initiation in Pisum sativum (Haupt 1969), and occurred as response to physiological ageing of the apical meristem, which was related to the activity of the meristem in initiating leaf primordia. Horridge & Cockshull (1979) showed that the transition to reproductive development in Chrysanthemum was associated with a particular size of apex.

From the results obtained in this study, it is impossible to determine whether critical size or ageing of the apex is the primary cause of female cone differentiation in lodgepole pine. It seems unlikely that critical size or ageing of the bud as a whole is related to female cone differentiation, as male buds often develop for longer than female, have faster growth rates, especially over the latter part of the growing season and may end up longer and broader at the end of the season. If, however, trees are receptive to female cone initiation stimuli for only a short period, as indicated by the results of the field survey where female coning occurred mainly in July and August (Table 3.1), any factors which stimulate early growth of a bud, may also increase the likelihood that the bud apex will reach the critical size, or the stage in the ageing process necessary for female cone initiation, when conditions for cone initiation are favourable, whereupon female cones may form. Buds which begin to grow later may reach the necessary apical size or stage in the ageing process, but too late in the season, when growing conditions are less favourable for coning. This may account for the Newport and North Bends, Oregon trees being so exclusively male coning in Scotland, for although they initiated many primordia and had large buds at the end of the growing
(clones 8979 & 8987; Figs 3.13-3.17), they did not begin development until up to a month after the clones which initiated female cones.

The role of photoperiod in determining the timing of male cone differentiation seems unclear especially as field survey results show that some male cones developed at the same time as female cones on the same tree. However, if the ABs which differentiate into male cones are committed to a particular sequence of development from their initiation, as opposed to some later stage in their development like the point at which ABs become morphologically distinct from each other, male cone initiation may be restricted to daylengths above a critical value. Male and female cone initiation might then be interpreted as occurring at different times on the same tree with male cones normally initiated before female cones, although differentiation of cones would still occur at the same time.

It is possible that bud size and growth rates are involved, along with photoperiod in determining the timing of male cone initiation. In the early part of the season, when male cone lry cataphylls are initiated, male buds are normally narrower than female buds. The buds also tend to have smaller apical domes. If an apical dome size or growth rate, smaller than a critical value, is essential for male cone initiation, coning may be restricted to the early part of the bud development season.

If an AB is committed to a specific sequence of differentiation from the initiation of the lry cataphyll which subtends it, this may also mean that male cones are initiated in increasing daylengths and female cones in decreasing daylengths, as proposed by Giertych (1967). Because of the difficulty in determining when cone initiation as opposed to differentiation takes place, no details are available of when in the bud development cycle cone initiation starts. To allow the factors controlling the timing of cone initiation to be understood more clearly, it is essential to determine when an AB is committed to a particular sequence of development.
7.3 Morphological changes in buds leading to coning

Cones do not appear to be initiated until buds have reached a minimum, but variable, size and stage of development. In female buds, cones were initiated after about 120 ABs had formed, and none were found with less than 80 ABs. Differentiation of female cones may be recognised by the increase in size of the apex of the AB from 100 μm to 200 μm. This may precede the initiation of the (up to 25) extra 2ry cataphylls which will ultimately surround the female cone, but is normally concurrent with an increase in length of the outermost 2ry cataphylls so that the cone apex is covered (Section 3.1). Male cone development did not appear to be restricted by the number of ABs initiated. Instead, cones did not differentiate until the ABs which would form the cones were at least 0.5 mm in length and six 2ry cataphylls had been initiated.

Until cones begin to form, no obvious morphological differences can be identified between potentially male, female or vegetative buds. Cone differentiation can be recognised accurately only from the point where 2ry cataphylls surrounding potential cones increase in size faster than similar 2ry cataphylls on potentially vegetative buds (Section 3.1). It may be possible to pinpoint the timing of cone initiation earlier by identifying ultrastructural or biochemical changes in buds which may occur before the already described morphological changes. These changes may include differences in the sizes of cells in potentially coning ABs, or to different amounts of assimilate or growth regulator/inhibitor in the proximity of ABs which appear to have different development potentials. Evidence of this kind would allow firstly, the timing of developmental changes to be pin pointed more accurately and secondly, may provide evidence to support or refute hypotheses of the causes of particular kinds of AB differentiation. It may also be possible to determine whether ultrastructural or biochemical changes occur in the apex before ABs are initiated, and thus to determine whether an AB is committed to a particular sequence of
differentiation from the initiation of the 1ry cataphyll which will subtend it, or from a later stage in its development. The difference in the timing of initiation and differentiation of cones may then be measured, making easier the identification of factors which affect the growth and development of buds on lodgepole pine.

7.4 Effects of environment and experimental treatments on bud development and coning

Variations in the sequence and rates of differentiation and growth of lodgepole pine buds in different seasons suggests that environmental variables are important in determining the outcome of bud development and cone formation. The outcomes of the controlled environment experiments support this assertion, as bud growth and differentiation were affected both by temperature, with more female cones initiated at 10°C compared to 20°C and male cones at 20°C compared to 10°C on unsampled trees, and also by photoperiod, differentiation of male cones apparently being promoted in LDs and female cones in SDs.

7.4.1 Temperature

In mature trees, the effects of temperature on developmental morphology of meristems or on the physiology of essential metabolic processes like carbohydrate metabolism, photosynthesis, water and nutrient uptake, transpiration and growth substances, are not well understood. If buds are limited in their growth potential by their growth rate, then increasing temperatures may allow buds to reach the threshold levels of growth rate necessary for particular sequences of development to be followed.

Although much useful information may be obtained, little work has been done to identify the effects of temperature on physiological processes leading to bud differentiation and coning. Matthews (1963) proposed that the temperature necessary for floral bud differentiation was higher than that needed for vegetative bud development. This response to temperature has been noted for Fagus (Matthews 1955), Betula (Longman 1976) and Pinus (Daubenmire 1960), although in some flowering
citrus trees, high summer temperatures are inhibitory to floral initiation (Jackson & Sweet 1972).

In experiment 1, higher temperatures increased the bud diameter growth rate and lry cataphyll initiation rate, while growth rate in length was unaffected by treatment. Therefore, if temperature is affecting bud development by increasing the rate of growth of bud parameters, the effect is not general to all parameters, or the method of recording results was not sensitive enough to identify all the effects of treatment.

Apices of buds of lodgepole pine grown at 20°C initiated sterile lry cataphylls earlier, and in greater numbers, than buds grown at 10°C, and many buds grown at 20°C became temporarily dormant (Section 4.3), a developmental stage which is linked with female cone initiation (Owens et al 1980). Therefore, higher temperatures did appear to have triggered part of the sequence of development which leads to female cone initiation. However, the environmental trigger normally associated with female cone differentiation did not cause many female cones to form, and female cone differentiation was preferentially stimulated at 10°C rather than 20°C. This may be because other conditions necessary for successful female cone development, like more than 80 short-shoots differentiating on buds to provide the photosynthetic for continued female cone development, had not been fulfilled. Therefore, although there is evidence that as the temperature increases, more cones may be initiated (Fober 1976, Philipson 1983), other factors must be able to modify bud development and affect the numbers of cones initiated even if the bud has begun to follow a sequence of development associated with female cone differentiation. It is possible that female cone initiation was triggered at the same time as temporary dormancy, but growing conditions supplied after initiation were not suitable for female cone differentiation. If the high temperature treatment had been applied for longer, or at a different time, the coning response may have been different because either more ABs may have been initiated on buds, or potential cones which did not differentiate because of unsuitable growing conditions might have differentiated fully.

Male cone development appears to be fairly sensitive
to changes in temperature, as unsampled trees growing at 10°C initiated no male cones while male cones developed on unsampled trees grown at 20°C and outside (Fig. 4.8). If the outcome of AB differentiation is related to growth rates of buds with higher rates promoting female cone differentiation at the expense of male cone differentiation (Tompsett 1977), the expected outcome would be for male cones to differentiate at 10°C rather than 20°C, as higher temperatures may be expected to increase the growth rate beyond that associated with male cone differentiation. Unfortunately, as more female cones were initiated at 10°C, the results of the temperature experiment do not support this hypothesis.

There may be a survival advantage in male cone development being temperature dependent. If male cones differentiate only in good growing conditions where female cones are also likely to differentiate, little energy will be wasted producing unused pollen.

The effects of temperature on rates of growth and differentiation of buds were far less important than intrinsic factors like size of the shoot the bud was growing on and height of the tree (Section 4.7). This relative lack of response to temperature highlights the intrinsic nature of the control of bud development in lodgepole pine. It also highlights the need, when designing experiments to study the effects on bud development of environmental variables or applied factors, to monitor both the immediate responses to treatment, and those which will appear over a longer period and have a continued effect. The short-term results may allow both the receptive area and the rate of response to be determined. The longer-term results may provide evidence of how the tree continues to respond to treatment even after the treatment has been withdrawn. For example, needle extension rates were faster and needles grew longer with larger photosynthetic areas (the needles were of similar widths) at 20°C than at 10°C. These larger needles, when they become exporters of assimilate, may be able to provide more substrate to their developing buds. If some developmental pathways are assimilate limited, increasing the amount of available assimilate may affect the sequence of development in buds. Therefore, environmental factors
which have affected the growth of trees in the past may continue to have an effect on tree growth and development.

The effects of temperature on bud development and coning were mediated by the tree to such an extent that treatment did not have much immediate effect. This may be a survival mechanism, whereby changes in environmental conditions alone cannot determine bud development and coning, but can have a modifying effect. The size and rate of growth of buds from the different treatments were similar on the sampling dates (cf. Figs 4.2-4.4 & Section 4.5), but by the following year the effect of varying temperatures on bud differentiation was more marked (Section 4.5).

Wareing (1953) noted that when terminal buds of Pinus sylvestris had been damaged by insects, more seed cones differentiated on the damaged branches the following season. When a debudding experiment was subsequently carried out, pollen cone initiation was enhanced. In the present study, removal of buds when sampling noticeably increased the number of male cone clusters developing (Section 4.5). Removal of buds may have led to an increased allocation of photosynthetic to the buds which were left. If male cone differentiation occurs only in buds which have more than a threshold amount of assimilate available to them, bud removal may have increased the level of assimilate available to remaining buds above that necessary for male cones to develop. This hypothesis could also be used to explain the increased numbers of female cones noted by Wareing (1953). Although this explanation is speculative, it would be useful to carry out experiments to test the effects of bud removal on bud development in a range of conditions. Other possible explanations for the effects of pruning include the concentration of promoter of male cone differentiation being increased above a threshold level necessary for coning to occur or the micro-environment of the remaining shoots and buds on a tree being changed by, for example, increased light being available to shoots which were likely to initiate male cones increasing the vigour of the bud to that associated with male cone development, or increased temperatures in the bud’s...
vicinity stimulating an increased growth rate which stimulated male cone formation. In conifers, increases in cone production as a response to thinning are well documented (Florence & McWilliam 1956, Bilan 1960). Indirect evidence is available which suggests that branches exposed to high light intensity tend to flower more than shaded branches (Owens & Blake 1985). The effects of bud removal on the light quality or temperature around individual branches on trees is not known. As no records of the changes in temperature, or light intensity or quality were made in the present study on trees growing outside, any hypothesis of the stimulatory effects of sampling on male cone initiation is purely speculative. Follow-up experiments are needed to determine precisely why bud development is affected by the removal of some of the buds on trees.

