PHYSIOLOGICAL FACTORS INFLUENCING THE ROOTING OF LEAFY STEM CUTTINGS OF *TRIPLOCHITON SCLEROXYLON* K. SCHUM

by

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DECLARATION

I declare that this thesis has been composed by me. The work therein is entirely my own and has not been presented in any thesis.

Theresa Nketiah
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ABSTRACT

Triplochiton scleroxylon K. Schum (Obeche) has until recently been one of the most important timber species both in West Africa and in world markets. However the high degree of exploitation has resulted in a considerable reduction in natural populations. Reforestation efforts have been hindered by problems of seed availability. As a contribution to addressing these problems, studies of multiplication by means of vegetative propagation were carried out on saplings and mature trees planted in the field in Ghana. A low technology non-mist propagation system was used in all experiments, the first time such a system has been used in Ghana with this species.

The effect of two stockplant irradiances (direct sunlight and 50% light interception) on rooting ability of leafy stem cuttings of Triplochiton scleroxylon was studied, in combination with different cutting leaf areas and cutting lengths and variation in fertiliser (NPK 15:15:15) application to stockplants. In addition to this, the effect of the origin of the cuttings was investigated.

Shading of stockplants was found to increase percentage rooting and number of roots. Larger leaf areas (100 cm²) were associated with higher rooting percentages under both high and low light. Higher cutting length (15 cm) was also observed to increase rooting and was associated with a higher rooting percentage. Fertiliser application enhanced rooting of cuttings from stockplants grown under direct sunlight more than under shade. The heights at which tree stumps are allowed to produce coppice shoots prior to propagation was also found to have an effect on rooting. A stump height of 0.5 m produced the highest rooting percentage.

Age of the stockplants was found to have an effect on rooting of cuttings from mature tree and coppiced shoots. Coppiced shoots from mature trees rooted more readily than cuttings from branches of the same trees. The source of plant material used for propagation by cuttings also influenced rooting. In a comparison of coppiced shoots from saplings, grafted/budded and mature trees, cuttings from saplings had the highest rooting percentage.

The implications of these results for the development of reliable vegetative propagation methods for Triplochiton scleroxylon were discussed.
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CHAPTER ONE

INTRODUCTION

1.1 Status of tropical forests
Tropical forests cover roughly 50% of the total forested land area of the world, and occupy a great variety of edaphically and climatically heterogenous sites (UNESCO/UNEP/FAO, 1978). These forests probably contain about 40-50 per cent of all the world's animals and plant species, and include many genera and families which are unknown to the rest of the world (Anderson, 1990). Tropical forests exhibit a great diversity of architectural structure and of species composition. The high degree of diversity is possibly a major condition for the sustained functioning of the tropical forest ecosystem. The forest has also been a feature of man's environment from time immemorial, taken for granted, sometimes preserved, but more often cleared. It has provided many of his basic needs; shelter and water, food from wild animals and protection for his crops (Repetto, 1988a). It is only in the last twenty five years that disappearance of the world's forests has been recognised as an imminent danger to the whole global environment (FAO, 1972; Winpenny, 1991).

Forest exploitation for timber started on a small scale in the late 18th century but has become a powerful force for change since the Second World War (Repetto, 1988a). The colonial governments established forest departments in the 19th century in Asia and in the early 20th century in Africa. The legislation of these departments, and of their successors in the independent nations that subsequently emerged, included the conservation and management of a wide variety of forest resources (Poore and Sayer, 1991; Kemp et al., 1993).

By 1977, 42% of the world's tropical forest had been destroyed, leaving barely 12 million km² (Last and Leakey, 1985). At that time, the rate of continuing deforestation was estimated between 20 and 50 ha per minute. The arid and semi arid areas were expanding at a similar speed and they now amount to one third of the world's land
surface compared with about one eight at the turn of the century (Last and Leakey, 1985; Winpenny, 1991). Many of these problems are due to the use of modern techniques (for example chainsaws, tractors and sawmills) which facilitate rapid harvesting and utilization of large trees and clearing the land for agriculture, urban development and many other purposes linked to infrastructural development and colonization.

There have been numerous studies on tropical forest resources, both globally and regionally, over a number of years. The focus has been primarily on determining the availability of timber for commercial uses, rather than on the problems of deforestation, degradation and marginalization of land facing the world today. It seems clear that scant regard is often paid to the continuing value of either biotic or soil resources. Meanwhile, the relentless decline in the area of tropical forest has become more rapid, greatly exceeding both natural regeneration and reforestation (Repetto, 1988a). Thus the world is now suddenly faced with the reality of an impoverished forest resource base.

1.1.1 Importance of the tropical forest

The importance of tropical forests resides in the multiple goods and services they provide. Forests are essential for sustaining human life in the tropics. Their function in regulating the flow of water is well known - they lower the intensity of peak floods and maintain the base flow of rivers in the periods of low rainfall. According to Repetto (1988a) and Poore and Sayer (1991), a well-managed tropical forest is a constantly self-renewing resource.

Among the goods and services provided by the tropical forest as mentioned by Whitmore (1975); UNESCO/UNEP/FAO (1978); Salati (1987); Shuttleworth (1988); Repetto (1988a; b); Falconer (1991); Winpenny (1991), Longman (in press), the following are included:

1) Conservation of soil and water. For example, shading the surface soil and so reducing excessive heating and evaporation. Forests are important in the
2) Production of non-timber products. Sufficient forest and tree cover provides - fuelwood, fodder and forest produce for the people. These provide a life support system for forest-dwellers.

3) Forests provide a potentially sustainable yield of wood products for domestic use both by local communities and for regional and international markets. This is important for the generation of export earnings.

4) Maintenance of biological diversity and genetic storage.

5) Regulation of climate. Forests moderate the climate both locally and globally.

6) Recreation, tourism and aesthetic beauty. To millions of forest dwellers trees are vested with spiritual values.

7) Resources for education and research.

8) Preservation of cultural heritage.

9) Reserves of potentially fertile land for agricultural development, support for rural development and options for the future.

According to Whitmore (1975) East African savanna woodlands and their huge animal populations became internationally popular from the early 1960's, showing the potential economic value of conserving wildlife resources and eco-tourism.

1.1.2 Deforestation in the tropics

Deforestation, in the strict sense of the term, refers to the transfer of forest land to non-forest uses and includes all land where the forest cover has been stripped off and the land converted to such uses as permanent cultivation, shifting cultivation, human settlements, mining and building of dams. The term degradation refers to a reduction in the extent and quality of the forest cover due to such factors as indiscriminate logging, inappropriate agricultural management, road making methods and forest fires. According to Poore and Sayer (1991), it has been estimated that shifting agriculture has accounted for 70% of deforestation in Africa, 50% in Asia and 30% in Latin America.
Logged-over areas are generally more easily accessible and topographically more suitable for cultivation, and are easier to convert to agricultural fields (Leakey, 1986; Repetto, 1988a). In some areas, for example in Africa, ranching and cattle grazing have been the main causes of deforestation, while in others, factors like permanent agriculture, flooding for hydroelectric dams, industrial development, rapidly increasing populations and urbanization have been the causes (Winpenny, 1991). Governments impelled to raise foreign exchange earnings and employment and to finance economic development programmes, turn to the forests as a resource that can readily be exploited (Repetto, 1988a;b). Anderson (1990) mentioned that the total area deforested in the Amazon region of Brazil in the 1980's, based on LANDSAT data estimates, was 600,000 km² by the end of 1987.

Deforestation and degradation of forest resources constitute a severe threat to the ability of tropical forests to perform their vital roles of soil, water and ecological conservation and the supply of wood for fuel and other forest products to the community (Repetto, 1988a, Quarrie, 1992). A report on the state of the tropical forest cover and trends of deforestation and forest degradation shows that during 1981 to 1990, the observed deforestation was 16.9 million ha annually at a global level (annual rate of change: -0.9%). However, plantation area in the tropics is 43.9 million ha (2.5% of the total forest area) with a distribution of 8.6 million ha in Latin America, 32.3 million ha in Asia and 3.0 million in Africa. The net plantation area is estimated at 30.7 million ha (70% of the reported area). Annually planted area is reported to be 2.6 million ha and the estimated net area 1.8 million ha (Forest Resources Assessment 1990 project).

1.1.3 The consequences of deforestation and land degradation

While deforestation results in a radical change of the woody components of the forest, most forms of degradation introduce progressive changes which are not so easy to detect and quantify. Logging is a major cause of degradation in closed forests as loggers extract the best shaped trees of a few of the more valuable species without there being any guarantee that these will be replaced either in the short, medium or
long-term (Repetto, 1988a). This allows entry by shifting cultivators who do more severe damage. This type of degradation brings in its wake other more serious problems such as erosion or landslides in areas where the terrain is steep, and the ground exposed (Anderson, 1990). Inevitably, structural diversity and complexity will be reduced and modifications will occur in the process of energy and matter exchange and circulation in the boundary layers between atmosphere and vegetation (Winpenny, 1991). In many cases this will create an imbalance in the functioning of the ecosystem (UNESCO/UNEP/FAO, 1978; Anderson, 1990; Kemp et al., 1993).

Forest fires probably represent one of the most serious factors for degradation in open forest. After a severe drought in 1982-1983 in Ghana, it was estimated that as much as 50% of the growing stock in the forest reserves had been damaged by fire (Longman and Jénik, 1987), while a great fire in Borneo burned 4 million hectares of forest in the late 1980's. Fires set by farmers in Brazil burned 20 million hectares of forest in 1987 and a similar amount in 1988 (Poore and Sayer, 1991).

Throughout the recent centuries man has reduced the area of natural tropical forest by at least one third. According to UNESCO/UNEP/FAO (1978) and Anderson (1990) the current reduction rate is accelerating rapidly in South-East Asia, West Africa and Amazonia. The world resource of potentially arable land is $3.2 \times 10^9$ ha or roughly 25% of the land surface of the earth; $1.4 \times 10^9$ ha are already agriculturally or silviculturally utilized. At the present rate of forest destruction in the tropics this remaining area will have been converted later or before the end of the century, leaving natural forest only in inaccessible or extremely poor sites. Repetto (1988a;b) and Anderson (1990) reported that 95% of the soil remaining under tropical forests is infertile and easily degraded through erosion, or other processes if the vegetative cover is removed.

The annual area of forest which is destroyed by shifting cultivation and settled agriculture is estimated to be in the order of 30 to 50 million ha in South America. Timber exploitation and shifting cultivation alone will remove all primary tropical
forest on easily accessible sites within the next 20-30 years (UNESCO/UNEP/FAO, 1978). The commercially harvested industrial timber between 1960-1962 was 6.1% of the estimated tropical growing stock in Central America, 0.3% in South America, 5.6% in Africa and 2.7% in Asia (Repetto, 1988a;b; Anderson, 1990). Deforestation and degradation have become a major problem in the Ghanaian environmental scene. Small-scale mining is destroying the high forest in the exploitation of gold and diamond reserves. In fact the "gold-craze" miners are more of a threat than loggers. The scenes they leave behind are ghastly and almost irreparable. Large-scale surface-mining also poses a threat to vegetation cover in almost all high forest regions of the country.

1.1.4 The need to rebuild forest resources
There has been a considerable growth in recent years in our knowledge and understanding of tropical ecosystems and of the causes and effects of their degradation and destruction (Poore and Sayer, 1991, Quarrie, 1992). Rapid deforestation has promoted an awareness of the importance of tropical forests and is now stimulating initiatives to restore renewable natural resources through means other than the slow process of natural regeneration. In most African States, the vast majority of plantations have been established by the Government with or without bilateral or multilateral assistance (Repetto, 1988b). These plantations have usually been established with exotic species.

In West Africa, developments have been aimed at the production of cellulose or fuelwood by introducing exotic species of genera such as *Eucalyptus, Pinus* and *Gmelina* (Last *et al.*, 1984; Last and Leakey, 1985). For every 35 ha of forest cleared, only 1 ha of plantation is established. Hence, little effort has been made to replenish the indigenous species which are important in the international and domestic timber trades, which are now suffering from severely depleted resources (Last *et al.*, 1984; Last and Leakey, 1985). There is therefore a great need for silvicultural approaches to tackle the general problem with indigenous rather than exotic species, particularly because indigenous species are more valuable, of which some have been listed in
<table>
<thead>
<tr>
<th>SPECIE</th>
<th>TRADE NAME</th>
<th>USES</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Terminalia ivorensis</em></td>
<td>Emire</td>
<td>General utility and construction timber, furniture, plywood, exterior and interior joinery, mines sleepers and shingles</td>
</tr>
<tr>
<td><em>Terminalia superba</em></td>
<td>Ofram/Afara</td>
<td>Furniture, interior joinery, lamellated wood, plywood and veneers</td>
</tr>
<tr>
<td><em>Nesorgordonia papaverifera</em></td>
<td>Danta</td>
<td>Furniture, floors, interior joinery, veneers and carpentry</td>
</tr>
<tr>
<td><em>Distemonanthus benthamianus</em></td>
<td>Ayan</td>
<td>Exterior and interior joinery, flooring, cladding, naval construction and heavy carpentry</td>
</tr>
<tr>
<td><em>Nauclea diderrichii</em></td>
<td>Kusia</td>
<td>Exterior and interior joinery, floors, carpentry, marine and freshwater installations and veneers</td>
</tr>
<tr>
<td><em>Aningeria altissima/robusta</em></td>
<td>Asanfona</td>
<td>Furniture, interior joinery carpentry and veneers.</td>
</tr>
</tbody>
</table>


Silviculture should be urgently directed to the creation and maintenance of forests. The approaches generally adopted to promote reforestation are:

i) raising of commercially useful trees through Government funding and management;
ii) promoting tree planting for rural communities on communal lands, not necessarily those under the ownership of the Forest Department, organised through the involvement of the local communities;

iii) encouraging individual farmers to grow tree crops in agroforestry systems;

iv) ensuring that logging companies regenerate or replant their concessions.

Since 1980, great efforts have been made to encourage the conservation of tropical forests (Repetto, 1988b; Gillis, 1988; Poore and Sayer, 1991). A very significant event was the publication by FAO (1981) of the report of the Tropical Forest Resource Assessment Project, which gave a reasonably complete and well-documented report of the present status of the tropical forest and estimates of the rate of deforestation. Several landmark studies have already been undertaken by International Tropical Timber Organisation (ITTO), on fundamental resource conservation issues. These include the extent to which sustainable forest management for timber is practised anywhere in the tropics, the development of guidelines for sustainable forest management, and a study of the way in which incentives could encourage sustainable management (Poore and Sayer, 1991).

Furthermore, during the Earth Summit in 1992, among the various important issues discussed was combating deforestation (Quarrie, 1992). Four major areas were deliberated on;

i) To sustain the multiple roles and functions of all types of forest, forest lands and woodlands. In this regard the establishment and strengthening institutions for forest education and training, for developing an adequate cadre of trained and skilled staff at the professional, technical and vocational levels, with emphasis on youth and women was one of the activities considered.

ii) Enhancing the protection, sustainable management and conservation of all forests, and the greening of degraded areas, through forest afforestation, reforestation and other rehabilitative means. In this context, some of the objectives were to integrate programmes and or plans such as preparing the implementation of a national forestry action plans for the management,
conservation and sustainable development of forests with other land uses. And to maintain and increase the ecological, biological, climatic, socio-cultural and economic contributions of forest resources.

iii) To promote efficient utilization and assessment to help recover the full valuation of the goods and services provided by forests, forest lands and woodlands. Among the objectives considered here is to promote more efficient and sustainable use of forest and trees for fuelwood and energy supplies.

iv) To establish and/or strengthen capacities for the planning, assessment and systematic observations of forests and related programmes, projects and activities, including commercial trade and processes. The basis for action was the need to rectify this situation for a better understanding of the role and importance of forests and to realistically plan for their effective conservation, management, regeneration, and sustainable development. One of the objectives was to provide economists planners, decision makers and local communities with sound and adequate updated information on forests and forest land resources.

During the last few years there has been growing political concern about climatic change (Kemp et al., 1993). There is evidence that deforestation may contribute between 7 and 20 per cent to the total of world emission of greenhouse gases. Conversely, it is recognised that increasing the standing volume of woody biomass is one known way of withdrawing carbon from the atmosphere. This has added another element to the movement to reverse tropical deforestation.

### 1.1.5 Problems associated with reforestation

There are a number of reasons why particular care has to be taken in making decisions about land use in areas formerly covered with tropical forests. Foresters traditionally seek to improve a tree crop by exploiting genetic differences between seed collections from various places over the natural range of species (Last and Leakey, 1985). Modern methods of renewable natural resources management, which emphasize conservation as a dynamic element in the planned development, have yet to be applied
in most tropical ecosystems (Kemp et al., 1993). Kemp et al. (1976) mentioned that the desirability of conserving the genetic resources of currently uneconomic and little known species is increasingly recognised, but this can only be achieved by setting aside sufficiently large areas of natural forest for this purpose. For this to be effective much more information is needed on the areas and species most urgently in need of conservation and on the methodology through which it may be achieved (Kemp et al., 1993). Problems still remain in maintaining the genetic composition of the material, through the sampling of genotypes in the original population, their survival and growth in the conservation stands, and the mating between the genotypes ex situ (Kemp et al., 1976).

For some indigenous hardwoods there are major constraints affecting the availability of seeds. Many tropical trees flower irregularly and produce few seeds, and these often have short periods of viability, for example the West African hardwood Triplochiton scleroxylon. Even if seeds are available there is often little knowledge about their storage and germination as well as their resistance to pests. Seeds of many tropical species, particularly some trees of the evergreen wet tropical high forests, germinate immediately after seed fall and may lose their viability within weeks or even days. Attempts to overcome these and other problems have not so far been completely successful (Longman and Jénik, 1987). In addition, commitment to tend young trees and protect them is often poor, so that maintenance is not accomplished.

Another factor hampering reforestation is the time taken for a tree to reach maturity. In addition, the price paid for timber extracted from natural forest does not reflect the cost of growing the trees (Leakey, 1991). The long-term investment required by most potentially sustainable forms of land use is unattractive when inflation is high and tenure insecure. Both of these factors discourage the investment of capital in forest plantations, and as a result, most regional investment is geared toward short-term profits that can be generated either by simply clearing the forest or selectively extracting its components (Anderson, 1990).
1.2 The Importance of Tree Improvement

Since the world demand for forest products is predicted to increase greatly over the next few decades, there is an urgent requirement for large numbers of improved, fast-growing trees of shortened rotation. Although traditional forest improvement programmes have begun to pay dividends in the industrialized world, efforts have lagged behind in the developing countries where some of the most acute shortages are likely to be felt (Biondi and Thorpe, 1981).

Tree improvement is one aspect of the process of domestication. It seeks to increase the genetic quality of plants and so to achieve their greatest productivity and quality when good silvicultural practices are followed (Zobel and Talbert, 1984). Since the natural forest can no longer be expected to meet the raw material supplies of timber, wood, pulp and various minor forest products, adequate and sustained supplies of these can only be ensured through planting of improved cultivars of selected tree species on a limited land base with minimal inputs and maximal outputs (Zobel and Talbert, 1984; Kemp et al., 1993). Management of planted stands and natural forest for sustained yield rather than exploitation is needed for both indigenous and exotic species, particularly those species and forms which are useful or potentially useful to man (Longman, 1976).

Tree improvement is usually associated with artificial regeneration from genetically improved seed often derived from sources remote from the planting site (Zobel and Talbert, 1984). Forest trees which form the main sources of raw material for wood-based industries, are by nature difficult material for genetic studies and breeding. This is partly because they have a long juvenile phase and usually take many years to reach reproductive maturity. Therefore the turn-over of generations so essential for genetic studies and traditional breeding research is extremely slow. Hence forest tree improvement projects are, as a rule, long-term ones (Zobel and Talbert, 1984; Longman and Jénik, 1987).

Forest plantations are most productive when trees are tall, straight and fast-growing.
With this in mind tree breeding aims to enhance growth rate and stem straightness; to widen branch angles; to encourage smaller branch sizes in narrow, compact crowns; to improve intrinsic wood properties and to select for resistance to specific pests or diseases (Wood, 1976). The characteristics vary in their heritability and hence can be improved correspondingly to a greater or lesser extent through tree breeding. Tropical trees are usually out-breeding, and hence through the segregation of alleles during meiosis and recombination during the process of fertilization, tree progenies are genetically very variable.

More recently the role of vegetative propagation in tree improvement programmes has been emphasized by several researchers, for example Libby (1974a); Leakey (1985); Mason and Gill (1986); Hartmann et al. (1990). Workers in tree improvement are looking at vegetative multiplication of young trees as an alternative way of raising planting stock (Longman, 1976), as this allows both the capture of the heritable (additive) and non-heritable (non-additive) genetic variation. Rapid and greater improvements can be achieved by vegetative propagation and clonal selection as has been shown for some *Eucalyptus* species (Campinhos and Ikemori, 1977). Coupled with procedures to identify superior genotypes, this can result in large genetic gains in both yield and quality (Leakey, 1986). Such an approach was set up in the early 1970's as a collaborative venture by the West African Hardwoods Improvement Project, as a cooperative programme of research by the Nigerian and British Governments. Twin projects were run at Ibadan and in tropicalised glasshouses at the Institute of Terrestrial Ecology, Edinburgh. The main purpose of the project was to examine methods of providing improved planting stock of several indigenous hardwoods, but particularly for *Triplochiton scleroxylon* (Longman and Jénik, 1987). In the 1980s it was hoped that the work would be expanded as a Regional Programme involving up to seven countries West and Central Africa, as well as Britain and France (Leakey and Grison, 1985). However funding for this was never found.

Progress on improving some indigenous tropical species by cloning techniques and the selection of superior genetic stock for timber has been dramatic. *Triplochiton*
scleroxylon is among those indigenous species where advances have been made through basic research to provide a foundation for tree improvement programmes (Grijpma, 1976; Styles and Khosla, 1976; Leakey et al., 1982a; Leakey and Ladipo, 1987). Triplochiton scleroxylon is one of the major economic indigenous hardwood species of West Africa. However, it is known for its irregular flowering and erratic fruiting; in addition this species produces relatively few germinable seeds (Taylor, 1960; Jones, 1976). Since this deficiency cannot be easily compensated for it has become important to employ vegetative propagation in order to ensure a predictable supply of planting stock (Howland, 1975; Leakey et al., 1982b; Leakey, 1983; Last et al., 1984). Among the progress made so far concerning vegetative propagation is an understanding of pre- and post-severance factors which have been shown to affect rooting of Triplochiton scleroxylon (Leakey et al., 1982b); the position of the shoot from which the cutting is taken with respect to other shoots on the stockplant (Leakey, 1983; 1985); the position of the cutting within a shoot (Leakey and Mohammed, 1985) and the interactions between stockplant irradiance, light quality and nutrients (Leakey and Storeton-West, 1992). Other areas where studies have been made are ecology (Hall and Bada, 1979), clonal selection (Longman, 1978; Ladipo et al., 1983; Leakey and Ladipo, 1987) and the influence of environmental factors on growth, which is particularly related to apical dominance and branching habit (Leakey and Longman, 1986; Ladipo et al., 1991a;b) and the morphological responses of Triplochiton scleroxylon to light (Igboanugo, 1991).

