VEGETATIVE PROPAGATION OF CENTRAL AMERICAN HARDWOODS

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CHAPTER 1

Introduction

CONTEXT OF RESEARCH

The forestry situation in Central America

Tropical forests are biotically richer than any other ecosystem on Earth. Unfortunately, they are also being destroyed more rapidly than any other ecosystem (Cook *et al.* 1990). Tropical forests lie along a broad belt, 25° south and north of the Ecuator. Those in Latin America merit special concern, both because the region contains more than half - 57 per cent - of the world's remaining tropical forests, and because they are being depleted at increasingly higher rates (FAO 1993; Myers 1988; World Resource Institute 1990). The long-term costs of deforestation will be high; soil erosion, high sedimentation rates in dams and loss of unique habitats for animal and plant species are only some of the problems resulting from deforestation.

Experience has shown that agricultural activities in forest soils are often not sustainable, threatening the welfare of two thousand million people who live in this area (Myers 1988; AID 1989; World Resource Institute 1990). The rapid loss of soil productivity results in abandonment of such areas, which are then taken over by weeds that can grow on the poor, now compacted soil. All this starts a degradation process, seriously affecting both the social and economic welfare of human populations. Less obvious, but beginning to be a source of concern, are climate changes (Myers 1988; Cook *et al.* 1990; World Resource Institute 1990). Nowadays it is accepted that forests have a major effect on climate; their presence serves to temper extremes of global climatic variables such as precipitation and the reflectiveness of the Earth's surface, but perhaps the most vital connection between forests and climate is their role in the global carbon cycle (Myers 1988; Cook *et al.* 1990; World Resource Institute 1990). When the forests are cleared, their capacity to withhold carbon from the atmosphere is lost. As the atmospheric content of CO_2 and other gases increases, thermal radiation which would otherwise radiate into space is trapped. Such trapped radiation, re-emitted downwards, raises the temperature of the Earth's surface and atmosphere, causing the so called "greenhouse effect" (Cook *et al.* 1990). This general warming, together with the decrease in the amount of moisture being returned to the atmosphere by evapotranspiration from the forest itself, also causes changes in distribution and rates of precipitation (Myers 1988). This could trigger a selfreinforcing cycle of growing desiccation of the remaining forest cover, with declining moisture stocks followed by yet more desiccation. Eventually, the repercussions could extend outside the tropics (Myers 1988). There is already growing concern that climatic warming is probably well under way (Detwiler and Hall 1988; Schneider 1989), and it is believed that climatic dislocations will surely be experienced by the first quarter of the next century (Myers 1988). Indeed, there is increasing evidence that decline in forest cover is already showing its impact on local climates in many parts of the world (Myers 1988).

Until recently, the estimated rate of loss of natural tropical forests in the world was 11 million hectares per year (Hartshorn 1982). More recent studies, however, suggest that deforestation rates may be higher (FAO 1993). If these studies are accurate, the world is losing 15.4 million hectares of tropical forests annually. In Central America alone, 370 000 hectares of tropical forests are cut annually (Hartshorn 1982; Heckadon 1991). In some areas, particularly in the dry Pacific region, deforestation has already been extensive, to the point of severe genetic erosion and even extinction of particular forest populations. There are many reasons for this situation, including: i) the increasing human population and the unequal distribution of land ownership, ii) the expanding agricultural frontier, iii) the increasing demand for high-valued timber and other forest products, and iv) the fact that governments have not enforced their forest protection laws (Hartshorn 1982; AID 1989; World Resource Institute 1990; Heckadon 1991).

In Costa Rica, during the last decade there has been an increasing awareness of the need to stop massive deforestation, to protect valuable forest resources and to stimulate tree planting in degraded land, both through public and private programmes. The establishment of reserves and other protected areas has been relatively successful, and at present, approximately 24 per cent of the national territory falls within these categories (Hartshorn 1982). The deforestation rate in unprotected areas, on the other hand, is still alarmingly high: whilst roughly 60,000 hectares of forest are cut per year, only 7 000- 12 000 are reforested, many of which fail to yield acceptable products because of poor nursery stock, bad management practices and/or use of germplasm of poor genetic quality (Boshier and Mesén 1987). If these trends continue, it has been estimated that the natural forest outside natural parks and protected areas in Costa Rica will be exhausted by the end of the century, with subsequent environmental, social and economic problems (Flores 1985). The World Resource Institute (1990) has estimated that in Costa Rica, although three quarters of the best agricultural land is devoted to pastures, the amount of land devoted to agriculture continues to increase in unsuitable areas, provoking erosion losses as high as 726 metric tonnes of soil per hectare per year in some areas.

Plantation forestry is part of the solution to this critical situation, by supplying the forest products that the population requires and by reducing the pressure on the remaining natural forests. It is clear that forest plantations are urgently needed in Central America with both timber and multipurpose species. The importance of reforestation is set out explicitly in the Tropical Forestry Action Plan for Costa Rica, which aims for the reforestation of 133 000 ha over the period 1989-1994, consisting of 118 000 ha in block plantations and 25 000 ha of agroforestry systems, and the development of the capacity to establish 45 000 ha annually (TFAP 1989). Given the land tenure situation in Central America, it is clear that most planting will be undertaken by farmers with small to medium-sized holdings, either as individuals or in their organized groups, using both timber and multipurpose species. To be successful, however, plantation forestry must utilize the best possible genetic material, combined with the use of appropriate silvicultural practices (Zobel and Talbert 1984). The use of poorly adapted material of unknown genetic potential only contributes to give reforestation a poor image and hinders further reforestation efforts (Cornelius *et al.* 1992; Mesén and Dvorak 1992; Ugalde *et al.* 1992). Most tree breeding programmes in the tropics have usually relied on the introduction and evaluation of species and provenances, followed by progeny testing and establishment of seed production areas and seed orchards with the best individuals of the best provenances (Zobel and Talbert 1984). A number of tree improvement programmes using traditional techniques are currently in progress (Mesén *et al.* 1992). However, it is now widely appreciated that vegetative propagation and clonal selection offers the means to achieve the greatest genetic gains in the shortest possible time. As Zobel (1992) quoted, the question is not if vegetative propagation has a future in forestry, but when and how.

Experience with clonal forestry in Latin America is limited, a prime example being the Aracruz operation in Brazil using eucalypts (Zobel 1992). The approaches adopted by this and other forestry companies in Latin America, and some general aspects of clonal forestry are discussed in the following section.

Clonal forestry

The development of clonal techniques for the establishment of commercial plantations is a relatively new approach, but it has rapidly become a sophisticated one based on agricultural and horticultural experience (Libby and Rauter 1984; Leakey 1988). As some foresters now realize, one of the main advantages of clonal forestry is the ability to capture and exploit both the additive and non-additive components of the total genetic variance, allowing for large genetic gains in very short periods of time. Many other advantages have been listed by Libby and Rauter (1984), the following being the most important:

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

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

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

iv) the ability to use clones that are well adapted to specific sites or to a wide range of sites,

v) the greater simplicity and flexibility of managing clonal gardens as compared to seed orchards,

vi) the greater ease of preventing inbreds from being planted in production populations, and

vii) the ability to choose clones that rarely or never produce flowers, thus reallocating significant amounts of photosynthates from pollen and seed production to stemwood growth.

The main disadvantages most commonly associated with clonal forestry are those relating to the genetic homogeneity of plantations, which may increase the risk of epidemics, and the possibility of the root system of cuttings being inferior to that of seedlings (Leakey 1985).

Concerning the former, there is certainly a temptation to use a few or even a single, outstanding clone over the whole plantation area (Longman and Jeník 1987; Burdon 1989). However, as the dependance on a single clone is widely recognized as extremely hazardous (Burdon 1989), security procedures are routinely taken in every clonal programme. These include the use of a minimum number of unrelated clones for the planting needs: normally more than about 20 (Burdon 1989), but in some cases as many as 250 (Lambeth and López 1988) or more (Zobel 1992); the use of clonal block plantings in which no clone is planted in more than one block each particular year; and the continuous renewal of the operational clonal population by discarding some clones and introducing new ones (Libby and Rauter 1984). In many situations, there may be even greater genetic variability in a clonal plantation than in one derived from seed. For example, when a small seed production area is used continuously over the years to supply the seed needed for the entire plantation area or when the seed is derived from only one or a few trees, the consequent plantations will have limited genetic diversity compared with a clonal planting of even 10 unrelated clones. Furthermore, the pedigree control, which is a normal practice in every clonal programme, allows for a careful deployment of clones in the field to maximize genetic variation over the forest, which is not feasible when using open-pollinated seed.

With regard to the root system, experience from many clonal programmes shows that the root systems of cuttings are not intrinsically inferior (Leakey 1985). In any case, clones should be selected, among other things, for their capacity to form adequate root systems. In addition, different treatments can be tested to improve the quality of the root system formed by the cuttings and those cuttings that produce only one or two roots are normally discarded (Leakey 1985).

Other disadvantages of clonal forestry are related to the higher propagation costs of using cuttings in comparison to seedlings (Leakey and Mesén 1991). This is not necessarily true, as has been shown, for instance, for clonal *Eucalyptus* planting in Congo (Leakey 1985), but in any case, the genetic gains achieved through the use of superior clones should more than compensate for any higher initial costs.

A further disadvantage associated with the use of cuttings, particularly when compared to micropropagation, is the low multiplication rate and the need for a larger space associated with the former (Leakey and Mesén 1991). The relevance of these disadvantages is also arguable: one hectare of *Eucalyptus grandis* clonal multiplication garden in Cartón de Colombia, for instance, produces over 2 million rooted cuttings per year, sufficient to plant about 1 800 ha (Lambeth and López 1988). Any reforestation company with a plantation rate of this magnitude will indeed have little trouble in sparing a hectare of land for the multiplication stage. With regard to economy of space, this is undoubtedly true during the first stages of the micropropagation programme; however, microcuttings must also go through a weaning and nursery stage, just as rooted cuttings do, and therefore, this initial advantage of micropropagation is subsequently lost.

One of the main practical constraints on clonal silviculture is that by the time a tree matures and shows its phenotypic worth, it is difficult to propagate vegetatively. Various practices have been used to overcome this problem, for example: i) to fell the tree and utilize the coppice shoots rising from the stump, ii) to stimulate sprouting from the base of the tree, and iii) serial grafting on juvenile rootstock to rejuvenate the adult scion (Leakey 1988). The latter has serious technical problems, as it may take a long time to develop truly juvenile material, and it is difficult to know when "true juvenility" has been achieved. The first method has been adopted by some large private companies, mainly in South America (e.g., Cartón de Colombia, Aracruz Celulose (Brazil) and Florestas Rio Doce (Brazil)) (Mesén 1990b). The strategy varies slightly between companies, but in every case, it starts with the selection and felling of plus trees. In Cartón de Colombia, 1 100 trees were initially selected and felled. A first selection reduced this number to 950, based on the coppicing ability of the stumps. A further selection based on the rooting ability of cuttings reduced the number of clones to 460, all of which were included in clonal field tests, using a randomized block design with ten blocks and line plots of ten trees per clone. After the first evaluation in year one, the top 30 clones were selected for inclusion in a clonal multiplication garden. All trees from one block were cut and the coppice from the selected clones, together with that from the original mother tree, used to bulk the clonal population required for the clonal garden. Those clones which did not rank within the first 30, but performed better than the control lots, were used to produce the material needed for commercial planting during the first year. The clones which ranked below the control lots were discarded. As some of the selected clones did not remain the best in further evaluations, replacements were made every year in the clonal garden, keeping always the best 30 after each evaluation. This system allows for a dynamic renewal of the selected clonal population and for increasingly higher genetic gains as the trials get older and the clones reflect more precisely their genetic worth.