7.4.2 Photoperiod

Photoperiod, unlike temperature, does appear to affect directly the rate and sequence of development in buds, although the time at which buds are receptive to photoperiodic treatments may vary depending on the stage of development reached by the bud, and possibly also the growing conditions and the clones involved.

The effects of photoperiod on needle and bud growth and development were significant with both needles and buds growing for longer in LDs than SDs (Sections 5.1, 5.2 & 6.2). Rates of growth of bud parts were sometimes affected by photoperiod with stem unit length increasing faster in SDs compared to LDs (Section 5.7). The photoperiodic response also varied to a limited extent, depending on when treatment was applied.

When buds were grown in LDs, initiation and growth of buds as a whole, and of bud parts, continued at a reasonably constant rate throughout the experimental period. In SDs, the rate and sequence of development changed markedly when compared to buds grown in LDs. These changes in SD included:

(i) Fertile 1ry cataphyll initiation stopping within a month of treatment being applied;

(ii) Fewer ABs being initiated per bud;

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(iii) Increases in the PR and API and decreases in the MRD of bud apices;

(iv) Differences in the rate of growth of ABs in different areas of buds, with ABs in sub-basal and sub-apical positions growing and differentiating faster than ABs in other areas;

(v) Buds became cylindrical in shape rather than remaining conical.

The major difference in response between the two photoperiodic experiments was that when treatment was applied from the middle of the bud development cycle, both male and female cones were initiated but when treatment was applied from the start of the bud development cycle, no male cones differentiated. It is unclear whether the variation in the type and number of cones initiated was truly related to the time of application of treatment, or to either the adjunct treatment (application of GA 4/7) or to the clones used in the second photoperiodic experiment being more receptive to cone initiation stimuli.

The results of the photoperiod experiments provide evidence that daylength may be, at least indirectly, involved in cone differentiation in lodgepole pine (Longman 1982). Although buds grown in SDs did initiate male cones, significantly more buds initiated male cones in LDs, and, in general, more cones were initiated per bud. It is possible that the male cones which developed on buds grown in SDs were initiated before the photoperiodic treatment was applied. If this were the case, male cone initiation may occur only when daylength is greater than a critical (unmeasured) number of hours. No buds grown in LDs initiated female cones. Therefore, in normal circumstances, photoperiods below a critical (unmeasured) value may be the trigger which causes the apices of buds to become dormant for the winter, with simultaneously, if buds are vigorous, the development of female cones. It is less clear from the photoperiod experiments, how much direct effect photoperiod has in determining the rates of growth in different parts of
buds. Photoperiod may act solely as a trigger for apical dormancy, with other factors, both environmental and intrinsic, determining the fate of individual ABs in buds. As buds growing in LDs were able to enter dormancy, and as a few buds growing in SDs were able to restart initiating fertile lry cataphylls after becoming temporarily dormant, SD photoperiods are not the only criterion controlling apical dormancy in lodgepole pine.

By determining, for any clone being studied, the critical photoperiod at which the apex of a bud enters dormancy, a method could be developed to predict the timing of female cone initiation in lodgepole pine. This information may then be used in controlled conditions to produce the growing conditions necessary for stimulation of female cones. The prediction of the timing of female cone initiation might be very accurate as initiation is nearly always concurrent with apical dormancy. However, as apical dormancy is not dependent on female cone initiation, only some of the buds entering the dormancy phase would produce female cones. Also, the results from the field survey indicated that the timing of female cone differentiation was related more to the size of a bud and its growth rate. Therefore, the time at which the apex of a bud responds to SDs by becoming dormant may vary depending on environment. If the critical daylength can vary, any experiments designed either to measure it or to stimulate female cone development would require strictly controlled growing conditions.

The results of the photoperiod experiments provide some evidence for the role of photoperiod in controlling bud development and coning. By triggering apical dormancy and effecting the subsequent release of ABs from apical dominance, which may allow them to compete more effectively for available assimilate, a decreasing photoperiod may be essential for female cone differentiation.

There is no evidence that the photoperiodic response is phytochrome based, with needles the receptor sites for the stimulus. Far-red light breaks have been used in conifer flowering experiments (Durzan et al 1975), with female cone initiation being inhibited in white spruce by red light treatment during the night under field
conditions. If it is the needles which perceive the photoperiodic stimulus, a means of transferring the stimulus to the apex is necessary. Therefore, a growth regulator may also be involved. Because the apex of the bud is surrounded by scale leaves, the photoperiodic stimulus may be perceived by these dry cataphylls rather than needles, in which case the stimulus would not need to be transferred very far. If a chemical messenger is involved in relaying the photoperiodic stimulus, a good chemical contender for the role may be GA 4/7. It may affect apical growth and development by redirecting assimilate to axillary meristems (Ross et al 1984). As this is exactly what appears to happen when plants respond to SDs, this may be the role of the growth regulator in bud development and coning. Response to the photoperiodic treatment was observed first as changes in the growth rate at the apex of the bud. Any attempt to determine the mode of action of photoperiod should be aimed towards determining which part of the tree is the receptor site for the photoperiodic stimulus, how the stimulus is carried to the apex of the bud and what the sequence of events in the apex of the bud are. These studies need to be carried out at the anatomical and biochemical level to allow the timing of events to be pinpointed with accuracy.

It is not clear why the response of the apex to photoperiod should vary depending on when the stimulus is applied. It was suggested that the effects of photoperiod may be mediated depending on the size and growth rates of buds, which vary between the beginning and in the middle of the bud development cycle. It is possible that the effects of photoperiod on cone initiation differ from those on cone differentiation and it is the outcome of these different effects which are measured when photoperiodic treatments are applied at different times. The application of short photoperiods at the beginning of a bud developmental cycle was not an event which would occur under normal circumstances, so the result obtained may have little bearing on the normal response to bud development and coning in lodgepole pine.

7.4.3 Application of GA 4/7

GA 4/7 application to trees caused an increase in the
rate of growth of the buds and stimulated the production of more bud parts. GA 4/7 did not appear to affect the overall sequence of differentiation in buds in the same way as photoperiod, as male, female and vegetative buds differentiated on trees injected with GA 4/7, and the growth regulator had only a limited effect on apical dormancy. The role of GA 4/7 in LDs appeared to be to enhance the response to treatment which had been already determined by the photoperiod. Buds grew longer. The MRD and bare area of the bud apex were larger. Basal ABs grew faster and more male cones differentiated. In SDs, the response to application of GA 4/7 was often for buds to grow in a similar fashion to buds grown in LDs without GA 4/7. For example, the rate of growth in bud length and final bud diameter were very similar in buds grown in LDs without GA 4/7 and buds grown in SDs with GA 4/7. GA 4/7 may also have increased the time taken by buds in SDs to enter dormancy and so, in the last few collection dates, have allowed more male cones to develop on buds grown in SDs. The growth regulator may be enabling buds growing in SDs to compensate for the reduction in daylength.

GA 4/7 may play an important role in any attempt to manipulate the outcome of bud development and coning as only when it was applied as an adjunct treatment with photoperiod did cones develop in any great quantities. GA 4/7 does not, at the concentrations used in this study, determine the types of cones which will be initiated on buds as it appeared to stimulate female cone development on large buds in SDs and male cone development in LDs.

GA 4/7 may be affecting the number of cones initiating on a bud by increasing the amount of assimilate available to developing ABs. Sachs and Hackett (1977) suggested that meristem development may be controlled by the amount of assimilate available to them. If this is the case in lodgepole pine, GA 4/7 may have its effect on cone development by redirecting assimilate from the bud apex to AB apices thus increasing the amount of available assimilate above the threshold levels necessary for cone differentiation (Ross et al 1985). GA 4/7 has been shown to cause a significant reallocation of dry matter and photosynthate to female cone primordia in Pinus radiata (Ross et al 1984). Techniques need to be developed which will allow both the concentration of GA 4/7 in buds and
the effect of GA 4/7 in redirecting assimilate within a differentiating bud to be measured with greater accuracy. It may then be possible to test the nutrient diversion hypothesis as a means by which coning is controlled in lodgepole pine.

7.5 Possible controls of changes in bud morphology at the physiological/biochemical level

Any hypothesis for the control of bud development and coning, in conifers in general, and lodgepole pine in particular has got to take into account the complexity of tree structure and physiology, necessary for the evolutionary survival of species. Romberger and Gregory (1974) first highlighted this complexity when they suggested that a number of factors and variables were involved in controlling coning in conifers, and all had to be "permissive" for coning to occur. Therefore, as the results of this study suggest, no one single factor is likely to be controlling bud development and coning. There does appear to be a role for environmental variables like temperature and photoperiod. The first, possibly in conjunction with irradiance and water availability, may be able to limit the outcome of bud development by affecting the growth rate and amount of assimilate available to buds. The second may cause a switch in the sequence of development of buds from the initiation of male cones in days longer than a critical daylength to the development of female cones in daylengths shorter than the critical daylength. Because of the apparent complexity of the control processes, it is impossible, from this study, to determine categorically whether there is a critical daylength and if there is, what it is. Further studies in controlled environments are required to determine precisely the effects of photoperiod on bud development and coning.

It is likely that under field conditions, the critical daylength will vary, both between clones and years, as the timing of cone initiation was dependent on both these factors (Section 3.4). The critical daylength for male and female cone differentiation may be different, as some
female cones may differentiate on a tree before buds which will produce male cones have initiated all the male cone primordia. Female cones may be initiated in longer days if other conditions, like temperature and irradiance favour coning. As the timing of cone differentiation may also vary within a tree depending on the growing conditions, the effect of photoperiod may be quantitative rather than determined by a critical photoperiod.

In the bud itself, development may be related to the size of primordia, especially at initiation (Rombetger & Gregory 1974), and/or their ability to compete for assimilate (Sachs & Hackett 1977). There is evidence from the photoperiod experiments that in SDs the growth of ABs towards the apex of buds is faster and ABs become larger than in LDs where growth of ABs towards the base of buds is promoted (Section 5.6). Factors like temperature, photoperiod and time of year may affect the size at initiation of ABs on buds, such that in LDs, or early in the year, smaller ABs are initiated when compared to later in the year or in SDs. If larger ABs have a competitive advantage over smaller ABs in terms of their ability to obtain larger quantities of assimilate and grow faster, as suggested by Rombberger and Gregory (1974), and if this increased amount of assimilate affects the outcome of AB differentiation (Sachs & Hackett 1977), a possible explanation for the effects of time of year, photoperiod and temperature on bud development and cone initiation and or differentiation in lodgepole pine could be proposed.