1.3 The use of cloning in tree improvement
Vegetative propagation is a generally accepted tool of tree improvement programmes particularly in the production of clonal seed orchards using mature tree (Libby, 1985). In clonal propagation the vegetatively produced plants arise from a single tree of seedling origin. The unique characteristics of any individual plant are preserved by cloning, which in effect makes new plants which are genetic copies of their parent (Longman, 1993). Clonal differences are very common indeed with rooting experiments. Longman (1993) reported that although the ramets are similar to each other, their growth in many other fields, such as shoot growth, branching and
reproductive behaviour are still affected by environment, competition and chance of damage.

According to some foresters one of the main advantages of cloning is the ability to capture and exploit both the additive and non-additive components of the total genetic variance, allowing for large genetic gain in very short periods of time. Many other advantages have been listed by Biondi and Thorpe (1981); Libby and Rauter (1984) and Libby (1985), and these include:

i) the mass production of those rare individuals that have two or more favourable characteristics which are usually negatively correlated,

ii) the ability to mass propagate outstanding genotypes produced by hybridization or genetic engineering,

iii) the ability to select and utilise greater genetic diversity than is normally found in a single progeny,

iv) the ability to use clones that are well adapted to a specific site,

v) the greater simplicity and increasing flexibility of managing a set of stockplants than seed orchards,

vi) the ability to programme the planting sequence, with thinning and other activities planned by clone and position thereby reducing negative competitive interactions and increasing productivity.

Some of the disadvantages include:

i) the risk of using few clones leading to genetic homogeneity of plantations which may increase the risk of epidemics,

ii) the possibility of the root system being inferior, this will have an effect on the reliability and general usefulness of the clone,

iii) increased risk of plagiotropic growth,

iv) low initial multiplication rate and the need for a larger space associated with cloning.
1.4 Integrating vegetative propagation by means of cuttings into Community Forestry

High population growth rates with their accompanying demand for food, fuelwood, timber, non-wood products and environmental benefits have led to the removal and destruction of most of the forest resources in many areas, with increased pressures on land resources in general (UNESCO/UNEP/FAO, 1978; Quarrie, 1992). By considering all uses of land in an integrated manner, it becomes possible to make the most efficient trade-offs and to link social and economic development with environmental protection and enhancement, to achieve the objectives of sustainable development (Quarrie, 1992; FAO, 1993). The participation and involvement of individuals, communities and community organisation is essential to the effective conservation and development of forest resources.

Community forestry is not aimed at full scale timber production but deals with ways to supply customary local needs by the participation of local communities in reforestation (TEDB, 1991). Vegetative propagation by cuttings can be incorporated into community forestry programmes, where the local people use woody shrubs for tools, fuelwood and charcoal. Farmers who rely on their modest earnings from selling charcoal and fuelwood to feed the fuel-hungry urban centres, as well as the non-governmental organisations who rely on fuelwood and charcoal for their various local activities such as tree planting can use the method.

These people when educated on how to use the coppice shoots from the cut stumps to multiply their stock will benefit from a sustained yield and maintain the quality of their produce. In addition, those who use fuelwood domestically will not need to walk long distances to search for fuelwood, but rely on their local stock managed on sustainable basis. Vegetative propagation using stump sprout could be applied to multipurpose trees as well.
1.5 Objectives of study

The practical objectives of the work of this thesis are to contribute to developing technology for increased, long-term productivity of *Triplochiton scleroxylon*, by applying vegetative propagation and clonal selection techniques.

The research objectives are:

i) To study the effects of light regimes and fertilizer applications to stockplants of *Triplochiton scleroxylon* on:
   a) water status and
   b) rooting ability of leafy cuttings.

ii) To study the effect of stockplant light environment and its interaction with leaf area and cutting length on adventitious root formation of *Triplochiton scleroxylon* cuttings.

iii) To study the effects of ontogenetic age and coppice shoots from mature trees on rooting ability of leafy stem cuttings of *Triplochiton scleroxylon*.

iv) To study the rooting ability of leafy stem cuttings from different stump heights of *Triplochiton scleroxylon*. 
2.1 Characteristics of *Triplochiton scleroxylon* k. Schum

2.1.1 Distribution

*Triplochiton scleroxylon* K. Schum is a deciduous tree which belongs to the family Sterculiaceae. It grows within moist semi-deciduous to dry semi-deciduous forests from Sierra-Leone in the West to the Central African Republic in the East (Fig. 2.1). It is most abundant in Nigeria, Ghana and Ivory-Coast, (Jones, 1976; Hall and Bada, 1979). Available information varies from territory to territory within the range. The barriers constituted by the Dahomey gap and the Cameroon Highlands partition the range into three sections.

2.1.2 Botanical description

The species is moderately fast-growing and regarded as a colonizer of disturbed forest (Howland *et al.*, 1977). *Triplochiton scleroxylon* reaches a height of 50 m and girth approaching 6 m, with buttresses. The lower stem is usually self-pruned and so free from branches such that clear timber in large dimensions can be obtained. The crown is rounded, wide and dense. The leaves are palmately lobed and fruits winged. The leaves of saplings and coppice shoots are often larger and more deeply lobed than the crown leaves (Keay, 1989).

The optimal temperature at which *Triplochiton scleroxylon* grows is in the range from 20°C to 35°C (Hall and Bada, 1979). The species has been noted normally to occur in clusters of ten or more and isolated trees are uncommon. This is one of the species characteristic of large areas of West African high forest that is called the Triplochiton-Celtis Association. It is said to be the commonest tree in this association representing up to 13% of the forest. Richards (1952) suggested that shifting cultivation
Fig. 2.1. The distribution of *Triplochiton scleroxylon* (stippled). Areas of greater abundance (heavier stippling) based on Aubréville (1957) for Ivory Coast, Kinloch (1945) and Taylor (1960) for Ghana, and Redhead (in press) for Nigeria.

Source: Hall and Bada (1979).
in West Africa has also influenced the natural distribution of *Triplochiton scleroxyylon*. Jones (1976) also suggested that the pattern may have developed either because of shifting cultivation or because the species may naturally occur in clusters. *Triplochiton scleroxyylon* is known for its irregular flowering and erratic fruiting. MacGregor (1934), Mackenzie (1959), Taylor (1960) and Jones (1974) reported that mast years occur in the months following an exceptionally short dry season (July - August). According to Mackenzie (1959), attempts have been made to correlate heaviness of crop with the previous weather patterns. Out-of-season flowering has been reported on mature potted grafts in forest nurseries (Howland and Bowen, 1977) and in tropicalised glasshouses in Edinburgh (Leakey et al., 1981). In both instances seeds have been found to be viable. *Triplochiton scleroxyylon* flowers can be found annually in limited quantities (Danso, 1970). Phenological studies conducted on *Triplochiton scleroxyylon* in Nigeria have confirmed that flowering can be an annual event, although the overall intensity is extremely variable from area to area and at different times during the flowering season (February-April) (Richards, 1952; Jones, 1974;1976). This might be the result of conditions (for example drought) for flowering occurring at different times from area to area. The few fruit collections were found to be of poor quality and short lived. This might be attributed to biological factors such as ovule abortion and pathogen infections. Ovule abortion could be related to the loss of suitable conditions for flower bud development. Pest and pathogen attacks are usually due to *Apion ghanaense* and *Mycosyrinx* Beck respectively. The viability of the seeds of *Triplochiton scleroxyylon* has been found to be only 2 - 3 weeks at room temperature, but with drying to 8% moisture and storage at -18°C they remain viable for at least 18 months (Bowen et al., 1977; Howland and Bowen, 1977).

According to Jones (1974; 1976) reforestation with the species is hampered by lack of seeds, but recently, progress has been reported with the development of cloning techniques (Leakey et al., 1982a; Leakey and Ladipo, 1987). Interest has been stimulated in deriving clonal planting stock through vegetative propagation techniques.
2.1.3 Timber properties and uses

*Triplochiton scleroxylon* produces the timber known as Obeche (Nigeria), Wawa (Ghana), Samba (Ivory Coast), or Ayous (Cameroon). This timber became popular during the second world war (Anon., 1966) and is a Class 1 timber. The excellent working qualities of the timber and the plentiful supply of logs made *Triplochiton scleroxylon* a leading export timber of West Africa, constituting about 60% of roundwood exported between 1950 and the late 1960's. It is an easy-to-work, light, general-purpose whitewood, which is used locally for construction, match splints, boat building and plywood (Farmer, 1975). The species have been heavily exploited, and as a result, exportation of the timber has been banned from several West African countries, for example Nigeria (Igboanugo, 1991).
CHAPTER THREE
VEGETATIVE PROPAGATION METHODS

3.1 Introduction
Vegetative propagation methods are used to maintain specific characteristics since each propagated individual has all the genetic traits of the plants from which it was derived. However their growth can also be affected by other factors such as environment, competition and chance of damage. In the absence of a reliable seed source, vegetative propagation also offers an alternative way of providing planting stock (Howland and Bowen, 1977; Hartmann and Kester, 1983). The different methods of vegetative propagation are described below.

3.2 Grafting and budding
In grafting methods a piece of shoot (scion) is transferred from the desired tree and fused to the rootstock of another plant to produce a complete new plant (Komissarov, 1969). Grafting and budding are methods used to join parts of two or more different plants together. In grafting, a small piece of shoot is used, while in budding only a single bud is used. However, in both cases the growth of the scion or bud is encouraged to form a new tree. Thompson (1984) and Zobel and Talbert (1984) described grafting as:

i) a well-understood method for vegetative propagation that avoids the problems of inducing root formation.

ii) a method for the establishment of clonal archives and clonal seed orchards.

iii) useful in situations where early flowering and seed production is required as in horticulture. Often mature tissue is used as the scion for fruit tree species.

For satisfactory results, grafting should be done at a time when conditions are favourable and the tissues are active. Scions can be stored for a short period, without being cut up into the desired lengths, in a cool, moist place, to protect them against desiccation and changes of temperature. However, it is necessary to keep them as fresh as possible and to retard their development.
Hartmann and Kester (1983) reported that the major problem with grafting is incompatibility between the stock and the scion. Loss of whole clones can be serious, especially if the lost clone happens to be one of the best genotypes. However, grafting success is greatest between closely related plants (Hartmann and Kester, 1983), and the success of a graft depends on the matching of the cambial layers of the scion and root stock. Because it is labour intensive, grafting has not been used in reforestation programmes, although it is used commercially for rubber production.

3.3 **Tissue culture**

In tissue culture, cells in almost all parts of a plant can be induced to form callus, however, greatest success has been with juvenile tissue, and if given proper stimulus a new plantlet could be initiated. This technique can enable the plant breeder to produce and multiply valuable clonal stock and hybrids for testing and for production planting. Again, failure to produce a number of desirable interspecific and intergeneric crosses due to inadequate development of ovules or ovaries to form viable seeds has raised interest in the technique of embryo culture (Konar and Nagmani, 1974; Hulse, 1992). The practical applications of plant organ, tissue and cell culture include:

i) shoot tip cultures to give virus-free stock,

ii) rapid propagation of stock to produce very large clonal populations, for example, strawberries or bananas,

iii) for propagation when normal macro-techniques are not available, for example oil palm, coconut palm,

iv) storage and genetic preservation of plants with poor seed viability,

v) production of homozygous plants for plant breeding using anther culture, for example wheat,

vi) easy transfer from one country to another.

Various tissue organ culture techniques have been used for propagating forest trees. These include shoot tip culture and nodal bud culture (micropropagation), callus culture (organogenesis), and somatic embryogenesis. The most commercially used in
vitro methods of micropropagation are shoot tip and nodal bud cultures (Murashige, 1974; Biondi and Thorpe, 1981; Suttle, 1986).

Many plant species have been propagated using tissue and organ culture. Among them are some tropical trees, which have been successfully micropropagated by these methods. They include *Theobroma cacao* (Orchard *et al*., 1979; Passey and Jones, 1983), *Mitragyna parviflora* (Roy *et al*., 1988), *Tectona grandis* (Gupta *et al*., 1980) *Khaya ivorensis* and *Nauclea diderrichii* (Mathias, 1988).

Advantages of using tissue and organ culture include:

i) large numbers of plants can be multiplied more rapidly,

ii) there could be restrictions on the starting material, these include: a) phase change, b) getting the tissue free from microorganisms c) persuading the cells to de-differentiate,

iii) less space is required,

iv) by treating shoot tip cultures plants can sometimes be produced free of viral infections,

v) limited numbers of stockplants need to be maintained,

vi) plantlets can be stored indefinitely if necessary.

Some disadvantages of tissue culture are:

i) there is the need for skilled labour and more specialised facilities than are required for conventional propagation methods,

ii) much research work is usually required to propagate a new species successfully,

iii) techniques are more complex and less well understood than conventional vegetative propagation,

iv) it is capital-intensive,

v) there are greater risks of problems, such as widespread contamination of the cultures.

vi) there is the chance of losing everything (example by power cut) except where
electricity supplies are very stable.

However, these techniques are likely to be increasingly used in the future because (a) theoretically plants can be produced in large numbers (b) the cost of using suspension cultures could be very low as plantlets do not have to be handled or subcultured individually, (c) in vitro propagation may be economically acceptable particularly if planting large areas, (d) actually it is well-known that tissue cultures quite often involve sizeable genetic changes - partly why micro-propagation with meristem culture is favoured by some workers. Recently, phenotypic variation has been observed in plants regenerated via embryogenesis (Evans and Bravo, 1986).

3.4 Air layering
Air layering is the stimulation of roots on an intact branch. This is done by girdling the stems, and removing a ring of bark and applying auxins. This method produces new propagules directly on their own root systems so avoiding incompatibility.

A number of tree species have been propagated successfully with air layering, for example Pinus taeda (Hare, 1975); Pinus oocarpa, Pinus caribaea (Lowery, 1980); Cryptomeria japonica and Pinus patula (Lahiri, 1981), Bamboo (Ramanuja Rao et al., 1992). Stoltz and Hess (1966); Thompson (1984) mentioned that stem girdling promotes accumulation of current photosynthate in the shoot so increasing carbohydrate reserves and increasing the rooting response. Air layering can be used in plants in which success with rooted cuttings is marginal.

However, this technique is usually slow and laborious and has rather low rates of multiplication. Even though pretreatments have shown promise, the added expense has prevented the use of air-layering in large-scale propagation schemes.

3.5 Rooting of leafy stem cuttings
Vegetative propagation can be achieved by rooting stem, root or leaf cuttings. Rooting of leafy stem cuttings is the vegetative propagation method currently most used for
deciduous and evergreen tree species. The techniques and methods developed are numerous but in all these a portion of stem is cut from the parent plant, and placed under humid environmental conditions to form roots. This produces a new independent plant, which is genetically identical with the parent plant. There are many systems used to provide an optimum propagating environment for cuttings to root, among them are: mist (high technology) and non-mist (low technology) systems.

3.5.1 Propagation environment

i) Types of propagation systems

a) Mist

Propagation of leafy stem cuttings requires that the cuttings are kept free from drought. To solve this problem the cuttings must be maintained without wilting until roots are produced. Intermittent misting has become the standard method of providing these conditions for cutting propagation. An electrically timed system provides a film of water droplets over the leaves, which lowers leaf temperature and increases humidity, reduces transpiration and respiration. This mist technique has made it possible to propagate plants previously found to be difficult to root. Intermittent mist keeps slow-rooting cuttings alive for a long period of time, giving them a chance to root before they die from desiccation (Hartmann and Kester, 1983; Dirr and Heuser, 1987). In addition, intermittent-mist reduces the volume of water applied to the cuttings and may avoid problems of excess moisture. The mist system has many components and usually involves capital investment with foreign currency to create the propagation facility.

Large numbers of cuttings with leaf areas of 30-200 cm² can be rooted by the use of mist propagation techniques. Many tropical tree species have been propagated by this method such as *Triplochiton scleroxylon* and *Lovoa trichiliodes* (Leakey, *et al.*, 1982b; Tchoundjeu, 1989).

b) Non-mist

In order to extend rooting of cuttings into rural areas of tropical countries, a low
technology system has been developed by the Institute of Terrestrial Ecology in Edinburgh in association with its field projects (Leakey et al., 1990). A non-mist propagating method was used in Nigeria by Howland (1975) and later modified by Leakey and Longman (1988) and Leakey et al. (1990). Basically the non-mist propagator does not require daily watering or a piped water supply (or electricity supply). The frame is made of wood or metal, and the entire frame is enclosed with clear polythene film (Leakey, 1989). The base of the propagator is covered with fine sand followed by larger stones to a depth of 10-15 cm. These stones are then covered by successive layers of small stones and gravel to a depth of 20 cm (Leakey et al., 1990). The rooting medium is supported by the gravel, while the air spaces of the stones are filled with water.

Root initiation in leafy stem cuttings requires environmental conditions that minimise physiological stress in the cuttings. In the non-mist system this is achieved by shading (using either plastic shade cloth, or other materials such as palm leaves) which also lower the air temperature and increase the relative humidity, so reducing transpiration losses. In addition, this ensures that the vapour pressure of air in the atmosphere surrounding the cutting is maintained close to that in the intercellular spaces of the leaf (Leakey et al., 1990). This method had proved successful for many tropical tree species, such as *Triplochiton scleroxylon*, *Lovoa trichiliodes* and *Nauclea diderrichii* but is especially good for dry zone species such as *Acacia tortilis* and *Albizia guachapele*.

3.5.2 The capacity of stem cuttings to form roots

There are several ways to assess the status of cuttings: the colour of the leaves and stems, firmness of wood and stage of bud development are suitable indices for different plant species (Dirr and Heuser, 1987). The variables often used to describe the capacity of stem cuttings to form roots as listed by Leakey (1985) are as follows: (i) the percentage of cuttings rooted, (ii) the number of roots per rooted cutting, and (iii) the rate at which roots emerge and grow. The ability of cuttings to root varies with the tree species, between clones within species and among plants within clones,
and between shoots within plants.

Thompson (1984) mentioned that hardwoods are generally easier to root than conifers, with the exception of Japanese cedar (*Cryptomeria japonica*) and coastal redwood, which are comparatively easy conifers to root, and black walnut (*Juglans nigra*) and the oaks (*Quercus* spp.) which are difficult hardwoods to root. These differences may be due to many factors:-(a) lack of endogenous auxins, phenolic or other co-factors, (b) lack of enzymes, (c) presence of inhibitors or, (d) the presence of enzymes that oxidise or degrade auxins or their co-factors. The variations among plants within clones may be due to the physiological condition of the stockplant. These conditions can be influenced by (i) the environment and season, (ii) the position of the harvested shoots on the plants, (iii) the age and size of the tree and (iv) incidence of pathogens, virus particles and mycorrhizal organisms (Howard, 1972; Rauter, 1982; Hartmann and Kester, 1983; Leakey, 1983). Among these, age of the tree is perhaps the largest limiting factor because rooting response is greatest in young seedlings and declines with age.

Treatments applied directly to cuttings strongly influence the ability to develop roots in the following ways: (a) chemically, by application of auxins, other growth regulators, rooting co-factors, minerals and fungicides; (b) physically, either by influencing the size of the cutting, its leaf area, or by wounding or damaging the base, and (c) environmentally, by manipulation of humidity, light, temperature and the type of rooting medium used (Rauter, 1982; Leakey, 1985).

### 3.6 Factors affecting rooting in leafy stem cuttings

#### 3.6.1 Stockplant management

Attention has been focused on the effects of stockplant environment on the successful rooting of cuttings. Heide (1964;1965); Hansen (1975); Andersen (1986) reported that the stockplant environment exerts a strong influence on root formation in stem cuttings, on root and bud formation in leaf cuttings. The physiological condition of the stockplant is the result of the interaction between genotype and environmental factors.
3.6.2 Environmental factors affecting stockplants

i) Light

Light influences many physiological processes which subsequently affect plant growth and development as well as being the source of energy in photosynthesis. The importance of light depends on the quantity of irradiance, the photoperiod and the light quality.

Increased irradiance may inhibit or delay rooting, in for example *Hedera helix* (Poulsen and Andersen, 1980), *Pinus sylvestris* (Hansen *et al.*, 1978; Hansen and Ernsten, 1982), *Pisum sativum* (Hansen and Eriksen, 1974; Andersen *et al.*, 1975), *Populus tremula* (Eliasson and Brunes, 1980). On the other hand, increased irradiance has been shown to promote rooting, for example in *Begonia x Cheimantha everet* (Heide 1965) and *Campanula isophylla* (Moe, 1976; 1977), or to have no effect on rooting, as for example in *Hibiscus rosa-sinensis* (Andersen, 1986). Cuttings from trees of temperate and tropical origin root more readily when stockplants are kept at irradiances well below the photosynthetic saturation point, for example *Pinus sylvestris* (Hansen *et al.*, 1978), *Populus* and *Salix* (Eliasson and Brunes, 1980). High irradiance rather than low was found to increase auxin transport and accumulation of auxin at the base of pea plants (Baadsmand and Andersen, 1984). It has been shown by Eliasson (1971) and Tillberg (1974) that concentrations of certain natural inhibitors are higher in plants grown under high light than in etiolated plants. These inhibitors may reduce rooting, although this effect has not been proved (Hartmann and Kester, 1983).

Studies on the effects of light intensity on *Khaya ivorensis* stockplants on rooting have shown that high stockplant irradiance had an inhibitory effect on rooting ability while low irradiances were associated with higher rooting percentages (Tchoundjeu, 1989). Similar results were obtained with *Triplochiton scleroxylon* (Leakey and Storeton-West, 1992). The above experiments were performed under artificial light, which has a different spectral composition from natural light.
Photoperiodic responses are particularly important for the promotion of flowering and bud dormancy. However, it seems that there are also effects of stockplant photoperiod on rooting. Short day treatments to stockplants inhibited rooting of cuttings, while long days promoted rooting (MacDonald, 1969). Smith and Wareing (1972) concluded that short days given to stockplants of a "long-day" plant like *Populus* do indeed reduce the number of roots in *Populus* cuttings, compared to long days. In "short-day" *Begonia* species (Heide, 1965) and *Chrysanthemum* species (Heins et al., 1980), there were more roots on cuttings from stockplants grown in long days than those exposed to short days. This may be due to auxin transport since auxin is known to be influenced by irradiance (Baadsmand and Andersen, 1984) and also cuttings without auxin supply form few roots if any (Eriksen, 1973).

Sources of light with a high Red to Far-Red ratio stimulate lateral branching of stockplants (Moe and Anderson, 1988). However, rooting is enhanced when cuttings are taken from the lower rather than from upper portion of both herbaceous and woody stockplants (Loreti and Hartmann, 1974; Roulund 1973; Moe, 1973; Poulsen and Andersen, 1980; Leakey, 1983). This effect may partly be attributed to the shading effect of the canopy, which filters out the Red radiation leaving a proportionally higher flux density of Far-red radiation at the bottom of the canopy. Light quality may regulate regeneration and development of roots and shoots through its effects on the balance between cytokinin and auxin. Studies of light quality on *Triplochiton scleroxylon* stockplants and its effect on rooting ability have shown that increasing the Red to Far-red ratio decreased both the rate of shoot growth and rooting ability (Leakey and Storeton-West, 1992).

ii) **Temperature**

Temperature changes control the metabolic processes of plants and so affect growth and development of stockplants. Heide (1964) carried out an experiment with different air temperatures, and showed that a temperature range of 12 °C to 22 °C had relatively little influence on the mass of roots produced on single leaf cuttings of *Begonia* species. However, bud initiation on these leaf cuttings was severely inhibited by high
stockplant temperature. Similarly, Moe (1977) reported that although rooting was unaffected by stockplant temperature, leafy stem cuttings of *Campanula isophylla* had a higher dry weight if taken from stockplants grown at higher (18-22 °C) temperatures than those grown at 15 °C. These investigations based on experiments with a few herbaceous species point to a minor role of stockplant air temperature in subsequent rooting of cuttings.

iii) Water

The water status of a stockplant plays an important role in several physiological and biochemical processes related to root formation. Too much or too little water may be harmful to the rooting process (Loach, 1988; Andersen, 1986). Evaluating the effects of stockplant drought on rooting include growing the plants under various drought regimes. Exposure of stockplants to a long period of drought resulted in poor rooting under both high and low irradiance and these results have been shown to be attributed to the effect of abscisic acid (Rajagopal and Andersen, 1980a; Rasmussen and Andersen, 1980). However, Kawase (1965) proposed that ethylene produced in the shoot may be responsible for the induction of roots.