This strategy is similar to that adopted by Aracruz Celulose, varying basically in the figures. Here, 7 000 trees were initially selected and felled, most of which were planted out in the field, using large monoclonal blocks. Based on the performance of the clones, the best 80 were selected for inclusion in the clonal multiplication gardens and for the production of the material needed for future planting. In Florestas Rio Doce, 7 800 trees were initially selected and felled. A first selection based on coppicing ability reduced this number to 3 600 clones, all of which were included in clonal field trials. After evaluation, the best 500 clones were regarded as 'commercial', and planted operationally. Based on the performance of these clones in plantations, the best 250 were finally selected for the future planting needs. These clones were not planted in clonal gardens, but after harvesting, the trees were managed as coppice shoots directly in the field.

Planting in every case is carried out in monoclonal blocks of 10-20 ha each. No clone is planted in more than one block each particular year, but if it were necessary to do so, they are planted separated by the maximum possible distance. Although genetic variation is absent within a single block, this system creates genetic variation along the forest as a whole, reducing the risks of epidemics. In addition, if a particular clone were severely attacked, harvesting and replacement operations are carried out easily, without the inconvenience associated with intimate mixtures of clones (see Libby and Rauter 1984).

The Centre for Tropical Agricultural Research and Education (CATIE)

CATIE is a nonprofit making, autonomous, scientific and education institute. It carries out, promotes and stimulates research, training and technical cooperation in agriculture and natural renewable resources in the American Tropics, particularly the countries of the Central American isthmus and the Antilles. CATIE is located in the Turrialba Valley, Costa Rica, at an altitude of 600 m.a.s.l., with a mean annual precipitation of 2 600 mm and a mean annual temperature of 22 °C.

CATIE was created in 1973 by a contract between the government of Costa Rica and the Inter-American Institute for Cooperation in Agriculture (IICA), which operated in Turrialba from 1943-73. At present, it has seven member countries: Costa Rica, Dominican Republic, El Salvador, Guatemala, Honduras, Nicaragua and Panama.

CATIE has a staff of approximately 800, of whom 23 per cent are professional personnel. To supply teaching services (many short courses and an M.Sc. Course) and carry out research, the Centre has 1 035 hectares of land and laboratories for soil science, plant physiology, phytopathology, animal nutrition and tissue culture. In addition, it holds a data processing centre, a germplasm bank and a tropical agriculture and forestry library with over 80 000 specialized volumes, the most complete of its kind in Tropical America.

CATIE is subdivided into two major research programmes: the Sustainable Agricultural Production and Development Programme, which deals with promising tropical annual and perennial crops, and the Natural Resources Management Programme, which deals with plantation forestry and management of natural forests, wildlife areas and watersheds. Each programme is made up of a number of projects, which undertake specific actions. One of the projects within the Natural Resources Management Programme is the Tree Improvement Project, within which the present study was undertaken.

The CATIE Tree Improvement Project

The CATIE Tree Improvement Project was created in 1977, with the general objective of contributing to regional forestry development through the identification and development of superior planting material and the conservation of endangered populations, as well as strengthening national institutions in the area of forest genetic resources. The Project concentrates technically on the evaluation and selection of germplasm sources, working with 18 species of regional importance. Activities are carried out on the selection of provenances and superior trees, establishment of progeny tests and seed orchards, and vegetative propagation studies aimed at the establishment of clonal plantations.

The Project offers an annual three-month course on genetics and tree improvement for students under the M.Sc. Programme and an annual short course on the basics of tree improvement, as well as technical assistance and in-service training to Central American foresters. It also publishes a twice yearly newsletter, concerning forest genetic resource activities in Central America.

Funding is provided mainly by the Royal Norwegian Ministry of Foreign Affairs and the Overseas Development Administration (ODA) of the United Kingdom, partly through a link project with the Institute of Terrestrial Ecology. The Project runs a forestry nursery with facilities for raising plants for genetic improvement trials, one large greenhouse equipped with a mist irrigation system and one working room for vegetative propagation work. In addition, ten non-mist propagators were built at the onset of this project.

DETAILS OF THE EXPERIMENTAL SPECIES

Much of the gene conservation effort of the Tree Improvement Project is carried out in collaboration with the Central America and Mexico Coniferous Resource Cooperative (CAMCORE), and rather than working on the traditionally studied conifers, it concentrates on six broadleaved species native to Central America (*Albizia* *guachapele*, *Alnus acuminata*, *Bombacopsis quinata*, *Cordia alliodora*, *Sterculia apetala* and *Vochysia guatemalensis*). These were selected because all are well known as valued timber species, cover a wide range of climates and potential users and all have populations in danger of extinction or genetic erosion (Mesén 1990a; Tree Improvement Project 1987). The TIP decided to adopt both traditional improvement techniques and clonal approaches for the genetic conservation and genetic improvement of these species. Explorations were carried out in Costa Rica, Guatemala, Honduras and Colombia and seed was collected from phenotypically superior trees (Corea *et al.* 1992; Mesén and Dvorak 1992). Following the first approach, seed was distributed to cooperating countries for the establishment of gene conservation banks and provenance/progeny trials.

In order to incorporate these species into a clonal programme, new strategies and vegetative propagation techniques had to be developed or adapted. The approaches and techniques developed by South American companies, as described above, were clearly inappropriate for the Central American region. CATIE works with small and medium scale farms, does not have large forest plantations, and the selected trees are scattered in small private farms all over the region. Felling of superior trees and periodical harvesting of coppice shoots appeared totally impractical, and unlikely to be accepted by the farmers. Similarly, the conventional, expensive and relatively high-technology mist propagation systems were also inappropriate for most situations commonly encountered in the Central American countries. Techniques had to be developed or adapted to vegetatively propagate the best ideotypes in an efficient way and at a cost that was affordable by small forestry development programmes in the Central American region.

For these reasons, for the clonal tree improvement work in CATIE, it was decided to start cloning from seedlings. Two other arguments put forward favouring this approach were i) it would be easier for participating countries to exchange seed rather than vegetative material, and ii) the use of seed would capture the full range of genetic variation from the undoubtedly dysgenic Central American populations.

Two of the species, *Albizia guachapele* (Kunth) Dug. and *Cordia alliodora* (Ruiz & Pavon) Oken, offered unique challenges and opportunities for research. Both are particularly important and well-known as plantations species throughout the region. However, all previous attempts to root *Cordia alliodora* cuttings had failed (Dyson 1981; Mesén, unpublished internal report), whilst apparently, no previous work on vegetative propagation had been carried out on *Albizia guachapele* (Leakey *et al.* 1990). Furthermore, the contrasting ecological conditions where each of these species grow naturally (dry and rain forest, respectively) offered the possibility to relate the observed responses to the ecological conditions prevailing in their natural environments and help to develop basic propagation principles. This would greatly contribute to overcome some of the technical problems faced when approaching propagation of new species.

Albizia guachapele (Family: Leguminosae; Mimosaceae) (Plate 1.1) is a fast-growing, drought-tolerant tree with large excurrent crown and good quality timber, now fairly rare in natural conditions and with a severely depleted gene pool (Tree Improvement Project 1987). A native of the dry tropical forest from 0 to 800 m a.s.l., it occurs naturally in Central America, Colombia and Venezuela. It reportedly fixes nitrogen, and is used as fuelwood, posts, live fences and timber (Tree Improvement Project 1987).

In Costa Rica, the area planted with *A. guachapele* is increasing greatly, as a result of its inclusion on the list of recommended species for reforestation under the Costa Rican Forestry Directorate reforestation schemes (Ortiz 1990). The species has also shown excellent growth in progeny trials established in Honduras (personal observation), indicating its potential for plantations at a larger scale. Information on this species is very scarce, consisting basically of reports on germination and early growth in Colombia (Carreno and Martínez 1984), Mexico (Foroughbakhch and Penaloza 1988) and Malawi (Ngulube 1989). Superior planting material is, at present, not available.

Cordia alliodora (Family: Boraginaceae) (Plate 1.2) is widely distributed in continental tropical America from central Mexico to northern Argentina and parts of the Caribbean. It is found on a wide range of sites at altitudes from sea level to 2000 m (Greaves and McCarter 1990). In Central America it is found in both the Atlantic (0-900 m a.s.l.) and the Pacific regions (0-1800 m a.s.l.), but is most common on the Atlantic side (wet tropical forest), where the tallest and best-formed trees are found, sometimes in small, pure stands (Boshier and Mesén 1987; 1988). On dry sites the tree seldom exceeds 12 m in height and 20 cm in diameter (Greaves and McCarter 1990), whereas in the Atlantic side heights of 30-40 m and diameters of 40-60 cm are common (Boshier and Mesén 1987). On these sites the bole has a monopodial growth habit, essentially straight, cylindrical and clear of branches for 50-60 percent of the total tree height.

Plate 1.1 A typical tree of *Albizia guachapele* in Guanacaste, Costa Rica

Plate 1.2 A typical tree of *Cordia alliodora* in Turrialba, Costa Rica

C. alliodora is a light-demanding species that readily colonizes exposed fertile soils (Greaves and McCarter 1990). It is a well-known and valued tree in tropical America and parts of the Caribbean, and has been introduced to other tropical countries where, in some cases (e.g., Vanuatu), it has become the major reforestation species (Hudson 1984; Neil and Jacovelli 1985). Because of its value in Costa Rica, natural regeneration is often maintained by farmers in combination with various perennial crops. It is also one of the Costa Rican General Forestry Directorate's recommended species for reforestation, and one of the most planted species in the humid lowlands, both in agroforestry systems and in pure plantings (Ortiz 1990). However, both in natural and in planted stands, the trees show a high variation both in growth and form, indicating the potential for improvement through genetic improvement techniques (Boshier and Mesén 1987).

AIMS AND OBJECTIVES OF THE RESEARCH

The aims of the present study were to develop the techniques required for the genetic improvement of *Cordia alliodora* and *Albizia guachapele* by cloning and early selection of superior genotypes.

The specific objectives were:

1. To determine the effects of physiological factors on the rooting process in both species.

2. To identify the optimal conditions for root initiation in leafy-stem cuttings of both species using non-mist propagators.

EXPERIMENTAL APPROACH

Physiology of rooting

Rooting is known to be influenced by a large number of interrelated factors, both pre- and post-severance. An understanding of these factors is fundamental to successful and sustained vegetative propagation. In the present study, the fundamental physiological factors affecting the rooting process of leafy stem cuttings of *Albizia guachapele* and *Cordia alliodora* were investigated. The study also aimed to identify the optimal conditions for root initiation in the cuttings, including the role of stockplant pre-conditioning treatments and propagator microclimate.

In order to make results directly applicable to the low-capital input situation encountered in most Central American countries, the study concentrated in the use of low-technology, non-mist propagators, as described in the following chapter. This can allow the use of clonal propagation techniques in simple tree nurseries, without the need for the expensive and relatively sophisticated equipment normally used in clonal forestry operations.

Among the factors affecting root initiation and development, those related to the photosynthetic and water relations of the cuttings and the stockplants are thought to have a critical influence in the rooting process. Therefore, the study concentrated on the evaluation of these factors, based on the hypothesis that different treatments affect the rooting ability of cuttings by affecting their water relations and photosynthetic capacity.

ADVENTITIOUS ROOT DEVELOPMENT IN LEAFY STEM CUTTINGS

Root initiation

In most plants, the formation of adventitious roots takes place after the severance of the cutting (Hartmann and Kester 1983). Usually, callus develops at the basal end of the cutting when placed under environmental conditions favourable for rooting. The callus arises from cells in the region of the vascular cambium and adjacent phloem, although various cells of the cortex and pith may also contribute to its formation (Hartmann and Kester 1983). Callus development, however, does not assure root initiation and sometimes can be excessive (Haissig 1974b; Hartmann and Kester 1983).

Root primordium initiation begins with dedifferentiation of cells, which attain a meristematic ability and become the primordium initials. Primordium development occurs through division of the initial cells and cells adjacent to the primordium (Haissig 1974b).