An hypothesis which related the outcome of AB development to the size of the primordium (especially the longitudinal distance between successive primordia and the diameter of the stem at the base of the AB) could explain the outcome of bud development. This hypothesis would correlate AB development in increasing order of internodal length or diameter at initiation with: weak vegetative (short-shoot); male cone; vegetative (short-shoot); female cone; strong vegetative (short-shoot or long-shoot). Thus, in the early part of the growing season, when internodes, especially on less vigorous buds are short, male cones would be differentiated. As the bud increases in size, the internodes would increase in length so that short-shoots
differentiate. This could be the pattern of development in most male buds. In female buds, the diameter of the apex and size of internodes early in the season would be too large for male cone development and short-shoots would differentiate. If the buds are in a favourable environment, shoot diameter and internode length may reach the threshold necessary for female cone initiation and or long-shoot initiation. Development of female cones may affect the rate of growth in length of buds such that cataphylls, initiated after female cones and long-shoots, do not have ABs developing in their axils. If growing conditions are favourable, and the season is not ended, fertile 1ry cataphyll initiation may recommence. As polycyclic buds are likely to have large diameters and thus probably large internodal lengths at initiation, no male cones will be initiated at the start of the second developmental cycle- short-shoots would form instead. Only if growth of buds is greater than the necessary threshold, will it be possible for internodal lengths to reach the size necessary for more female cones to differentiate at the top of the second cycle of bud parts.

This hypothesis relates the outcome of AB development to the cross-sectional area of the main bud associated with the AB at initiation. It does not suggest reasons for the variation in cross-sectional area in different buds during the season. Romberger and Gregory suggested that increased size gave some meristems the potential to out-compete others leading to differential development. Sachs and Hackett suggested that it was assimilate supply which limited the development of meristems. Both may play a part with increased size leading to development of a better translocation system to some ABs and thus more assimilate. Any factor which affects the size and/or rate of growth of buds would affect the development of ABs, but the response would be mediated internally because the response to most environmental and structural changes would be gradual. Only treatments like pruning and short-shoot removal would be likely to produce immediate effects on bud growth, and even the effects of these treatments may be modified by the tree in order to retain balanced growth throughout the tree.
7.5.1 Endogenous GAs

Pharis et al. (1976) suggested that GA 4/7 was directly involved in the control of morphogenesis. The results from this study do not support this hypothesis as the role of GA 4/7 was to stimulate coning per se, and not the production of one type of cone. A more likely hypothesis for the effect of GA 4/7 on cone initiation and differentiation, is that that GA 4/7 increased the number of cones initiated by increasing the internodal lengths of ABs at and after initiation. This may affect the rate of growth or supply of assimilate to those larger ABs such that male cones differentiated instead of weak short-shoots and female cones instead of only short-shoots. The increased distance from a source of AB inhibition caused by larger internode lengths may have reduced the effects of apical dominance. It is also possible to hypothesise that GA 4/7 directly affects either the rate of growth or the flow of assimilates to ABs. If there is a threshold growth rate below which cones do not differentiate fully, GA 4/7 may affect the coning potential of buds by increasing the growth rate of ABs beyond this threshold. Because of the inaccuracy associated with predicting the type of structure which will differentiate from any AB, and the difficulty in pinpointing the timing of cone initiation, no comparative studies have been done to test the hypothesis that GA 4/7 affects the coning potential of buds by increasing AB growth rates directly. This is a research area which requires further study. There is some evidence to support the hypothesis that GA 4/7 affects the number of female cones initiating on a bud by increasing the amount of assimilate available to developing ABs. When exogenous GA 4/7 was applied to Pinus radiata it enhanced flowering and also caused a significant reallocation of photosynthate to female cone primordia (Ross et al. 1984). It is essential that more detailed studies of the effects of exogenous GA 4/7 are done with the aim of clarifying how the growth regulator affects the coning potential of buds.

7.5.2 Vigour of buds

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There is a great deal of circumstantial evidence to suggest that female cones are initiated on more vigorous buds i.e. those on larger shoots which have faster rates of shoot and needle extension early in the season. But, analyses of the growth rates of different sexes of bud on a tree did not provide substantive evidence that sex determination was related to specific differences in growth rate. Female buds could grow faster in length, but male buds tended to have a faster growth rate in bud diameter (section 3.5.1). As bud volume growth rates were not measured during the field survey, and as no accurate estimate of volume growth rates could be made from measurements of bud length and basal diameter, it was impossible to determine whether there were variations in volume growth rates in buds of different sexes which could be related to differences in the rates of cell division or cell enlargement in buds.

Male and female cones are initiated in different areas on a tree, with female buds developing in the more vigorous regions (Fig. 1.1). In this study, no attempt was made to identify the causes of cone zonation. It may be related to environmental limitations (eg. irradiance) having a direct effect on the potential for differentiation of a particular type of cone by limiting or enhancing growth rates in different parts of the crown. The control of bud zonation may be indirect, with variations in shoot size and position, which are related to the previous history of the tree, determining the growth potential of buds, possibly by regulating the amount of assimilate which is available to the bud (the adjacent shoot may be the primary supplier of assimilate to the bud (Ericsson, 1978)).

As the rate of bud growth and development did not vary significantly on male and female buds from the same tree (section 3.3), variations in growth rates per se do not seem to be the cause of different sexes of bud differentiating unless the means of measuring rate of differentiation in this study were not sensitive enough.

Zonation in the types of buds developing on a tree may be controlled by the amount of assimilate being exported from the shoot a bud is growing on. Female buds developed on shoots which were longer and had more needles than shoots on the same tree which had male buds. If the
amount of carbohydrate exported from large year-1 shoots to their buds is greater than that exported from smaller shoots, it is possible to hypothesize (based on Sachs 1977) that more assimilate is available for use in buds growing on larger shoots. If availability of assimilate can limit bud development so that male buds differentiate from ABs with a restricted supply of assimilate available to them when compared to female buds, ABs developing on buds on larger shoots may not be limited in their growth potential in the way that buds on smaller shoots with less available assimilate may be. It is not known whether larger shoots export more assimilate to their buds, or if that assimilate is directed to ABs in different parts of the bud. There is also no evidence to support the hypothesis that either male or female cone differentiation is limited by the amount of available assimilate. Experiments which could accurately measure the flow of assimilate to ABs on buds in different positions on trees, may allow the nutrient diversion hypothesis to be tested, at least tentatively.

A mechanism whereby female cone development is restricted to vigorous branches on fast growing trees would be one way of controlling the number of cones initiated on a tree, especially as this mechanism would be dependent on both the environment the trees are growing in and the vigour of the parent shoots. There would also be a survival advantage in restricting male cone differentiation to the lower, smaller, more heavily shaded shoots on a tree since these shoots may not provide enough assimilate for the continued development of female cones. They are also in a position where the photosynthetic rate of needles may be lowered by shading effects. Therefore, the loss of photosynthetic area associated with the production of male cones may be of less importance to the overall growth of the tree.

Competition between ABs on buds in different positions on trees provides a useful explanation for the control of bud development in trees with both male and female cones, where the male cones all differentiate on buds on shorter, lower order shoots which tend to have slower growth rates in the early part of the season than shoots which produce female buds. On trees which are almost exclusively male coning, very vigorous shoots may
initiate male cones. Therefore, the vigour of shoots and competition between buds cannot be the sole determinant of the outcome of bud development. Other factors, possibly genetic variations in the way clone responds to environmental variables like photoperiod or temperature must be involved.

Variations in shoot size may be important also to the outcome of bud development, as the intrinsic relationship between the size and the rate of development of bud parts appears to be extended to the size of the shoots on which the buds are growing and the position of the shoot on the tree. Although the number of bud parts initiated per bud is extremely variable, this number correlates highly in any bud, with the number of short-shoots and cones on the shoot on which the bud is growing. This number is, itself, related to the length and diameter of the shoot and its position on the tree. One outcome of the relationship between the size of the shoot and the size of the bud, may be that accurate methods can be developed to predict the final size and the type of bud which will differentiate on any lodgepole pine shoot.

The close relationship between shoot structure and bud development implies that when developing an hypothesis for the control of bud differentiation in lodgepole pine the previous history of the tree should be taken into account, especially the factors which affected the structure and morphology of the tree. These factors, whether environmental or applied, may have more effect over bud development than the tree's present environment or any newly applied factors. It should be possible, for an individual clone of a specified age, to develop an equation which relates the outcome of bud development to the size and position of the shoot and the bud's growth rate, and thus to predict the type of bud which will develop. It may then be possible to model the effects of environmental variables on bud development in buds of different sizes in a variety of positions on trees. This type of statistical modelling may provide some understanding of the balance of control of bud development between the environment the tree is growing in and the intrinsic control systems.
7.5.3 Growth rates

Bud development in lodgepole pine is highly regulated, with the intrinsic control of development of major importance. This is shown by the rate of growth of the bud in length and diameter, although showing some variation (e.g. dissected bud length and diameter do not always respond to growing conditions in the same way), being paralleled by the rate of growth of bud parts.

Within the bud, the rate of growth and development of ABs parallels the rate of growth and development of cataphylls both 1ry and 2ry, although there may be differences in the rate of growth of ABs at the base compared to the apex of a bud, and initiation and growth rates of 2ry cataphylls may show some variation, especially towards the end of the 2ry cataphyll initiation period.

Cone initiation does not appear to be related to the initial growth rates of the ABs from which cones will develop. ABs which appeared likely to develop into male cones had an only slightly slower rates of growth than ABs which appeared likely to develop into short-shoots, although after male cone differentiation began, the rate of growth of male cone ABs increased rapidly compared to that of short-shoots.

The ABs likely to develop into female cones may begin initiating 2ry cataphylls earlier than ABs on potentially vegetative or male buds, but the rate of initiation and growth of all 2ry cataphylls was very similar. Thus, when all the buds sampled in the field study were taken into account, evidence suggests that cone differentiation was not related to differential growth rates of ABs, although within one tree or clone differential growth rates may be more important. More detailed studies are needed to determine whether the apparently slight differences in the rates of growth of ABs are of more importance to AB differentiation than appears likely from the field survey results, especially as the results from experiment 2dc suggest that the rates of growth of ABs in different positions on buds could be affected by photoperiod.