Drought was, however, found to reduce rooting in Norway spruce, (Stromquist and Eliasson, 1979). Leakey (1983) found that low leaf water potentials of lateral shoots of *Triplochiton scleroxylon* stockplants were related to less extensive rooting, although in this experiment cutting length was the major factor affecting rooting. Although water stress influences rooting, the type of response probably depends on more general environmental influences to which stockplants are exposed since drought also arises due to high temperature and high irradiance. A close relationship between rooting of a number of species and the measured foliar water potential has been found (Darbyshire, 1971; Hsiao, 1973; Loach, 1977; 1988a; Grange and Loach, 1985).

iv) Nutrients

The importance of using healthy and vigorous stockplants has been reported by Haun and Cornell (1951) who showed that availability of mineral nutrients influences rooting
of cuttings. Dirr and Heuser (1987) showed that nutrient deficiencies in the stockplant are detrimental to rooting. Cuttings of *Juniperus chinensis* and *Ilex crenata* collected from plants grown for two years under severe boron, iron, manganese and zinc deficiencies generally rooted with lower percentages than cuttings taken from plants grown with complete nutrient solutions.

Nutrient application to stockplants at the optimum rate for maximum shoot growth has rarely been mentioned to be optimal for production of well rooted cuttings, since some evidence suggests that lowering the mineral nutrition status of stockplants encourages rooting. Depending on the species and environmental conditions, rooting may be increased if stockplants are supplied with a combination of low to moderate nitrogen availability together with other macro- or micro- nutrients ordinarily required for satisfactory plant vigour (Haun and Cornell, 1951; Eliasson, 1978). Leakey (1983) found that when fertilizers were given to *Triplochiton scleroxylon* stockplants they enhanced the rooting ability of only the suppressed shaded basal shoot. Leakey and Storeton-West (1992) showed that stockplants of *Triplochiton scleroxylon* grown at high irradiance with fertilizer application rooted better than stockplants with high irradiance but without fertilizer. On the other hand, at lower irradiance those without fertilizers rooted better than those, with fertilizers.

Veierskov *et al.* (1982a) studied the carbon/nitrogen (C/N) ratio in different pea plants and obtained cuttings where the C/N ratio varied from 0.9-1.6, but no significant correlation to root number was observed. Nitrogen fertilization altered the C/N ratio within the plant, and which therefore modified the carbohydrate pool. Many authors, including Hansen *et al.* (1978), Davis and Potter (1981), and Veierskov and Andersen (1982), have shown that whereas the content of amino acids in the cutting changes a little during rooting, a large accumulation of carbohydrates occurs. However, cuttings are normally unable to increase the content of nitrogen after excision, at least until new roots are formed, but they may alter the different pool concentrations of carbohydrates. Hence, rooting may be related to specific pools rather than to total contents (Haissig, 1986).
Mineral nutrition probably influences a variety of endogenous biochemical responses that cause variation in stockplant growth. However, mineral nutrition of stockplant that maximises vegetative growth may inhibit rooting, and further increases in mineral nutrition may be even more inhibitive to rooting (Haissig, 1986). Excessive nitrogen in particular has been shown to reduce the rooting of cuttings. For example, the results obtained for *Khaya ivorensis* (Tchoundjeu, 1989) were contrary to those reported for *Triplochiton scleroxylon* by Leakey (1983), and showed that different concentrations of complete fertilizer had little effect on rooting ability until very high amounts were applied. In this case low rooting percentage was associated with increased cutting mortality.

While fertilizer application to stockplants does seem to affect the rooting process, it is also clear (Struve, 1980) that there can be benefits to the cutting after rooting has occurred. Fertilizer-treated cuttings have been shown to have more profuse root systems and grow better when transplanted.

### 3.6.3 Physiological Influences within stockplants

1) **Plant hormones**

Plant development is influenced by interactions between several plant growth regulators, of which auxin is one. Auxins have long been known to play an important role in cellular differentiation and to influence root initiation in stem cuttings. Andersen *et al.* (1975) reported that auxins comprise the only group of chemicals which consistently enhance root formation in naturally responsive or easy-to-root cuttings. Auxins usually induce the formation of a greater number of roots per cutting (Wightman and Thimann, 1980; Wightman *et al.*, 1980; Hartmann and Kester, 1983), hasten rooting and increase the numbers of cuttings rooted. Young leaves and active buds are sources of auxin and may enhance rooting in some cuttings. Auxin is transported preferentially in a basipetal direction, an observation consistent with the polarity of root formation (Haissig, 1974).

Work has been done on non-woody cuttings to establish endogenous concentrations
of auxin in the region of root regeneration. Weigel et al. (1984) suggested that the number of roots initiated per cutting may be a function of the amount of auxin-like substances in the region of regeneration. Studies on intact apical cuttings of *Chrysanthemum morifolium* to determine the auxin contents during root regeneration showed that the greater the endogenous auxin content of cuttings at the time they were taken, the greater the number of roots formed (Biran and Halevy, 1973). However, both free and total indole-acetic acid contents increased markedly from the time cuttings were taken, although declining contents were evident after roots became visible. Moreover, it is still not known whether maximum auxin content of the cuttings precedes, coincides with or follows initiation. The presence of buds is actually an essential pre-requisite for the cutting rooting in most plant species, and is often needed just to keep it alive. Presence of buds on cuttings has little or no effect on rooting for instance in *Chrysanthemum* (Fischer and Hansen, 1977). The influence of buds on root formation is probably due to the supply of auxin. Active shoots on cuttings, on the other hand, can act as competition for assimilates and so reduce rooting (Hansen, 1988).

Gibberellins are growth substances which do not appear to be required for the initiation of adventitious rooting in cuttings. The irradiance at which stockplants are grown have been considered to influence the rooting response to gibberellin in *Pisum sativum* cuttings (Hansen, 1975; 1976). Nanda et al. (1967) reported that a low irradiance or short photoperiod during stockplant growth or a short photoperiod during rooting may provide the basis for a stimulation of root formation by gibberellin. Hartmann and Kester (1983) noted that gibberellin prevent the early cell divisions involved in transformation of mature stem tissues to a meristematic condition. However, according to Hansen (1988) changes in response to gibberellin during the process of root formation indicate that different developmental stages of root formation have different degrees of sensitivity to gibberellin.

Abscisic acid has been reported to oppose the action of gibberellins or cytokinins which inhibit adventitious rooting (Javis, 1986). Davis and Sankhla (1988) mentioned
that abscisic acid may also influence stress tolerance of plants which may be significant in cuttings, because cuttings undergo considerable stress upon excision from the stockplant. Stomatal closure is induced by abscisic acid which might thus influence photosynthesis and transpiration by the cuttings.

Many researchers have reported the effect of abscisic acid, which may promote (Rasmussen and Andersen, 1980), inhibit (Heide, 1968), or have no effect (Biran and Halevy, 1973) on rooting. Rasmussen and Andersen (1980) revealed that factors such as abscisic acid concentration, length of rooting period, and stockplant growth conditions all influence the response of pea (*Pisum sativum* L.) cuttings to abscisic acid.

ii) **Carbohydrates**

The carbohydrate status of the stockplant may influence rooting, although high initial carbohydrate contents have not always been associated with high rooting potential. Correlation of rooting responses with irradiance is in itself inadequate evidence of an involvement of any carbohydrate in adventitious root development (Javis, 1986). For example, a negative correlation between carbohydrate contents and rooting was observed where increased stockplant irradiance caused an increased content of carbohydrates but diminished root number (Hansen *et al.*, 1978; Veierskov *et al.*, 1982b). It has been found that the failure of *Populus tremula* to root is not caused by insufficient carbohydrate reserves, and that starch content was not related to rooting (Nanda and Anand, 1970; Okoro and Grace, 1976). It was noted in these cases that the initial carbohydrate content must be sufficient to supply the cutting with energy reserves throughout the rooting period for optimum rooting to occur under conditions when photosynthesis is limiting. The role of carbohydrate is most evident in leafless cuttings from etiolated material (Davis and Potter, 1981) which have very low contents of stored reserves and hence root poorly. Thus, it is clear that the carbohydrate content of the stockplant has some important effects on the rooting ability of cuttings at least under some conditions. However, there is still an incomplete understanding of all the aspects of a cutting's carbohydrate budget and the interactions with other factors.
affecting rooting. For example, Baadsmand and Andersen (1984) reported that altering the level of irradiance to stockplants grown in growth chambers causes an increase in leaf temperature, and altered auxin transport and accumulation in the subsequent cuttings. Sometimes carbohydrate content and rooting may be positively correlated, such as when the supply of current photosynthate is insufficient to support optimal rooting.

iii) Position of shoots on stockplants

Rooting ability has been found to vary between cuttings from different parts of the same plant (Roulund, 1973; Loreti and Hartmann, 1974; Hartmann and Kester, 1983). This might be attributed to the poorly understood concept of juvenility. During the development of plants, their life cycle passes through a series of qualitatively different stages. These changes are then transmitted to new cells, resulting in a phased diversity of qualities of the tissue along the stem. The differences in ontogenetic age of the tissues from the top to the base may influence rooting of cuttings taken from different part of the plant (Roulund, 1973). An alternative hypothesis is that these effects of position involve the combination of many factors such as internode length (Veierskov, 1978), carbohydrate and auxin status (Nanda et al., 1970), lignification, leaf age (Leakey, 1985), and topophysis (Dirr and Heuser, 1987) and competition between shoots (Leakey, 1983). A study of the within-shoot variation in rooting ability in *Triplochiton scleroxylon* showed that cutting length and probably cutting diameter explained most of the variation in rooting ability (Leakey and Mohammed, 1985). A study of the between-shoot variables in *Triplochiton scleroxylon* cuttings showed a relationship between percentage rooting and the number of shoots on the stockplant, with the highest rooting percentage obtained from plants with the fewest shoots (Leakey, 1983). Subsequently, using 2-shoot stockplants, cuttings from the basal lateral shoots rooted better than those from apical shoots, this apparently being the effect of position on the shoot's microclimate, especially light. A similar pattern was observed in *Lovoa trichilioides*, but the reverse was recorded in *Khaya ivorensis* (Tchoundjeu, 1989).
iv) Juvenility
Cuttings taken from chronologically young (juvenile) stockplants are much more liable to root than cuttings from older plants. Juvenility may be defined as a transition phase between germination and flowering and fruiting (reproductive maturity). Juvenility is attributed to many causes but the actual mechanisms are largely unknown. It has been postulated that as the plant matures the concentration of root inhibitors increase. Paton et al. (1970) reported this in *Eucalyptus* species. Similarly, phenolics decrease in some plants with age and this may also affect rooting response since they are thought to function as co-factors with auxin in the rooting process (Hess, 1961; 1963). Other growth regulators such as cytokinins and gibberellins have also been shown to affect rooting when sprayed on various species, and have also introduced a measure of "juvenility" (Dirr and Heuser, 1987). Most nurserymen delay maturation by using stockplant management regimes that maintain juvenility. This is done by frequent pruning.

v) Physiological ageing
The formation of adventitious roots in cuttings depends on external conditions and in particular on the physiological condition of the cuttings. This is influenced by the age of the maternal plant as a whole and that of its individual parts taken for propagation by cuttings. The capacity of both intact trees and cuttings for producing adventitious roots is correlated with their age. After some critical tree age is reached, rooting capacity declines rapidly (Kozlowski, 1971). Ageing involves reduction in vigour and associated changes which occur as the tree gradually increases in size and complexity (Wareing, 1987). The major practical consequences for vegetative propagation relate to the effect of the age of the parent plant and location of the propagule on the parent plant and its ability to grow as a new tree (Clark, 1981). Among the major manifestations of ageing include a decrease in metabolism, reduced growth of vegetative tissue, and an increase in dead branches.

There is frequently much confusion between changes associated with reproductive maturity (phase change) and physiological ageing. The transition from the juvenile to
the mature flowering state is accompanied by a range of changes, which are not readily reversible if cuttings or scions are taken from the mature parts. This phenomenon is known as phase change (Brink, 1962). Changes related to phase change may be distinct to changes associated with physiological ageing. Many authors such as Schreiber and Kawase (1975), Poulsen and Andersen (1980) and Leakey (1983) have observed that ease of adventitious root formation declines with the physiological age of the stockplants. The effect of ageing on shoot growth varies with species, environmental fluctuations and site conditions. Moorby and Wareing (1963) studied ageing in shoot growth of *Pinus sylvestris* and concluded that ageing involves increased competition among shoots for nutrients, as a tree or branch system increases in complexity, such that intact leading shoots obtained larger quantities of mineral nutrients than did higher order laterals. Leakey (1983) in his studies on *Triplochiton scleroxylon*, also found that cuttings from uppermost shoots root better than those of lower shoots. This he attributed to inter-shoot competition, but how competition is imposed is not known. However it has been observed that there are a number of different factors that independently affect rooting and at the same time change as trees increase in size and complexity (Leakey, 1985). Therefore, the critical characteristics of shoots for vegetative propagation have not been sufficiently defined.

### 3.6.4 Factors which affect rooting during propagation

I) Environmental factors

a) Rooting media

Cuttings of many plant species will root easily in a wide range of rooting media. Plants which are more difficult to root may be influenced more strongly by the rooting medium. The optimal requirements for rooting of cuttings can be specified in terms of the physical characteristics of the medium, and the relative proportion by volume of air and water. The rooting medium has three functions (Hartmann *et al.*, 1990) namely:

i) to hold the cuttings in place during the rooting period,

ii) to provide moisture for the cutting,

iii) to allow air to the base of the cutting,
Therefore, the best media should allow adequate air to the base of the cutting to prevent waterlogging and rotting, provide porosity, have a high water holding capacity and be well-drained (Andersen, 1986). Comparative trials of different rooting media show that, while the nature of the medium can substantially influence rooting, results differ between species and are not necessarily reproducible in repeated propagations (Loach, 1988). Many rooting media have been proposed over the years but gravel and sand are the most popular both for mist and non-mist propagators (Howland and Bowen, 1977; Leakey et al., 1990).

b) Temperature

Temperature is one of the important factors influencing cambial activity and could therefore be expected to be a factor influencing the rate of root formation in cuttings. Generally, longer roots are formed with higher propagation temperatures, for example in Begonia (Heide, 1964). Experiments have shown that the optimum temperatures for the formation of callus and of roots in cuttings of the same plant are not the same and may depend on the plant's ecological features (Komissarov, 1969).

Rooting of cuttings usually requires a higher temperature than that at which plants normally develop during the growing season. Temperature during propagation also influences rooting time and modifies the number of roots (Andersen, 1986). Tropical and sub-tropical plants usually require a higher temperature for their rooting than do cuttings of plants from temperate and cold countries (Hartmann and Kester, 1983). A daytime temperature range of 21-27 °C and about 15 °C at night enhances rooting for many temperate species (Hartmann and Kester, 1983). Excessively high air temperature inhibits root formation, tends to promote bud growth and increases water loss from the leaves. Excessively high temperature in the rooting media can destroy growth promoters, such as auxins and their co-factors. It has been suggested that the optimum temperature for the rooting of cuttings varies with their degree of lignification (Komissarov, 1969). Low temperatures increase the time required for rooting, but also increase the root number, perhaps because of a slowing down in metabolic activity and a prolongation of the cell division cycle (Brown, 1951). A
propagating bed temperature of about 25-30 °C has been recommended for optimal rooting in *Triplochiton scleroxylon* (Leakey et al., 1982a).

c) Nutrients

When cuttings have been severed from the stockplant, there is a fixed mineral nutrient pool available for rooting, which the first roots to emerge depend on for their growth. This mineral nutrient pool may decline as a result of leaching during propagation so it is preferable to set cuttings under conditions that promote rapid rooting (Blazich et al., 1983), such as higher temperatures (White and Biernbaum, 1984). However, mineral deficiencies in the rooting zone may be overcome by redistribution of minerals within the cutting (Haissig, 1986). Applied nutrients may not generally be needed during rooting because endogenous nutrients are basipetally transported from the shoot (Eliasson, 1978). It is possible (section 3.6.1a) (iv)), however, that rooting may be influenced by mineral nutrition of the stockplant or of cuttings during propagation. For example, some nurserymen provide nutrients through the mist. This may be advantageous if cuttings remain in the propagator a long time, since nutrients can be leached from the cutting and the medium.

Good and Tukey (1967), working on *Chrysanthemum morifolium* cuttings, found that measurements of nitrogen, phosphorus and potassium in their cuttings indicated a redistribution particularly of potassium from developing tissues, including the rooting zone. In a similar experiment, nitrogen, phosphorus, potassium, calcium and magnesium were found not to be redistributed in holly (*Ilex crenata*) cuttings (Blazich and Wright, 1979). In addition, the influence of applied IBA on mineral distribution during propagation on holly was studied by Blazich et al. (1983). They found that nitrogen, phosphorus, calcium and magnesium were not redistributed to the rooting zone of untreated or IBA-treated cuttings during primordium initiation. However, minerals were redistributed to the upper stem from the leaves and rooting zone. This took place only after primordium initiation and bud break. In contrast, IBA treatment enhanced movement of nitrogen, phosphorus and potassium to the rooting zone (Blazich et al., 1983).
d) **Light**

Irradiance should be sufficient for assimilate production to be in excess of respiration. The irradiance needed by cuttings to root depends on the carbohydrate reserves of the cuttings. Increased irradiance may either promote or inhibit rooting. Many authors have reported that increasing irradiance to the leaves of cuttings enhances the number of roots formed on the cuttings (Eliasson, 1980; Rasmussen and Andersen, 1980; Davis and Potter, 1981; Javis and Ali, 1984). This indicates that rooting usually may be limited by the availability of current assimilates. This was also evident in leafy pea cuttings (Davis and Potter, 1981; Veierskov et al., 1982a), Pine (Hansen et al., 1978) and Rhododendron (Davis and Potter, 1987). Eliasson (1980) mentioned that illumination of the base of the cuttings will inhibit rooting, especially if the tops of the cuttings are exposed to low irradiance (Eliasson and Brunes, 1980).

e) **Water status**

The water content of the cuttings is determined by the balance between the rates of uptake and loss of water. Separation of the cutting from its original root system influences cell volume, turgor pressure and cell water content, and physiological processes (Komissarov, 1969) including the activity of enzyme systems. Therefore, in order to maintain the normal metabolic processes of the cuttings and for them to form adventitious roots, maximum amount of water is necessary in their cells and tissues.

The rate at which a cutting loses water is mainly dependent on the vapour pressure difference between the leaf and the surrounding air, which is influenced by the extent of incoming radiation which creates a temperature gradient (Andersen, 1986). To reduce the transpiration from the leaves of the cuttings to a minimum, the vapour pressure of the water in the atmosphere surrounding the leaves should be maintained nearly equal to the water vapour pressure in the intercellular spaces within the leaves (Hartmann and Kester, 1983). In addition, water loss can be restricted by shading to reduce irradiance and temperature, by enclosing the cuttings in propagators to increase the water content of the surrounding air, or by misting to reduce water loss from the leaves (section 3.5 b) (Komissarov, 1969; Hartmann and Kester, 1983; Andersen,
1986). Andersen (1986) reported that water uptake is reduced during the first week of rooting as a result of xylem blockage in the basal part of the stem. However, in some species the blockage can be reduced by trimming the stem base (Andersen, 1986).

The irradiance experienced by a cutting inside a propagator is a major factor in determining its water status (Eliasson, 1978; Stromquist and Eliasson, 1979; Rajagopal and Andersen, 1980a;b). The stomatal aperture and, to some extent the cuticular conductance, control leaf conductances. This is because the rate at which water is lost from a cutting is determined by the vapour pressure gradient between leaves and the surrounding air, and further by the conductance of the leaf to water vapour (Gay and Loach, 1977). Studies on pea (*Pisum sativum*) cuttings by Eliasson (1978) indicated that they may require a source of inorganic ions with which to adjust their osmotic balance and thereby maintain turgor. Commonly the stomata close immediately after severance which reduces water loss, but also reduces the cutting's rate of net photosynthesis, which has an impact on the cuttings carbohydrate status (Gay and Loach, 1977; Eliasson and Brunes, 1980; Davis and Potter, 1981). Severe drought causes wilting, and also results in the accumulation of other metabolites which may affect hormone metabolism (Haissig, 1986).

**li)** **Physiological and morphological factors of cuttings on rooting**

a) **Cutting size**

A correlation between cutting size and adventitious root formation has been reported in many species (Leakey, 1983; Leakey and Mohammed, 1985; Dick *et al.*, 1991). In single-node cuttings the length of the cutting depends on the internode length of the stem which is determined by the vigour of shoot growth. The importance of cutting length *per se* was demonstrated in an experiment with *Triplochiton scleroxylon* in which sequential cuttings were taken with both the natural gradient of internode length and an inverse gradient. In both instances, higher rooting percentages were recorded in the longest cuttings (Leakey, 1983; Leakey and Mohammed, 1985).

With respect to the node position on the stockplant, rooting percentage was found to
show a basipetal decline in *Triplochiton scleroxyylon*, perhaps reflecting an increasing stem diameter and volume, and thereby an increasing storage capacity of assimilates (Leakey and Mohammed, 1985). In *Khaya ivorensis*, basal cuttings rooted more successfully, but cutting length (internode length) was not closely related to node position (Tchoundjeu and Leakey, (in press)).

b) Leaf area
Leaves play an important role in rooting success of leafy stem cuttings, because this organ is the major site of photosynthesis. However, leaves are not only importers of assimilates during much of their period of development, but also retain much of their photosynthate (Thrower, 1962) and other substances required for the rooting process (Porlingis and Therios, 1976). A balance should be sought between the positive effects of photosynthesis and the negative effects of water loss (Leakey and Coutts, 1989). This balance is needed because large leaves are more prone to desiccation in unrooted cuttings, whereas small leaves may not produce enough sugar and other compounds needed for the cutting to survive and root. Leaf area can be optimised by trimming the leaves. The practice of trimming the leaf aims at minimising water loss while allowing sufficient photosynthesis for the rooting process (Leakey and Coutts, 1989).

The optimum leaf size for rooting varies between species. For example, in *Triplochiton scleroxyylon* cuttings the optimum was found to be 50 cm² (Leakey *et al.*, 1982b), but the leaf area was found to be less critical in easy-to-root species such as *Nauclea diderrichii* (Leakey, 1990). By contrast, the optimum leaf area for rooting of *Khaya ivorensis* cuttings was about 30 cm² (Asanga, 1989), while that of *Lovoa trichiliodes* was 200 cm² (Tchoundjeu, 1989). In many species the total removal of leaves prevents rooting except when hardwood cuttings of species such as *Populus* are taken (Okoro and Grace, 1976). Failure of leafless cuttings to root has been shown for *Eucalyptus camaldulensis* (Geary and Harding, 1984) and *Terminalia spinosa* (Muthoka, 1990).

c) Carbohydrates
Carbohydrates serve as a source of energy in cell division for adventitious root
formation. They are also required for elongation and root growth. Haissig (1986) mentioned that it is possible that carbohydrate accumulation in the rooting zone may influence osmoregulation, cellular solvent capacity, and other physiochemical phenomena. Few studies have critically evaluated the role of carbohydrates in rooting. Accumulation of osmotically active solutes such as sugars may have a significant effect on metabolic processes which are involved in rooting (Veierskov et al., 1982b; Veierskov, 1988). However, some studies have revealed that there are many instances where carbohydrate-rich cuttings failed to root (Okoro and Grace, 1976). According to Haissig (1984) supply and distribution of carbohydrates within cuttings can sometimes limit their capacity to root.