Induced root primordia most commonly arise near the base of the cutting, as the result of stimulation from auxin and other factors that translocate to the basal cut surfaces. Induced root primordia have been initiated in a wide range of tissues. Buds, epidermis, cortex, pericycle, phloem, cambium, xylem, pith, parenchyma and other tissues contain cells capable of dedifferentiation and therefore capable of initiation of root primordia. Cambium, phloem and pericycle have most often been observed as the seats of primordia initiation, whereas cortex, pith and xylem have been the least important (Haissig 1974b). Root primordia are frequently initiated near vascular elements because of the readily available nutrient supply (Haissig 1974b). As cell division continues each group of cells takes on the appearance of a root tip. A vascular system develops in the new root primordium and becomes connected to the adjacent vascular bundle. The root tip continues to grow outward, through the cortex, emerging from the epidermis of the stem (Hartmann and Kester 1983).

The time at which root initials develop after the cuttings are placed in the propagating bed varies greatly, from a few days to several weeks depending on the species, the environmental conditions and physiological status of the cuttings (Hartmann and Kester 1983).

Physiological activity of the cuttings

Water relations and adventitious rooting

In vegetative propagation from leafy cuttings, it has long been recognized that loss of turgor can reduce rooting (Evans 1952; Hess and Snyder 1955; Kemp 1952; Hartmann and Kester 1983). Evans (1952) remarked that even a slight water deficit which may be insufficient to cause any visual symptoms of distress results in considerable delay or reduction in the rooting response. The degree of water deficit developed by the cuttings affects rooting in a number of ways (Loach 1988a): the most immediate effect of water deficit is on limiting carbohydrate gain through photosynthesis, cell growth, cell wall synthesis and cell division, and translocation of photosynthates to developing root primordia (Hsiao 1973; Loach 1988a). Conceivably, water deficit in the foliage may also diminish the supply of other naturally occurring substances, usually called 'leaf cofactors' (Loach 1988a), known to synergise the action of auxin in promoting adventitious root formation (Hess, 1969; Haissig 1974a; Hartmann and Kester 1983) (see section "The effect of variation in foliar area on adventitious rooting", Page 25).

However, little quantitative evidence is available concerning the physiological behaviour of leafy cuttings during propagation, and the critical thresholds of water status for root development are still poorly defined (Loach and Grange 1983; Loach 1988a; Newton and Jones 1993b; Newton *et al.* 1992b).

Several approaches have been utilized to relate the water status of the cutting to its physiological role in rooting, consisting mainly of measurements of leaf water potential, stomatal conductance and relative water content of leaves and stems. All these approaches will be reviewed separately.

Foliar water potential

In cuttings of *Ceanothus thyrsiflorus*, Loach (1977) recorded values of foliar water potential as low as -3.9 MPa under mist and -1.2 MPa under polythene, low values being associated with high irradiances. In a study on the rooting of 81 species from leafy cuttings in open and polythene-enclosed mist systems, Grange and Loach (1984) recorded values of foliar water potential between -0.65 MPa and -1.2 Mpa, lower values being generally associated with open systems. In leafy cuttings of three ornamental species, Grange and Loach (1985) found values of foliar water potential of -0.2 MPa and -1.8 MPa, with lower values recorded under higher irradiances. In *Forsythia* leafy cuttings under polythene tents, Grange and Loach (1985) found values between -0.2 MPa and -0.7 MPa. Newton and Jones (1993b) recorded foliar water potential values of leafy cuttings of four tropical species (*Albizia guachapele, Cordia alliodora, Terminalia spinosa* and *Triplochiton scleroxylon*) under mist and non-mist systems. Mean values for *A. guachapele* and *C. alliodora* were in the range -0.4 MPa to -0.85 MPa and, generally, lower values were recorded under mist (Newton and Jones, 1993b). Mean values of *T. scleroxylon*, a species from the tropical moist forest, were lower (-1.1 MPa) than those of *T. spinosa*, a species from the semi-arid lands (-0.45 MPa), with a minimum value of -3.1 MPa recorded in *T. scleroxylon* (Newton and Jones, 1993b). Similarly, water potentials of these two species were generally lower under mist. Mean water potential values for the latter species are similar to the range of -0.4 MPa to -1.2 MPa reported previously for *T. scleroxylon* under open mist (Leakey and Coutts 1989) and the range of 0 MPa to -0.25 MPa reported for *T. spinosa* under non-mist (Newton *et al.* 1992a).

Low foliar water potentials have sometimes been associated with poor rooting. Loach (1977; 1988a) and Grange and Loach (1985) found a close relationship between rooting percentages of a number of species and the measured foliar water potential.

Similarly, Darbyshire (1971) and Hsiao (1973) have also remarked that root initiation and development are severely curtailed by low foliar water potentials. However, in other instances (Grange and Loach 1984; Newton and Jones 1993b), rooting ability has shown no relation to foliar water potential in the cuttings. One possible explanation for this lack of consistency may lie in the nature of the process involved. It is now widely appreciated that rooting is affected by a large number of inter-related factors (Leakey 1983; Leakey and Mohammed 1985; Leakey *et al.* 1992; 1993; Newton *et al.* 1992b), and suboptimal treatments to the cuttings or the stockplants may result in poor rooting in spite of no apparent water deficits (Newton and Jones 1993b). In other instances it is possible that the water deficits developed by the cuttings under traditional propagation systems is seldom as severe as to negatively affect rooting (Grange and Loach 1984). This is supported by the findings of Newton *et al* (1992a) and Newton and Jones (1993b), in which leafy cuttings from a number of tropical species were able to recover from high water deficits and eventually rooted successfully.

In some instances, foliar water potential may not necessarily be the most pertinent measure (Loach 1988a). The water potential consists of two major components: osmotic potential, arising from the presence of dissolved solutes in the cell, and turgor potential (P), arising from pressure exerted on the cells by their walls (Beadle *et al.* 1985). In some studies, P has been shown to be more relevant and more closely related to rooting than water potential (Hsiao *et al.* 1976; Grange and Loach 1984; Loach 1988a).

A further problem is that roots are initiated in the stem base of the cutting, whose water status may differ from that of the leaves (Loach 1988a). In *Pilea grandis*, for instance, the cuttings were allowed to dry slowly, but the tissues at the base remained fresh and produced roots long after the internodal tissues had severely shrunk (Kemp 1952). Very few measurements of stem water potential have been carried out, and its influence on rooting ability has been even more inconsistent (Loach 1988a).

Stomatal conductance

Few relevant studies on stomatal conductance during propagation have been reported (Loach 1988a). Gay and Loach (1977) found that the leaf conductances of *Cornus* and *Rhododendron* leafy cuttings fell to a low value within a few hours of preparation and remained low (approximately between 0 cm^{-1} s^{-1} and 0.2 cm^{-1} s^{-1}) until a measurable length of root had been produced. The authors suggested that stomata of these species remain virtually closed until roots have emerged. However, these measurements were taken in controlled environment cabinets, and the rapid flow of air needed to achieve a stable temperature may have inadvertedly encouraged stomatal closure (Loach 1988a). Further investigations with *Hebe elliptica* suggested that the stomata of cuttings were not fully closed during propagation, but adopted a degree of opening related to ambient evaporative conditions (Loach 1988a). More recent work by Newton and Jones (1993b) showed that stomatal conductance of a number of tropical tree species were generally over 0.4 cm^{-1} s^{-1} prior to root formation, with a maximum of around 1.4 cm^{-1} s^{-1} recorded for *Terminalia spinosa* under mist. For *Albizia guachapele* and *Cordia alliodora*, conductances tended to increase with time after insertion but most cuttings eventually rooted, highlighting the ability of the cuttings to recover from water deficits incurred after severance (Newton and Jones 1993b; Newton *et al.* 1992b). The ability to recover from water deficits also seems to vary among different species. Newton and Jones (1993b) found that conductances tended to be higher in an arid-zone species than in a species from humid areas, reflecting the greater ability of the former to tolerate water deficits.

Foliar relative water content

Foliar relative water content (RWC), being based in measurements of fresh, dry and turgid masses, requires relatively simple and cheap equipment (Beadle *et al.* 1985). Many metabolic processes, such as photosynthesis, protein synthesis and nitrate reduction have been found to be correlated with RWC (Sinclair and Ludlow 1985). However, very little relevant information is available on the relationship between relative water content and the rates of these physiological processes (Beadle *et al.* 1985). Newton and Jones (1993b) measured RWC in four tropical tree species during propagation under mist and non-mist systems. No clear differences were found between propagation systems, but there were large differences between species: relative water content of two species from the tropical moist forest, *Cordia alliodora* and *Triplochiton scleroxylon*, was consistently higher than those of *Albizia guachapele* and *Terminalia spinosa*, two species from the semi-arid lands. RWC generally remained above 80% during the first three weeks after the insertion of the cutting in the propagator, with the exception of *A. guachapele*, which showed values as low as 56% during the first week. These initial values may represent a shock response to propagation (Gay and Loach 1977), but the cuttings were able to recover in both propagation systems and eventually showed rooting percentages above 60% (Newton and Jones 1993b).

Although the water status of cuttings has been repeatedly associated with the rooting ability of leafy cuttings (Loach 1977; Grange and Loach 1985; Loach 1988a), the question of how much water deficit cuttings can tolerate and still root has yet no clear answer, and it will probably be dependent on the species and propagation system used. From the above results, it seems that most species are able to recover from severe water deficits and eventually root successfully. However, it is clear that more precise information is required on the interaction between water potential of leafy cuttings during propagation and its impact on root development.

Photosynthesis and adventitious rooting

The role of photosynthesis in severed leafy cuttings has been subject of considerable debate (Davis 1988; Leakey and Coutts 1989). Not all cuttings depend on current photosynthesis for rooting; some leafy cuttings from a number of species can form at least some roots when placed in the dark, and many plants can be propagated from the stored reserves of non-photosynthetic leafless hardwood cuttings, which shows that photosynthesis by cuttings of some species is therefore not an absolute requirement for rooting (Davis 1988). In *Cornus* and *Rhododendron* cuttings, leaf conductances did not increase until a measurable length of root had been produced, which led the authors to suggest that root formation must depend on reserves rather than current photosynthesis (Gay and Loach 1977). Sometimes, rooting has been associated with redistribution of carbohydrates within the cuttings. In *Populus*, for example, Okoro and Grace (1976) concluded that the increasing rooting ability of cuttings was more dependent on the downward transport of carbohydrates within the cutting than on current photosynthesis.

In other instances, the ability of cuttings to photosynthesize whilst in the propagation medium has been considered to favour rooting (Davis 1988). With *Pisum sativum* 'Alaska', for example, Davis and Potter (1981) found that root formation was reduced by about 50% when net photosynthesis was adjusted to the compensation point by shading, by reducing the CO_2 concentration, or by blocking CO_2 exchange with an antitranspirant. Also, with the tropical hardwood *Triplochiton scleroxylon*, it has been shown that photosynthesis occurs in cuttings during the propagation process and this has been correlated with the rooting ability of the cuttings (Leakey and Storeton-West 1992). In *Terminalia spinosa* cuttings, removal of the leaf prevents rooting (Newton *et al.* 1992a), supporting the suggestion that carbohydrates produced by the leaf are vital for root development. This species has been shown to actively photosynthesize during propagation, with rates of 2-6 μmol m^{-2} s^{-1} being recorded using infra-red gas analysis (Newton *et al.* 1992b). It must be taken into account, however, that the absence of leaves also affects many other variables, such as water balance in the cutting, stored reserves and synthesis of rooting promoters or inhibitors, all of which influence rooting ability (Haissig 1974a; Hartmann and Kester 1983; Loach 1988a).

Conclusive evidence that current photosynthesis influences root formation in leafy cuttings is difficult to obtain. Ideally, one should utilise an experimental system where photosynthetic rate can be varied independently of all other factors which may affect rooting, but this is impossible to achieve (Davis 1988). Different approaches have been used, such as rooting cuttings under different photosynthetic active radiation (PAR), altering ambient CO_2 (Davis 1988), and covering the leaves with different areas of aluminium foil (Leakey *et al.* 1982a). Such treatments, however, may also influence auxin content, water relations, anatomy and other factors that affect rooting, making the experiments difficult to interpret (Davis 1988).