Once ABs begin differentiating into cones, the rate of growth of these ABs (and sometimes other ABs in the same area of the bud which are not differentiating into
cones) increases. In male buds, the increase in AB growth rate may coincide with a decrease in the rate of differentiation of short- and long-shoots on the same bud when compared to the rate on female and vegetative buds. In female buds, the increased growth rate coincides with the beginning of apical dormancy and often with an increase in the rate of differentiation of both short and long-shoots, when compared to the rates on male or vegetative buds. Long-shoot primordia appear to differentiate on most buds, but there is a significant positive correlation between bud vigour as measured by the number or rate of initiation of 1ry cataphyll primordia and long-shoot development, with few long-shoots differentiating immediately after initiation, except on the largest, most vigorous buds. Long-shoot primordia on small, less vigorous buds often remain latent or abort. As variations in the rates of growth of ABs may determine the outcome of AB differentiation, it is essential that further studies are carried out to identify any variation in the initial growth rates of potential male or female cones and long- or short-shoots which could be determining the outcome of AB development.

7.5.4 Partitioning of assimilate between apical dome and primordia

Apical dimensions

Apical dimensions were not measured directly in the field survey, but the high positive correlation between shoot length and diameter and apical meristem diameter would lead to the conclusion that more vigorous female coning buds on lodgepole pine trees have larger apical meristems than less vigorous male or vegetative buds. The size of the apical meristem does not alter the type of primordia initiated in lodgepole pine directly, as all the primordia which are initiated differentiate into scale leaves. If apical meristem diameter and height correlate positively with the stem unit length associated with a 1ry cataphyll at initiation, then, as suggested by Romberger and Gregory (1974), stem unit length may be very relevant in determining the type of AB which differentiates. The 1ry cataphylls which will have male
cones differentiating in their axils may have smaller initial stem unit lengths than those with short-shoots which are in turn smaller than those with female cones differentiating in their axils. Romberger & Gregory (1974) proposed that a difference in size of this type was enough to affect the competitive status and thus the growth rates and sink strengths of meristems, leading to the development of different axillary structures.

Another possible explanation for the relationship between apical size and cone initiation is that as the apex increases in size to a 'critical size' the growth rate in the apical region of the bud also increases, and it is this high growth rate which is important for female cone differentiation. Below the 'critical size' growth rates may be more favourable for male cone or short-shoot initiation. The difference between the two explanations proposed above is that the first relies on differences in the initial sizes of structures affecting the development of ABs, while the other relates differences in rates of growth of bud parts to the outcome of AB differentiation. Both size and rate of growth may be involved.

Apical dormancy and female cone initiation

As in other conifer species studied (Owens et al 1980), the initiation of female cones on a bud and the start of apical dormancy of the bud in lodgepole pine coincide, although the dormancy which occurs does not always mark the cessation of growth of the bud apex for the season.

Although there appears to be a strong link between female cone initiation and apical dormancy, irrespective of whether the dormancy is temporary or marks the end of growth for a season, dormancy may occur without female cones being initiated. Therefore, female cone initiation is not a prerequisite for dormancy.

Although there is no evidence to prove that female cone initiation is inhibited until apical dormancy occurs, it may be that female cone initiation is dependent on the apex of the bud becoming dormant. If the apex of a bud, while it is initiating fertile lry cataphylls, has an inhibitory effect over the growth and development of ABs close to the apex, and if that inhibitory effect is reduced when the apex enters
dormancy, female cone development may be caused, at least in part, by a release from apical dominance. The potential cone's release from apical dominance may be related to an increase in the amount of assimilate available to the AB. As less assimilate will be needed by the apex of the dormant bud, there may initially be an excess of assimilate in the apical region of the bud, which can be redirected to the potential cone, allowing cone differentiation to occur. This explanation presupposes that cone differentiation is limited by the amount of assimilate available to ABs. Other explanations may be proposed, which link cone development to changes in the concentration of a growth regulator, possibly GA 4/7, or to growth inhibitors.

Apical dominance, which may be responsible for the ABs on buds differentiating only slowly, appears to be reduced as the apex enters temporary dormancy, as axillary meristems (both female and vegetative) develop more rapidly (unpublished visual observation). As the release occurs, the ABs near the apex of the bud may be more able to compete, possibly because they are closer to the translocation pathway taking assimilate to the apex of the bud, giving them a competitive edge which allows them to grow faster and differentiate into long-shoots or female cones. As more female cones differentiate on longer buds with more ABs, the number of cones differentiating on a bud may be proportional to the size of the bud and to the amount of assimilate available to ABs as the apex of the bud becomes dormant.

In conclusion, female cone initiation may occur as a result of early, fast bud development in favourable conditions, where temporary apical dormancy is triggered by some intrinsic factor. Female cone development may also occur in vigorous buds as the final stage of bud differentiation at the end of the season, possibly triggered by some environmental factor eg. decreasing photoperiods or lower temperatures. The number of female cones which differentiate appears to be related to the size and number of bud parts (and by inference the size of the shoot the bud is growing on), and possibly to the amount of assimilate available when the cones begin to differentiate.

The start of male cone differentiation does not appear
to be related to apical dormancy as male cones begin developing before the apex enters dormancy. It is possible that male cone initiation is linked to apical dormancy, with no male cones being initiated after the apex has entered dormancy. The results of this study provide no evidence for a link between the cessation of male cone initiation and apical dormancy.

7.5.5 Summary

Bud development and coning in lodgepole pine appears to be under the control of a variety of factors, both inherent and environmental, which have varying effects depending on time of year and tree structure. There is a great deal of variation in the way trees respond to variations in temperature, photoperiod and application of GA 4/7. However, from the results obtained in this study, it is possible to identify some of the effects of these factors on bud differentiation.

Variations in temperature, when applied for only a short time (42 days) have only a minor effect on the rate of growth of buds and bud parts. The sequence of differentiation in buds was affected more, with male cones differentiating preferentially at 20°C and female cones at 10°C. Photoperiod also affected the sequence of differentiation in buds, with male cone differentiation possibly preferentially stimulated in LDs and female cones in SDs. Photoperiod may affect the sequence of differentiation in buds by increasing the rates of growth of ABs in different areas of buds such that basal ABs grow faster in LDs and apical ABs faster in SDs. Female cone differentiation may be dependent on apical dormancy being stimulated as coning very rarely occurs (Black 1961) unless the apex of the bud has stopped initiating fertile lry cataphylls. GA 4/7 does not appear to affect the sequence of differentiation in buds as the effects of GA 4/7 application vary depending on the photoperiod in which it is applied. There is evidence that GA 4/7 may increase the rate of growth of ABs towards the base of buds in LDs, and towards the apex of buds in SDs. If the outcome of AB differentiation is limited by the rate of
growth of ABs, GA 4/7 application may increase the number of cones differentiating by increasing growth rates above the threshold. As other evidence suggests that GA 4/7 may affect cone differentiation by increasing the supply of assimilate to potential cones (Ross et al 1984), studies need to be carried out to determine exactly how and when GA 4/7 affects the growth and development of ABs.

It is possible to hypothesise that either the small internode length or the slow initial growth rate associated with ABs at their initiation may lead to the development of male cones. As the size or rate of growth of the apex increases, the internode length/rate of growth may also increase and short-shoots may differentiate. In buds developing on vigorous shoots, the size/growth rate of the apex at the start of the season may be larger than that normally associated with the initiation of male cones, and only needles may differentiate. As the size of the apex increases, the critical size necessary for female cone initiation may be reached and cones will form. Differentiation of ABs may be limited by either the rate of growth or the amount of assimilate available to ABs in different parts of buds. Therefore, although an AB may be likely to follow one sequence of development because of the size of the shoot it is developing on and the time of year at which it was initiated, changes in the competitive status of the bud caused by either environmental variations or cultural practices may enable the AB to follow a different sequence of differentiation. The threshold levels necessary for different developmental pathways to be followed probably differ with clone, so may be very difficult to determine. Male cone development does appear to be linked with a slow initial rate of AB initiation and development when compared with short-shoot development in female buds. Any ABs which do not differentiate the year they are initiated, almost invariably differentiate as needles, so there also appears to be a time limit, possibly related to environmental differences, which affects the outcome of AB differentiation.

As neither the initial lengths of internodes nor growth rates of apices were measured in buds collected in the field survey, no information is
available to prove or disprove either explanation.

Although significant effects on bud development were recorded in each of the controlled environment experiments, the inherent close relationship between shoot size and structure and bud size and sequence of development must be emphasised. As shoot length and needle number appear to be more important to the outcome of bud development than either variations in environmental factors or application of GA 4/7, an understanding of the mode of action of the inherent processes controlling bud development and coning in trees is essential.

7.6 Control of flowering in trees, especially lodgepole pine; problems of investigation and how they may be overcome

7.6.1 Field survey

Trees used in the field survey were extremely variable in growth form. Therefore, accurate comparisons between buds of different sizes, in different stages of development from different clones were difficult to make. As the stages of bud development most likely to provide information about why cones differentiate were those which occurred before the cones had begun to form (stages 1-4), there was an added difficulty that the predicted outcome of any bud's development was not guaranteed. Uniform tree material which has a predictable developmental sequence needs to be used in future studies.

No record was made of the positions on trees from which buds were sampled, or of the variations in environmental parameters at the sampling sites during the survey. Therefore, it was impossible to carry out accurate covariance analyses which may have provided some understanding of how the cone zonation pattern is maintained in trees. Future studies may benefit by looking at small trees of only one or two clones in more detail, and by building factors like position of buds on the tree into the analysis, along with accurate environmental data. It would then be easier to recognise differences in rates of growth and sequences of development in buds, and to determine the causes of them. The methods of analysis of buds also need to be modified.
so that data on the size and growth rate of the apex, the rate of movement of assimilate from the shoot to the bud and concentration of assimilate and growth regulators, especially GA 4/7, in bud parts, can be collected. Information obtained may be used to build mathematical models so that the effects of changes in environmental variables on bud development and coning could be tested without having to set up costly and time-consuming experiments.

### 7.6.2 Controlled environment experiments

In experiment 1, growth of the outside control trees was not directly comparable with the trees growing in the controlled environments because of diurnal and seasonal variations in their growing conditions. Surprisingly, growth of buds on outside trees was more consistent than on trees grown in the growth rooms. This suggests that the conditions supplied in the growth rooms, although as good as were possible, were far from natural. It is possible that the stimulation of polycyclic bud formation in buds grown in the controlled environments was the result of stress caused by supplying unsuitable growing conditions.

Stresses induced when the growth rooms broke down may have affected the differentiation of buds, although no obvious difference in the sequence or rate of growth and development occurred at the same time as the temperature stress was applied.

In experiment one, the two temperature treatments were only applied for 42 days. This was probably not long enough to initiate the full effects of varying temperature. The 10°C and 20°C temperature regimes caused buds to follow different sequences of development, but these developmental pathways were probably modified by growing conditions after the trees were placed outside.