Work on *Triplochiton scleroxylon* has suggested that while the carbohydrate content at severance is unrelated to rooting, the rooting ability of single-node cuttings is related to their carbohydrate content after four weeks on the propagation bed (Leakey and Coutts, 1989; Leakey and Storeton-West, 1992). Studies on *Khaya ivorensis* have shown that there are considerable changes in starch, soluble and reducing sugars during the period of propagation but these may have minimal effect on subsequent rooting (Tchoundjeu, 1989).

d) **Role of auxins**

Auxins are known to be the principal promoters of adventitious root formation (Leakey, 1985; Davis, 1988). The synthetic commercially-available auxins which are most reliable are indole-3-butyric acid (IBA) and naphtlene acetic acid (NAA). Phenoxy compounds such as 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid are less useful (Hartmann and Kester, 1983; Blazich, 1988). Although IBA and NAA had been judged to be superior to IAA, IBA is however normally used in most species as compared to NAA (Blazich, 1988). Recently, however equimolar concentrations of aryl- and phenyl- esters of IAA and IBA have been reported to outperform the unmodified acids in *Pinus banksiana* (Haissig, 1979;1983), but these have not yet been widely tested.
Leakey *et al.* (1982a) studied the effect of auxin concentrations on clones of *Triplochiton scleroxylon* using IBA and NAA in equal proportion. The result was that 40 μg IBA per cutting gave a high proportion of rooting in the clones, despite the considerable genetic variation in optimal concentration. High concentrations (200 μg per cutting) tended to increase the number of roots per cutting but inhibited root production in some clones. When applied separately it was found that IBA was more effective than NAA in most clones. Similarly, 200 μg IBA per cutting has been found to be optimum for *Khaya ivorensis*, but in contrast, *Lovoa trichiliodes* did not respond to any of the auxin concentrations applied (Tchoundjeu, 1989).

Reduced root promoting activity of the naturally occurring indoleacetic acid (IAA) has been attributed to the fact that plant tissues possess several enzymes that reduce the effectiveness of IAA (Leopold and Kriedemann, 1975; Gasper and Hofinger, 1988), by conjugating it with other compounds or by destroying it (Blazich, 1988).
CHAPTER FOUR

MATERIALS AND METHODS

4.1 Source of plant materials
Clonal stockplants of *Triplochiton scleroxylon* were not available in Ghana at the commencement of the experiments. However, it was possible to obtain stockplants that were not of clonal origin namely:

i) 3-year old saplings raised from seeds from Abetifi in Ghana (altitude 465 m and rainfall 1273 mm);

ii) 2-year old budded (mature plus-tree) material from South Formangso in Ghana (altitude 210 m and rainfall 1520 mm); and

iii) 13-year old mature trees from Mesewam in Ghana (altitude 300 m and rainfall 1520 mm).

4.2 Stockplant management
4.2.1 Coppice shoots
Saplings 3 years of age were cut back to a height of 0.5 m, 1 m, 1.5 m and 2 m from the ground to allow them to coppice for an experiment involving different stump heights. Other saplings were cut at 1 m for fertiliser, leaf area, cutting length and light experiments. A commercial fertiliser, Grofol (N-29 P-30, K-10, Agrofarma Mexicana, S. A. de C. V. Mexico) of concentration 2.5 g per litre of water was applied weekly for four weeks after emergence of shoots from the coppiced stumps. Three 13 year-old mature trees were also cut at a stump height of 1 m for coppice shoot production (Plate 4.1a;b) after using the branches for an experiment involving different branch positions.

4.3 Propagation unit
At the start of the project there were not sufficient vegetative propagation facilities at the Forestry Research Institute of Ghana. Therefore twelve low-technology, non-mist
Plate 4.1  a) A stump of a mature (13 year old) tree.
Plate 4.1b Coppice shoots on a mature (13 year old) tree.
propagators were built following the design of Leakey et al. (1990) (Fig. 4.1). This was the first time such propagators had been used on a large scale in Ghana. These were placed under shade screens made from palm fronds to protect the cuttings from direct sunlight (Plate 4.2).

The non-mist propagator does not require daily watering or an electricity supply. The frame is made of wood, and the entire frame is enclosed with clear polythene so that the base is watertight. The base of the propagator is covered with fine sand followed by a larger of medium-sized stones to a depth of 20 cm. These stones are then covered with gravel to a depth of 5 cm. The rooting medium is supported by the gravel while the air spaces of the stones are filled with water. A number of reasons favour the use of these propagators: they are cheap to construct, and have proved very effective for the propagation of a large number of tropical species (Leakey et al., 1990). They have no requirement for either piped water or electricity, making them ideal for rural or small developmental programmes.

4.4 Preparation of cuttings

The cuttings were taken from coppice stumps in the nursery of the Forestry Research Institute of Ghana (Mesewam, altitude 300 m, rainfall 1520 mm). The cut shoots were kept in humid, closed polythene bags, protecting them from direct sunlight, and were then transported to the preparation site as rapidly as possible. The leaves were trimmed using templates tested with a leaf area meter (Delta-T Devices, Burwell, Cambridgeshire, UK) to the required leaf area. The uppermost node was discarded, and except where otherwise stated, cuttings of a uniform stem length of 5 cm and leaf area of 50 cm² were taken (Plate 4.3). Due to the limited amount of material available and the fact that shoot lengths were also variable, it was decided to use every cutting available and randomise node position within the experiments. The bases of the cuttings were treated with auxin; 4 g of indole-3-butyric acid (IBA) (Sigma Chemical Company, St. Louis, U. S. A.) was dissolved in 1 litre of industrial methylated spirit and applied in a 10 μl dose with a precision micro pipette. This is the quantity of auxin (40 μg) found to be optimal for the rooting of this species by Leakey et al.
Fig. 4.1. A non-mist propagator.
Plate 4.2 Non-mist propagators used in Ghana for the experiments.

Plate 4.3 Prepared cuttings of *Triplochiton scleroxylon*.
(1982b). The methylated spirit was evaporated off quickly in a stream of cold air from a fan before cuttings were inserted in the propagating medium (coarse sand) at a depth of 1.0 - 1.5 cm (Plate 4.4).

The propagating beds were treated with systemic fungicide (Dithane M.45, Rohm and Hans, France, S.A.) at 25 g per 10 litres of water and insecticide (Cymbush 10EC, Imperial Chemical Industries Plc, Plant Protection Division Fernhurst, England) at 21 ml per 10 litres of water a day before the experiment was set up, to prevent cuttings from fungal infection. To protect cuttings from drying when they were inserted, or whenever the propagator lid was opened, a fine spray of water was applied using a hand sprayer (Tecnom Pulsar 2, Epernary, France).

The propagator bed and air temperatures were measured using a mercury-in-glass thermometer (THL-210-051R, Gallenkamp Express, Loughborough England), air humidity (using a Thermohygrometer, HYT-705-010G, Gallenkenamp Express, Loughborough England) and irradiance (using a Lux Meter M x 4, Metrawatt GmbH Nurnburg, Germany). These were recorded at 2 pm each day.

The cuttings were assessed weekly after insertion in the propagator. Each cutting was assessed for:-

i) presence of roots;
ii) cutting mortality;
iii) number of roots;
iv) leaf abscission;
v) maximum and minimum root length.

4.5 Weaning of cuttings

The rooted cuttings (Plate 4.5) were potted in a black polythene bag of size 32 x 25 cm using humus soil collected under a Triplochiton scleroxylon plot at Mesewam nursery of the Forestry Research Institute of Ghana. The potted plants were kept for two weeks in a closed propagator under shade using palm fronds. They were then
Plate 4.4 Cuttings inserted in a propagator.

Plate 4.5 Rooted cuttings ready for potting.
placed under a shade for six weeks.

4.6 Insect and fungal attack
The coppiced shoots became infected with psyllids at the start of the experiments. The attack occurred on both the stems and the leaves and so all the stockplants were treated with insecticide (Cymbush 10EC, Imperial chemical Industries Plc, Plant Protection Division Fernhurst, England) at 7 ml in 3 litres of water. This was applied four times at an interval of two to three weeks depending on the extent of the infection. All the cuttings that were set were also infected with fungus and psyllids and therefore had to be treated with fungicide (Dithane M.45, Rohm and Hans, France, S.A.) and insecticide (Cymbush 10EC, Imperial Chemical Industries Plc. Plant Protection Division Fernhurst, England). Nevertheless all experiments had to be repeated two to three times since initially all the cuttings died within two to three weeks from these infections or from the subsequent leaf abscission. The fungi *Botryodiplodia* spp. and *Rhizoctonia solani* were isolated from the diseased cuttings. These were identified by the pathology section of the Forestry Research Institute of Ghana.

4.7 Relative water content (RWC)
The relative water content of the leaf was determined using the method described by Beadle *et al.* (1987). The measurement was based on fresh mass (F), turgid mass (T), (obtained after floating the leaf in a distilled water for 24 hrs.), and dry mass (D) taken after drying the leaf in an oven at 80 °C for 2 days (48 hrs). RWC was then calculated as: \[ \text{RWC} = \frac{(F-D)}{(T-D)} \times 100\% \].
A precision standard electronic balance (Model TSV20S, OHAUS Europe Ltd, England) was used to weigh the samples.

4.8 Statistical analysis
The cuttings were randomly set and treatments replicated in the propagators. The percentages and standard errors of the variables were calculated following the procedure described by Snedecor and Cochran (1980) for binomial data using SAS
(1980). The percentage data were transformed using an arcsin transformation, and analysis of variance was performed using SAS (1980). Root numbers and lengths were analysed by t-tests.
CHAPTER FIVE

EFFECT OF STOCKPLANT LIGHT ENVIRONMENT AND DIFFERENT LEAF AREAS ON ROOTING ABILITY OF LEAFY STEM CUTTINGS OF TRIPLOCHITON SCLEROXYLON

5.1 Introduction

The influence of irradiance on stockplants and the subsequent rooting of cuttings from them has been the subject of controversy. Higher irradiance has been shown to inhibit or delay (Hansen and Eriksen, 1974; Andersen et al., 1975; Hansen et al., 1978; Eliasson and Brunes, 1980; Poulsen and Andersen, 1980; Hansen and Ernsten, 1982), promote (Moe, 1976; 1977), or have no effect on rooting (Lovell et al., 1972). In general, reduced irradiance has more often been found to enhance rooting (Moe and Andersen, 1988; Leakey and Storeton-West, 1992).

The presence of leaves on stem cuttings exerts a strong stimulatory influence on adventitious root formation, but at the same time leads to water loss. Thus, for leafy stem cuttings to achieve rooting, a balance between the processes of photosynthesis and transpiration must be sought, and this can be achieved by the presence of an optimum leaf size (Okoro and Grace, 1976; Eliasson and Brunes 1980; Leakey et al., 1982b; Leakey and Coutts, 1989; Newton et al., 1992a; Leakey et al., 1994). The aim of this experiment was to study the interaction of the light environment of the stockplants and the leaf area of the cuttings on adventitious root formation of Triplochiton scleroxyylon cuttings.

5.2 Materials and methods

Ideally, clonal stockplants would have been used for this study. Lack of available clonal stockplants restricted this experiment to the use of coppice shoots from 3 year old seedlings derived from one seedlot from Abetifi as mentioned in Section 4.1. Two light treatments were used: direct sunlight and a shade treatment made by using fabric Agroscreen (LBS Group, Lancashire, UK) (Plate 5.1). An integrating light meter and
Plate 5.1 Light treatments: Stockplants grown under, a) direct sunlight, b) 50% light interception using fabric material (Agroscreen).
Parsum Reader (DIDCOT, UK) were used to measure the irradiance. When the readings were calculated and compared, the shade treatment was found to be approximately 50% of direct sunlight. The allocation of plants to either direct or diffused sunlight as well as other treatment posed problems for analysis, because the former were all grouped together, and so were replicates within each treatment are not strictly independent. An alternative design with many shaded and unshaded replicates would have been preferable but this would have required more plants and shade cloth which were not available. The stockplants were cut back to a height of 1 m for coppicing, and were grown for four months before cuttings were taken (the above also is applicable to experiments in chapters 6 and 7).

Cuttings were taken with a standard length of 5 cm, with four different leaf areas (30 cm², 50 cm², 80 cm² and 100 cm²) obtained by trimming. They were set randomly on the propagating bed in twelve replicate blocks. Two propagators were used, each having six replicate blocks. Eight cuttings per treatment per block were taken, giving a total of 96 cuttings per treatment for the experiment. Forty cuttings (5 cuttings per each treatment) were sampled randomly at day 0, 7 and 49, for relative water content measurements. Assessments of rooting and other variables were carried out weekly as described under preparation of cuttings (Section 4.4). Standard errors and rooting percentages were calculated following the procedure described by Snedecor and Cochran (1980) using SAS (1980). These data were arcsin transformed prior to analysis of variance, which was performed using SAS (1980) (Table A1 in the appendix). The correlations between leaf area, percentage rooting, root length and root number were calculated.

5.3 Results

5.3.1 Microclimate

Differences existed between the air and bed temperatures inside the propagator. The higher (32.4 °C) was recorded for the air temperature. There was also difference between the irradiance inside and outside the propagator with the higher (5501 Lux) (104 μmol m⁻² s⁻¹) recorded outside (Table 5.1).
Table 5.1: Temperatures (of the bed and air inside the propagators) and irradiance (inside and outside the propagators) measured during the experiment involving stockplant light environment and leaf area. (Mean ± SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>32.4 ± 2.0</td>
<td>28.5 - 35.0</td>
</tr>
<tr>
<td>Bed</td>
<td>28.5 ± 2.1</td>
<td>24.0 - 30.0</td>
</tr>
<tr>
<td>Irradiance (Lux) (µmol m⁻² s⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside propagator</td>
<td>1705 ± 720</td>
<td>800 - 3000</td>
</tr>
<tr>
<td></td>
<td>(32 ± 14)</td>
<td>(15 - 57)</td>
</tr>
<tr>
<td>Outside propagator</td>
<td>5501 ± 171</td>
<td>5140 - 5950</td>
</tr>
<tr>
<td></td>
<td>(104 ± 3)</td>
<td>(97 - 113)</td>
</tr>
</tbody>
</table>

5.3.2 Rooting
The first root began to appear in the second week. From week 3 onwards the rate of rooting increased for almost all of the leaf areas (Fig 5.1). The highest percentage rooting (52.9%) at week 10 was obtained with a leaf area of 100 cm² from stockplants grown under shade, and the lowest (25.3%) for a leaf area of 30 cm² from stockplants grown under direct sunlight. There was no significant difference (P > 0.05) in rooting between direct sunlight and shade, however there was a significant difference (P < 0.05) between the leaf area of 100 cm² and the other treatments. There was no significant difference (P > 0.05) between rooting of the other leaf areas. The number of roots per rooted cutting was higher in cuttings from stockplants grown under direct sunlight than those from stockplants grown under 50% light interception (Table 5.2). In addition, the number of roots per rooted cutting increased with decreasing leaf area of the cuttings, while root length per rooted cutting did not show any particular trend. Leaf area showed a negative trend with root number but this was not significant (r = 0.81, P > 0.05). Percentage rooting also showed a positive trend with leaf area but this was also not significant (r = 0.78, P > 0.05).

5.3.3 Mortality
The percentage mortality increased in all the leaf area treatments from week 2 to week 10. The highest percentage (80.5%) was recorded for the leaf area of 30 cm² in
Fig. 5.1. Effect of stockplant light environment and leaf areas on rooting of leafy stem cuttings of *Triplochiton scleroxylon*. Vertical bars show standard error.
cuttings from stockplants grown in full sunlight and the lowest (49.4%) for 100 cm² leaf area for cuttings from stockplants grown under shade (Fig 5.2). The high percentage leaf abscission was probably linked to the high mortality rate recorded at a leaf area of 30 cm² of cuttings from stockplants grown under full sunlight. Generally, the high mortality recorded in all the treatments was caused by rotting of the cuttings, which started from the top of the cutting, causing leaf abscission and finally death.

Table 5.2: Effect of stockplant light environment and leaf area on mean number of roots and root length per rooted cutting in cuttings of *Triplochiton scleroxylon*, 10 weeks after insertion. (Mean ± SE).

<table>
<thead>
<tr>
<th>Leaf Area (cm²)</th>
<th>Mean number of roots per rooted cutting (Mean ± SE)</th>
<th>Mean root length per rooted cutting (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sunlight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>4.5 ± 0.6 a</td>
<td>25.3 ± 2.6 a</td>
</tr>
<tr>
<td>50</td>
<td>3.2 ± 0.6 a,b</td>
<td>13.9 ± 2.1 a</td>
</tr>
<tr>
<td>80</td>
<td>2.9 ± 0.3 a,b</td>
<td>20.7 ± 2.6 a</td>
</tr>
<tr>
<td>100</td>
<td>3.1 ± 0.4 a,b</td>
<td>19.6 ± 2.2 a</td>
</tr>
<tr>
<td>50% light interception</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3.0 ± 0.4 a,b</td>
<td>22.9 ± 3.0 a</td>
</tr>
<tr>
<td>50</td>
<td>2.6 ± 0.3 b</td>
<td>17.3 ± 2.4 a</td>
</tr>
<tr>
<td>80</td>
<td>2.5 ± 0.4 a,b</td>
<td>17.2 ± 3.4 a</td>
</tr>
<tr>
<td>100</td>
<td>2.5 ± 0.3 b</td>
<td>21.9 ± 3.0 a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P >0.05).

5.3.4 Leaf abscission

Leaf abscission began at week 1. The proportion of cuttings which shed their leaves increased rapidly between week 1 and week 3, and altered less between week 3 to week 10 (Fig 5.3). The leaf area treatment of 80 cm² of cuttings from stockplants
Fig. 5.2. Effect of stockplant light environment and leaf areas
  (○ = 30 cm² (Light), □ = 50 cm² (Light), △ = 80 cm² (Light), ▼ = 100 cm² (Light), ● = 30 cm² (Shade), ★ = 50 cm² (Shade), ▲ = 80 cm² (Shade) and ▼ = 100 cm² (Shade)) on mortality of *Triplochiton scleroxylon* leafy stem cuttings. Bars show standard error.
Fig. 5.3. Effect of stockplant light environment and leaf areas on leaf abscission of *Triplochiton scleroxylon* leafy stem cuttings. Bars show standard error.
grown under full sunlight produced the highest percentage (64.8%) leaf abscission, and leaf area of 100 cm$^2$ from stockplants grown under shade the lowest (28.2%).

5.3.5 Relative water content
The relative water content of the cuttings varied with time in all the treatments. At day 0, the mean value recorded was higher for a leaf area of 80 cm$^2$ for cuttings from stockplants grown under full sunlight and the lowest for a leaf area of 100 cm$^2$ for cuttings from stockplants grown under shade. Cuttings from stockplants under direct sunlight displayed higher values of relative water content than those from stockplants grown under shade at day 14. The same trend was observed on day 49; however, the difference was less pronounced at this time. Relative water content declined continuously throughout the experiment with minimum readings recorded at day 49 (Table 5.3).

Table 5.3: Effect of stockplant light environment and leaf area (cm$^2$) on foliar relative water content (%) of leafy stem cuttings *Triplochiton scleroxylon.*

<table>
<thead>
<tr>
<th>Day</th>
<th>Leaf area (cm$^2$)</th>
<th>Light</th>
<th>Shade</th>
<th>Light</th>
<th>Shade</th>
<th>Light</th>
<th>Shade</th>
<th>Light</th>
<th>Shade</th>
<th>Light</th>
<th>Shade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>50</td>
<td>80</td>
<td>100</td>
<td>30</td>
<td>50</td>
<td>80</td>
<td>100</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Light  Shade</td>
<td>79.4 ±8.4 a</td>
<td>74.5 ±1.9 b</td>
<td>84.3 ±1.9 a</td>
<td>79.4 ±2.6 a</td>
<td>81.9 ±9.2 a</td>
<td>67.6 ±14.8 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Light  Shade</td>
<td>76.6 ±12.7 a</td>
<td>62.3 ±4.9 a</td>
<td>73.6 ±4.9 a</td>
<td>63.5 ±6.5 a</td>
<td>76.7 ±5.4 a</td>
<td>62.4 ±11.5 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>Light  Shade</td>
<td>43.8 ±11.3 a</td>
<td>48.7 ±9.4 a</td>
<td>60.5 ±21.0 a</td>
<td>49.8 ±21.0 a</td>
<td>59.5 ±12.9 a</td>
<td>57.2 ±11.3 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P>0.05).

5.4 Discussion
Leaves export much of their assimilate production to the rest of the plant parts during the period of plant growth. These assimilates and other substances are important for
the rooting process (Porlingis and Therios, 1976). The presence of leaves on stem cuttings has been found to exert a strong stimulatory influence on adventitious root formation by experiments with leafless cuttings (Vardar, 1968). This is due to the role the leaf plays in the production of carbohydrates through photosynthetic activities (Reuveni and Raviv, 1980) and therefore in the carbon budget of the cutting, and partly because the leaf supplies auxins and other compounds which stimulate rooting (Wightman et al., 1980; Stanley and Toogood, 1981). The leaves also affect the cutting through their influence on water status. Stomatal closure minimises water loss and also limits photosynthesis by reducing CO₂ intake. For leafy stem cuttings to achieve rooting successfully, a balance between the process of photosynthesis and transpiration must be sought and this can be achieved by ensuring the presence of a leaf area of an optimum size (Okoro and Grace, 1976; Eliasson and Brunes, 1980; Leakey et al., 1982b; Leakey and Coutts, 1989; Newton et al., 1992b; Leakey et al., 1994).

The practice of trimming the leaf aims at minimising water loss while allowing sufficient photosynthesis to occur during propagation to enable root development (Leakey and Coutts, 1989). These processes are influenced by the amount of light received by the cuttings, both through its direct effects on photosynthesis and its indirect effects on air and leaf temperature, vapour pressure deficit and stomatal conductance (Loach, 1988a). Trimming of the leaf has, however, been found to stimulate rooting and induce the flushing of new leaves, as found for example in *Eucalyptus camaldulensis* (Geary and Harding, 1984).

An optimum leaf area per cutting for rooting has been found with *Triplochiton scleroxylon* and other tropical hardwoods (Leakey et al., 1982b; Leakey, 1985; Asanga, 1989; Tchoundjeu, 1989; Newton et al., 1992b). These studies suggest that leaf areas of 50 cm² and 10-30 cm² are optimal for *Triplochiton scleroxylon* and *Khaya ivorensis* respectively. Low rooting percentages have been found to occur in leafless cuttings of *Populus nigra* and many other species, whether or not treated with auxin (Nanda et al., 1971). Reuveni and Raviv (1980) studying the importance of
leaves on rooting of Avocado, reported that not all the effects of leaves are beneficial to rooting and they also presented evidence of optimal leaf areas for rooting.