The influence of photosynthesis on rooting may depend upon several characteristics of the cutting, for example, the amount of carbohydrate reserves in the cutting, the length of the rooting period and the amount of shoot growth during rooting (Davis 1988). It is clear, however, that current photosynthesis may influence root formation on some types of leafy cuttings and therefore, it seems sensible to try to optimise photosynthesis during the rooting process (Davis 1988). Surprisingly, only a few direct measurements of photosynthesis have actually been made in cuttings during the period of propagation (Davis 1988; Newton *et al.* 1992a; 1992b).

Carbohydrates and adventitious rooting

The role of carbohydrates on adventitious rooting has been the subject of controversy for over 70 years (Veierskov 1988). Most studies dealing with this subject have focused on the effects of C/N ratios on rooting, and in general, high C/N ratios have been associated with increased rooting ability (Hartmann and Kester 1983; Veierskov 1988). Since the first studies dealing with this matter early in this century, this hypothesis has been widely accepted and used to explain differences in rooting ability (see Veierskov 1988). Growing stockplants under conditions of limited N supply was recommended as a way to increase the C/N ratio in the shoots and hence, to stimulate rooting (Hartmann and Kester 1983). Few studies, however, have critically evaluated the exact role of carbohydrates in the rooting process, and their relationship with rooting remains obscure (Veierskov and Andersen 1982; Veierskov *et al.* 1982a; 1982b; Veierskov 1988). The reasons for this may lay in the impossibility of designing an experimental approach to alter the endogenous carbohydrate content without altering many other parameters, such as auxin content, water status and anatomy, that may also affect rooting. Consequently, the possible role of carbohydrates on rooting has usually been confounded by other factors (Veierskov 1988). It is now accepted that C/N ratio is but one of many variables which may affect rooting and to ascribe a determinant role to it is probably misleading (Hartmann and Kester 1983; Veierskov 1988). Every nutrient involved in the multitude of metabolic processes associated with dedifferentiation and root meristem formation are indeed essential for root initiation and development (Blazich 1988).

It is clear, however, that carbohydrates play an important role in rooting, by serving as an energy source and yielding the carbon skeletons needed for new metabolic products (Van't Hoff 1968; Veierskov 1988). If stockplants are depleted of carbohydrates and subsequent cuttings are rooted under conditions where photosynthesis cannot occur, the amount of available carbohydrates will be too low to support rooting (Veierskov 1988). The ability of cuttings to supply carbohydrates, either through stored reserves or through current photosynthesis, to the base of the cuttings, appear to be important for root development (Moe and Andersen 1988). This is supported by evidence of changes in pools of carbohydrates during the rooting period in *Pinus sylvestris* (Hansen *et al.* 1978), *Pisum sativum* (Veierskov and Andersen 1982; Veierskov *et al.* 1982a; 1982b), *Pinus banksiana* (Haissig 1989), *Triplochiton scleroxylon* (Leakey and Coutts 1989; Leakey and Storeton-West 1992) and *Eucalyptus grandis* (Hoad and Leakey 1992).

In leafless hardwood cuttings (Vieitez *et al.* 1980) and etiolated stockplants (Nanda *et al.* 1971), suboptimal rooting has been obtained when the initial carbohydrate content was too low. In pea cuttings, external supply of carbohydrates to the rooting medium has also been shown to greatly increase the rooting ability of cuttings (Eliasson 1978; Loach and Whalley 1978). However, caution must be taken in interpreting these results, since applied sugars affect the osmotic relations and hence the water balance in the cuttings (Veierskov 1988). In other studies, poor rooting has not been associated with insufficient carbohydrate reserves in the cuttings (Okoro and Grace 1976), and negative correlations have also been found between initial content of total carbohydrates and rooting ability (Veierskov *et al.* 1982a,b; Leakey and Storeton-West 1992). In *Pisum* cuttings from stockplants grown at high irradiances, for instance, the lack of root formation was attributed to the supraoptimal carbohydrate content in the cuttings (Ericksen 1973). Similarly, the poor rooting found in *Triplochiton scleroxylon* cuttings from stockplants grown at high irradiance was attributed to suppressed photosynthetic ability in the shoots, resulting from end product inhibition (Leakey and Storeton-West 1992). In a wide range of species, the low rooting obtained under high irradiances during propagation has also been associated with an accumulation of soluble sugars in the cutting (Lovell *et al.* 1972; Hansen and Eriksen 1974; Loach and Whalley 1978; Loach and Gay 1979; Christensen *et al.* 1980; Strömquist and Hansen 1980; French and Lin 1984; Grange and Loach 1985).

Carbohydrate accumulation in the rooting zone may also influence other physiological phenomena such as osmoregulation and cellular solvent capacity, which may have a profound effect on metabolic processes which are involved in rooting (Veierskov 1988).

Thus, carbohydrate content apparently has some effect on the rooting ability of cuttings, at least under some circumstances. The supply of carbohydrates during the rooting period may regulate the number of roots to be developed and their subsequent growth, but their exact role in the process remains unclear (Veierskov 1988). Consequently, the optimum carbohydrate content for rooting has not been critically defined under any conditions (Veierskov 1988).

The role of stockplant condition on adventitious rooting

The physiological status of the stockplant, which results from the interaction between genotype and environmental factors, has also been shown to exert a strong influence on the subsequent root formation in stem cuttings (Moe and Andersen 1987; Menzies 1992). Light quality and intensity, photoperiod, temperature, CO_2, nutrient availability and relative humidity are known to affect the cuttings subsequent rooting ability (Moe and Andersen 1988; Menzies 1992). In addition, in *Triplochiton scleroxylon* stockplants, it has been shown that the number and position of shoots on stockplants play an important role in the determination of rooting ability (Leakey 1983; Leakey *et al.* 1992). The effects of such factors are complex and remain controversial, but have been related either to the photosynthetic ability of the shoot or to the metabolic rate of the cutting. Moe and Andersen (1988) suggested that the ability of cuttings to supply carbohydrates to the area where roots appear, either from stored reserves or through current photosynthesis, seems to be important for root development but not for root initiation, which is hormonally controlled. In this regard, adverse stockplant growth conditions may limit subsequent rooting by a lack of carbohydrates and auxins at the base of the cutting during rooting.

The loss of juvenility as the stockplants get larger and more mature tends to be blamed for any unexplained decline in rooting ability over time, or in shoots originating from upper portions of a tree or stockplant (Leakey *et al.* 1992). However, in *Triplochiton scleroxylon* stockplants, various manipulative experiments have shown that differences in subsequent rooting ability can be attributed to many contributing factors operating in the stockplants (Leakey 1983), which are apparently not related to the loss of juvenility (Leakey *et al.* 1992).

Mineral nutrition

The effect of stockplant mineral nutrition on subsequent rooting is also controversial. In *Triplochiton scleroxylon*, the application of complete fertilizer (NPK) to pruned stockplants considerably enhanced the rooting ability of cuttings from lower lateral shoots, but did not affect the rooting of cuttings from apical lateral shoots, although their growth was increased by the added nutrients (Leakey 1983). Application of different concentrations of NPK to *Khaya ivorensis* stockplants did not affect rooting ability of subsequent cuttings, while high N concentrations caused an increase in cutting mortality (Tchoundjeu 1989). In further experiments with *Triplochiton scleroxylon*, the addition of fertilizers improved rooting of cuttings from stockplants grown at high (650 μmol m^{-2} s^{-1}) irradiance, but had an opposite effect on cuttings from stockplants grown under low (250 μmol m^{-2} s^{-1}) irradiance (Leakey and Storeton-West 1992). In several studies with herbaceous species, fertilization suboptimal for stockplant growth resulted in cuttings that root best (Moe and Andersen 1988). This is probably associated with undesirable morphological and physiological characteristics shown by stockplants grown under supraoptimal rates of fertilization. It is also likely that the nutritional status of the cuttings influences rooting through its effects on the production of root promoting substances (rooting cofactors or auxin synergists), but this possibility has not been investigated to any great extent (Blazich 1988).

Irradiance

The irradiance under which stockplants are grown is also known to influence cutting production and rooting capacities, but again, results have been quite inconsistent (Moe and Andersen 1988). Increased irradiance on stockplants of a wide number of species, from herbs to trees, has been shown to inhibit or delay rooting, promote rooting or have no effect at all (Moe and Andersen 1988). Generally, however, low stockplant irradiance has enhanced rooting ability in a wide variety of plants (Hansen and Ericksen 1974; Hansen *et al.* 1978; Eliasson and Brunes 1980; Poulsen and Andersen 1980; Moe and Andersen 1988). It is evident that stockplants require a certain minimal level of light to produce cuttings that root well, but the optimal irradiance may vary from species to species and under the various environmental factors to which the stockplant is exposed (Moe and Andersen 1988). Howard *et al.* (1985) presented a stockplant response curve to irradiance which appears to be valid for most plant species (Moe and Andersen 1988). It shows that light is necessary, but either too little or too much hinders rooting. Rooting is maximum when stockplant irradiance reaches an optimal level, which differs for different species, to decrease again when irradiance is higher than the optimal. At stockplant irradiance below optimum, lack of carbohydrates and auxin supplies to the base of the cutting may limit rooting (Moe and Andersen 1988). Accumulation of auxin at the base of cuttings from stockplants grown at high irradiances has been found in *Chrysanthemum* spp. (Weigel *et al.* 1984) and *Pisum* cuttings (Baadsmand and Andersen 1984). At supraoptimal irradiance, carbohydrates are probably occurring at too high a concentration (Moe and Andersen 1988), which may inhibit current photosynthesis (Leakey and Storeton-West 1992), and there may be photodestruction of auxin, changes in water relations and rooting inhibitors and/or promoters (Moe and Andersen 1988). It may also result in altered anatomical and morphological characteristics in the stockplant tissues where roots later will appear (Moe and Andersen 1988).

Light quality

Light quality has also been shown to affect subsequent rooting independently of intensity (Hoad and Leakey 1992; Leakey and Storeton-West 1992; Leakey *et al.* 1992). However, it has received very little attention and the ways it affects root initiation remain unclear (Baadsmand and Andersen 1984; Moe and Andersen 1988). Generally, low red:far-red (R:FR) ratios have been found to improve rooting in a number of herbaceous (Heins and Wilkins 1979; Hagen and Moe 1981; Moe 1985) and tree species (Hoad and Leakey 1992; Leakey and Storeton-West 1992). The highest rooting ability of cuttings taken from the lower rather than the upper portions of the stem, reported for many species, has been usually associated with decreasing R:FR ratios found down in the stem, due to the filtering of red wavelength radiation by the overlying leaves (Moe and Andersen 1988). The influence of light quality on the leaf morphology and physiology of stockplants is also important, as has recently been demonstrated for *Eucalyptus grandis* (Hoad and Leakey 1992). These authors found that low R:FR ratios (0.4 and 0.7) were associated with longer cuttings, showing greater leaf areas, specific leaf areas and stem volumes than those from stockplants grown at higher R:FR ratios (3.5 and 6.5), and such cuttings showed eventually higher rooting percentages. On the other hand, Dick and East (1992) found in *Acacia tortilis* that although height growth increased with decreasing R:FR ratios, cuttings from shade-grown stockplants were not found to root more readily than those from plants grown under natural R:FR ratios. The authors suggested that, possibly, the lowest R:FR ratio tested (0.61) was not sufficiently low to be beneficial to rooting. The effects of light quality on rooting have also been associated with changes in the balance between auxin and cytokinin in the shoots (Moe and Andersen 1988). That auxin content increases under FR irradiation has been found in tomato shoots (Tucker 1977). FR light may also retard or prevent sucrose and/or auxin transolcation to the base of the cuttings (Moe and Andersen 1988).