Problems in analysing the data from experiment 1 were increased because the trees used in the experiment were not very uniform in structure. To get enough material, it was essential to sample shoots of different ages from different positions on trees. The likelihood of these buds having a uniform rate of growth and similar outcome of bud development is very small, yet because of the
costs involved in growing suitable experimental material, the buds had to be treated as if they did respond to treatment in the same way.

Some of the trees used in experiment 1 had been used in a previous experiment, and the residual effects of the earlier experiment were in some cases more significant than the effects of temperature. Therefore, determining the effects of temperature on bud development and coning was made very difficult. Although it is costly, labour intensive and time consuming, it is essential that more uniform tree material is used in any future experiments, so that any small, but important, differences in the growth of buds at different temperatures can be identified.

There were fewer problems associated with experiments 2 and 3 because trees were maintained in growth cabinets for the whole of the experimental period, the trees were more uniform in shape (although they varied in height by a factor of 2), only the results for terminal bud development are given, and the trees had not been used in previous experiments. The trees used, however, were much younger and smaller, and therefore were not physiologically mature. This made the study of cone development hazardous. Also, in both experiments 2 and 3, towards the end of the experimental period trees appeared to be suffering from stress. The needles on some trees became brown and necrotic, and a few needles were shed. It seems likely that the growing conditions provided both in the growth rooms and the growth cabinets were far from ideal for growing lodgepole pine trees.

This study depended almost entirely on destructive sampling as a means of collecting information on bud development from trees. It is possible to collect some information retrospectively, by applying treatments and then recording the results when buds have extended the following year. Although this technique means that the structure of the tree is not modified during the experiment, there is a built in time-lag before any results are obtained, and other, uncontrolled factors may affect the outcome of the experiment before the results are recorded. If destructive sampling is used, the balance of assimilate and growth regulators and the microenvironment of trees is disturbed when buds are
removed, and this disruption may have more effect on bud development and coning than the applied factors. Also the structure of the tree is irrevocably changed, which may make the tree useless for further experiments. The gain from carrying out destructive sampling is that more information may be obtained about the mode of action of factors which affect development. New techniques, possibly involving soft X-rays, which will allow the development of buds to be monitored without the bud being dissected, need to be developed. The sequence of differentiation of individual buds could then be followed, providing a much clearer understanding of how buds of lodgepole pine develop.
The timing of bud development and coning in lodgepole pine varies with tree, clone and season. In Scotland, buds began developing in May. Cones began differentiating between early July and late September with female cones generally differentiating earlier than male cones, although male cone primordia tended to be initiated first.

Environmental variables like temperature and photoperiod and cultural treatments like the addition of GA₁₇ and bud removal affect bud and cone development. The effect of growing trees at 10°C was to increase female cone development and inhibit male cone development when compared to growth at 20°C. Pruning buds on trees grown at 10°C caused those buds left on the trees to initiate male cones although the temperature treatment on its own inhibited male cone development.

SD photoperiods caused apices of buds to enter dormancy while ABs grew faster than on buds grown in LDs. The sequence of development of buds in LDs was similar to male bud development while buds grown in SDs had a similar sequence of development to female cones. Few cones, however, developed on buds grown in different photoperiods, except when GA₁₇ was applied as an adjunct treatment. GA₁₇ application stimulated the initiation of primarily male cones in LDs and female cones in SDs. The treatment did not appear to affect the sequence of differentiation in buds except to increase the size of bud parts and number of cones differentiating, and therefore it seems unlikely that GA₁₇ plays a direct morphogenetic role in bud development and coning in lodgepole pine.

Growth and development of buds is under a great deal of inherent control, with the rate and sequence of development of bud parts highly correlated with each other and with the size and rate of growth of the shoot the bud is on and the position of the bud on the tree. All responses to treatment are mediated by the inherent control processes within trees, and these responses to externally applied factors are often less important to the outcome of bud development than the inherent positional and structural effects.
REFERENCES


APPENDIX ONE

Listing of the computer program ANGLES.

For a description of the program see p 38.
ANGLES

THE APICAL DIAMETER, HORIZONTAL DOME AREA, DIVERGENCE ANGLE AND
PLASTOCHEONE RATIO WILL BE CALCULATED FROM THE RECTANGULAR
COORDINATES OF A NUMBER OF PRIMORDIA.

DIMENSION ODATA(100,3),TEMP(100,100),TEMP2(100)
DIMENSION ORDER(100,100),DATA(100,100)
DIMENSION CALCENT(100,100),RESULT1(100,100),RESULT2(100,100)
COMMON RAWDATA(100,3)
REAL PI

PRINT*, 'ANGLES' PRINT*, 'ANGLES' PRINT*, 'ANGLES'
PRINT*, 'A PROGRAM DESIGNED TO MEASURE THE PLASTOCHEONE RATIO, APICAL
DIAMETER, DIVERGENCE.'
PRINT*, 'ANGLE AND RELATIVE RADIAL GROWTH RATE OF APICAL MERISTEMS
USING AN ITERATIVE TECHNIQUE.'

INPUT THE REFERENCE CODE FOR THE BUD BEING MEASURED

PRINT*, 'INPUT THE REFERENCE CODE FOR THE BUD BEING MEASURED'
PRINT 2
FORMAT(' INPUT THE REFERENCE CODE FOR THE BUD BEING MEASURED')
READ(1,*)ICODE
READ IN THE UNFORMATTED DATA FROM A REMOTE TERMINAL IN THE ORDER:
X COORDINATE, Y COORDINATE RETURN
THE FIRST THREE POINTS INPUTTED MUST BE IN THE ORDER
OLDEST, SECOND OLDEST, THIRD OLDEST PRIMORDIA TO BE USED,
I.E. THE THREE POINTS FURTHEST AWAY FROM THE APICAL DOME.

PRINT*, 'INPUT DATA IN THE ORDER "X-COORD,Y-COORD" RETURN'
PRINT 3
FORMAT(' WHEN YOU HAVE INPUT ALL YOUR DATA INPUT 999.0,999.0')
PI=3.14159
DO 10 I=1,100
READ(1,*)X,Y
IF(X.EQ.999.0)GOTO20
RAWDATA(I,1)=I
RAWDATA(I,2)=X
RAWDATA(I,3)=Y
CONTINUE

X IS THE X-COORDINATE, Y IS THE Y-COORDINATE, I IS THE IDENTIFIER
PRINT*, 'YOU HAVE INPUT TOO MANY DATA POINTS'
PRINT*, 'REDIMENSION THE PROGRAM ARRAYS AND ALL LINKED STATEMENTS'
N=I-1

N IS THE NUMBER OF DATA POINTS BEING USED

PRINT 4
FORMAT(' IF YOU WANT TO CHECK YOUR DATA INPUT 1, IF NOT INPUT 2')
READ(1,*)IANSWER
IF(IANSWER.EQ.2)GOTO30
CALL CHECK(N)

THE RAWDATA CHECK HAS BEEN COMPLETED AND ALL COORDINATES
HAVE BEEN INPUT CORRECTLY INTO THE COMMON BLOCK.

PRINT 5
FORMAT(' INPUT THE SCALE FACTOR')
READ(1,*)CONV

DOES THE DATA NEED TO BE ORDERED? OR IS IT ALREADY ORDERED

PRINT*, 'DOES THE DATA NEED TO BE ORDERED?'
PRINT 6
FORMAT(' INPUT "1" FOR YES AND "2" FOR NO')
READ(1,*)IALTER
The first calculation of the centre point will now be made using the X and Y coordinates of the first three points.

\[ \text{AX} = \text{RAWDATA}(1, 2), \quad \text{AY} = \text{RAWDATA}(1, 3), \quad \text{BX} = \text{RAWDATA}(2, 2), \quad \text{BY} = \text{RAWDATA}(2, 3), \quad \text{CX} = \text{RAWDATA}(3, 2), \quad \text{CY} = \text{RAWDATA}(3, 3) \]

Print*, 'AX = ', AX, ' AY = ', AY
Print*, 'BX = ', BX, ' BY = ', BY
Print*, 'CX = ', CX, ' CY = ', CY

Firstly, the lengths of DISTA, DISTB, and DISTC are calculated. Then, the angle Alpha is calculated by finding the angle Beta and subtracting its value from \(2 \times \pi\).

The cosine rule is used to calculate the length of \(R2\) (radius 2) and the sine rule to calculate the size of AngleP.

\[ \text{CALL CENTRE}(\pi, \text{AX}, \text{AY}, \text{BX}, \text{BY}, \text{CX}, \text{CY}, \text{RAD2}, \text{ANGLEP}) \]

To calculate the coordinates of the centre, we need to know if the primordia spiral in a clockwise or anticlockwise orientation around the apical centre.

\[ \text{CALL TOPOLAR}(\pi, \text{AX}, \text{AY}, \text{BX}, \text{BY}, \text{RAD}, \text{THETA}) \]
\[ \text{CALL TOPOLAR}(\pi, \text{CX}, \text{CY}, \text{BX}, \text{BY}, \text{RADC}, \text{THETAC}) \]
\[ \text{ANGLE} = \text{THETAC} \]

Print*, AX, AY, BX, BY, CX, CY, RAD, THETA, RADC, THETAC

If (THETAC.GT.3*PI/2.AND.THETAC.LT.PI/4) GOTO 40
If (THETAC.LT.3*PI/4.AND.THETAC.GT.3*PI/2) GOTO 50
GOTO 60

40 \[ \text{THETACTHETAC+}(2*\pi) \]
GOTO 50
50 \[ \text{THETAA} = \text{THETAA+}(2*\pi) \]
60 \[ \text{IF(THETAC.GT.THETAA)} \]
GOTO 70

If statement 60 is true, the points are arranged in a clockwise orientation around the centre. If it is false, the points are arranged in an anticlockwise order.

(Spiral arranged from oldest to youngest primordia)

We will deal with the anticlockwise case first.

\[ \text{ZED} = 1 \]
\[ \text{P} = \text{ANGLEA - ANGLEP} \]
Print*, P
Call ANGCHECK(\pi, P, \text{THETAP})
GOTO 80

We will now deal with the clockwise case.

70 \[ \text{ZED} = 2 \]
\[ \text{P} = \text{ANGLEA + ANGLEP} \]
Print*, P
Call ANGCHECK(\pi, P, \text{THETAP})

We now have the polar coord. form of the centre \(P = (\text{RAD2}, \text{THETAP})\).

\[ \text{CALL TORECT}(\pi, \text{BX}, \text{BY}, \text{RAD2}, \text{THETAP}, \text{QX}, \text{QY}) \]

We now have the rectangular coordinates of the centre "QX, QY" from the first three points.

The next part of the program has to arrange the original RANDATA so that the primordia are ordered from oldest to newest. The ordered data is then put into array ODATA.