In the present study the highest rooting percentage were obtained in cuttings with leaf areas of 100 cm². In this respect, these results differ from those of Leakey et al. (1982b) who recorded an optimum leaf area of 50 cm² in this species. The difference in optimum leaf areas may be due to different pre-severance treatments applied to the stockplants before cuttings were taken and also different propagation conditions. Although large leaf areas are assumed to photosynthesize more, they may be more susceptible to water stress. Furthermore, a larger leaf area may be associated with greater auxin production which might have increased transport of sugars to the region of root primordia development and root production. However, the fact that an increase in leaf area resulted in a decrease in root number suggests that this process did not occur in this experiment. The low percentage rooting recorded for smaller leaf areas is probably due to a lack of assimilate production, either pre or post-severance. Many researchers such as Scott and Briggs (1963); Heide (1968) and Vardar (1968) have reported low rooting in leafless cuttings and those with small leaf areas; they attributed their results to reduced auxin transport and carbohydrate synthesis. Thus it may be that the rates of photosynthesis and reserve mobilization in the leaves were insufficient to support much root development.

The rooting ability of cuttings from stockplants exposed to different irradiances has been the subject of controversy. Higher irradiance of the stockplants has been shown to inhibit or delay rooting, (Moore et al., 1972; Biran and Halevy, 1973; Lovell et al., 1974; Hansen, 1976; Fischer and Hansen, 1977; Moe, 1977; Hansen et al., 1978; Veierskov, 1978; Eliasson and Brunes, 1980; Rajagopal and Andersen, 1980a; Rasmussen and Andersen, 1980). Reduced irradiance generally enhances rooting (Hansen and Eriksen, 1974; Hansen et al., 1978; Eliasson and Brunes, 1980; Moe and Andersen, 1988; Leakey and Storeton-West, 1992). The ways light affects root development are not clear. According to Baadsmand and Andersen (1984) the main effect of light on rooting in pea is via an effect on photosynthesis and assimilate
production and hence on the initial carbohydrate content of the cuttings. However, indirect effects could also occur. Studies on *Pisum sativum* (Baadsmand and Andersen, 1984) showed that high stockplant irradiance enhances the transport of auxin to the base of subsequently collected cuttings and as a result of higher tissue temperatures at high irradiance. The effect of high irradiance in their study was the formation of fewer roots per cutting. Rooting percentage increased with increasing leaf area both in cuttings from high and low irradiance. Similar results were obtained in the current experiment; whilst rooting percentage tended to increase with increasing leaf area, a reduction in root numbers per cutting in direct sunlight was also observed.

The relationship between pre- and post-severance treatments may depend on the level of assimilate production both before and during propagation. The pre- and post-severance rates of photosynthesis and respiration will determine the relative availability of carbohydrates produced both pre- and post-severance. Hoad and Leakey (1992) reported that the production of carbohydrate, by photosynthetic activity of the leaves, depends upon leaf size, nutrient status and water status of the cutting. These may each be affected by the pre-severance light environment. In this study the highest percentage rooting was found to be with leaf area of 100 cm² using cuttings collected from stockplants grown under 50% light interception. Although photosynthesis was not measured in this experiment, one would expect that under higher light the pre-severance rate of photosynthesis would be higher, so more carbohydrates would be produced. The fact that rooting percentage of cuttings from direct sunlight was relatively low is supported by Leakey and Storeton-West (1992) and Mesén (1993), who suggested that, if too much carbohydrate is produced, rates of photosynthesis and therefore rooting may be reduced. In this study, it was evident that increasing leaf area with reduced pre-severance irradiance, had a greater influence on percentage rooting as well as number of roots produced, in contrast to results reported by Mesén (1993).

Pre-severance irradiance influences the water status of cuttings, either by increasing or reducing the rate of transpiration of the stockplant. The importance of maintenance of turgor in the cutting is well known. Even slight water deficits can be sufficient to
cause water stress, even without any visual symptoms, and so result in considerable delay or reduction in rooting response (Evans 1952). Then again, a large leaf area could be detrimental in systems where water losses and temperatures are high, for such cuttings will lose more water than cuttings with smaller leaf areas (Grange and Loach, 1984).

The result obtained for RWC as well for rooting percentage associated with the different treatments show that the light treatments (both shade and direct sunlight) encountered with the stockplants are such as to induce water stress. This supports what Rajagopal and Andersen (1980b) reported on pea. The results indicate that cuttings in this study did not recover from the shock after severance as the RWC declined in subsequent measurements. This stress might have affected subsequent rooting. Grange and Loach (1984) reported that lower rooting may be the result of water stress. This may impose severe restrictions on metabolism and correspondingly restrict photosynthesis, resulting in lower percentage rooting of cuttings from direct sunlight than cuttings from shaded stockplants. Relative water content of the cuttings declined after their insertion in the propagator. A similar trend was reported by Newton and Jones (1993a) in Terminalia spinosa and Triplochiton scleroxylon cuttings in which RWC declined with time. Moreover, a relationship between RWC and irradiance was recorded suggesting that to minimise water stress and thus promote rooting in cuttings, shading of the stockplants is essential.
CHAPTER SIX
EFFECT OF STOCKPLANT LIGHT ENVIRONMENT AND LENGTHS OF CUTTINGS ON ROOTING ABILITY OF LEAFY STEM CUTTINGS OF _TRIPOCHITON SCLEROXYLON._

6.1 Introduction

The stockplant light environment is one of the main pre-severance factors influencing adventitious root formation in leafy cuttings (Section 5.4). High irradiance inhibits subsequent rooting in many species (Hansen and Eriksen, 1974; Eliasson and Brunes, 1980; Poulsen and Andersen, 1980). Reduced irradiance generally enhances rooting (Moe and Andersen, 1988; Leakey and Storeton-West, 1992). This has been attributed to the accumulation of sugars and also starch in the leaves of cuttings under high irradiance, to the extent that post-severance rates of photosynthesis are inhibited (Leakey and Storeton-West, 1992).

A correlation between cutting size and adventitious root formation has been reported in many species (Roulund, 1973; Loreti and Hartmann, 1974; Leakey, 1983; Leakey and Mohammed, 1985; Dick et al., 1991). For example, Veierskov (1978) reported that in pea the number of roots formed on a cutting is dependent on the length of the internode. The importance of cutting length in _Triplochiton scleroxylon_ has been demonstrated by an experiment in which sequential cuttings were taken with both the natural gradient of internode length and an inverse gradient. In both instances, the longest cuttings were associated with the highest rooting percentage (Leakey, 1983; Leakey and Mohammed, 1985). These results were attributed to increased stem volume, and thereby storage capacity of assimilates, in the longer cuttings.

The aim of this experiment was to study the effect of light environment of the stockplants and cutting lengths on adventitious root formation of _Triplochiton scleroxylon_, and to test the hypothesis that the relationship between cutting length and rooting ability of leafy stem cuttings is influenced by the pre-severance light environment.
6.2 Materials and methods

Due to the lack of plant material, one seedlot was used, as mentioned in Chapter 5. The stockplants were managed as mentioned in Section 5.2. The same pre-severance light treatments as in Chapter 5 were applied to the stockplants and post-severance treatments of cutting lengths of 5 cm, 10 cm, and 15 cm were used. A uniform leaf area of 50 cm² was employed. The design used was a factorial design in randomised blocks with twelve replicate blocks per treatment. A total of 6 cuttings per treatment per block were taken. Assessment of rooting and other variables as mentioned earlier (Section 4.4) was made and the microclimate was also recorded daily. Percentage rooting and standard error were calculated following the procedure described by Snedecor and Cochran (1980) for binomial data using SAS (1980). These data were transformed using Arcsin transformation, and analysis of variance was performed using SAS (1980) (Table A2 in the appendix).

6.3 Results

6.3.1 Microclimate

The microclimatic conditions measured during the experimental period showed that the air temperature recorded inside the propagator was higher (27.2 °C) than that of the bed. The irradiance outside the propagator was also higher than that inside the propagator (Table 6.1).

Table 6.1: Temperatures (air and bed) and irradiance (inside and outside propagators) recorded during the experiment under Chapter 6.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>27.2 ± 1.5</td>
<td>24.0 - 31.0</td>
</tr>
<tr>
<td>Bed</td>
<td>25.0 ± 1.2</td>
<td>240 - 28.0</td>
</tr>
<tr>
<td>Irradiance (Lux) (μ mol m⁻² s⁻¹)</td>
<td>Inside propagator</td>
<td>807 ± 292 (15 ± 6)</td>
</tr>
<tr>
<td></td>
<td>Outside propagator</td>
<td>5668 ± 247 (107 ± 5)</td>
</tr>
</tbody>
</table>
6.3.2 Rooting

Overall rooting was significantly (P < 0.05) higher (35-50%) in cuttings from stockplants grown under shade than from under direct sunlight, with nearly three times as many cuttings rooting on average at week 8. The highest percentage rooting (48.6%) was recorded for a cutting length of 15 cm (from stockplants grown under shade) whilst the lowest percentage (13.9%) was recorded for cutting length 10 cm (from stockplants grown under light) (Fig 6.1). Although rooting percentages tended to increase with increasing cutting length, there were no significant differences (P>0.05) between cutting lengths 5 cm, 10 cm and 15 cm within either light treatment. Not surprisingly, treatments with a higher percentage of leaf abscission and mortality produced lower rooting percentages (Figs. 6.2; 6.3). Overall, cuttings from stockplants grown under the high light treatment tended to have the greatest mean root length, while cuttings from stockplants grown under shade had slightly more roots (Table 6.2).

Table 6.2: Effect of stockplants light environment and cutting length (cm) on the number of roots and root length (mm) per rooted cutting of *Triplochiton scleroxylon* leafy stem cuttings. (Mean ± SE).

<table>
<thead>
<tr>
<th>Cutting lengths (cm)</th>
<th>Mean root length per rooted cutting (mm)</th>
<th>Mean number of roots per rooted cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>33.3 ± 10.4 a</td>
<td>1.5 ± 0.5 a</td>
</tr>
<tr>
<td>10</td>
<td>29.0 ± 14.0 a</td>
<td>3.0 ± 1.5 a</td>
</tr>
<tr>
<td>15</td>
<td>18.3 ± 3.3 a</td>
<td>2.3 ± 0.4 a</td>
</tr>
<tr>
<td>Shade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26.8 ± 11.3 a</td>
<td>3.6 ± 1.5 a</td>
</tr>
<tr>
<td>10</td>
<td>20.7 ± 3.9 a</td>
<td>2.4 ± 0.3 a</td>
</tr>
<tr>
<td>15</td>
<td>20.7 ± 5.9 a</td>
<td>1.7 ± 0.5 a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P>0.05).
Fig. 6.1. Effect of stockplant light environment and cutting lengths (○ = 5 cm (Light), ● = 5 cm (Shade), △ = 10 cm (Light), ▲ = 10 cm (Shade), ▼ = 15 cm (Light) and ▼ = 15 cm (Shade)) on rooting of *Triplochiton scleroxylon* leafy stem cuttings. Bars show standard error.
6.3.3 Mortality
Percentage mortality increased rapidly between weeks one and two, and thereafter more slowly (Fig. 6.2). The highest percentage mortality (84.7%) was recorded for a cutting length of 10 cm from stockplants grown under direct sunlight whilst a cutting length of 10 cm from stockplants grown under shade (50% light interception) recorded the lowest percentage (50%). The higher percentage mortality recorded in the former treatment may have been due to higher leaf abscission of the cuttings.

6.3.4 Leaf abscission
Percentage leaf abscission increased rapidly during the first two weeks in all treatments (Fig. 6.3). After this time, very few cuttings abscised their leaves. The highest percentage abscission (63.9%) was recorded for a cutting length of 5 cm from stockplants grown under direct sunlight and the lowest (20.8%) for a cutting length of 15 cm from stockplants grown under shade.

6.4 Discussion
The relationship between cutting length and rooting is thought to relate to the importance of stored carbohydrate reserves in promoting adventitious root development. The stem is the primary carbohydrate storage organ in a cutting. Relationships between rooting and stem length have been reported in species such as Norway spruce (Roulund, 1973), Olive (Loreti and Hartmann, 1974), Sesbania sesban (Oduol and Akunda, 1988), Lovoa trichiloides (Tchoundjeu 1989), Eucalyptus camaldulensis (Reuveni et al., 1990), Prosopis juliflora (Wilson et al., 1989; Dick et al., 1991), Triplochiton scleroxylon (Leakey, 1983; Leakey and Storeton-West, 1992; Leakey et al., 1994) and Eucalyptus grandis (Hoad and Leakey, 1992). Higher percentage rooting was obtained for a length of 50 mm than 15 mm for pea cuttings (Veierskov, 1978). Furthermore, higher rooting percentages with increasing size of cutting have been found in Tilia americana L. over a size range of 12 - 60 cm (Morsink and Smith, 1974). Similar results have been reported for Acer saccharum (Donnelly, 1974), some Poplar species (Heilman and Ekuan, 1979; Deol and Khosla, 1983), and for Eucalyptus species (Geary and Lutz, 1985). Longer cuttings gave rise
Fig. 6.2. Effect of stockplant light environment and cutting lengths (○ = 5 cm (Light), ● = 5 cm (Shade), ▲ = 10 cm (Light), ▲ = 10 cm (Shade), ◢ = 15 cm (Light) and ◢ = 15 cm (Shade)) on mortality of leafy stem cuttings of *Triplochiton scleroxylon*. Bars show standard error.
Fig. 6.3. Effect of stockplant light environment and cutting lengths (○ = 5 cm (Light), ● = 5 cm (Shade), △ = 10 cm (Light), ▲ = 10 cm (Shade), ▼ = 15 cm (Light) and ▼ = 15 cm (Shade)) on leaf abscission of *Triplochiton scleroxylon* leafy stem cuttings. Bars show standard error.
to more roots per cutting in Norway spruce (Volna and Radosta, 1985). Poulsen and Andersen (1980) found in *Hedera helix* that cuttings with longer stem lengths produced a higher percentage rooting than shorter cuttings. In *Triplochiton scleroxylon*, cutting size, as determined by the normal pattern of internode lengths, was closely correlated with percentage of cuttings rooted (Leakey, 1983). A higher percentage rooting has also been reported with cutting lengths > 15 cm than cutting length < 10 cm in Sitka spruce (Mason et al., 1992).

The role of irradiance in rooting of cuttings was described in the previous Chapter (Section 5.4). Higher irradiance has been shown to inhibit or delay, promote or have no effect on rooting (Hansen et al., 1978; Eliasson and Brunes, 1980; Poulsen and Andersen, 1980; Hansen and Ernsten, 1982). Low stockplant irradiance enhanced rooting and changed the dominance relationships between the shoots of 2-shoot stockplants of *Triplochiton scleroxylon* (Leakey, 1985). Furthermore, high irradiance may have resulted in lower pre-severance rates of net photosynthesis than low irradiance in this study, perhaps owing to end product inhibition. Rooting was greatest in cuttings taken from stockplants grown under low irradiance which seemed to be related to the fact that they had low starch contents (Leakey, 1985). Light is known to inhibit adventitious root formation if the root-forming part of the stem is illuminated before the excision of the cutting (Biran and Halevy, 1973). Baadsmand and Andersen (1984) reported that the ways light affects root initiation are not clear. The probable main influence is through direct effects of photosynthesis on assimilate production and hence on the initial carbohydrate content of the cuttings. According to Hansen and Eriksen (1974) a threshold concentration of carbohydrates must exist in the cutting to provide nutrition for root development. This concentration may differ for different species and it may also depend on different photosynthetic capacity.

In this experiment, rooting percentages were significantly higher in cuttings from stockplants grown under shade than full sunlight, regardless of cutting length. The light treatments used in this study presumably affected rooting, by affecting the cutting morphologically and physiologically as described by Leakey et al. (1994). At
stockplant irradiances below optimum, rooting may be limited by a lack of carbohydrates and auxin supplies to the base of the cutting. It is also known that stockplants grown under high irradiance have a higher light compensation point and a higher respiration rate than those grown under low irradiance (Moe and Andersen, 1988). There is the possibility that higher concentrations of carbohydrates, the photodestruction of auxin, and concentrations of rooting inhibitors or promoters may have affected the rooting of cuttings from stockplants under direct sunlight in this study. However, these aspects were not investigated in this study. The possibility of greater water deficits in cuttings from stockplants grown under direct sunlight, is supported by the generally higher proportions of leaf abscission recorded in cuttings from this treatment.

Hansen and Eriksen (1974) also reported a higher percentage rooting in pea when stockplants were cultivated at a lower irradiance than at a higher irradiance. They attributed this to the nutritional status of the stockplant and possibly the role of carbohydrates and the growth promoters in the process of root formation. Effects of irradiance on stockplant to rooting have been also reported by Biran and Halevy (1973) on *Dahlia variabilis* and by Poulsen and Andersen (1980) on *Hedera helix*. Under artificial irradiance it is difficult to obtain the exact intensity similar to that of natural irradiance and conditions. However, the results obtained in this study using natural irradiance did not deviate much from what was obtained by Leakey and Storeton-West (1992) on *Triplochiton scleroxylon* using artificial light.

Although the irradiance treatments of the stockplant is important in regulating adventitious root formation, root formation is also correlated with cutting length. Studies on pea by Veierskov (1978) using two different levels of irradiance 16 and 38 Wm$^{-2}$, with internode lengths of 35 - 40 mm showed that a cutting length of 40 mm produced a higher number of roots under 16 Wm$^{-2}$ and 38 Wm$^{-2}$ than 35 mm, and therefore concluded that although the irradiance treatments of the stockplant is important in regulating adventitious root formation, root formation is also correlated with cutting length, independently of irradiance treatment of the stockplants. Grange
and Loach (1984) in their studies on comparative rooting of 81 cultivars in open and enclosed mist reported that irrespective of light treatments, larger size cuttings rooted better. In the current experiment, longer cuttings (15 cm) were associated with the highest percentage rooting, and shorter cutting lengths (5 cm) the lowest. Under high light, a higher rooting percentage was recorded in the 15 cm cuttings, and the lowest percentage rooting was recorded in the 10 cm cuttings, although these differences were not significant. In the present study also, higher rooting percentages were obtained for cuttings with sizes of 15 cm, 10 cm and 5 cm from stockplant grown under shade (50% light interception) than those from direct sunlight.

Shedding of leaves by cuttings resulted in high mortality rates, with consequent effects on rooting percentage of the cuttings. As in Chapters 5 and 7 with *Triplochiton scleroxylon*, leafless cuttings died within a few days. The symptoms associated with failure of a cutting to root may be difficult to attribute to a particular physiological process (Leakey et al., 1994), because a wide range of factors are involved. These include lower light intensity, changed temperature, mineral deficiency and water stress. The high leaf abscission recorded in this study may have been caused by the existence of water stress during the first few days after severance and the relatively high air temperatures recorded in the propagators.
CHAPTER SEVEN
INTERACTIONS BETWEEN STOCKPLANT LIGHT ENVIRONMENT
AND FERTILISER APPLICATION ON ADVENTITIOUS ROOT
FORMATION OF LEAFY STEM CUTTINGS OF
TRIPLOCHITON SCLEROXYLON

7.1 Introduction
Fertiliser application enhances shoot growth by increasing the internal concentration of nutrients in the leaves and thereby potentially increasing rates of metabolic processes, such as photosynthesis. Root formation and root growth are influenced by a deficiency of mineral nutrients (Eliasson, 1978). Therefore, percentage rooting depends on maintaining an adequate mineral nutrition before and during rooting (Good and Tukey, 1967).

The irradiance under which stockplants have been grown preceding propagation affects the subsequent rooting of the cuttings (see previous sections). For example, shading of stockplants of Dahlia variabilis increased the number of roots formed in cuttings (Biran and Halevy, 1973). However, how irradiance regime and fertiliser treatments interact to influence rooting is not clear. The aim of this experiment was to study the influence of light and fertiliser application on stockplants and their effect on rooting of Triplochiton scleroxyton cuttings and to test the hypothesis that the effect of fertiliser application on adventitious root formation is influenced by the stockplant light environment.

7.2 Materials and methods
Saplings from a single seedlot as mentioned in Chapter 5 were used for this experiment, and were managed as mentioned in Section 5.2. Two light treatments were used: direct sunlight and shade (50% light interception) using agro-screen (LBS Group, Lancashire, England). Complete commercial fertiliser (NPK, 15:15:15, Norsk Hydro a.s., Norway) (nil or 7.5 g) was applied to the stockplants. Fertiliser was applied using the band method—thus digging around the stockplants at a distance of 12
cm and 6 cm deep and then applying the fertiliser. This was done two weeks after pruning the stockplants to a height of 1 m. The fertiliser was applied once to the plants, and water was added daily except when it rained.

Due to attack by psyllids the plants were sprayed once every two to three weeks with insecticide (Cymbush 10EC, Imperial Chemical Industries Plc, Plant Protection Division Fernhurst, England). A total of 7 cuttings per treatment were taken and arranged in nine replicate blocks. Sixteen cuttings (4 cuttings per treatment) were sampled at both day 0 and 21 for relative water content measurements. Assessments on cuttings were made weekly and variables considered were as described under preparation of cuttings (Section 4.4). The microclimatic conditions under which the experiment was conducted were also recorded (Table 7.1). The method for the analysis was the same as mentioned in the previous experiments (Section 4.8). The percentage data was transformed and analysis of variance was performed using SAS (1980) (Table A3 in the appendix).

7.3     Results
7.3.1     Microclimate
The measured microclimatic conditions during propagation are illustrated in Table 7.1. The air temperature inside the propagator recorded the higher reading (27.3 °C) as compared to bed temperature. The irradiance outside the propagator also recorded the higher reading compared to that of the inside (Table 7.1).
Table 7.1: Temperatures (air and bed) and irradiance (inside and outside propagators) recorded during experiment 3 under Chapter 7.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>27.3 ± 1.7</td>
<td>24.0 ± 33.0</td>
</tr>
<tr>
<td>Bed</td>
<td>24.8 ± 1.7</td>
<td>24.0 ± 29.5</td>
</tr>
<tr>
<td>Irradiance (Lux)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside propagator</td>
<td>821 ± 192</td>
<td>560 - 2500</td>
</tr>
<tr>
<td>(µ mol m⁻² s⁻¹)</td>
<td>(16 ± 4)</td>
<td>(11 - 47)</td>
</tr>
<tr>
<td>Outside propagator</td>
<td>5311 ± 1151</td>
<td>1800 - 6200</td>
</tr>
<tr>
<td></td>
<td>(101 ± 22)</td>
<td>(34 -117)</td>
</tr>
</tbody>
</table>

7.3.2 Rooting

At week 8 the highest percentage (50%) rooting was recorded for cuttings from stockplants grown under direct sunlight with fertiliser, and the lowest (21.8%) for cuttings from stockplants grown under direct sunlight without fertiliser (Fig. 7.1). Overall, percentage rooting was higher in cuttings from stockplants grown under direct sunlight with fertiliser than the other treatments. There was no significant difference (P>0.05) between cuttings from stockplants grown under direct and diffuse sunlight with fertiliser, but there was no significant difference (P<0.05) between light and shade treatments without fertiliser. There was a significant difference (P<0.05) between fertiliser and no fertiliser in the full sunlight treatment. The interaction was not significant from appendix.