Between-shoot variation

A series of manipulative treatments applied to *Triplochiton scleroxylon* stockplants has shown that competition between shoots plays a preconditioning role in the rooting ability of cuttings (Leakey 1983; Leakey *et al.* 1992). In small *T. scleroxylon* stockplants, a negative relationship was found between the mean number of shoots per stockplant and the rooting ability of cuttings from the top shoot, suggesting an apparent effect of intershoot competition (Leakey 1983). In a further experiment (Leakey 1983), decapitation and debudding were used to induce lateral shoot formation from apical and/or basal shoots. Basal shoots showed a higher rooting percentage than apical shoots, irrespectively of the presence of absence of apical shoots. However, rooting of cuttings from apical shoots was reduced by the presence of competing basal shoots, suggesting that, in addition to competition, other factors were affecting the rooting ability of cuttings. When basal and apical shoots were grown under the same light environment, subsequent cuttings rooted equally well (Leakey 1983; Leakey *et al.* 1992). Therefore, in addition to intershoot competition, changes in the light environment were also shown to be important in the determination of the rooting ability of cuttings (Leakey 1983; Leakey *et al.* 1992).

Post-severance treatments applied to cuttings

The effects of variation in foliar area on adventitious rooting

The physiological factors affecting the rooting process of photosynthesizing cuttings are likely to be those relating to the role of the leaf, ie., the photosynthetic capacity and water relations of cuttings pre- and post-severance (Leakey and Coutts 1989), as described in the previous sections.

The strong stimulatory influence of the leaf on root initiation of some species has been generally associated with the photosynthetic activity of the leaves, by supplying photosynthates to the developing root primordia (Hartmann and Kester 1983; Leakey and Coutts 1989; Newton *et al.* 1992b). The production of other substances, usually called leaf cofactors, which undergo basipetal transport in the cutting, also allow or enhance root primordium initiation and development (Haissig 1974a; Hartmann and Kester 1983). The type and amount of cofactors, in fact, seems to partially determine whether cuttings initiate root primordia easily, with difficulty or not at all (Haissig 1974a). Compounds such as cathecol (Hess 1962; Hackett 1970), chlorogenic acid (Hess 1965), phloroglucinol (James and Thurbon 1981) and oxygenated terpenoids (Hartmann and Kester 1983) have been claimed to have some stimulatory effect on rooting. Abscisic acid has also been considered as a rooting cofactor, perhaps by antagonizing gibberellic acid which, at certain concentrations, inhibits root formation (Chin *et al.* 1969). Many other substances, thought to be formed in the leaves, have been found to have root-promotion activity, at least under certain conditions, but they remain to be properly identified (Hartmann and Kester 1983). The effect of phenolic compounds in root promotion could be, at least partly, in protecting indolacetic acid from destruction by the enzyme, IAA-oxidase (Donoho *et al.* 1962; Hackett 1970; Hartmann and Kester 1983). In many studies, no correlation has been found between rooting response and rooting cofactors and to date, their exact role in the process remains controversial (Hartmann and Kester 1983).

Not all the effects of the leaves are beneficial, however, since water loss by transpiration may cause water deficits in the cuttings (Gay and Loach 1977; Grange and Loach 1983a, 1983b; Leakey 1985; Loach 1988a; 1990), to levels that can impair rooting (Loach 1988a; 1990) or cause the cuttings to die before root formation can take place (Hartmann and Kester 1983). The traditional practice of leaf trimming aims at minimizing water deficits caused by transpiration, while still allowing the cutting to carry out photosynthesis during the propagation process. This trade-off between photosynthesis and water loss is reflected by the tendency of some species to display optimum leaf areas for rooting (Okoro and Grace 1976; Leakey *et al.* 1982a; Leakey and Coutts 1989; Tchoundjeu 1989). The achievement of this critical balance seems to be more important in difficult-to-root species (Leakey 1985).

Grange and Loach (1984) evaluated the rooting of 81 species and invariably found higher rooting percentages when cuttings with larger leaf areas were used. This was attributed to either increased carbohydrate reserves or increased photosynthate from the larger leaf area, but also to possible differences in the supply of root promoters or inhibitors from the different leaf areas. The deleterious effects of sub- or supra-optimal leaf areas seem to be greater in difficult-to-root species (Leakey 1985). For instance, a leaf area of 50 cm^2 was found optimal for *Triplochiton scleroxylon* (Leakey *et al.* 1982a) and *Cleistopholis glauca* (Leakey 1985); *Khaya ivorensis* showed higher rooting percentages with leaf areas of 10-30 cm^2 (Tchoundjeu 1989), while the rooting of *Lovoa trichiliodes* was higher when a leaf area of 200 cm^2 was used (Tchoundjeu 1989). On the other hand, *Terminalia spinosa* (Newton *et al.* 1992a), *Nauclea diderrichii* and *Terminalia ivorensis* (Leakey 1990) are apparently less sensitive to variation in leaf area. In most tropical species, removal of the leaf prevents rooting (Leakey and Mesén 1991; Newton *et al.* 1992a), which suggests that current photosynthesis, and perhaps endogenous auxins produced by the leaves, are important for root development in these species. Species from dry areas, such as *Terminalia spinosa*, have been shown to tolerate higher irradiances and higher VPD's during propagation than those from humid areas (Newton and Jones 1993b; Newton *et al.* 1992b), a fact which probably explains why such species are less sensitive to variation in leaf area.

The effects of node position on adventitious rooting

Cuttings taken sequentially down a stem are known to vary in their rooting ability (Hartmann and Kester 1983; Leakey 1983; Leakey *et al.* 1992). This is not surprising considering that tissues along a stem are subjected to differential gradients of development, growth regulators, water and nutrient contents, state of differentiation and lignification, illumination and photosynthetic efficiency of their leaves, among other factors (Hartmann and Kester 1983; Leakey 1983; Leakey *et al.* 1992). In hardwood cuttings, the highest rooting percentages have usually been obtained with cuttings from the basal portions of the shoot (Hartmann and Kester 1983). Cuttings prepared from shoots of the highbush blueberry (*Vaccinium corymbosum*), for instance, showed a significantly higher rooting percentage if taken from the basal portion of the shoot rather than from terminal portions (Hartmann and Kester 1983). This was attributed to the increasing number of preformed root initials from the tip to the base found in such shoots, and to the greater accumulation of carbohydrates at the base of the shoot (Hartmann and Kester 1983). Softwood shoots usually behave differently; cuttings from the tip usually show higher rooting percentage than those taken from the basal portions (Hartmann and Kester 1983). In this type of shoots, preformed root initials and carbohydrate storage are normally absent; therefore, their higher rooting ability has been explained by the possibility of higher concentrations of endogenous root-promoting substances arising in the terminal bud of such shoots (Hartmann and

Kester 1983). There is also less differentiation in the terminal cuttings, with more cells capable of becoming meristematic (Hartmann and Kester 1983). In single-node, leafy cutting of *Triplochiton scleroxylon*, Leakey (1983) found that final rooting percentage after 10 weeks decreased sequentially from about 70% for cuttings taken from the youngest apical nodes to about 10% from the oldest basal nodes. Cuttings from the upper parts of the stem usually have longer internodes (Leakey 1983), and therefore have higher volumes and perhaps a higher storage capacity for assimilates, which may favour rooting (Grange and Loach 1984; Hoad and Leakey 1992; Leakey *et al.* 1992). In addition, long internode cuttings are able to keep their leaves well clear of the propagating medium, which favours the leaf's access to light and thus, a higher photosynthetic activity (Hoad and Leakey 1992).

Rooting results have not always followed this straightforward pattern. With leafy stem cuttings of *Prosopis juliflora*, for instance, Dick *et al.* (1991) found that cuttings originating lower in the stockplant had a higher probability of rooting. The possible reasons for this result were not discussed, but the authors suggested that cutting diameter was obviously involved. Also, with *Triplochiton scleroxylon*, rooting of cuttings taken sequentially down the stem differed from those reported above as a result of variations in the number of competing shoots, pruning regime, orientation, application of fertilizers and light environment on the stockplants (Leakey 1983). It became clear, therefore, that the production of easily-rooted cuttings is a complex process with many interacting factors influencing rooting ability (Leakey 1983; Leakey and Mohammed 1985; Leakey *et al.* 1992; Newton *et al.* 1992b).

The effects of cutting volume on adventitious rooting

Very little work has been done to test the effects of cutting volume on rooting. Most related work has focused on the effects of cutting length, as it has been shown to be important on the rooting ability of single-node cuttings in *Pisum* (Veierskov 1978), *Hedera* (Poulsen and Andersen 1980) and more recently in *Triplochiton scleroxylon* (Leakey 1983; 1985). In the latter species, the percentage rooting of sequential single-node cuttings has been found to be strongly correlated with their length, as both cutting length and rooting ability decreases down the length of the shoot (Leakey 1983; Leakey and Mohammed 1985). When morphological differences were separated experimentally from other node position-related characteristics by inverting the gradient of cutting length at harvest time, it became clear that cutting length *per se* has a major effect on rooting ability (Leakey and Mohammed 1985; Leakey *et al.* 1992). This is supported by the higher rooting percentage of two- rather than one-node cuttings found in *Triplochiton scleroxylon* (Howland 1975) and also by the higher rooting percentage obtained in *Eucalyptus grandis* when cuttings longer than 5 cm were used (Hoad and Leakey 1992). When cutting length was evaluated independently of their position within the shoot, by using standard-length cuttings, basal cuttings with larger diameters rooted best, supporting the suggestion that cutting volume, and thus stored carbohydrate reserves, may be critical for root development. It is also possible that the factors enhancing the rooting of cuttings from basal nodes, when not constrained by their normally limited internode lengths, may result from effects of shading (Leakey and Mohammed 1985), as low irradiance is known to enhance rooting of many species (Hansen *et al.* 1978; Christensen *et al.* 1980; Eliasson and Brunes 1980; Leakey and Mohammed 1985). These results highlight the importance of removing or quantifying all these sources of variation in experimental work, to enhance repeatability of results and ensure that rooting results are not confounded by other sources of variation not accounted for in the experimental protocol.

The effects of auxins in adventitious rooting

The application of auxins has been shown not only to increase the percentage of cuttings which form roots, but also to hasten root initiation, to increase the number and quality of roots produced per cutting and to increase uniformity of rooting (Hartmann and Kester 1983; Blazich 1988). This has obvious advantages in the propagation process, since the quick formation of a good root system permits uptake of water to compensate for that lost by the leaves (Hartmann and Kester 1983).

It seems that there is no simple relationship between auxin concentration in cuttings and rooting, and, to date, the exact role of auxins in stimulating adventitious root formation remains unclear (Gaspar and Hofinger 1988). In addition to the direct effects of auxins on cell division, the effects of auxins on rooting have been associated with an increased transport of carbohydrates and leaf cofactors to the base of the cuttings, where they enhance root primordium initiation and development (Haissig 1974a). It is now well established that metabolites and other growth factors are translocated to auxin-treated regions of the stem, either by the creation of a metabolic sink or as a result of a stimulus provided by auxin other than its sink activity (Phillips 1969; 1975). When there is little photosynthetic activity, auxin transport and/or synthesis may be reduced; thus, photosynthesis by the cuttings may also indirectly influence rooting, by affecting auxin supply to the base of the cutting (Davis 1988). Exogenous auxins may also induce rooting by stimulating DNA synthesis in suitable cells (Gaspar and Hofinger 1988). It is likely that the effect of auxins in stimulating adventitious root formation is a result of a complex interaction of these and probably other processes.

Although indole-3-acetic acid (IAA) is the auxin naturally found in plants, two related synthetic compounds, indole-3-butyric acid (IBA) and α-napthalenacetic acid (NAA), have been found most reliable in stimulating adventitious root formation in cuttings. The former has proved to be the best material for general use, because it is nontoxic over a wide concentration range and is effective in promoting rooting of a large number of species. It is also much more light-stable than IAA and, being insoluble in water, remains longer in the site of application and then maintains its effectiveness over longer periods of time (Hartmann and Kester 1983). In addition, plants possess several mechanisms which operate to reduce and/or nullify the effectiveness of IAA, by conjugating it with other compounds or destroying it, but this is not so with IBA or NAA (Blazich 1988).