The polar coordinate form of the data is calculated with the centre of the circle at QXQY. The coordinate data is stored in array TEMP1.
PRINT*,QX,QY
PAUSE 128
DO 90 I1,N
   AX=RAWDATA(I,2)
   AY=RAWDATA(I,3)
   CALL TOPOLAR(PI,AX,AY,QX,QY,RAD,ANGLE)
   PRINT*,AX',AX,AY',AY,QX',QX,QU',QU,RAD',RAD,ANGLE',ANGLE
   TEMP(I,1)=I
   TEMP(I,2)=RAD
   TEMP(I,3)=ANGLE
   PRINT*,TEMP(I,1),TEMP(I,2)
90 CONTINUE
PAUSE 129
WE NOW HAVE A SAFETY CHECK TO MAKE SURE THAT THE ORIENTATION
OF POINTS HAS BEEN CALCULATED CORRECTLY
C C
THETAA=TEMP(1,3)
THETAB=TEMP(2,3)
   ANGLE2=THETAA+2.57453
   CALL ANGCHECK(PI,ANGLE2,ANG2)
   ANGLE3=THETAA+2.2529
   CALL ANGCHECK(PI,ANGLE3,ANG3)
C IF(ANG2.GT.ANG3)GOTO100
   IF(THETAB.GT.ANG3.AND.THETAB.LT.(2*PI)))GOTO115
   IF(THETAB.LT.ANG2.AND.THETAB.GE.0.0)GOTO115
GOTO110
100 IF(THETAB.LT.ANG2.AND.THETAB.GE.ANG3)GOTO115
110 ZED1=2
   PRINT*,ZED1=',ZED1
GOTO120
115 ZED1=1
C C
   ZED1=1:- ROTATION IS ANTICLOCKWISE
   ZED1=2:- ROTATION IS CLOCKWISE
   PRINT*,ZED1=',ZED1
   PAUSE 130
   IF(ZED.EQ.ZED1)GOTO130
   IF(ZED.EQ.2)GOTO105
C C
   ORDERING ANTICLOCKWISE POINTS
135 PT1=TEMP(1,3)
   TEMP(1,3)=888.0
   ORDER(1,1)=1
   DO 190 J=2,N
      L=0
      BEAR1=PT1+2.04204
      BEAR2=PT1+2.74017
      CALL ANGCHECK(PI,BEAR1,BEAR1O)
      CALL ANGCHECK(PI,BEAR2,BEAR20)
      IF(BEAR20.LT.BEAR1O)GOTO145
      DO 140 I=1,N
      IF(TEMP(I,3).GT.BEAR1O.AND.TEMP(I,3).LT.BEAR2O)GOTO136
140 CONTINUE
136 L=L+1
   TEMP2(L)=TEMP(I,1)
140 CONTINUE
   M=TEMP2(1)
   A=TEMP(M,2)
   DO 145 K=1,L
      M=TEMP2(K)
      IF(TEMP(M,2).LT.A)GOTO145
      A=TEMP(M,2)
      FLAG=M
145 CONTINUE
GOTO180
WE WILL NOW DEAL WITH THE BEAR1.GT.BEAR2 CASE

DO 160 I=1,N
IF(TEMP(I,3).GT.BEAR10.AND.TEMP(I,3).LE.(2*PI))GOTO155
IF(TEMP(I,3).LT.BEAR20.AND.TEMP(I,3).GE.0.0)GOTO155
GOTO160

L=L+1
TEMP2(L)=TEMP(I,1)
CONTINUE
M=TEMP2(1)
A=TEMP(M,2)
DO 165 K=1,L
M=TEMP2(K)
IF(TEMP(M,2).LT.A)GOTO165
A=TEMP(M,2)
FLAG=M
CONTINUE
ORDER(J,1)=FLAG
PT1=TEMP(FLAG,3)
TEMP(FLAG,3)=868.0
CONTINUE
GOTO200

ORDERING CLOCKWISE POINTS

PT1=TEMP(1,3)
TEMP(1,3)=868.0
ORDER(1,1)=1
DO 250 J=2,N
L=0
BEAR1=PT1-2.04204
BEAR2=PT1-2.74017
CALL ANGCHECK(PI, BEAR1, BEAR10)
CALL ANGCHECK(PI, BEAR2, BEAR20)
PRINT*, BEAR10, BEAR20, BEAR1, BEAR2, PT1
PAUSE 305
IF(BEAR20.GT.BEAR10)GOTO210
DO 200 I=1,N
IF(TEMP(I,3).LT.BEAR10.AND.TEMP(I,3).LE.(2*PI))GOTO215
IF(TEMP(I,3).LT.BEAR10.AND.TEMP(I,3).GE.0.0)GOTO215
GOTO200

L=L+1
TEMP2(L)=TEMP(I,1)
CONTINUE
M=TEMP2(1)
A=TEMP(M,2)
DO 205 K=1,L
M=TEMP2(K)
IF(TEMP(M,2).LT.A)GOTO205
A=TEMP(M,2)
FLAG=M
CONTINUE
GOTO240

WE WILL NOW DEAL WITH THE BEAR10.LT.BEAR20 CASE

DO 220 I=1,N
IF(TEMP(I,3).GT.BEAR20.AND.TEMP(I,3).LE.(2*PI))GOTO215
IF(TEMP(I,3).LT.BEAR10.AND.TEMP(I,3).GE.0.0)GOTO215
GOTO220

L=L+1
TEMP2(L)=TEMP(I,1)
CONTINUE
M=TEMP2(1)
A=TEMP(M,2)
DO 225 K=1,L
M=TEMP2(K)
IF(TEMP(M,2).LT.A)GOTO225
A=TEMP(M,2)
FLAG=M
CONTINUE
ORDER(J,1)=FLAG
PT1=TEMP(FLAG,3)
TEMP(FLAG,3)=868.0
CONTINUE
DO 330 I=1,N
FLAG =ORDER(I,1)
ODATA(I,1)=FLAG
ODATA(I,2)=RAWDATA(FLAG,2)
ODATA(I,3)=RAWDATA(FLAG,3)
CONTINUE
GOTO335

DO 275 I=1,N
ODATA(I,1)=RAWDATA(I,1)
ODATA(I,2)=RAWDATA(I,2)
ODATA(I,3)=RAWDATA(I,3)
CONTINUE

THE VALUE OF THE CENTRE WILL NOW BE CALCULATED ACCURATELY IN AN ITERATIVE FASHION USING A RUNNING SET OF THREE POINTS

DO 340 I=1,N-2
AX=ODATA(I,2)
AY=ODATA(I,3)
BX=ODATA(I+1,2)
BY=ODATA(I+1,3)
CX=ODATA(I+2,2)
CY=ODATA(I+2,3)
CALL CENTRE(PI,AX,AY,BX,BY,CX,CY,R&DZ,ANGLEP)

THIS WILL GIVE US THE RADIUS AND ANGLE PART OF THE CENTRE CALCULATION

CALL TOPOLAR(PI,AX,AY,BX,BY,D1,THETAA)
IF(ZED.EQ.2)G0T0350

DOING ANTICLOCKWISE CASE

THETAP=THETAA-ANGLEP
GOTO360

DOING CLOCKWISE CASE

THETAP=THETAA+ANGLEP
CALL ANGCHECK(PI,THETAP,THETAQ)
CALL TORECT(PI,BX,BY,RAD2,THETAQ,QX,QY)
CALCENT(I,1)=I
CALCENT(I,2)=QX
CALCENT(I,3)=QY
CONTINUE

X=0.0
Y=0.0
DO 370 I=1,N-2
X=X+CALCENT(I,2)
Y=Y+CALCENT(I,3)
CONTINUE
AVCENTX=X/(N-2)
AVCENTY=Y/(N-2)

USE ACCURATELY CALCULATED CENTRE FROM 3 POINTS TO CALCULATE ALPHA, THE PLASTOCHRONE RATIO AND RAD3.

DO 380 I=1,N-2
AX=ODATA(I,2)
K=ODATA(I,1)
AY=ODATA(I,3)
BX=ODATA(I+1,2)
BY=ODATA(I+1,3)
CX=ODATA(I+2,2)
CY=ODATA(I+2,3)
QX=CALCENT(I,2)
QY=CALCENT(I,3)
CALL TOPOLAR(PI,AX,AY,QX,QY,RA,
THETAA)
CALL TOPOLAR(PI,BX,BY,QX,QY,RA,
THETAB)
CALL TORECT(PI,CX,CY,QX,QY,RC,
THETAC)

FOR CLOCKWISE ROUTINE

IF(ZED.EQ.1)GOTO390
IF(THETAA.GT.THETAB)GOTO400
THETAA=THETAA+(2*PI)
400 ALPHA=THETAA-THETAB
GOTO410

IF(THETAB.GT.THETAA)GOTO420
THETAB=THETAB+(2*PI)
420 ALPHA=THETAB-THETAA
CALCULATING THE 'PLAStOCHRONE RATIO

\[ PR = \frac{(RA/RB) + (RB/RC)}{2} \]

CONVERT RA, RB AND RC TO THEIR PROPER DIMENSIONS

\[ RA1 = \frac{RA}{CONV} \]
\[ RB1 = \frac{RB}{CONV} \]
\[ RC1 = \frac{RC}{CONV} \]

RESULT1(I, 1) = K
RESULT1(I, 2) = QX
RESULT1(I, 3) = QY
RESULT1(I, 4) = ALPHA
RESULT1(I, 5) = PR
RESULT1(I, 6) = RA
RESULT1(I, 7) = RB
RESULT1(I, 8) = RC
RESULT1(I, 9) = RA1
RESULT1(I, 10) = RB1
RESULT1(I, 11) = RC1
CONTINUE

\[ \alpha = \frac{\alpha_{AB}}{R} = \frac{(PR A/B + PR B/C)}{2}, RC = RADIUS FROM CENTRE TO POINT C, THE THIRD POINT IN THE GROUP AND THUS THE SMALLEST RADIUS \]

CALCULATING ALPHA AND THE PLAStOCHRONE RATIO AROUND THE AVERAGE CENTRE

DO 430 I = 1, N - 1
\[ AX = ODATA(I, 2) \]
\[ AY = ODATA(I, 3) \]
\[ BX = ODATA(I + 1, 2) \]
\[ BY = ODATA(I + 1, 3) \]
\[ K = ODATA(I, 1) \]
CALL TOPOLAB(PI, AX, AY, AVCENTX, AVCENTY, RA, THETAA)
CALL TOPOLAB(PI, BX, BY, AVCENTX, AVCENTY, RB, THETAB)