In this experiment fertiliser application increased root numbers in both light and shade treatments. The mean length of the roots was between 21 mm - 28 mm, while the root number per rooted cutting was between 2.0 and 4.5 (Table 7.2).
Fig. 7.1. Effect of stockplant light environment and fertiliser application on rooting of *Triplochiton scleroxylon* leafy stem cuttings. (○ = Light with fertiliser, △ = Light without fertiliser, ● = Shade with fertiliser and ▲ = Shade without fertiliser). Vertical bars show standard error.
Table 7.2: Effect of light and fertiliser application on stockplant on mean accumulated number of roots and root length (mm) per rooted cutting of *Triplochiton scleroxylon* cuttings. (Mean ± SE).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of roots per rooted cutting</th>
<th>Root length (mm) per rooted cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light with fertiliser</td>
<td>4.2 ± 0.9 a</td>
<td>27.3 ± 4.5 a</td>
</tr>
<tr>
<td>Light without fertiliser</td>
<td>2.3 ± 0.4 a</td>
<td>22.3 ± 1.9 a</td>
</tr>
<tr>
<td>Shade with fertiliser</td>
<td>4.4 ± 0.7 a</td>
<td>27.7 ± 2.9 a</td>
</tr>
<tr>
<td>Shade without fertiliser</td>
<td>4.3 ± 1.4 a</td>
<td>21.8 ± 5.7 a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P>0.05)

7.3.3 Mortality

The percentage mortality increased rapidly in all treatments between weeks 1 and 3. The highest percentage (74.5%) mortality was recorded for cuttings from stockplants grown under direct sunlight without fertiliser and the lowest (50%) for cuttings from stockplants grown under direct sunlight with fertiliser (fig 7.2). Mortality of the cuttings was associated principally with leaf abscission.

7.3.4 Leaf abscission

The percentage leaf abscission increased rapidly during the first two weeks, then remained roughly constant for the remainder of the experiment. The highest percentage (61.5%) leaf abscission was recorded for cuttings from stockplants grown under direct sunlight without fertiliser, while the lowest (30.4%) was recorded for cuttings from stockplants grown under direct sunlight with fertiliser (fig 7.3).

7.3.5 Relative water content

The relative water content (RWC) increased from day 0 to day 21 (Table 7.3) in all the treatments. Cuttings from stockplants grown from high light with fertiliser had rather lower RWC than the other three treatments. At day 21 values tended to be higher in cuttings from high light plus fertiliser (Table 7.3).
Fig. 7.2. Effect of stockplant light environment and fertiliser application on mortality of *Triplochiton scleroxylon* leafy stem cuttings (○ = Light with fertiliser, △ = Light without fertiliser, ● = Shade with fertiliser and ▲ = Shade without fertiliser). Vertical bars show standard error.
Fig. 7.3. Effect of stockplant light environment and fertiliser application on leaf abscission of *Triplochiton scleroxylon* leafy stem cuttings. (○ = Light with fertiliser, △ = Light without fertiliser, ● = Shade with fertiliser and ▲ = Shade without fertiliser). Vertical bars show standard error.
Table 7.3: Effect of light and fertiliser application on relative water content (%) of leafy stem cuttings of *Triplochiton scleroxylon*.

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light with fertiliser</td>
</tr>
<tr>
<td>0</td>
<td>75.1 ± 3.5 a</td>
</tr>
<tr>
<td>21</td>
<td>87.1 ± 4.4 a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P>0.05).

7.4 Discussion

Mineral nutrients are presumably involved in the metabolic processes which are associated with differentiation and root meristem formation and adequate contents may even be essential for root initiation (Blazich, 1988). According to Haissig (1986) mineral nutrition influences a variety of endogenous biochemical responses that are sensitive to or cause variations in stockplant growth and adventitious root formation of cuttings taken from these stockplants. Mineral nutrition may also be of significance by influencing the production of various root inducing factors and affecting post propagation growth and vigour. Cuttings have a fixed mineral nutrient pool, after separation from the stockplant. Root initiation is highly dependent on initial nutrients contents within that portion of the cutting where roots are to form. However the mineral nutrient pool in the cutting may decline as a result of leaching during propagation (Blazich *et al.*, 1983). Therefore higher rooting percentages depend on adequate mineral nutrition before and during rooting (Good and Tukey, 1967).

The results obtained here showed that there was an effect of fertiliser application on rooting. Leakey (1983) found that fertiliser application to stockplants of *Triplochiton scleroxylon* accelerated and increased the percentage rooting of cuttings. However, in *Khaya ivorensis* the use of different concentrations of fertiliser did not increase the rooting ability, but relatively high concentrations of nitrogen significantly lowered rooting (Tchoundjeu, 1989). Increased rooting was observed in *Lovoa trichilioides*
following fertilisation (Tchoundjeu, 1989). Tchoundjeu (1989) attributed the response of *Khaya ivorensis* to fertiliser additions to the low nutritional status of tropical soils. This is because tropical soils are inherently of low fertility (Bennema, 1977; Lundgren, 1981) with large amounts of nutrients coming from organic matter, rainfall and rainwash (Nye and Greenland, 1960) and the rapid decomposition and recycling of plant and mineral debris (Golley, 1983; Jordan, 1985).

The higher percentage rooting obtained in this experiment by the cuttings from stockplants supplied with fertiliser and grown under high light indicates that the stored mineral nutrients absorbed by the stockplant may limit rooting, when fertilisers are not applied. This supports the results obtained by Eliasson (1978) on *Pisum sativum* and by Nanda et al. (1971) on *Populus nigra*. The fact that nutrient availability may become a limiting factor for root formation by cuttings from stockplants grown without fertiliser has been shown for other species, such as *Sinapis* (Moore and Lovell, 1970) and *Phaseolus vulgaris* (Altman and Wareing, 1975). Experiments conducted on *Salix tetrasperma* by Nanda and Jain (1971) showed that the ability of stem cuttings to root is determined by a balance between mineral nutrition and chemical regulators. They further concluded that rooting may not occur even when the concentration of one of the above factors is very high.

There has been a great deal of discussion about the interactions between irradiance and nutrient application on stockplants and subsequent rooting. Previous results have indicated that there does not seem to be any consistent response in all tree species. This is because trees vary in their light and nutrient requirement both in time and space. Leakey and Storeton-West (1992) studied the interactions between irradiance and nutrients, and found that rooting ability of *Triplochiton scleroxylon* was strongly correlated with the cutting's ability to photosynthesise prior to rooting. Furthermore, these authors reported that in cuttings from stockplants grown at low irradiance, with or without nutrients, rooting percentages were relatively high, whereas in cuttings from high irradiance stockplants, and in those without nutrients rooting percentage were very low. Moreover, when *Lovoa trichilioides* stockplants were grown in full sunlight
or shade, the rooting of cuttings was lowest when fertiliser was added (Tchoundjeu, 1989). This was attributed to reduced photosynthetic activity as there was a strong relationship between carbohydrate concentration in the stem and rooting percentage. Furthermore, Mesén (1993) reported the interaction between two irradiances and two concentrations of fertiliser on *Albizia guachapele*, that stockplants grown at low irradiance with a low nutrient supply produced higher percentage rooting than cuttings from stockplants grown under high light and high nutrient supply.

In this experiment, higher percentage rooting was obtained in cuttings from stockplants grown under 50% light interception without fertiliser than direct sunlight without fertiliser. In this respect, these results are consistent with those of other experiments carried out as part of this study (Chapters 5 and 6). Reduced light intensity applied to stockplants has been found to increase the rooting of cuttings in a range of species, for example *Sinapis alba* (Lovell and Moore, 1969), *Populus tremula* (Eliasson, 1971; Biran and Halevy, 1973), Pea (Eriksen, 1973; Tillberg, 1974), Scots pine (*Pinus sylvestris* L.) (Hansen et al., 1978) aspen and willow (Eliasson and Brunes, 1980; Stromquist and Hansen 1980, Chestnut (Vieitez, 1981; Moe and Andersen, 1988). Similar results were obtained on Sitka spruce by van den Driessche (1985). The results obtained also conform to those obtained with *Triplochiton scleroxylon* using artificial irradiance without nutrient addition (Leakey and Storeton-West, 1992). However, in this experiment the highest rooting percentage was recorded from fertilised stockplants grown under high light. In *Triplochiton scleroxylon*, current photosynthetic capacity, as influenced by light and nutrient regimes, is thought to be the main pre-determinant of rooting capacity (Leakey and Storeton-West, 1992). Relatively high pre-severance photosynthetic rates would be expected in fertilised plants grown under high light.

It has been reported by many researchers (Loach, 1977; Grange and Loach, 1984; Leakey and Coutts, 1989; Newton and Jones, 1993a) that severe water stress results in low rooting percentage. Rajagopal and Andersen (1980b) reported that a longer exposure of pea stockplant to water stress (16 Wm⁻² and 38 Wm⁻²) resulted in reduced
rooting. The results obtained in this study indicate that RWC did not show the same trend as that obtained for the percentage rooting. It has been found that water stress in cuttings may have less direct effect on the rooting metabolism than hormone and carbohydrate metabolism (Loach, 1977; Gay and Loach, 1977; Grange and Loach, 1984). As noted by Newton and Jones (1993b) cuttings are in some cases able to recover after suffering water deficits, and are able to subsequently root successfully.
CHAPTER EIGHT

EFFECT OF COPPICE SHOOTS FROM DIFFERENT STUMP HEIGHTS
(0.5 M, 1 M, 1.5 M AND 2 M) ON ROOTING ABILITY
OF LEAFY STEM CUTTINGS OF TRIPLOCHITON SCLEROXYLON.

8.1 Introduction
Shoots of the same chronological age can also be different physiologically, as expressed by their rooting ability. According to Rauter (1982) when a parent tree (ortet) is cut back to a height of about 0.5 m - 1 m above ground, the reduced distance for transport between the potential cutting and the root system may affect juvenility in cuttings. Initial results from Sitka spruce (Morgan and Mason, 1992) showed that rooting ability of cuttings declines with increasing height of hedges. Lyrene (1981) working on Blueberry shoots reported that juvenility persists indefinitely in dormant buds at the seedling or base of plants. According to Hartney (1980) the base of the seedling could be the site for the accumulation of substances essential for rooting, or alternatively gradients in growth regulators and growth inhibitors may exist. Howard and Ridout (1991a) studying the rooting potential in plum cuttings reported that the shoots nearest to the stockplant roots have the highest rooting potential. A factor derived from the root system of the stockplant enhances rooting potential in the shoot system especially in shoots close to the roots.

The aim of this experiment was to study the effect of stump heights on rooting of Triplochiton scleroxylon coppice shoots and to test the hypothesis that varying stump heights influences rooting.

8.2 Materials and methods
The same seedlot as mentioned in Chapter 5 was used in this study. Saplings (3-year old) were cut back to different heights (0.5 m, 1 m, 1.5 m and 2 m) for coppicing.
The treatments were randomised and replicated twelve times. 5 cuttings per treatment per block were taken. Twenty cuttings (5 cuttings per treatment) were randomly sampled at both day 0 and 21 for relative water content measurements. Rooting assessments were made and variables were recorded as mentioned previously under Section 4.4. The percentage rooting and standard errors were calculated as mentioned under Section 4.8. The data were transformed and analysis of variance was performed using SAS (1980) (Table A4 in the appendix).

8.3 Results

8.3.1 Microclimate

The recorded values of microclimatic conditions during the experimental period showed that air temperature inside the propagator was higher (29.2 °C) than that of the bed. The irradiance outside the propagator was higher ((5271 Lux)(100 μmol m⁻² s⁻¹)) than that inside the propagator (Table 8.1). The relative humidity recorded was higher (94.1%) inside the propagator than outside.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>29.2 ± 2.0</td>
<td>26.1 - 31.6</td>
</tr>
<tr>
<td>Bed</td>
<td>27.0 ± 1.4</td>
<td>26.0 - 28.5</td>
</tr>
<tr>
<td>Irradiance (Lux)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol m⁻² s⁻¹) Inside</td>
<td>994 ± 356 (19 ± 7)</td>
<td>450 - 1850 (9 - 35)</td>
</tr>
<tr>
<td>Outside</td>
<td>5271 ± 185 (100 ± 4)</td>
<td>5110 - 5700 (97 - 108)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>94.1 ± 2.4</td>
<td>90.1 - 99.1</td>
</tr>
<tr>
<td>Outside</td>
<td>68.5 ± 4.9</td>
<td>59.5 - 79.2</td>
</tr>
</tbody>
</table>

8.3.2 Rooting

By week 5, rooting percentages were clearly different between treatments, decreasing
with increasing stump heights. The rooting percentage reached 58.3% in cuttings with 0.5 m stump height compared with 30.8% for 2 m stump height at week 5 (Fig. 8.1). Percentage rooting showed a highly negative trend but was significant ($r = 0.99$, $P < 0.05$) with increasing stump height. There was a significant difference ($P < 0.05$) between 0.5 m stumps and 2.0 m stumps, however, there was no significant difference ($P > 0.05$) between stump heights of 0.5 m, 1.0 m and 1.5 m. The rate of rooting was slower for the cuttings from 2 m stumps, starting a fortnight later and they had less than half the number of roots. Stump height showed a negative trend which was not significant ($r = 0.89$, $P > 0.05$) with mean number of roots (Table 8.2). The root length per rooted cutting decreased with increasing stump height. The stump height also showed a significant negative correlation which was significant with mean root length ($r = 0.96$, $P < 0.05$).

### Table 8.2: The mean root number and root length (mm) per rooted cutting of *Triplochiton scleroxylon* leafy stem cuttings at week 5. (Mean ± SE)

<table>
<thead>
<tr>
<th>Stump heights (m)</th>
<th>number of roots per rooted cutting</th>
<th>root length (mm) per rooted cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>$3.6 \pm 0.5$ a</td>
<td>$14.8 \pm 1.5$ a</td>
</tr>
<tr>
<td>1.0</td>
<td>$3.3 \pm 0.5$ a</td>
<td>$12.8 \pm 1.8$ a</td>
</tr>
<tr>
<td>1.5</td>
<td>$3.1 \pm 0.9$ a,b</td>
<td>$10.4 \pm 2.2$ a,b</td>
</tr>
<tr>
<td>2.0</td>
<td>$1.5 \pm 0.2$ b</td>
<td>$4.1 \pm 1.3$ b</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different ($P > 0.05$).

#### 8.3.3 Mortality

The percentage mortality of the cuttings was relatively high, and increased from week 2 to week 5 (Fig. 8.2). The highest percentage mortality (69.1%) was recorded for a stump height of 2 m and the lowest (41.7%) for a stump height of 0.5 m.

#### 8.3.4 Leaf abscission

Leaf abscission started at the first week with further leaf abscission from week 2 to
Fig. 8.1. Effect of different stump heights (○ = 0.5 m, ▲ = 1.0 m, ● = 1.5 m, △ = 2.0 m) on rooting of leafy stem cuttings of *Triplochiton scleroxylon*. Vertical bars show standard error.
Fig. 8.2. Effect of different stump heights (○ = 0.5 m, ▲ = 1.0 m, ◦ = 1.5 m, △ = 2.0 m), on mortality of leafy stem cuttings of *Triplochiton scleroxylon*. Vertical bars show standard error.
week 4; the proportion of cuttings with their leaves abscised then remained constant to week 5. Cuttings from the 2 m stump height had the highest percentage leaf abscission (32.4%), 0.5 m stump height recording the lowest (16.7%) at week 5 (Fig 8.3).

8.3.5 Callus production
The percentage of cuttings producing callus increased from week 2 to week 4 in all the treatments. There was no callus formation after week 5 (Fig 8.4). The callus started turning black after week 4.

8.3.6 Relative water content (RWC)
At day 0, the relative water contents (RWC) for all the treatments were above 80% (Table 8.3). The RWC increased from day 0 to day 21 with the exception of the stump height 1.5 m in which the RWC decreased. However there was no significant difference between the treatments.

Table 8.3: Relative water content (RWC (%)) of shoots from different stump heights on day 0 and day 21 of Triplochiton scleroxylon leafy stem cuttings.

<table>
<thead>
<tr>
<th>Day</th>
<th>Height of stump (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>0</td>
<td>90.4 ± 5.9 a</td>
</tr>
<tr>
<td>21</td>
<td>93.0 ± 0.9 a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P >0.05)

8.4 Discussion
When a parent tree is cut back to a height of 0.5 m - 1.0 m above ground, it reduces the distance from the potential cutting to the root system and thus affect juvenility in cuttings (Rauter, 1982). The results of this study confirms the thought that the base of the tree is juvenile in terms of rooting ability. The low percentage rooting for 1.5 m and 2 m recorded here conforms to a report by Porlingis and Therios (1976) that
Fig. 8.3. The rate of leaf abscission of different stump heights (○ = 0.5 m, ▲ = 1.0 m, ● = 1.5 m, △ = 2.0 m) of Triplochiton scleroxylon leafy stem cuttings. Vertical bars show standard error.
Fig. 8.4. Callus formation of different stump heights (○ = 0.5 m, ▲ = 1.0 m, ● = 1.5 m, △ = 2.0 m) of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
rooting ability was high in cuttings taken from shoots at the base of an olive tree and gradually decreased to a constant value from shoots taken at a height of 1.5 - 2.0 metres.

The results obtained in this study may be associated with a phase change (juvenility) gradient with distance from the ground. Reports by Fortainer and Jonkers (1976), Wareing and Frydman (1976), Paton et al. (1980) and Reuveni et al. (1990) indicated that juvenility appears to be related in part to the distance of the plant parts from the roots. However, it is also possible that it is due to physiological ageing associated with stockplant height, as reported for *Triplochiton scleroxylon* by Leakey (1983). In many tree species juvenility tends to persist in the lower part of the plant. Therefore cuttings taken from different parts of an individual plant at various stages of development differ considerably in their rooting ability (Reuveni et al., 1990).

Rooting ability of cuttings has also been found to decline with increasing height of hedges (Morgan and Mason, 1992). In their studies initial results from outdoor hedges of sitka spruce, a height of 0.75 m was considered to achieve the optimum balance between numbers and rootability of cuttings. Studies in root - shoot gradients in *Eucalyptus* by Paton et al. (1980) show that juvenility of the shoots appears to depend largely on proximity to the shoot base or root. These authors further explained that this spatial relationship implies the existence of a juvenility gradient that extends above the shoot base.

In this experiment the variation in rooting may also have been due to distribution of assimilates within the stem in relation to stump height from the ground. This could have a negative effect on shoot growth and hence subsequent rooting, because more energy is required for the production of more shoots. The gradient of nutrients, endogenous concentrations of growth regulators and other compounds were probably higher as stump height decrease. Shoots produced at a stump height of 2 m may have had a lower content of these substances which then may have affected rooting.
The percentage leaf abscission was relatively high among treatments throughout the experiment. This may have caused rooting difficulty, due to cessation of photosynthesis and other physiological activities. It is likely that the effect of leaf abscission resulted in the higher percentage mortality recorded, as in Chapter 8. Water loss through transpiration may cause desiccation problems in the cutting (Leakey, 1985) and cause them to die before root formation can take place as was seen in Chapter 8. Water loss also reduces current photosynthesis by closing the stomata (Loach, 1988a). It has also been reported by Loach (1977) and Grange and Loach (1984) that water stress reduces rooting. Water stress may cause changes in hormonal status and impose restriction on metabolism and also affect photosynthesis during rooting. The values of RWC recorded here show that significant water deficits occurred in these cuttings. This may account for the high percentage leaf abscission observed, and the associated high cutting mortalities.

Callus formation generally precedes adventitious root formation and roots may emerge through the callus (Hartmann et al., 1990). With this experiment, although most roots appeared through the callus, others either produced heavy callus without emergence of roots, or did not produce callus but rooted. Callus formation and adventitious root formation are therefore perhaps independent of each other even though they both involve cell division. However, they often occur simultaneously, due to their dependence upon similar internal and environmental conditions (Hartmann et al., 1990).
CHAPTER NINE

THE ROOTING ABILITY OF LEAFY STEM CUTTINGS OF MATURE TREES OF *TRIPOCHITON SCLEROXYLON*.

9.1 Rooting of *Triplochiton scleroxylon* cuttings in relation to different branch position.

9.1.1 Introduction

The age of the tree from which the cuttings are taken has been found to influence the rooting of cuttings, in a range of species such as Norway spruce (Johnsen, 1985). For example, previous studies in apple have shown that scion cultivars are more difficult to root than clonal rootstocks (Delargy and Wright, 1979). The differences between chronologically young and old shoots are both physiological and morphological (Leakey et al., 1992).

This experiment was aimed at studying the rooting differences between different types of branch cuttings and their comparison with later coppice shoots from the same tree.

9.1.2 Materials and methods

Three trees of age 13 years growing at the Mesewam nursery of the Forestry Research Institute of Ghana, were felled at a height of 1 m from the ground for coppicing and the branches were used for this experiment. The branches were grouped subjectively into six classes based on position in the tree crown (Fig. 9.1). The categories were as follows:

- **P** - At the top of the crown
- **S5** - Just below the primary branches (P)
- **S4** - Below S5
- **S3** - Below the secondary branch 4 (S4)
- **S2** - Below the secondary branch 3 (S3)
- **S1** - At the base of the crown.
Fig. 8.1 Grouping of branches on a mature (13 years old) tree of *Triplochiton scleroxylon* for vegetative propagation by cuttings.
Cuttings were then harvested from these tree branches for vegetative propagation. A randomised block design with nine replicates per treatment was used on the propagation bed. A total of 6 cuttings per treatment per block were taken. Thirty cuttings (5 per treatment) were sampled for RWC measurements at day 0 and 21. Rooting Powder containing 0.8% IBA (Seradix, Embetec crop protection, North Yorkshire, UK) was applied by dipping the cutting bases into the auxin powder prior to insertion. Weekly assessments were made of rooting performance: variables were recorded as stated previously under preparation of cuttings (Section 4.4). The relative humidity, bed and air temperature, and light (irradiance) were recorded daily in the propagators (Table 9.1.1).

9.1.3 Results
9.1.3.1 Microclimate
The microclimatic measurements measured during the experimental period showed that a higher temperature (29 °C) was recorded for the air within the propagator than the rooting medium. There was a large difference with respect to measurement of irradiance outside and inside the propagator, with the higher recorded ((5394 Lux)(102 μmol m⁻² s⁻¹ )) outside the propagator. The higher relative humidity (97.7%) was however recorded inside the propagator (Table 9.1.1).

Table 9.1.1 Temperatures (air and bed), irradiance (inside and outside propagators) and relative humidity recorded during the experiment on different branch position of Triplochiton scleroxylon leafy stem cuttings.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (± SE)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>29.0 ± 1.9</td>
<td>25.3 - 31.1</td>
</tr>
<tr>
<td>Bed</td>
<td>26.4 ± 1.4</td>
<td>24 - 28</td>
</tr>
<tr>
<td>Irradiance (Lux)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>1200 ± 629 (23 ± 12)</td>
<td>550 - 2800 (10 - 53)</td>
</tr>
<tr>
<td>Outside</td>
<td>5394 ± 262 (102 ± 5)</td>
<td>5120 - 5850 (97 - 111)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>97.7 ± 6.6</td>
<td>90.9 - 99.0</td>
</tr>
<tr>
<td>Outside</td>
<td>73.1 ± 11.3</td>
<td>59.9 - 97.3</td>
</tr>
</tbody>
</table>
9.1.3.2 Rooting

The rooting success under all the treatments was relatively low. There was no rooting in any of the cuttings from week 1 - week 4. By week 5, 2.3% of Treatment S2 and 4.9% of Treatment P had rooted (Table 9.1.2).