Optimum auxin concentration, however, varies greatly between species and even between clones from the same species (Leakey 1985). This variation has been attributed to the cuttings varying in their concentrations of endogenous auxins, rooting co-factors and inhibitors, anatomy, leaf retention and possibly, to interactions between these factors (Leakey 1985; 1990). Application of auxin at high concentrations may result in injury; this may merely inhibit bud development, or it may cause yellowing and dropping of leaves, blackening of the stem and eventual death of the cutting. Usually, a concentration just below the toxic point is considered the most favourable for root promotion (Hartmann and Kester 1983).

Numerous reports exist on the effects of auxin concentrations on the rooting of cuttings (See Hartmann and Kester 1983), but relatively few have examined these effects in terms of the impact of the hormone on the physiological activity of the cuttings and how these relate to the rooting ability of the cuttings.

The effects of mineral nutrition in adventitious rooting

The nutritional status of the cutting material itself is also important in the rooting process through its influence on root growth and development (Hartmann and Kester 1983; Blazich 1988). Very little is known about the importance of each individual nutrient in the rooting process, the possible mobilization of nutrients and its influence on rooting ability, the effects of nutrient leaching and the effects of supplemental nutrition during propagation (Blazich 1988).

One might suggest that all nutrients involved in the metabolic processes of a plant in general are important for root initiation and development. Sufficient experimental evidence now exists to confirm the need for N, P, K, Ca, B, Mn and Zn during root growth (Blazich 1988). In addition to the direct influences of nutrients on root initiation, root development and growth, they may also have indirect effects by influencing the production of various root inducing factors and affecting post propagation growth and vigour (Hartmann and Kester 1983; Blazich 1988).

Mobilization of nutrients to the base of the cutting would be an indication that these nutrients are needed for root growth and development (Blazich 1988). Mobilization of P into the base of the cuttings has been demonstrated in *Chrysanthemum morifolium* (Good and Tukey 1967) and *Ilex crenata* (Blazich *et al.* 1983). The importance of Ca during root growth and development has been shown in *Pisum sativum* (Eliasson 1978) and *Ilex crenata* (Blazich *et al.* 1983). Also, the presence of B has been shown to be critical for root growth in *Vigna radiata* (Middleton *et al.* 1978). However, many inconsistencies and conflicting results have also been reported. Blazich *et al.* (1983) found that some roots are initiated before mobilization occurs and that, despite extensive initiation in the base of a cutting, certain nutrients, such as Ca and Mg are sometimes mobilized from the base to support growth in the upper part of the cutting. Blazich (1988) suggested that nutrients have probably a greater impact on root growth and development than root initiation, and also, that root initiation as influenced by mineral nutrition is more dependant on initial nutrient concentrations within that portion of the cutting where roots are to be formed than on actual mobilization of nutrients.

Leaching of nutrients during mist propagation was suspected in early studies (Evans 1951; Sharpe 1955) and demonstrated later on (Good and Tukey 1966; Blazich *et al.* 1983). The extent of leaching may be dependant on the tissue maturity - in young, growing tissues nutrients are quickly metabolized and therefore, difficult to leach (Good and Tukey 1966) - and the relative leachability of particular nutrients (Tukey *et al.* 1958). Leaching of N, P, K, Ca and Mg during mist propagation has been clearly demonstrated and has led to research on the use of nutrient mist to replenish nutrient loss (Blazich 1988). However, results have generally been mixed. Whilst nutrient mist increased rooting in various herbaceous, softwood and hardwood cuttings (Wott and Tukey 1967), it caused foliage injure and delayed rooting in *Rhododendron* spp. cuttings (Keever and Tukey 1979) and also caused sanitation problems and encouraged growth of algae in the propagators (Wott and Tukey 1967; Coorts and Sorensen 1968). In general, it appears that supplemental nutrition has little or no positive influence on root initiation (Blazich 1988). Although nutrient leaching during mist propagation is a well established fact, the extent to which it prevents or retards rooting is presently unknown. It is possible that leaching may have some influence on rooting if cuttings are taken from stockplants growing under poor or marginal fertility (Blazich 1988).

The effects of the propagation environment on adventitious rooting

Propagation microclimate

The propagator microclimate has a major influence in the rooting of cuttings (Loach 1988b; Newton *et al.* 1992b). Tremendous variability in rooting or even complete failure may occur if environmental conditions are not properly controlled during propagation (Loach 1988b; 1990). The ideal propagator microclimate must maintain a satisfactory water balance in the cuttings, a proper irradiance, and suitable substrate, air and leaf temperatures (Loach 1988b). As explained in the preceding sections, all these variables are closely linked and interact with each other.

The water status of the cuttings is governed by the balance between evaporative losses from the leaves and water uptake by the cuttings (Gay and Loach 1977; Grange and Loach 1983a; 1983b). As cuttings lack roots at first, they must rely on retention of their turgor and water absorption through a cut stem base or through the leaf and stem surfaces (Loach 1988a). The absorption of water through the stem bases seems to be of great importance for most species, at least during the first days in the propagating bed, although foliar absorption may perhaps aid survival (Grange and Loach 1983a; Loach 1988a). The rate of water loss from foliage is determined by the vapour pressure gradient from the leaves to the surrounding air (VPD) and by the resistances to its transfer (comprising cuticular, stomatal and boundary layer resistances) (Gay and Loach 1977; Loach 1988a). A range of environmental factors (e.g., irradiance, carbon dioxide, humidity, temperature and wetting of foliage) operate on these variables. A rise in humidity has a major effect on the water status of the cuttings, reducing the rate of water loss by maintaining a high degree of saturation in the atmosphere (Loach 1988a). The wetting of foliage allows foliar absorption of water and reduces the temperature and the vapour pressure of the leaf by evaporative cooling (Grange and Loach 1983a; Loach 1988a). Most importantly, wetting of foliage provides insurance against severe tissue water deficit, by satisfying the evaporative demands of the environment by externally supplied water rather than water originating from within the leaves (Grange and Loach 1983a; 1984; Loach 1988a).

The tremendous sensitivity of rooting to apparently narrow changes in relative humidity has been recently demonstrated by Loach (1990). Cuttings from six species showed mean rooting percentages of 30 or 78% in response to a variation from 90 to 100% in relative humidity.

In the rooting medium, water content should be sufficient to allow water uptake by the cuttings, but not so excessive as to create aeration problems which can reduce rooting (Grange and Loach 1983b; Loach 1990).

Irradiance in the propagation environment has been shown to be a major factor influencing the rooting of leafy cuttings (Loach 1977; Loach and Whalley 1978; Loach and Gay 1979; French and Lin 1984; Grange and Loach 1985). The primary effects of irradiance in the propagation environment are on leaf turgidity and on the production of carbohydrates required for root initiation and growth (Grange and Loach 1985). A rise in irradiance raises the vapour pressure of the leaf, decreases the vapour pressure of the air and increases the rate of water loss from the cuttings (Loach 1988a). Even relatively low irradiance has been shown to reduce turgor pressure in inmature large-leafed material (Grange and Loach 1984).

High irradiance has also been found to inhibit rooting in a large variety of species (Lovell *et al.* 1972; Hansen and Eriksen 1974; Loach and Whalley 1978; Loach and Gay 1979; Christensen *et al.* 1980; Strömquist and Hansen 1980; French and Lin 1984; Grange and Loach 1985), through its effects on sugar accumulation and loss of turgor. However, other factors are probably involved (Grange and Loach 1985). For example, it has been shown that irradiance influences the hormonal status of the cuttings, through its effects on both auxin and synergistic co-factors (Christensen *et al*;. 1980). Irradiance must be sufficient, however, to generate photosynthetic production of carbohydrates for root initiation and growth (Grange and Loach 1985; Loach 1988b).

A rise in temperature promotes bud growth at expense of rooting (Loach 1988a; 1988b). However, too low a temperature may restrict the physiological activity of the cuttings and hence, reduce rooting (Loach 1988a; 1988b). The higher rooting percentages obtained under mist than under polythene, reported for a number of species, have been associated with the lower temperatures maintained by the evaporative cooling effect of the mist (Hess 1969).

For many years, mist propagation has been widely used for the rooting of cuttings of a large number of species, and probably remains the most widely used system for the propagation of leafy cuttings (Hartmann and Kester 1983; Loach

1988b). Many advantages are commonly associated with mist systems: the wetting of leaves that occur under this system may allow some degree of foliar absorption of water; evaporation of this applied film of water reduces leaf temperature and leaf vapour pressure and, more importantly, most of the water lost to the surroundings is not internal, but externally applied water, preventing severe tissue water deficits occurring (Grange and Loach 1983b; Loach 1988a; 1988b). It has also been argued that the higher irradiances which can be tolerated under mist increase photosynthesis in the cuttings (Hess and Snyder 1955).

Conventional mist systems, however, have not always compared favourably with other systems, such as polythene covers (Gay and Loach 1977; Grange and Loach 1983a) and non-mist systems (Leakey *et al.* 1990; Newton *et al.* 1992b). There is not enough evidence to conclude that photosynthesis is necessarily lower under polythene covers or non-mist systems than under mist. It has been found that very little light is needed to saturate photosynthesis in some species during propagation (Davis and Potter 1987) and leafy cuttings from some tropical species have been shown to actively photosynthesise during the propagation period in non-mist systems (Mesén *et al.* 1992; Newton *et al.* 1992b). The cooler substrate temperature maintained by mist systems is not always an advantage, since it may fall to levels which are inadequate for rooting, especially during winter months in temperate regions (Loach 1988b). Mist sprays which are not appropriately matched to changes in the weather may cause either cyclic water deficits or over-wetting (Loach 1988b; Leakey and Mesén 1991). Over-wetting reduces oxygen diffusion to developing root initials (Loach 1988a), but it may also bring other problems, such as nutrient leaching from the leaves (Blazich 1988; Loach 1988a), reduced CO_2 entry caused by extensive water films on the leaf surface (Grange and Loach 1983b; 1984) and spread of diseases (Loach 1988a). The spray seldom reaches the leaf under-surfaces where a majority of the stomata are usually located, resulting in loss of some tissue water from exposed portions (Loach 1988b). When the mist jets are located in towers along the propagation bed, the circular patterns of irrigation that they produced usually result in both dryer corners in the bed and over-wet patches in the overlapping zones (Leakey and Mesén 1991). When the bed sides are unprotected, as is usually the case under tropical conditions, prevailing winds deflect the spray to one side of the propagating bed, resulting in large watering variations within the bed (Leakey and Mesén 1991). In some studies, cuttings under mist showed a higher water deficit than those under polythene and eventually showed lower rooting (Loach 1977). Possibly, the cyclical fall in ambient humidity that occurs between mist applications, which is absent under polythene, resulted in more water loss from the drier leaves in the canopy (Gay and Loach 1977). In addition, failures in the electricity or water supply may create major problems during the propagation period

(Leakey *et al.* 1990; Leakey and Mesén 1991).

Taking the above considerations in mind, it seems reasonable to think that a more convenient propagation system would be one which combines some of the advantages of mist with those of polythene covers, resulting in leaf wetting during the day and a high humidity and saturation at night (Grange and Loach 1983a). This idea is not new, and in fact, mist within polythene enclosures has been used since the 1950's (Templeton 1953). The mist system ensures that most of the upper leaf surfaces are wet and this must result in lower rates of transpiration (Grange and Loach 1983a). Also, the enclosure maintains a high air saturation by preventing the escape of water vapor which has been transpired by the cuttings or evaporated from wet surfaces beneath the covers (Loach 1988a). Because the system is sealed, heat loss by air exchange is impeded and air temperature rises if irradiance is high (Grange and Loach 1983a; Loach 1988b). This, as discussed above, would increase VPD and water loss from the leaf (Gay and Loach 1977; Grange and Loach 1983a; 1983b; Loach 1988a). Supra-optimal temperature within the enclosure is considered, in fact, the main disadvantage of enclosed systems (Grange and Loach 1983a; Loach 1988b). For instance, when outdoor temperature was 27 °C, Deen (1971) reported temperatures of up to 43 °C under a polythene tunnel. Similarly, with outdoor temperatures of 35.5 °C, Lewandowski and Gouin (1982; 1985) reported temperatures of 65.5 °C under the tunnel. While many cuttings will survive temperatures in excess of 40 °C, sustained exposure may cause cell injury and tissue water deficits sufficient to retard metabolic activity (Loach 1988b). Soil temperatures in excess of 35-40 °C have been found to inhibit root growth of woody species (Wong *et al.* 1971).