FOR CLOCkWISE ORIENTATION

IF(ZED.EQ.1) GOTO 440
IF(THETAA.GT.THETAB) GOTO 450
\[ \alpha = \alpha_{AA} - \alpha_{BB} \]
\[ \alpha = \alpha_{TB} - \alpha_{TA} \]
PR = \( \frac{RA}{RB} \)
RA2 = RA/CONV
RB2 = RB/CONV
RESULT2(I, 1) = K
RESULT2(I, 2) = ALPHA
RESULT2(I, 3) = PR
RESULT2(I, 4) = RA
RESULT2(I, 5) = RB
RESULT2(I, 6) = RA2
RESULT2(I, 7) = RB2
CONTINUE

CALCULATING THE AVERAGE ALPHA AND PR USING RESULT1

\[ \alpha = 0.0 \]
\[ PR = 0.0 \]
DO 480 I = 1, N - 2
\[ \alpha = \alpha + RESULT1(I, 4) \]
\[ PR = PR + RESULT1(I, 5) \]
CONTINUE
\[ \alpha = \frac{\alpha}{N-2} \]
\[ PR = \frac{PR}{N-2} \]
CALCULATING THE AVERAGE ALPHA AND PR USING RESULTS AND AVCENTRE

\[
\text{ALPHA2} = 0.0 \\
\text{PR2} = 0.0 \\
\text{DO 490} \ I = 1, N-1 \\
\text{ALPHA2} = \text{ALPHA2} + \text{RESULT2}(I, 2) \\
\text{PR2} = \text{PR2} + \text{RESULT2}(I, 3) \\
\text{CONTINUE} \\
\text{AVALPHA2} = \text{ALPHA2} / (N-1) \\
\text{AVPR2} = \text{PR2} / (N-1) \\
\text{OPEN(9, FILE='ANGOUT', STATUS='UNKNOWN')} \\
\text{WRITE}(1,9501) \\
\text{WRITE}(9,9501) \ ICODE \\
\text{WRITE}(1,9500) \ ICODE \\
\text{WRITE}(9,9500) \ ICODE \\
\text{WRITE}(1,9502) \\
\text{WRITE}(9,9502) \\
\text{WRITE}(1,9989) \\
\text{WRITE}(9,9989) \\
\text{WRITE}(1,9999) ((\text{ODATA}(I,J), J=1,3), I=1,N) \\
\text{WRITE}(9,9999) ((\text{ODATA}(I,J), J=1,3), I=1,N) \\
\text{WRITE}(1,9988) \\
\text{WRITE}(9,9988) \\
\text{WRITE}(1,9998) ((\text{RESULT}(I,J), J=1,5), I=1,N-2) \\
\text{WRITE}(9,9998) ((\text{RESULT}(I,J), J=1,5), I=1,N-2) \\
\text{WRITE}(1,9979) \\
\text{WRITE}(9,9979) \\
\text{WRITE}(1,9978) ((\text{RESULT}(I,J), J=9,11), I=1,N-2) \\
\text{WRITE}(9,9978) ((\text{RESULT}(I,J), J=9,11), I=1,N-2) \\
\text{WRITE}(1,9987) \\
\text{WRITE}(9,9987) \\
\text{WRITE}(1,9997) ((\text{RESULT2}(I,J), J=1,7), I=1,N-1) \\
\text{WRITE}(9,9997) ((\text{RESULT2}(I,J), J=1,7), I=1,N-1) \\
\text{WRITE}(1,9996) \\
\text{WRITE}(9,9996) \\
\text{WRITE}(1,9996) \ AVPR1, AVALPHA1, AVPR2, AVALPHA2 \\
\text{WRITE}(9,9996) \ AVPR1, AVALPHA1, AVPR2, AVALPHA2 \\
9501 \text{FORMAT(3X, 'BUR IDENTIFICATION CODE')} \\
9500 \text{FORMAT(3X, 'I15/')} \\
9502 \text{FORMAT(3X, '**********************************/')} \\
9989 \text{FORMAT(5X, 'PRIMORDIUM NO.', 5X, 'X-COORDINATE', 5X, 'Y-COORDINATE', /)} \\
9999 \text{FORMAT(3X, F14.2, 3X, F14.2, 3X, F14.2, /)} \\
9998 \text{FORMAT(F5.1, 3/)} \\
9979 \text{FORMAT(F8X, 'RADIUS A', 8X, 'RADIUS B', 8X, 'RADIUS C', /)} \\
9978 \text{FORMAT(F3F15.3/)} \\
9997 \text{FORMAT(2X, 7F14.4)} \\
9986 \text{FORMAT(10X, 'AVERAGE PR1', 2X, 'AVERAGE ALPHAL', 2X, 'AVERAGE ALPHA2')} \\
9986 \text{FORMAT(6X, 4F14.6)} \\
\text{CLOSE(9)} \\
\text{CALL COMND('COPY-FILE LINE-PRINTER ANGOUT', '')}}</code>
SUBROUTINE CHECK(N)
COMMON RAWDATA(100,3)
INTEGER IDATA, IANSWER
DO 610 I=1,N
WRITE(1,*)('THE FLAG IS',RAWDATA(I,1),', THE X-COORD. IS ',
*RAWDATA(I,2),', THE Y-COORD. IS ',RAWDATA(I,3))
PRINT*, 'IF THE DATA IS CORRECT INPUT 2, IF NOT INPUT 1'
READ(1,*)IDATA
IF(IDATA.EQ.2)GOTO610
PRINT*, 'INPUT CORRECTED DATA IN THE ORDER X,Y ',
READ(1,*)X,Y
RAWDATA(I,2)=X
RAWDATA(I,3)=Y
CONTINUE
PRINT*, 'THIS IS THE EDITED DATA.'
PRINT*, 'DO YOU WANT TO RECHECK YOUR DATA?'
PRINT*, 'INPUT 1 FOR YES AND 2 FOR NO.'
READ(1,*)IANSWER
IF(IANSWER.EQ.2)GOTO620
GOTO630
620 RETURN
END

SUBROUTINE CENTRE(PI,AX,AY,BX,BY,CX,CY,RAD,ANGLEP)
C THIS SUBROUTINE TAKES THREE POINTS FROM THE MAIN
C PROGRAM AND USES THE CENTRE CALCULATION TO ESTABLISH
C THE ANGLE AND RADIAL DISTANCE OF THE CENTRE FROM B,
C THE MIDPOINT OF THE THREE DATA POINTS.
C
C DISTAB=SQRT(((AX-BX)*(AX-BX))+((AY-BY)*(AY-BY)))
C DISTBC=SQRT(((BX-CX)*(BX-CX))+((BY-CY)*(BY-CY)))
C DISTAC=SQRT(((AX-CX)*(AX-CX))+((AY-CY)*(AY-CY)))
C
C FIND THE DIVERGENCE ANGLE ALPHA BETWEEN COORDINATES
C D=ACOS(C)
C ALPHA=PI-D
C
C THE ANGLE ALPHA IS IN RADIANS
C THE RADIUS FROM POINT B TO THE CENTRE WILL BE CALCULATED
C
C R1=(DISTAB**2)*(DISTBC**2)
C R2=((DISTAB**2)+(DISTBC**2))-(2*DISTAB*DISTBC*COS(ALPHA))
C R=R1/R2
C RAD=SQRT(R)
C
C NOW FIND THE ANGLE BETWEEN CHORD B-A AND THE RADIUS TO CENTRE
C ANGLEP=PI-ALPHA-(ASIN(RAD*SIN(ALPHA)/DISTAB))
RETURN
END
SUBROUTINE TOPOtAR(PI, AX, AY, BX, BY, RAD, ANGLE)
C THE POINT TO BE CONVERTED IS AX, AY
C COORDINATES OF THE CENTRE AROUND WHICH COORDINATES ARE REQUIRED
C ARE BX, BY
C
RAD = SQRT(((AX-BX)**2)+((AY-BY)**2))
C FIND OUT WHICH QUADRANT CHORD B-A IS IN
C
IF((AX-BX).GE.0.0.AND.(AY-BY).GE.0.0)GOT0510
IF((AX-BX).LT.0.O.AND.(AY--BY).GE.O.0)G0T0520
IF((AX-BX).LT.0.0.AND.(AY-BY).LT.0.0)G0T0530
1F((AX-BX).GE.0.0.AND.(AY-BY).LT.0.0)G0T0540
PRINT*, 'ERROR NUMBER 1'
STOP
510 ANGLE = ASIN((AY-BY)/RAD)
GOTO550
520 ANGLE = PI-ASIN((AY-BY)/RAD)
GOTO550
530 ANGLE = PI+ASIN(ABS((AY-BY)/RAD))
GOTO550
540 ANGLE = (2*PI)-ASIN(ABS((AY-BY)/RAD))
550 RETURN
END

SUBROUTINE TORECT(PI, AX, AY, RAD, ANGLE, BX, BY)
IF(ANGLE.GE.0.0.AND.ANGLE.LE.(PI/2))GOTOT10
IF(ANGLE.GT.(PI/2).AND.ANGLE.LE.PI)GOT0720
IF(ANGLE.GT.PI.AND.ANGLEJ.E.(3*(PI/2)))GOT0730
IF(ANGLE.GT.(3*(PI/2)).AND.ANGLE.LE.(2*PI))GOT0740
PRINT*, 'ERROR NUMBER 2'
STOP
710 BX = AX+(RAD*COS(ANGLE))
BY = AY+(RAD*SIN(ANGLE))
GOTO750
720 BX = AX-(RAD*COS(PI-ANGLE))
BY = AY+(RAD*SIN(PI-ANGLE))
GOTO750
730 BX = AX-(RAD*COS(ANGLE-PI))
BY = AY-(RAD*SIN(ANGLE-PI))
GOTO750
740 BX = AX+(RAD*COS((2*PI)-ANGLE))
BY = AY-(RAD*SIN((2*PI)-ANGLE))
750 RETURN
END

SUBROUTINE ANGCHECK(PI, ANGIN, ANGOUT)
IF(ANGIN.GE.(2*PI))GOTO810
IF(ANGIN.LT.0.0)GOTO820
GOTO830
810 ANGOUT = ANGIN-(2*PI)
GOTO840
820 ANGOUT = ANGIN+(2*PI)
GOTO840
830 ANGOUT = ANGIN
840 RETURN
END
Editor-in-Chief:
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A Survey of the Timing of Bud Development and Cone Initiation in *Pinus contorta* Growing in Central Scotland

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ABSTRACT


Variation in the rates of bud and cone development over 4 years was recorded in three clones of *Pinus contorta* Dougl. (lodgepole pine), growing in Central Scotland and quantitative analysis of bud development was performed. Axillary bud initiation began in late May and cone differentiation began in early to mid-July for both male and female cones. Female cones could be initiated until the end of August and male cones until mid-September. All the female cones on a bud started to develop within a few days of each other, but male cones could take up to 6 weeks to all start developing. The implications of developmental differences are discussed in relation to the physiology of the bud.