Table 9.1.2: Rooting percentages of *Triplochiton scleroxylon* leafy stem cuttings taken from different branch positions.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Branch position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

9.1.3.3 Mortality

Death of cuttings began at week 2 with the highest (54.2%) recorded for S4 (Secondary branch 4) and the lowest (15.9%) for P (Primary branch). This increased up to week 5 with the highest (100%) recorded for secondary branches (S1, S4, S5) and lowest (95.1%) for primary branches (P) (Fig. 9.1.1).

9.1.3.4 Leaf abscission

Leaf abscission began during the first week of the experiment in all the treatments. Leaf abscission increased from week 1 to week 4 then remained constant until week 5 for all the treatments as shown in (Fig 9.1.2), with the highest percentage of leaf abscission recorded for secondary branch 1 (S1) (70.8%) and the lowest for primary branch (P) (46.3%).

9.1.3.5 Relative water content (RWC)

Relative water content increased in most treatments between day 0 and day 21.
Fig. 9.1.1. Mortality rate between different branch position (○ = S1, • = S2, △ = S3, ▲ = S4, □ = P), of a mature (13 years old) tree of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
Fig. 9.1.2. The rate of leaf abscission of different branch position (○ = S1, ⋄ = S2, △ = S3, ▲ = S4, ▼ = S5, □ = P), of a mature (13 years old) tree of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
Treatments S1, S2, S3 and P showed an increased from day 0 to day 21 while S4, and S5 showed a decrease. There was no relationship between treatments (Table 9.1.3).

Table 9.1.3: Relative water content of branch positions at day 0 and day 21.

<table>
<thead>
<tr>
<th>Day</th>
<th>Branch position</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>82.2 ± 6.9 a</td>
<td>86.2 ± 2.1 a</td>
<td>85.5 ± 1.6 a</td>
<td>86.8 ± 3.6 a</td>
<td>87.6 ± 3.0 a</td>
<td>78.8 ± 8.4 a</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>89.4 ± 5.3 a</td>
<td>87.7 ± 4.3 a</td>
<td>86.3 ± 3.1 a</td>
<td>81.6 ± 5.7 a</td>
<td>85.2 ± 6.2 a</td>
<td>86.8 ± 4.9 a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P > 0.05).

9.1.4 Discussion

Results from this study show that it was possible for cuttings from a mature (ontogenetically old) plant to root by week 5, but the rooting percentage was very low. These results conform to earlier studies (Chalupa, 1982; Davies et al., 1982; Dirr and Heuser, 1987; Hackett, 1988) indicating that adventitious rooting declines with age of the plant, and that cuttings taken from a mature crown are difficult to root. None of the cuttings from the mature crown of *Eucalyptus camaldulensis* rooted (Hartney, 1980; Heth et al., 1986; Reuveni et al., 1990). The physiological condition of the plant from which the cuttings are taken has been reported to affect rooting (Leakey et al., 1992) and to be one of the most important factors affecting rooting response of cuttings (Brix, 1974). The epigenetic and physiological mechanisms involved in the ontogenetic changes from juvenility to maturity are poorly understood and probably involve changes of physiologically influential substances (Robbins, 1959) through changes in genome activity. Studies in *Douglas fir* by Chalupa (1982) showed that the age of the ortet from which cuttings were taken had a pronounced influence on the formation of roots. According to Hartmann et al. (1990) the development of a continuous sclerenchyma ring between the phloem and cortex, exterior to the point of
origin of adventitious roots, is often associated with maturation and may inhibit root development. This, however, is probably more likely to be related to physiological ageing than ontogenetic ageing. To increase the rooting ability of older plant materials, hedging has often found to be effective. This supports what has been reported by Roulund (1981) and van den Driessche (1983).

The physiological condition of these branches in terms of their assimilate reserves, differs greatly from seedling or coppiced shoots. Mature cuttings may have lower endogenous contents of auxins and other co-factors. The very low rooting percentage recorded here may be due to a number of branch apices competing for water, nutrients and light.

In the present study, the cuttings shed their leaves as early as the first week. This might be attributable to water stress caused by excessive transpiration. The mortality rate of the cuttings from these plants was very high due to the shedding of the leaves. The leaf has strong stimulatory influence on root initiation besides also being generally associated with photosynthetic activity (Hartmann et al., 1990). This function of the leaf may have been restricted due to the leaf shedding, which may thereby have caused the high mortality rate.

According to Borchert (1994) assessment of water status is considerably more difficult in trees because of their large size and complexity. For instance, steep gradients in water potential may develop between trunk and outer branches (Hinckley et al. 1991). However, the results obtained in this study follow what Newton and Jones (1993a) found in Cordia alliodora and Albizia guachepele, where RWC increased with time during propagation, whilst they differed from the results obtained in Terminalia spinosa and Triplochiton scleroxylon, where RWC declined with time. The relative water content results show that the cuttings were suffering from water deficit which may account for the low rooting percentages observed, at least in part.
9.2 Differences in rooting ability between cuttings from coppiced shoots from mature (13 years old) trees of *Triplochiton scleroxylon.*

9.2.1 Introduction
Cuttings taken from older trees are generally more difficult to root than those from younger trees (MacDonald, 1986). In order to produce shoots which can root from older trees, several techniques have been developed. These include serial propagation by cuttings (Black, 1972; Dormling and Kellerstam, 1981), severe pruning of mature ortets (Black, 1972; Dormling and Kellerstam, 1981; Copes, 1983), and growing of mature ortets under various cultural treatments (Dormling and Kellerstam, 1981). Stem cuttings taken from vigorously growing coppice shoots near the base of a tree (even in a mature tree) retain the ability to form roots to a greater extent and generally root more successfully than those taken from branches of the same tree (Robinson and Schwabe, 1977; Hartney, 1980; Heth et al., 1986). It has been found that cuttings taken from coppiced shoots often root more easily than those taken from genetically identical unhedged plants of the same age (Libby and Hood, 1976; van den Driessche, 1983). The aim of this experiment was to study the effect of genotype on rooting ability of coppiced shoots from mature trees; thus testing the hypothesis that genetic differences between individual trees might affect the rooting ability of coppiced shoots.

9.2.2 Materials and methods
Coppiced shoots from three mature (13 years old) trees were used in this experiment. The stumps were cut at a height of 1 m above the ground. The design used was the same as mentioned in section 9.1 with nine replicates. The number of cuttings used for the experiment were 5 per block. The treatments were 1, 2 and 3 (representing tree number 1, 2 and 3) respectively. The procedure used for the assessment was as mentioned under preparation of cuttings (Section 4.4). Standard errors and percentages were calculated following the procedure described by Snedecor and Cochran (1980) using SAS (1980). The percentage data was transformed and analysis of variance was performed using SAS (1980) (Table A5 in the appendix). The microclimatic conditions under which the experiment was conducted were also measured (Table 9.2.1).
9.2.3 Results

9.2.3.1 Microclimate

The microclimatic conditions recorded during the experimental period are illustrated in Table 9.2.1, and showed that air temperature within the propagator was higher (29.6 °C) than that of the bed. The irradiance measured outside was higher (5363 Lux)(102 μ mol m⁻² s⁻¹) than that inside the propagator. The relative humidity was higher (97.2%) inside the propagator than outside.

Table 9.2.1: Temperatures (air and bed), irradiance (inside and outside propagators) and relative humidity recorded during the experiment on coppice shoots from mature (13 year old) trees.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>29.6 ± 2.4</td>
<td>27 - 34</td>
</tr>
<tr>
<td>Bed</td>
<td>26.4 ± 1.3</td>
<td>25 - 29</td>
</tr>
<tr>
<td>Irradiance (Lux)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>1792 ± 1275 (34 ± 24)</td>
<td>750 - 4800 (14 - 91)</td>
</tr>
<tr>
<td>Outside</td>
<td>5363 ± 1228 (102 ± 23)</td>
<td>5105 - 6000 (97 - 114)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>97.2 ± 5.3</td>
<td>91.4 - 97.8</td>
</tr>
<tr>
<td>Outside</td>
<td>68.5 ± 4.9</td>
<td>56.6 - 78.6</td>
</tr>
</tbody>
</table>

9.2.3.2 Rooting

Rooting of the cuttings began at week 4. At the end of the experiment at week 9, the highest percentage (65.8%) rooting was recorded for tree 2 and the lowest (32.4%) for tree 1 (Fig 9.2.1)

9.2.3.3 Mortality

Death of cuttings was recorded from week 1. The mortality rate of the cuttings increased for the subsequent weeks until week 4, after which it remained constant until week 8 and again increased at the end of the experiment at week 9, with the highest percentage (64.9%) of mortality recorded for tree 1 and the lowest (34.2%) for tree 2 (Fig. 9.2.2).
Fig 9.2.1. Rooting differences between coppice shoots from mature (13 years old) trees (○ = Tree 1, ▽ = Tree 2, □ = Tree 3) of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
Fig 9.2.2. Mortality rate of coppice shoots from mature (13 years old) trees (○ = Tree 1, ▼ = Tree 2, □ = Tree 3) of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
9.2.3.4 Leaf abscission

The percentage leaf abscission recorded at week 1 was very low for all the trees. At week 9 the highest percentage (25.0%) was recorded for tree 3 and the lowest (7.9%) for tree 2 (Fig 9.2.3).

9.2.4 Discussion

Vegetative propagation of rooted cuttings is usually restricted to juvenile material, such as seedlings, because ageing reduces growth vigour and ability to root. Libby et al. (1972) and Roulund (1981) reported that the rooting ability of older material can be increased by hedging stockplants. This is because coppicing induces delays in ontogeny so that loss of easy rooting ability is delayed. Hedging of a parent tree (ortet) may be assumed to reduce maturation (ontogenetic ageing). This method also reduces propagation problems caused by maturation in plants. The importance of hedging has been reported in Sitka spruce by van den Driessche (1983) and Morgan and Mason (1992).

The results obtained in this experiment show clearly that felling a tree and using the coppice shoots for vegetative propagation is effective and produces cuttings which are able to root. Cuttings taken from regrowth on hedges root more readily than those taken from genetically identical unhedged plants of the same age such as found in radiata pine (Libby and Hood, 1976; Bolstad and Libby, 1982; van den Driessche, 1983) and Douglas-fir (Copes, 1983). In this experiment, there was a pronounced difference in rooting capacity between tree 2 and the other two. Brix and Baker (1971) attributed differences in rooting to strong genotypic variation in rootability as well as environmental factors. Furthermore, the differences in rooting percentage obtained on these shoots may be due to physiological variations inherent in the individual trees. A similar results were observed in Eucalyptus camaldulensis clones (Reuveni et al., 1990) and in many other experiments on many forest tree species.

The retention of leaves on the cuttings may have played an important role in adventitious root formation. The cuttings were able to maintain their leaves and so
Fig. 9.2.3. The percentage leaf abscission of coppice shoots from mature (13 years old) trees (○ = Tree 1, ▼ = Tree 2, □ = Tree 3) of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
reduced their mortality rate with more cuttings rooting. However, the rooting was spread over a number of weeks.

Researchers have generally agreed that adventitious root formation involves a number of steps with each having different requirements for growth substances (Eriksen, 1974, Mohammed and Eriksen, 1974). Comparing the results of sections 9.1 and 9.2, cuttings from the crown of *Triplochiton scleroxylon* did not root as well as cuttings from their coppice stumps, which supports for instance what Porlingis and Therios (1976) reported on olive. The results also substantiate what was reported by Libby and Hood (1976) on radiata pine that cuttings taken from hedged ramets rooted faster and in greater percentage compared to cuttings from the upper crowns of the tree. The differences in rooting between these may be partially attributed to endogenous auxin contents. Davies and Joiner (1980) also found that mature *Ficus pumila* cuttings did not root as efficiently as juvenile material. They attributed their result to a lower endogenous auxin contents and/or other co-factors needed to stimulate root initiation in the mature *Ficus pumila* cuttings.

The observation of the cuttings from mature trees and coppice shoots from these mature trees indicated that maturation (ontogenetic ageing) has an effect on rooting ability of the former and that the shoots from the cut stumps are effectively more juvenile (physiological youth) in terms of rooting ability (Leakey et al., 1992) than the original tree.
CHAPTER TEN

THE EFFECT OF COPPICE SHOOTS FROM DIFFERENT ORIGINS (GRAFTED/BUDDED, MATURE AND SAPLING) ON ADVENTITIOUS ROOT FORMATION OF *TRIPLOCHITON SCLEROXYLON* LEAFY STEM CUTTINGS.

10.1 Introduction

As has been already been stated, cuttings taken from seedlings and new shoots arising near the base of the main stem can often root well, but those from adult trees rarely do (Hartney, 1980). Therefore superior trees are often felled to produce coppice shoots. Where trees cannot be felled they have to be rejuvenated by tissue culture or by reinvigoration of the adult tissue by grafting onto seedling rootstocks. In some species, cuttings from shoots that are produced after grafting, root (Heth *et al.*, 1986).

The aim of this experiment was to study the effect of coppice shoots from budded (grafted), mature (13 year old) trees and saplings on the rooting success of *Triplochiton scleroxylon* leafy stem cuttings, and to test the hypothesis that the type of plant materials used influences adventitious root formation.

10.2 Materials and methods

Three different types of plant materials were used in this experiment, namely:

i) coppice shoots from saplings (3-year old) growing in direct sunlight or shade (50% light interception),

ii) coppice shoots from a felled mature (13-year old) trees growing in a plantation, and

iii) coppice shoots from mature material (21-year old) budded/grafted onto rootstock (3-year old) and grown in direct sunlight and shade (50% light interception) at Mesewam nursery of Forestry Research Institute of Ghana.

The bases of the cuttings were dipped in rooting powder (Seradix 3, 0.8% IBA, Embetec Crop Protection, North Yorkshire, England) before inserting them on the
propagating bed. The design was a randomised block replicated nine times per each treatment. 8 cuttings per treatment per block were taken. Assessments were done as mentioned under Section 4.4. Standard error and rooting percentage were calculated following the procedure described by Snedecor and Cochran (1980) using SAS (1980) (Table A6 in the appendix).

10.3 Results

10.3.1 Microclimate

The microclimatic conditions under which the cuttings were propagated showed that the air temperature recorded the higher (31.1 °C) reading than the bed temperature. The irradiance outside the propagator was also higher \((5500 \text{ Lux}) \times (104 \mu \text{ mol m}^{-2} \text{ s}^{-1})\) than that inside (Table 10.1). The relative humidity was higher (80.9%) inside the propagator than outside.

Table 10.1: Temperatures (air and bed), irradiance (inside and outside propagators) and relative humidity recorded during rooting of different type of plant material.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>31.1 ± 1.9</td>
<td>27 - 34</td>
</tr>
<tr>
<td>Bed</td>
<td>26.9 ± 1.6</td>
<td>24 - 29</td>
</tr>
<tr>
<td>Irradiance (Lux) ((\mu \text{ mol m}^{-2} \text{ s}^{-1}))</td>
<td>Inside</td>
<td>815 ± 339</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(15 ± 6)</td>
</tr>
<tr>
<td></td>
<td>Outside</td>
<td>5500 ± 141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(104 ± 3)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>Inside</td>
<td>80.9 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>Outside</td>
<td>67.4 ± 4.9</td>
</tr>
</tbody>
</table>

10.3.2 Rooting

Large and significant differences were found between cuttings of different origins. The rooting percentage was statistically different between treatments \((P< 0.05)\). The rate of rooting in all treatments was low for the first three weeks, except that more rapid rooting was recorded for cuttings from saplings grown under shade. The highest
percentage (56.9%) rooting was recorded for coppiced saplings grown under shade, followed by coppiced shoots from saplings grown unshaded (39.7%), whereas none of the cuttings rooted from budded material (both from shade or unshaded) (Fig 10.1). The number of roots per rooted cutting was higher in saplings than shoots from mature trees at week 8. However, root length was higher in saplings grown under shade and the lowest in cuttings from saplings grown under direct sunlight (Table 10.2).

Table 10.2: Effect of coppice shoots from different types of plant material on mean number of roots per rooted cutting and root length of *Triplochiton scleroxylon* leafy stem cuttings. (Mean ± SE).

<table>
<thead>
<tr>
<th>Type of plant material</th>
<th>Mean number of roots per rooted cutting</th>
<th>Mean root length per rooted cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grafted budded (Light)</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Grafted budded (Shade)</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Saplings (Light)</td>
<td>2.0 ± 0.2 a</td>
<td>14.5 ± 2.5 b</td>
</tr>
<tr>
<td>Saplings (Shade)</td>
<td>1.9 ± 0.2 a</td>
<td>25.8 ± 2.2 a</td>
</tr>
<tr>
<td>Mature</td>
<td>1.4 ± 0.1 a</td>
<td>18.0 ± 2.5 a,b</td>
</tr>
</tbody>
</table>

10.3.3 Mortality

The mortality rate was very high in all the treatments. At week 3, 100% of the cuttings from the budded material both from plant grown under shade and direct sunlight were dead. The lowest mortality was recorded for cuttings from saplings grown under light and shade (Fig 10.2). The high mortality recorded was due to high leaf abscission and rotting of the cuttings.

10.3.4 Leaf abscission

The percentage of leaf abscission recorded in all the treatments was high. The percentage leaf shedding for almost all the different types of plant materials used remained constant from week 4 to week 8, with the highest percentage recorded for budded material under light (66%) and the lowest (13%) for saplings grown under shade (Fig 10.3).
Fig. 10.1. Rooting differences between shoots from different types of plant materials (○ = Grafted/budded (from light), • = Grafted/budded (from shade), □ = Saplings (from light), ▲ = Mature, ● = Saplings (from shade)), of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
Fig. 10.2. The rate of mortality of cuttings from different types of plant materials (○ = Grafted/budded (from light), ● = Grafted/budded (from shade), □ = Saplings (from light), ▲ = Mature, ■ = Saplings (from shade)), of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
Fig. 10.3 The percentage leaf abscission of different types of plant materials (○ = Grafted/budded (from light), ● = Grafted/budded (from shade), □ = Saplings (from light), ▲ = Mature, ■ = Saplings (from shade)), of *Triplochiton scleroxylon* leafy stem cuttings. Vertical bars show standard error.
10.4 Discussion

Different types of plant materials of the same species have been found to differ in their rooting capability (Reuveni et al. 1990). Rooting ability of cuttings is said to decrease with the ontogenetic development of a plant from the juvenile to the adult phase (Libby and Conkle, 1966; Paton et al., 1970; 1980; Brix, 1974; Libby, 1974a; 1974b; Libby and Hood, 1976; Brix and van den Driessche, 1977; Hartney, 1980; Roulund, 1981; Chalupa, 1982; Davies et al., 1982; Mason and Gill, 1986; Hartmann et al., 1990) but this is poorly understood (Leakey 1985). This is thought to be due to the effects of environmental conditions under which the shoots grew or their physiological status (Leakey et al., 1992; Leakey et al., 1994). The results obtained in the present study show that cuttings taken from young plants (coppiced shoots from saplings) rooted more readily than those from adult (shoots from mature) trees while those from the adult budded source did not root at all. It is not possible to determine the reasons for this from the present study, but it is clear that the viability of mature cuttings was low since they were handled similarly. This might be due the physiological state (ontogenetic age in the case of grafted/budded and chronological age for the mature tree) of the materials. Moreover, in the case of the grafted budded material, it might be due to physiological ageing or the position of the hierarchy of the buds from the parent plant. In addition, mature shoots retain their ontogenetic age (maturity) when grafted/budded and may have continued the process of phase change.

Buds taken from the upper (mature) part of the plant are likely to produce shoots that are fully mature, and after being grafted on to a rootstock, the shoots that emerge have increased growth vigour (reinvigoration). However, cuttings taken from them tend to loose their high rooting capacity due to ontogenetic ageing. Paton et al. (1970) reported that adult shoots do not root as stem cuttings under ordinary propagating conditions in Eucalyptus. In respect of this they linked this difficulty to the ontogenetic age at which a sufficiently high concentration of inhibitor is produced to prevent rooting. Furthermore, according to Ununger and Ekberg (1987) ageing in Picea abies leads to a low frequency of rooted cuttings, a loss of growth capacity and plagiotropic growth of cuttings originating from shoots collected from older trees.
Many researchers have found that fruit tree scion cultivars are often difficult to root from cuttings (Wilkinson and Withnal, 1970; Child and Atkins, 1980) but rootstocks usually root readily (Howard, 1971), although considerable variation was found between rootstocks (Delargy and Wright 1979; James 1983a;b). The grafted budded material used in this study is closely associated with the attainment of maturity and exhibiting loss of rooting ability due to its position on the original stockplant. Grafting on to a juvenile rootstock is assumed to physiologically reinvigorate the adult scion (Leakey, 1988); this was observed in the grafted plants of *Triplochiton scleroxylon* in this study. Van Dorsser and Fauld (1991) reported successful attainment of a sufficient number of rooted cuttings from physiologically mature cutting material of *Pinus radiata*. Their technique involved the pre-treatment of the trees by topping and ring barking four to six weeks prior to the removal of cuttings from the donor tree, then rooting in tunnel houses.

Coppice shoots from the mature tree and the sapling displayed differences in their rooting ability. The percentage rooting of cuttings from the sapling was higher than cuttings from coppice shoots from mature trees. In this experiment the shoots used as a source of cuttings were of the same chronological age, but apparently differed physiologically as reflected in their rooting abilities. Although coppiced shoots from mature stumps are also usually thought of as juvenile, their rooting capability was not as high as that of the saplings. The shoots from saplings rooted faster and formed more roots per rooted cutting than cuttings from mature shoots. These results confirm previous work with other species such as Olive that juvenile cuttings root faster and in higher percentages than mature cuttings (Porlingis and Therios, 1976).

Shoots of the same chronological age can also be very different physiologically, as expressed by their rooting ability. Hence it is likely that the physiological condition of these shoots in terms of their assimilate reserves differed greatly between seedling and mature coppice shoots (Leakey *et al.*, 1992). Physiological factors may have affected the rooting ability of the cuttings and therefore either cuttings from the mature shoots may require higher exogenous auxin contents and probably a longer time to
root as compared to those from the saplings. The differences in rooting ability of coppice shoots of the same chronological age perhaps indicate that physiological ageing is also influenced by factors other than the complexity and size of growing trees. The mature (coppice shoot) cuttings may also have lower endogenous auxin concentrations and/or other endogenous chemicals required to enhance root formation as was reported by Davies and Joiner (1980) in *Ficus pumila* juvenile and mature cuttings. Then again, Morgan and Mason (1992) reported that rooting ability of cuttings from spruce and larch hedges declines from 6 to 7 years from establishment, perhaps as a result of branches becoming more woody and the occurrence of self shading.

The low rooting during propagation in this experiment may be attributed to leaf abscission. Leaf shedding is a common cause of cutting mortality as leaves play an important role as a source of carbohydrates and also affect the cutting through their influence on water status (Leakey and Coutts, 1989). Leakey and Storeton-West (1992) reported that rooting ability in *Triplochiton scleroxylon* is strongly influenced by production of current assimilates during propagation and that those cuttings which rooted well are those with the highest rates of pre-severance net photosynthesis. The presence of leaves is essential for the above processes to take place. The relatively low humidity and high air temperature in the propagators might also have influenced the leaf abscission in this study.
CHAPTER ELEVEN

GENERAL DISCUSSION

11.1 Introduction
This chapter represents an attempt to evaluate the objectives outlined in Chapter 1, in the light of the experiments described in subsequent chapters, along with suggestions for future work. A schematic diagram showing how vegetative propagation by cuttings can be incorporated in community/rural afforestation programme is shown (Fig. 11.1).