However, as temperature depends on irradiance, shading can be used as an effective measure to prevent excessive substrate and air temperatures inside the cover (Grange and Loach 1983a; Loach 1988b; Leakey *at al.* 1990; Leakey and Mesén 1991). Using 81% shade, Lewandowski and Gouin (1985) obtained a temperature inside the cover only 2.3 °C higher than the corresponding temperature outdoors. Grange and Loach (1983a) obtained successful propagation of several broadleaf species by providing shade to keep irradiance below 100 W m^{-2}. In Costa Rica, the use of one layer of black plastic netting reduced irradiance from 1200 μmol m^{-2} s^{-1} to less than 400 μmol m^{-2} s^{-1} on average, which was found appropriate for the propagation of a number of tropical tree species (Mesén *et al.* 1992).

When propagation is carried out under conditions of low capital inputs, as those faced by rural projects in many tropical countries, automatic mist systems may become a serious limitation (Leakey *et al.* 1990; Leakey 1991; Leakey and Mesén 1991).

Therefore, a system which maintains a high humidity inside the polythene cover without the need for mist irrigation, offers a useful alternative. Such a system, described by Leakey and Longman (1988), Leakey *et al.* (1990), Leakey (1991), Newton and Jones (1993a) and in the following chapter, is currently being used in a number of tropical countries for the propagation of leafy cuttings of several broadleaf species (Leakey *et al.* 1990). A volume of water lying under the rooting medium maintains a high humidity inside the propagator and also provides water to the cuttings through capillary action (Leakey and Mesén 1991).

The microclimate inside non-mist propagators is comparable with other more sophisticated propagation systems. In a comparison of non-mist *vs.* mist systems, Newton and Jones (1993a) found lower values of VPD in the non-mist propagator than under mist at the same irradiance. Similarly, relative humidity and leaf and air temperatures were lower in the non-mist system (Newton *et al.* 1992b). Foliar water potential was found to be greater (less negative) under non-mist than under the mist system (Newton and Jones 1993a). Inside the propagators, the air saturates at night (Mesén *et al.* 1992). This gives a further advantage to the system, since condensation occurs on the cooler leaf surfaces, ensuring leaf wetting and reducing water loss (Loach 1988a). A large amount of water also condenses on the upper polythene sheet and eventually falls over the leaves, contributing to certain extent to the wetting of the upper leaf surfaces (Personal observation). If the system is adequately shaded during the day, the condensation on the leaves can provide some safeguard against excessive water loss (see Grange and Loach 1983a). These results suggest that the non-mist propagators are at least as effective as the mist system and highlight their suitability for a wide range of species (Newton *et al.* 1992b).

Rooting media

Rooting media have a considerable effect on the rooting of cuttings and must be considered as an integral part of the propagation system (Hartmann and Kester 1983; Loach 1988b). A large number of materials have been used for the rooting of cuttings, including water, sand, gravel, sawdust, peat, bark, leaf mould, *Sphagnum* moss, rice hulls, vermiculite, polystyrene, clay granules, soil, pumice, perlite and different mixtures of some of these materials (see Hartmann and Kester 1983; Loach 1988b; Leakey *et al.* 1990; Leakey and Mesén 1991; Mesén *et al.* 1992). Mixes of an organic component such as peat and an inert ingredient such as grit, pumice or perlite are most commonly used (Loach 1988b). Hartmann and Kester (1983) described a good rooting medium as one with sufficient porosity to allow good aeration, a high water holding capacity but good drainage and free from harmful pathogens or easy to sterilize. In addition, the rooting medium should offer no obstacles for root growth and of course, should have an adequate consistency to hold the cuttings in place. Ideally, the rooting media should be accessible at any time of the year (Leakey and Mesén 1991).

Peat is normally included primarily because of its ability to hold water, while the mineral ingredient increases the proportion of large air-filled pores and improves drainage (Loach 1986; 1988b). General recommendations have been made for different conditions and propagation systems; for instance, a higher proportion of peat in relation to the inorganic component is recommended for summer conditions in temperate areas, drier systems and soft cuttings and the reverse for winter conditions, wetter systems and mature cuttings (Loach 1988b).

The influence of rooting media on the water uptake by the cuttings has been shown by Grange and Loach (1983b). Compared to pure water, every other rooting media exerted a resistance to water uptake by the cuttings, probably because of the incomplete contact of the cutting base with water films around particles in the medium. Therefore, uptake was improved in direct proportion to increasing water content in the medium. The same authors suggested that the water content of the rooting medium should be greater than 0.5 m^3 water m^{-3} to allow sufficient water uptake. Excess water in the medium, however, can cause aeration problems and reduce rooting. Rooting experiments in grit:peat mixtures have shown that wetter media are appropriate for summer propagations in temperate regions, when water deficits are likely to appear. The same media, however, greatly reduced rooting during winter months, because of the detrimental effect of an over-wet medium (Loach 1990). The necessity of oxygen for adventitious root development was clearly shown in an experiment with willow cuttings in water (Hartmann and Kester 1983). When water was oxygenated, cuttings produced roots throughout their length, whilst in non-oxygenated water, roots were produced only near the water surface.

Rooting medium influences not only the number of cuttings that form roots, but also the type and quality of the roots produced (Hartmann and Kester 1983). Also, different species show large variations in rooting ability under different rooting media (Hartmann and Kester 1983; Loach 1986; Leakey *et al.* 1990; Mesén *et al.* 1992). Rooting results under different rooting media, however, are extremely variable and not necessarily reproducible in repeated propagations (Loach 1988b). The relative proportion of air and water has been commonly used to specify the optimal requirements for rooting (Leakey *et al.* 1990). However, the great differences between results reported in the literature suggest that other factors are involved (Loach 1988b). For instance, Matkin (1965) concluded that a rooting medium should have around 20% of its volume as air-filled pore space, and similarly, Puustjarvi (1969) suggested a minimum of 15%. A minimum of 10 ppm oxygen was recommended for the rooting of *Hedera helix* and rooting of carnation and chrysanthemum increased markedly as the oxygen in the water in which they were rooted increased from 0 to 21% (Hartmann and Kester 1983). However, O'Dell and Stoltz (1978) obtained high rooting in ornamental species in media with only 1% air-filed porosity; similarly, 1 ppm oxygen in the medium was found sufficient for the rooting of willow cuttings (Hartmann and Kester 1983).

The present level of knowledge precludes any specific recommendations and only some general principles are clear. The provision of sufficient water to prevent wilting is a prime requirement. However, the more water filling the pore space, the less air is available to the cutting base, so it is important to achieve a suitable balance between air and water contents (Loach 1986). Such a balance seems to differ according to the species involved, the type of cutting, the propagation system and even the weather (Loach 1986). In some studies, straightforward positive relationships have been found between water content of the medium and water gain by the cuttings and subsequent rooting abilities (Grange and Loach 1983b; Loach 1986). In others, rooting was inversely correlated to the volumetric water content of the medium (Loach 1986). Only occasionally have significant correlations between air content and rooting been found, and usually, neither the volumetric water nor air content are related to the rooting performance (Loach 1986).

One obvious reason for these discrepancies is the disparate methods used to measure the physical characteristics of the medium. It also suggests that our understanding of the principles involved are far from complete (Loach 1986).

As discussed in previous sections, rooting is influence by a large variety of interactive factors, both before and after severance. In addition, responses to these factors differ between species and even between clones within the same species (Leakey 1985; Newton *et al.* 1992b). Therefore, unaccounted sources of variation between different experiments may well be the reason for the lack of repeatability of results (Dick and Dewar 1992; Leakey *et al.* 1992). Experimenters should seek to either remove or quantify different sources of variation, in order to assess their impact accurately and assist in interpretation of results (Leakey *et al.* 1992; Newton *et al.* 1992b).

CHAPTER 2

Materials and methods

PLANT MATERIAL AND GROWING ENVIRONMENT

Institute of Terrestrial Ecology (ITE), Bush Estate, Scotland

Plants used in the experiments were derived from the open pollinated progeny of plus trees selected by the CATIE Tree Improvement Project in Costa Rica. Plants were grown in plastic pots (90 mm; this and subsequent figures refer to diameter of the top of the pot) in 7:3:1 peat-sand-loam mixture in tropicalized glasshouses at ITE. Except where otherwise stated, the plants were watered daily to field capacity and given a weekly application of 1% liquid fertilizer ('Solinure', Imperial Chemical Industries plc; 20%N, 20%P, 20%K). The glasshouses were maintained at a temperature of 25-30 °C, with a daylength of 19.5 hours throughout the year and a relative humidity of around 60%. Supplementary Mercury vapour (MBFRU-400 watts) lighting maintained a red:far red ratio of around 1.2 and a photosynthetically active radiation (PAR) always in excess of 50 μmol m^{-2} s^{-1}, even at night during winter months when these lights were the only source of irradiance. PAR increased to 1800 μmol m^{-2} s^{-1} during bright summer weather and to 200 μmol m^{-2} s^{-1} during bright winter weather. Vigorous *Cordia alliodora* and *Albizia guachapele* seedlings were potted into bigger containers (190 mm and 150 mm respectively), cut back to maintain a supply of coppice shoots and used as stockplants for building up clonal populations. Rooted cuttings were potted into plastic pots (90 mm) containing the standard mixture described above, and routinely repotted into increasingly larger containers (127 mm and 150 mm for *Albizia guachapele*, 127 mm and 190 mm for *Cordia alliodora*) as the plants grew bigger.

Centre for Tropical Agricultural Research and Education (CATIE), Turrialba, Costa Rica

Plants were derived from the open pollinated progeny of plus trees selected by the CATIE Tree Improvement Project (TIP) in Costa Rica. Original seedlings were grown 1 metre apart in beds at the TIP nursery at CATIE, Turrialba, cut back to maintain a supply of coppice shoots and used as a source of cuttings to build up clonal populations. Rooted cuttings were potted into black polythene bags (70 cm^2)

containing a 1:1:1 mixture of forest soil, sand and organic compost (decomposed residues from the bottom of an artificial lake) and kept under shade and decreasing watering (from automatic mist irrigation to once daily watering) during a 2-3 week period. After this weaning period, cuttings were planted in beds beside the original stockplant, at a spacing of 20 cm x 20 cm. The clonal plants were given fortnightly soil applications of a powder fertilizer 10-30-10 (FERTICA, Puntarenas, Costa Rica) containing 10%N, 30%P and 10%K, at a rate of approximately 30 g per plant. The beds themselves were made up of the potting mixture described above. Annual rainfall in Turrialba reaches 2600 mm, with no month below 50 mm. In consequence, watering was not usually necessary, but the plants were watered to field capacity when there was no rain for two consecutive days (typically in January and February).