INTRODUCTION

Natural variation in the timing of cone initiation and rate of cone differentiation in the Pinaceae has created difficulties in 1) identifying the factors controlling cone initiation; 2) interpreting the function of these factors in the physiological processes leading to cone differentiation; and 3) manipulating coning experimentally to give a predictable outcome. Although vegetative bud development in lodgepole pine (Van Den Berg and Lanner, 1971; Cannell and Willet, 1975; Cannell, 1976) and coning structure of pine buds (Doak, 1935; Duff and Nolan, 1958; Couper, 1985), have been described, very little is known about variation in the time-span of cone initiation and subsequent development in pine. Owens and Molder (1975) examined the timing of cone initiation in *Pinus contorta* growing in Western Canada and described the variation in timing in two sub-species. A detailed study has now been carried out in Central Scotland to discover the time scale over which cones can be initiated in one
season and to determine how this time scale varies from year to year. The variation in timing of cone initiation has been correlated with variation in the rates of development of other bud parts to discover if it is possible to predict when coning will occur.

MATERIALS AND METHODS

One tree of each of three naturally heavily coning clones, which were raised from seed sown in 1970 and planted out in 1971 in the nursery at The Institute Of Terrestrial Ecology, Bush Estate, Penicuik, Midlothian, was sampled. Clone 8987 was an almost totally male-coning tree from an Oregon provenance. Clone 8988 was almost completely female coning while clone 8989 had male and female cones in about equal numbers; both of these trees were from a British Columbian provenance. The trees were sampled at intervals from 1981 to 1984 with most samples collected during the cone initiation period from July to October. In total, 494 buds were examined. Buds were selected from positions on the tree where male or female cones were likely to be initiated and if they had a shape which is characteristic of male or female coning buds. Terminal buds were cut from the tree at their junction with the surrounding lateral buds, fixed in formalin aceto alcohol (Johansen 1940), and stored until dissection in 70% ethanol, when the sizes of bud parts and their stages of development were measured.

RESULTS

Female cones on the same bud were all initiated within a few days of each other while male cone initiation on the same bud continued for up to 6 weeks depending on season and clone.

Table 1 shows the times at which cones were initiated in the three clones between 1981 and 1984. The period of female cone initiation was similar in clones 8988 and 8989, although clone 8988 may have initiated cones earlier in 1982 and 1984. Male cones were initiated earlier on clone 8989 than on clone 8987. Female cone initiation took place earlier in 1982 and later in 1983 than in other seasons. Male cone initiation in clone 8987 started earlier and in clone 8989 finished earlier in 1982, while male cone initiation in clone 8989 finished later in 1983.

The rates of growth of male cones on clones 8987 and 8989 were compared over a season by measuring the increase in length of the outermost secondary (2ry) cataphylls surrounding the first formed axillary bud (AB), on each bud dissected in 1983 (Fig. 1). Increases in 2ry cataphyll length are highly correlated with increases in length and diameter of the cone (Couper, unpublished data). The increase in 2ry cataphyll length in both clones could be separated into three phases. In the first, when the undifferentiated AB was initiating 2ry
TABLE 1

The timing of cone initiation from 1981 to 1984 on clones 8987, 8988 and 8989 of Lodgepole Pine growing in Central Scotland

<table>
<thead>
<tr>
<th>YEAR</th>
<th>8987 MALE</th>
<th>8988 FEMALE</th>
<th>8989 MALE</th>
<th>8989 FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>Early August to September</td>
<td>Mid to late July</td>
<td>Mid July to early August</td>
<td>Mid to late July</td>
</tr>
<tr>
<td>1982</td>
<td>Late July to September</td>
<td>Early to mid July</td>
<td>Early to mid July</td>
<td>Mid July</td>
</tr>
<tr>
<td>1983</td>
<td>Mid August to mid September</td>
<td>Early to mid August</td>
<td>Mid July to late August</td>
<td>Early to mid August</td>
</tr>
<tr>
<td>1984</td>
<td>Early August to early September</td>
<td>Mid July</td>
<td>Early July to mid August</td>
<td>Late July to early August</td>
</tr>
</tbody>
</table>

Male cone differentiation was recorded as having begun when the secondary cataphylls surrounding the basal axillary buds (ABs) on a bud had increased in length from about 500 μm to 800 μm, while the diameter of the apex of the AB had increased from 100 μm to approximately 200 μm. (Male cone development stage 1 to 2 (Couper 1985)). Male cone initiation was recorded as having stopped when no AB on a bud was found at developmental stage 2; the male cones were all larger and more developed than the undifferentiated axillary buds. Female cone differentiation was recorded as starting when an AB near the top of the main bud increased in size until the secondary cataphylls covering it were longer and its apex was taller and broader than undifferentiating ABs lower down the bud. As female cone differentiation began, the apex of the main bud started to initiate sterile primary cataphylls.

cataphylls, the increase in length was approximately 10 μm per day for both clones. In the second phase male cones started to differentiate and 2ry cataphylls increased in length by approximately 36 μm per day for clone 8987 and 46 μm per day for clone 8989. In the third phase, growth of 2ry cataphylls slowed down as cones became completely covered in microsporophylls and microsporophyll initiation ceased. Although male cones on the two clones studied showed the same pattern of changes in their growth rate, they did not initiate cones at the same time. Clone 8987 was, in terms of size and development, between 20 and 30 days behind clone 8989 throughout most of the developmental season.

Female cone differentiation began almost immediately after the female cone AB had been initiated, and its newly formed 2ry cataphylls increased rapidly in length (Fig. 2). This was in contrast to the sequence in male cones, where the AB could be present on the bud for up to 6 weeks before cone differentiation began and where the growth rate of the ABs destined to become male cones was initially slow. As in male cones, the increase in length of female cone 2ry cataphylls slowed towards the end of the growing season as the cones became dormant. For both male and female cones, actual cone size and stage of devel-
Fig. 1. Mean length of outermost secondary cataphylls surrounding a basal axillary bud (AB), before and after the AB differentiated into a male cone in 1983. Mean and 95% confidence interval of 5 buds (until 1/9/83) or three buds (last three collections) are shown. Empty symbols — no buds were initiating cones; half filled symbols — some buds initiated male cones; filled symbols — all buds had initiated male cones.

Growth reached appeared to depend on the growing conditions during the season. In 1982 cones differentiated earlier, grew faster and were larger when they stopped growing than cones initiated in 1983.

Increases in bud length and number of ABs per bud in 1983 were compared (Fig. 3A and 3B). Potential female buds were initially significantly larger than male buds, and grew in length at a faster rate (approximately 75 µm per day for both female clones; 70 µm per day for clone 8987 and 63 µm per day for 8989 males). In all the clones examined, bud growth rate increased until the end of the cone initiation period, when growth slowed. For clone 8989, male buds were smaller than female buds for most of the growing season because of having initially a slower growth rate and because they apparently began to grow later than females, as further data suggest. A slow growth rate is not necessary for male cone initiation as clone 8987 male buds during their cone differentiation period had the fastest growth rate of any of the buds collected and also the longest mean length at the end of the study.

Over the first weeks of bud development, AB initiation rate correlated highly
with increases in bud length (cf Figs. 3A and 3B). At the start of the 1983 study, potential female buds had a significantly greater number of axillary buds than male buds. AB initiation continued in clones 8988 and 8989 females until about 25th July, in clone 8989 males until 2nd August and in clone 8987 until 1st September. The terminal meristems of female buds stopped initiating fertile cataphylls before the AB primordia which were to differentiate into female cones were formed. Male cone differentiation began before the terminal meristem had stopped initiating fertile cataphylls. The final number of ABs on buds from each of the clones showed a wide range depending on position but in clone 8989 there were significantly fewer ABs in males than in females.

DISCUSSION

The variation in timing of female cone initiation in different years, although significant, was not large. All female cones were initiated within a 6-week period from early July to mid August. There was greater variation in the timing of male cone initiation, possibly because trees from different provenances were
studied and possibly because as more male than female cones are initiated per bud, more time is needed for cone initiation to be completed. Seasonal variation in the timing of cone initiation appears to be related to conditions affecting the early growth and development of the bud. Cones were not initiated until buds had reached a certain size and stage of development. In female buds cones were initiated after about 120 ABs had formed, and none were found with less than 90 ABs. In male buds, cones did not differentiate until the ABs which would form the cones were at least 0.5 mm in length and six 2ry cataphylls had been initiated. In 1982 when the rate of increase in bud length and number of ABs initiated per day was greater than in other years, cone initiation took place earlier and cone differentiation rates were faster (Couper, unpublished work). It appears that factors which influence the rate of growth of buds do so through both the timing of cone initiation and rate of bud development. In 1983 the number of female buds which were only partially differentiated at the end of the season, or which had reverted to producing needle primordia, was much greater than in 1982, so growing conditions which affect the timing of cone initiation may also affect the numbers of cones which are fully formed.
Giertych (1967) suggested that buds which produced male cones were analogous to long day plants and buds which produced female cones were analogous to short day plants. Longman (1982) found some evidence for short days stimulating female cone initiation. The present study indicates that different parts of a tree must respond differently to any photoperiodic stimulus as male and female cones on clone 8989 were initiated in the same photoperiod. Of interest is that male cones started differentiating before the terminal meristem stopped initiating ABs, while female cones started differentiating as the terminal meristem started to initiate sterile cataphylls. In other experiments (Couper, unpublished data), it has been found that lodgepole pine buds respond to short photoperiods by ceasing to initiate ABs while in long days AB initiation continues. Photoperiodic sensitivity of buds may be modified by other factors like growth rate to allow different parts of a tree to respond independently to the same photoperiod. In keeping with this idea, male buds are smaller, have fewer ABs and usually grow more slowly than female buds on the same tree. However the male buds of clone 8987 had a faster growth rate and greater final length than other buds. The absolute value of growth rates may be less important than the value relative to other buds if male cones are to be initiated. It may be significant that male buds start to differentiate later than female buds. Timing and growth rate may interact to determine the sex of a coning bud.

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REFERENCES

Owens, J. N. and Molder, M. 1975. Development of long-shoot terminal buds of *Pinus contorta*

NOTE TO CONTRIBUTORS

A detailed Guide for Authors is available upon request and is also printed in the first volume to appear each year. You are kindly asked to consult this guide. Please pay special attention to the following notes:

Types of papers published in the journal
- papers reporting results of original research
- review articles
- short communications
- editorials
- book reviews
- news and announcements.

Language
The official language of the journal is English.

Preparation of the text
(a) The manuscript should include at the beginning an abstract of not more than 400 words. It should be typewritten with double spacing and wide margins. Words to be printed in italics should be underlined. SI units should be used throughout.
(b) The title page should include: the title, the name(s) of the author(s), and their affiliation(s).
(c) Submit original plus 2 copies of manuscript (original illustrations or computer printouts plus 2 photocopies - for photographs, 3 prints should be supplied).

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