11.2 Pre-severance effects on rooting

i) Stockplant light environment
Many environmental and physiological factors can influence adventitious root formation in leafy stem cuttings (Fernquist, 1966; Leakey et al., 1992; 1994). These factors may operate both pre- and post-severance (Leakey and Coutts, 1989, Leakey and Storeton-West, 1992) and may act in a complex and interrelated manner. The stockplant growth environment (for example, light, nutrient and water availability), the position of shoot on the stockplant and the age of the stockplants from which the cuttings are collected, are all known to affect the physiology and rooting ability of cuttings (Hansen, 1976; Hartney, 1980; Veierskov et al., 1982a,b; Leakey and Coutts 1989; Hartmann et al., 1990; Leakey and Storeton-West, 1992).

In all the experiments involving the two light treatments, direct sunlight and 50% light interception, the results showed that the direct sunlight was inhibitory to rooting; that is the percentage rooting was lower compared to results obtained from the shade treatment. According to Hansen and Eriksen (1974) high irradiance causes a high carbohydrate content in the cutting at the time of excision, which may be supra-optimal for the rooting process. The cuttings from stockplants grown under 50% light interception produced a relatively high percentage rooting. It seems therefore that
rooting in this species is positively affected when stockplants are grown under a certain degree of shade in a natural environment. Reduction of light intensity to stockplants has been observed to subsequently increase the rooting of cuttings in many species (Eliasson and Brunes, 1980, Stromquist and Hansen, 1980; Vieitez, 1981; Howard, 1972; Leakey and Storeton-West, 1992). Despite the infection of both stockplants and cuttings by psyllids with consequence application of insecticide and fungicide the results were however not biased. For although the rooting percentage were not much high, the results did follow the trend reported by other researchers such as Leakey and Storeton-West (1992).

It is widely accepted that root initiation and root development are influenced by a different set of conditions (Lovell and White, 1986). The number of roots produced by cutting, on the other hand, appears to be highly influenced by the cuttings' ability to supply carbohydrates, either from stored reserves or through current photosynthesis to the area where roots appear (Haaland, 1976; Moe and Andersen, 1988). The results presented here were consistent with the above statement. With the exception of Chapter 5, the number of roots per rooted cutting and root length was higher in cuttings from stockplants grown under direct sunlight, than in cuttings from stockplants grown under 50% light interception.

II) Effect of fertiliser application to stockplants on rooting
Mineral nutrition influences a variety of endogenous and biochemical responses associated with variation in stockplant growth (Haissig, 1986) and hence affects the rooting ability of cuttings prepared from them. Root primordium initiation is hormonally controlled (Veierskov et al., 1982a;b, Veierskov and Andersen, 1982; Lovell and White, 1986; Moe and Andersen, 1988) and not markedly influenced by excesses or deficiencies of particular mineral nutrients (Haissig, 1986). This therefore suggests that the nutritional status of the stockplant might be more important for root development and growth than for root initiation (Blazich, 1988).

Studies on Triplochiton scleroxylon by Leakey and Storeton-West (1992) showed that
application of nutrients to stockplants increased the rooting percentage of cuttings. According to Good and Tukey (1967) higher rooting depends on an adequate mineral nutrition before and during rooting. In Chapter 7, higher percentage rooting was obtained in cuttings from stockplants supplied with fertiliser grown under both direct sunlight and 50% light interception. However, the percentage rooting of cuttings from stockplants with fertiliser and grown under direct sunlight was higher than cuttings from stockplants with fertiliser and grown under 50% light interception. Whereas cuttings whose stockplants were treated with no fertiliser but were grown under similar light conditions gave lower percentage rooting. The fact that fertiliser addition increased rooting indicated that mineral nutrient contents may have limited rooting at lower nutrient availabilities.

11.3 Effect of post-severance treatments on rooting

I) Leaf Area

The presence of leaves on stem cuttings exerts a strong stimulatory influence on adventitious root formation. This appears to be partly due to the role the leaf plays in the production of carbohydrates through photosynthetic activities (Reuveni and Raviv, 1980) and therefore the carbon budget of the cutting, and partly because the leaf supplies auxins and other compounds which stimulate rooting (Wightman et al., 1980; Stanley and Toogood, 1981). The practice of trimming the leaf aims at minimising water loss while allowing sufficient photosynthesis to occur during propagation to enable root development (Okoro and Grace, 1976; Eliasson and Brunes, 1980; Leakey et al., 1982b; Leakey and Coutts, 1989; Newton et al., 1992a; Leakey et al., 1994). The stimulatory effects of leaves on rooting have also been demonstrated for instance by Altman and Wareing (1975) in Phaseolus vulgaris.

The rooting ability of cuttings is partly a function of their pre-severance ability to produce assimilates. In addition it has been clearly shown that Triplochiton scleroxylon cuttings are capable of assimilate production after severance (Leakey and Coutts, 1989), as well as those of Pisum sativum (Eliasson, 1978), indicating that the cuttings ability to produce assimilates after severance is important to the rooting process. To
achieve this the presence of leaf is generally essential.

In Chapter 5 the highest percentage rooting was obtained by the largest leaf area of cuttings from stockplants grown under shade. This contrasts with the results obtained by Leakey et al. (1982b). This may reflect the contrasting environments that the cuttings were propagated under; a mist system in the case of Leakey et al. (1982b) and a non-mist system in this investigation. Furthermore, larger leaf areas resulted in a decrease in root number, suggesting that rooting percentage and root numbers are affected by a different set of conditions. The low rooting percentage recorded for smaller leaf areas was probably due to lack of assimilate production during propagation.

II) Cutting length

According to Leakey et al. (1994) cutting length and leaf area influences the capacity of a cutting to store assimilates produced during pre- and post-severance. Leakey and Coutts (1989) reported that the relationship between cutting length and rooting ability seemed to be dependent on stored reserves. In Eucalyptus grandis (Hoad and Leakey, 1992), Triplochiton scleroxyylon (Leakey and Mohammed, 1985), Prosopis juliflora (Wilson et al., 1989) and basswood (Morsink and Smith, 1974), cutting length and diameter have been considered to be among the most important variables influencing the rooting ability of cuttings. Cutting length was usually more strongly correlated with the rooting percentage than with the number of roots per rooted cutting in Triplochiton scleroxyylon (Leakey and Mohammed, 1985). The highest rooting percentage has been obtained with large cuttings and the lowest with small ones in Sitka spruce (Mason et al., 1992).

In the experiment described in Chapter 6, longer cuttings produced the highest percentage rooting, and the shorter cuttings the lowest. The high rooting percentage of longer cuttings might be explained by their higher carbohydrate reserves. Furthermore, a higher percentage rooting was obtained for longer cuttings from stockplants grown under shade. This lower irradiance to the stockplants may have
avoided reduced rates of net photosynthesis caused by end product inhibition.

11.4 Effect of age on rooting

11.4.1 Effect of branch position and coppice shoots from a mature (13 years old) (chronological age) tree.

1) Branch position

Ageing of trees involves a number of developmental processes associated with loss of vigour and increased complexity taking place simultaneously. Three types of ageing have been distinguished by Fortanier and Jonkers (1976): chronological, physiological and ontogenetical. Chronological ageing does not give any information about the ontogenetical phase or physiological condition reached, but rather indicates the time elapsed since the plant germinated. Physiological ageing is caused by an increased disorganisation, exhaustion and stress, and it has no well defined localisation. Ontogenetic ageing is genetically programmed and is localised in the meristems and is irreversible.

Adventitious root formation typically declines with the age of the plant (Chalupa, 1982; Davies et al., 1982; Dirr and Heuser, 1987; Hackett, 1988), and cuttings taken from a mature crown are difficult to root, as found for example in Eucalyptus camaldulensis (Hartney, 1980; Heth et al., 1986; Reuveni et al., 1990). The fact that cuttings from mature trees are difficult to root presents a serious problem in propagation of many species (Johnsen, 1985).

The physiological condition (physiological age) of the plant from which the cuttings are taken has also been reported to affect rooting (Leakey et al., 1992) and to be one of the most important factors affecting rooting response of cuttings (Brix, 1974). The rooting percentage obtained in Chapter 8.1 was very low, which might be due to a large number of branch apices competing for water, nutrients and minerals. This is due to the fact that the internal transport path gradually becomes longer as the tree grows, and the balance between assimilation and between root and shoot activity becomes less favourable (Fortanier and Jonkers, 1976). Furthermore, since as the tree grows many
shoots decrease in vigour with its increased size and complexity, so age affects physiological processes like photosynthesis, respiration, overall growth patterns, resource allocation; and other measures of plant vigour (Fortanier and Jonkers, 1976). It has been reported by Clark (1983) that changes in the rates of photosynthesis and respiration occur during development, and on an organ level, photosynthetic rate often declines with age. For an individual leaf net photosynthetic rates decline with time, and respiration patterns also change with increasing age, reflecting the large increases in stem volume that take place (Clark, 1983). Although these processes were not measured due to lack of equipment, they might have been relevant to the rooting ability of the plant material used in the experiment under Chapter 8.1.

ii) Coppice shoots from mature (13 years old) trees
Stem cuttings taken from growing coppice shoots (physiologically juvenile) near the base of a tree retain a greater ability to form roots than those taken from branches of the same tree (Libby and Hood, 1976; Robinson and Schwabe, 1977; van den Driessche, 1983), as found in *Eucalyptus* (Hartney, 1980; Heth et al., 1986). The idea of pruning the branches was to promote shoot development from adventitious buds, these being more juvenile than those removed (Fortanier and Jonkers, 1976; Dormling and Kellerstam, 1981). The result obtained in Chapter 8.2 shows clearly that the coppice shoots rooted more readily than the branches of the same tree in Chapter 8.1. The differences in rooting between these two experiments might be due to physiological age of the plant materials; the coppice shoots were assumed to be physiologically juvenile while the branches were physiologically mature. Their physiological processes such as photosynthesis, respiration and assimilate production would also be expected to differ.

Many researchers, for example Haissig (1986), Haissig and Riemenschreider (1988) and Reuveni et al. (1990), consider that rooting by cuttings is genetically influenced, but little is known of the possible mechanisms (Leakey et al., 1982b). Again, Brix and Baker (1971) have attributed differences in rooting between and within species to strong genotypic variation as well as environmental factors. In Chapter 8.2, there were
differences in rooting between the coppice stumps of the mature trees. The variation in rooting might be due to inherent genetic factors as well as physiological variation between the individual trees, since the shoots produced were of the same chronological age. Similar results were observed in *Eucalyptus camaldulensis* clones (Reuveni *et al.*, 1990). Clonal variation in rooting is commonly observed, though the processes involved are still not fully understood.

### 11.4.2 Effect of different sources of plant material

The age of the tree has been found to influence rooting of cuttings and cuttings taken from older trees are generally more difficult to root than those from younger trees (MacDonald, 1986; Mason and Gill, 1986). This phenomenon is common in many tree species, such as *Eucalyptus* (Kormanik and Brown, 1974). According to Reuveni *et al.* (1990) different types of plant material of the same species differ in their rooting ability. This has been found to be due to the shoot's environmental conditions or physiological status (Leakey *et al*., 1992; Leakey *et al*., 1994). Different characters may respond variably to rejuvenation treatments. Rooting success, for example, may be indicative of a more juvenile state; other characters may indicate that only a partial rejuvenation has been achieved (Clair *et al*., 1985). Again, partial rejuvenation may be as a result of an improved physiological condition of the plant and not a true ontogenetical rejuvenation.

In Chapter 9 the rate of rooting was higher in coppice shoots from saplings (under shade), than the mature (13 year old) tree, while none rooted with respect to shoots from grafted/budded materials (from mature tree)(for both light and shade). Although all the shoots were of the same chronological age, they were different physiologically, due to the source of the individual material. The difficulty in rooting of the grafted/budded material was due to the ontogenetic age even after grafting unto a juvenile rootstock. According to Fortanier and Jonkers (1976) ontogenetic ageing is genetically programmed and localised in the meristems and is irreversible.
11.4.3 Effect of different stump heights on rooting

Juvenility is traditionally regarded as persisting near to the root system of trees or seedlings, and to be lost as the distance from shoot to the roots increases (Lyrene, 1981; Rauter, 1982; Howard and Ridout, 1991a,b). Thus, the bases of the seedlings or trees could be the site for the accumulation of substances essential for rooting and could indicate that the retention of juvenile characteristics is related to the position on the tree rather than to chronological age (Hartney, 1980). According to Morgan and Mason (1992) in Sitka spruce rooting ability of cuttings declines with increasing height of hedges.

In Chapter 10, although the coppice shoots were of the same chronological age, there were considerably rooting differences between the stump heights. The highest percentage rooting was obtained by stump height of 0.5 m and the lowest by 2 m. The results substantiates the proximity theory which has been proposed by the above researchers.

11.5 Overall variation in rooting ability between different experiments

The maximum percentage rooting was found to be different in each experiment. This may partly have been due to different sources of material used. For example, saplings were used in experiments under Chapters 5, 6, 7 and 10; branches from mature trees and coppice shoots from these trees were employed in Chapter 8 and in Chapter 9 saplings, grafted/budded as well as coppice shoots from mature trees were used. However, even when material from the same source was used (as in Chapters 5, 6, 7 and 10) rooting differences were recorded. These may be attributed at least in part to variation in propagation microclimate, or to different pre-severance treatments applied to stockplants in each experiment.

High temperature in a medium increases the metabolic activity and reduces the cell division cycle, thus probably affecting the rooting ability. In all the experiments the maximum air temperatures were higher than the maximum bed temperatures. The range of temperature differences might have resulted in the rooting variation in the
experiments. For example, experiments in Chapters 8, 9 and 10 with relatively high air temperatures and low bed temperatures gave higher rooting percentages as compared to experiments 1, 2 and 3 (Chapters 5, 6 and 7) with lower range of temperature differences.

Increased irradiance during propagation promotes the basipetal transport of auxin (Baadsman and Andersen, 1984), the accumulation of soluble carbohydrates at the base of the cuttings (Veierskov and Andersen, 1982) and the concentration of phenolic rooting inhibitors. The irradiance measured in the propagators also differed in the various experiments. These variations might have resulted in the differences in rooting ability between the experiments. The experiments in Chapters 6, 7, 8 and 10 with lower measured irradiance had relatively higher percentage rooting. Those in Chapters 5 and 8, with approximately twice the irradiance values to other experiments gave comparatively lower percentage rooting. In Chapter 8 the materials used were different physiologically (that is branches from mature trees as well as coppice shoots) which also influenced the percentage rooting.

A low relative humidity may result in water deficits, which may have an effect on rooting of cuttings. The relative humidities recorded in the various experiments were different and below 100%, indicating probably that the transpiration of the cuttings was high and therefore the cuttings were under water stress. Differences in the relative humidity between experiments may account for some of the variation in rooting, for the higher the relative humidity the higher the percentage rooting. For example, in Chapters 8, 10 and 9 with relative humidity in the order of 97%, 94% and 81%, their maximum percentage rooting at the final assessment was 66%, 58% and 57% respectively.

11.6 Practical Implications
The shading of stockplants prior to severance is important in vegetative propagation by cuttings, and under natural environment 50% light interception is recommended on the basis of these results. Fertiliser application to stockplants does not increase rooting
of stockplants grown under shade (50% light interception) but does to stockplants grown under direct sunlight. A leaf area of 100 cm$^2$ was found to be optimal for rooting of *Triplochiton scleroxylon* leafy stem cuttings from stockplants grown both under direct sunlight and 50% light interception under natural conditions. Cutting lengths of 15 cm and 10 cm gave the highest rooting percentages for cuttings from stockplants grown under shade than that from stockplants grown under direct sunlight. Therefore to maximise the number of cuttings, a cutting length of 10 cm is recommended.

The type of plant material used is important in determining the rooting ability of cuttings. For mature trees there is a need to prune and allow them to coppice in order to produce juvenile material which will have an increased rate of rooting ability during vegetative propagation by cuttings.

The height at which coppice shoots are produced also influences adventitious root formation. A stump height of 0.5 m displayed the highest percentage rooting. However, there was no significant difference between 0.5 m and 1.0 m stump heights. Therefore, for production of large numbers of cuttings for propagation a stump height of 1 m is recommended.

Moreover, to be able to assess the assimilate production in terms of biomass, and in terms of cuttings' rooting ability, it is important to have more information on the distribution of individual carbohydrates within the cuttings. It is therefore recommended that further studies be carried out to determine carbohydrate dynamics in cuttings derived from stockplants grown under different environmental conditions.

Further research is also required to define the appropriate microclimate during propagation to optimise rooting percentages.

**11.7 Conclusions and suggestions for further research**

In the natural environment, 50% light interception is recommended for the rooting of
cuttings harvested from stockplants grown under similar irradiance. For availability and maximisation of plant material for propagation, cutting length of 10 cm is recommended for both direct sunlight and 50% light interception. For stockplants grown under both direct sunlight and 50% light interception, leaf area of 100 cm² should be considered. Application of fertiliser is suitable for stockplants grown under direct sunlight but not for stockplants grown under 50% light interception.

For work of such nature, juvenile materials such as seedlings and coppice shoots, and stump height between 0.5 and 1 m are recommended. An improved propagation conditions should be required during propagation.

It is however suggested that further work be carried out to determine:

i) the quality of light under both direct sunlight and 50% light interception;

ii) Photosynthesis of stockplants and cuttings during propagation;

iii) to analyse carbohydrates before and during propagation;

iv) to determine stomatal conductances and specific leaf area.

It is further suggested that experiments be conducted under varying microclimatic conditions throughout the year. Again, experiments could be carried out first by putting stockplants under direct sunlight followed by shade or vice versa. And also it is important to train nursery staff in handling of cuttings.
Seeds from seed orchards or selected seed source

Selected mature trees (Plus trees selection)

Raising of seedlings

Coppice Shoots

Selection by progeny tests

Vegetative Propagation by cuttings

Raising of Clones

Selection by clonal Tests

Bulking up for Commercial Plantation eg. Timber

Multiplication of materials for community tree planting eg. Non-timber products

Fig. 11.1 Schematic diagram of Vegetative Propagation by cuttings in Tree Improvement.
REFERENCES


APPENDICES

Table A1. Analysis of variance to determine the influence of stockplant light environment and leaf areas on rooting percentage of *Triplochiton scleroxyylon* leafy stem cuttings at week 10.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIGHT</td>
<td>1</td>
<td>85.659</td>
<td>85.659</td>
<td>0.18</td>
<td>0.672</td>
</tr>
<tr>
<td>AREA</td>
<td>3</td>
<td>5640.271</td>
<td>1880.090</td>
<td>3.95</td>
<td>0.011</td>
</tr>
<tr>
<td>LIGHT*AREA</td>
<td>3</td>
<td>104.401</td>
<td>34.800</td>
<td>0.07</td>
<td>0.974</td>
</tr>
</tbody>
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Transformed treatment means

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transformed means</th>
<th>Least significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sunlight</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td>50% light interception</td>
<td>30.3</td>
<td>8.85</td>
</tr>
<tr>
<td>Leaf Areas (cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>27.9</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>42.3</td>
<td>12.5</td>
</tr>
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</table>

Table A2. Analysis of variance to determine the effect of light on stockplants growth and cutting lengths on rooting percentage of *Triplochiton scleroxyylon* leafy stem cuttings at week 8.

<table>
<thead>
<tr>
<th>Source</th>
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<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
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<td>LIGHT</td>
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<td>9180.918</td>
<td>9180.918</td>
<td>31.61</td>
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</tr>
<tr>
<td>LENGTH</td>
<td>2</td>
<td>1081.751</td>
<td>540.876</td>
<td>1.86</td>
<td>0.163</td>
</tr>
<tr>
<td>LIGHT*LENGTH</td>
<td>2</td>
<td>303.815</td>
<td>151.908</td>
<td>0.52</td>
<td>0.595</td>
</tr>
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</table>
### Transformed treatment means

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transformed means</th>
<th>Least significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sunlight</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td>50% light interception</td>
<td>41.8</td>
<td>8.02</td>
</tr>
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</table>

### Cutting Lengths (cm)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>26.3</td>
</tr>
<tr>
<td>10</td>
<td>29.6</td>
</tr>
<tr>
<td>15</td>
<td>35.7</td>
</tr>
</tbody>
</table>

### Table A3. Analysis of variance to determine the effects of light and fertiliser application on stockplants on rooting percentage of *Triphlochiton scleroxyylon* cuttings at week 7.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
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<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIGHT</td>
<td>1</td>
<td>0.035</td>
<td>0.035</td>
<td>0.00</td>
<td>0.984</td>
</tr>
<tr>
<td>FERT</td>
<td>1</td>
<td>1280.887</td>
<td>1280.887</td>
<td>15.12</td>
<td>0.001</td>
</tr>
<tr>
<td>LIGHT*FERT</td>
<td>1</td>
<td>276.314</td>
<td>276.314</td>
<td>3.26</td>
<td>0.080</td>
</tr>
</tbody>
</table>

### Transformed treatment means

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transformed means</th>
<th>Least significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sunlight</td>
<td>36.2</td>
<td></td>
</tr>
<tr>
<td>50% light interception</td>
<td>36.2</td>
<td>6.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fertiliser (g)</th>
<th></th>
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</tr>
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<tbody>
<tr>
<td>0</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>42.2</td>
<td>6.25</td>
</tr>
</tbody>
</table>
Table A4. Analysis of variance on the rooting percentage of different stump heights of *Triplochiton scleroxylon* leafy stem cuttings at week 8.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>M S</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREAT</td>
<td>3</td>
<td>2302.892</td>
<td>767.631</td>
<td>2.89</td>
<td>0.046</td>
</tr>
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</table>

Transformed treatment means

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transformed means</th>
<th>Least significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stump heights (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>51.9</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>45.7</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>39.6</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>33.3</td>
<td>13.40</td>
</tr>
</tbody>
</table>

Table A5. General linear model was used on the rooting percentage between coppiced shoots from mature trees.

<table>
<thead>
<tr>
<th>Source</th>
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<th>SS</th>
<th>M S</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREAT</td>
<td>2</td>
<td>2664.830</td>
<td>1332.415</td>
<td>4.93</td>
<td>0.017</td>
</tr>
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Transformed treatment means

Comparisons significant at the 0.05 level are indicated by '***'.

<table>
<thead>
<tr>
<th>Treatments comparison</th>
<th>Difference between means</th>
<th>Critical Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 - 3</td>
<td>20.1 ***</td>
<td></td>
</tr>
<tr>
<td>2 - 1</td>
<td>23.9 ***</td>
<td></td>
</tr>
<tr>
<td>3 - 2</td>
<td>-20.1 ***</td>
<td></td>
</tr>
<tr>
<td>3 - 1</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>1 - 2</td>
<td>-23.9 ***</td>
<td></td>
</tr>
<tr>
<td>1 - 3</td>
<td>3.7</td>
<td>2.07</td>
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</table>
Table A6. Analysis of variance on the rooting percentage of different type of plant materials of *Triplochiton scleroxylon* leafy stem cuttings at week 8.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREAT</td>
<td>4</td>
<td>18878.983</td>
<td>4719.746</td>
<td>105.43</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Transformed treatment means**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transformed means</th>
<th>Least significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saplings (Shade)</td>
<td>48.9</td>
<td></td>
</tr>
<tr>
<td>Saplings (Light)</td>
<td>38.9</td>
<td></td>
</tr>
<tr>
<td>Mature tree</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td>Budded/Grafted (Shade)</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Budded/Grafted (Light)</td>
<td>0.0</td>
<td>6.37</td>
</tr>
</tbody>
</table>