PREPARATION OF CUTTINGS

Cuttings were collected from vigorous, orthotropic shoots, 30-40 cm long. Shoots were harvested early in the day, placed in water filled containers or in polythene bags containing moist paper and transferred immediately to the propagation area. The uppermost node was discarded and the leaves trimmed to the appropriate area (as described for each particular experiment). Cuttings were then produced by making an oblique cut just above a node using a scalpel or sharp secateurs; except where otherwise stated, 5 cm long cuttings were used. A solution of indole-3-butyric acid (IBA) was prepared by dissolving a precise amount of auxin in methanol to obtain the desired concentration. To prevent excessive evaporation of the alcohol from the container and subsequent variation in the concentration, a micrometer syringe was used. The needle was inserted through a small hole in a piece of plastic which was used as a lid. The plastic lid was tied to the container with a rubber band. The solution was applied to the clean cut base of the cuttings in 10 μl droplets using the micrometer syringe. Immediately after applying the solution to the base of the cutting, the alcohol was quickly evaporated off in a stream of cold air from a fan before inserting the cuttings in the propagators. Prior to inserting cuttings, holes were made in the medium to a depth of 2 cm using a board with wooden pecks at regular spacing (5 cm x 5 cm) and the medium pressed firmly around the cutting. During the establishment of the experiments, cuttings were kept moist by spraying with water from a hand-held sprayer every time the propagator lid was opened.

THE NON-MIST PROPAGATOR

For the rooting experiments, 10 non-mist propagators (Plate 2.1) were available at ITE (Plate 2.2a), and 10 were built at CATIE (Plate 2.2b). The non-mist propagator design is basically a wooden or metal frame enclosed in clear polythene so that the base is water-tight. The basal 25 cm are covered by successive layers of large stones (6-10 cm), small stones (3-6 cm) and gravel, and topped with an appropriate rooting medium. The basal 20 cm are then filled with water. The rest of the frame is covered tightly with a single piece of clear polythene, and a closely-fitting lid is attached (Leakey *et al.* 1990). A number of reasons were put forward favouring the use of these propagators for the present study: they are cheap to construct, have proved very effective for the propagation of a large number of tropical species and have been found to be acceptable to foresters, managers and farmers in Costa Rica and other countries. In addition, they have no essential requirements for either piped water or electricity, which make them ideal for small/rural development forestry programmes.

Generally, three different rooting media, fine sand, gravel and sawdust, were used (as detailed in each experiment). The sand was obtained from a nearby river, the gravel from the CATIE quarrel and the sawdust from the CATIE sawmill. The latter was chosen from the oldest piles, which had been allowed to decompose in the field for at least one year. Both the sand and the gravel were carefully washed to remove dirt and soil particles, before they were placed in the propagator. No such treatment was given to the sawdust.

The propagators were placed in the Tree Improvement Project nursery, and a cover of black plastic netting (saran) was built two meters above them, with extensions towards the east and west sides.

MEASUREMENTS

Relative water content (RWC)

For the determination of foliar relative water content (RWC), the method described by Beadle *et al.* (1985) was used, using whole leaves in every case. This was based on measurements of fresh mass at time of sampling (*F*), turgor mass (*T*), obtained after floating the leaf on distilled water for 24 h, and dry mass (*D*), taken after drying the leaf in an oven at 80 °C for 24 h. RWC calculated as:

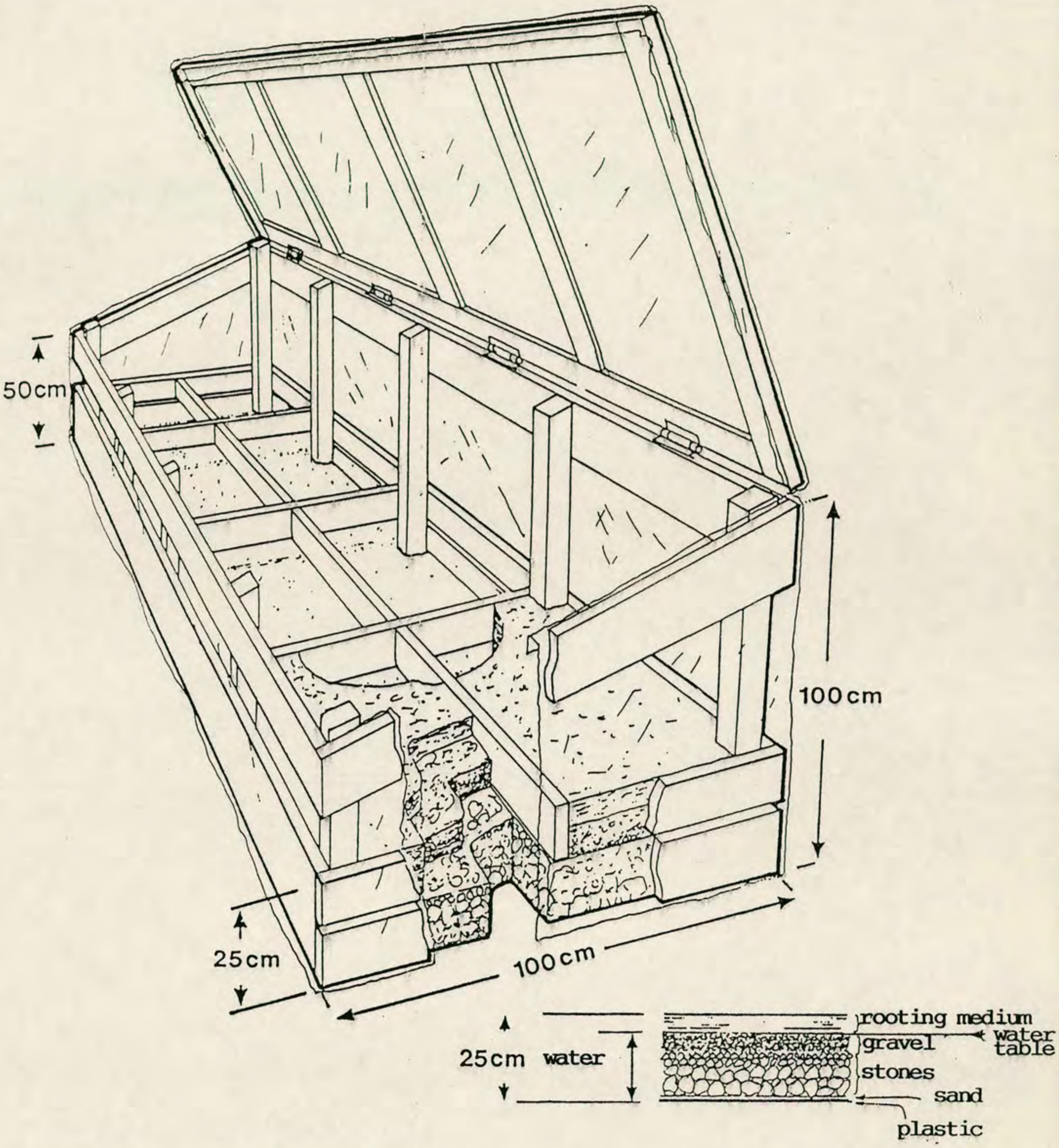


Plate 2.1 A non-mist propagator

Plate 2.2a,b Non-mist propagators at (a) the Institute of Terrestrial Ecology (ITE), Bush Estate, Scotland, and (b) at the Tropical Agricultural Research and Training Centre (CATIE), Turrialba, Costa Rica.

$$RWC = ((F-D)/(T-D)) \times 100\%$$

Determining RWC requires relatively simple, cheap equipment and many samples can be taken from a large number of treatments (Beadle *et al.* 1985). It has the disadvantage that it involves destructive sampling and there may be difficulties in arriving at turgor mass, as this may vary with species (Beadle *at al.* 1985). For this study, preliminary tests were conducted on a sample of leaves and no further changes in mass occurred after 24 h.

Specific leaf area

Specific leaf area (SLA) was determined as described by Beadle (1985). Measurements of leaf area (s) using an area meter (Delta T Devices, Burwell, Cambridgeshire, U.K.) and dry mass (D, oven dry mass after drying at 80 °C for 24 h) were taken. SLA was calculated as:

$$SLA = s/D \ (m^2 \ g^{-1})$$

Chlorophyll fluorescence

For measurements of chlorophyll fluorescence, a Plant Stress Meter was used (PSM Mark II, Bio Monitor S.C.I. AB, Sweden), set at an irradiance of 400 μmol photons m^{-2} s^{-1}, a run-time of 25 seconds and a period of foliar darkening of 20 minutes. The period of darkening must be long enough to make sure that the chlorophyll is completely in a non-excited state, while the irradiance must be as high as possible, to make sure that the photosystems are saturated when the light is switched on. These settings were determined after conducting a series of preliminary tests with the cuttings of both species. Dark adaptation periods of 15-30 minutes have been recommended in the literature (Bolhar-Nordenkampf *et al.* 1989).

Chlorophyll fluorescence has been widely used in understanding the mechanisms by which a range of environmental factors alter photosynthetic capacity (Bolhar-Nordenkampf *et al.* 1989). Measurements are both non-destructive and non-invasive, and thus have considerable potential for use in field situations (Bolhar-Nordenkampf *et al.* 1989). The theory behind chlorophyll fluorescence measurements has been described in detailed by Walker (1985) and Bolhar-Nordenkampf *et al.* (1989) and is summarised here. Each quantum of light absorbed by the chlorophyll molecule raises an electron from a ground, 'resting' state to an excited state 'one' or to an even higher orbit (excited state 'two'), depending on the wavelength of the light absorbed. Red and blue quanta cause electrons to raise to excited states one or two respectively, because the higher energy content of the blue quanta. However, electrons raised to state two fall almost immediately to state one, losing their energy as heat, too quickly to permit any useful chemical work. Therefore, whatever the quality of light absorbed, the electrons reach the same energy level (excited state one) more or less immediately after excitation. All subsequent events, including photosynthesis, derive from this energy level. The energy not used to drive photosynthesis is dissipated as fluorescence, as electrons return from excited state one to the ground state; therefore, if photosynthesis were constrained by some factor, more energy would be "wasted" and more fluorescence would be detected. If a leaf is kept for a few minutes in darkness and then brightly illuminated, fluorescence (normally measured in arbitrary units) rises to an initial level, termed initial fluorescence (F_O). If illumination is strong enough, fluorescence increases from F_O to a peak level, called F_m, and then decreases in a more or less simple fashion to a steady-state value. The difference between F_m and F_O is termed the variable component of F (F_v). The ratio F_v/F_m is highly correlated with the quantum yield of net photosynthesis of intact leaves and thus, a decline in F_v/F_m is a good indicator of a decline in the photosynthetic activity. The area over the curve between F_O and F_m is proportional to the pool size of electrons acceptors on the reducing size of photosystem II; therefore, if electron transfer from the reaction centres to the acceptors is blocked, this area is dramatically reduced. A simple indicator of this area is given by $t_{1/2}$, which represents one half of the time required for the rise from F_O to F_m.

Gas exchange

Photosynthetic rates and stomatal conductances were measured using a portable gas exchange system with infra-red gas analyser (portable IRGA, LCA-3, Analytical Development Company Ltd., Hoddesdon, Herts., U.K.), attached to a Parkinson leaf chamber (Analytical Development Co. Ltd., Hoddesdon, U.K.). Except where otherwise stated, a section in the propagator was not filled with rooting medium, so that the leaf chamber was placed in a lower position inside the propagator, to allow measurements to be taken without removing the cuttings from the rooting medium. Before every measurement, care was taken to ensure that the leaf surface was dry, to prevent inaccurate conductance readings due to free water on the leaf surfaces. Absorbent paper was used to dry the leaf. The leaf chamber was connected to the LCA through a small hole in the propagator, enabling the propagator lid to be kept closed whilst collecting gas exchange readings. The IRGA was checked and calibrated as necessary before each session of measurements. Measurements were taken after a steady differential of zero was obtained in CO_2 and relative humidity, normally after 20-30 minutes.

Measurement of CO_2 uptake by the cutting has important advantages over measurements of dry-weight change. It is instantaneous, non-destructive, allows separate investigation of individual leaves and allows separation of photosynthetic gain from respiratory losses (Long and Hallgren 1985). In an IRGA system, the leaf is enclosed in a chamber and a net flow of air is blown across the chamber. Changes in water vapour and CO_2 concentrations across the leaf are determined by comparison to the reference gas stream (Long and Hallgren 1985). The only heteratomic gas normally present in air with an absorption spectrum overlapping that of CO_2 is water vapour. This problem is overcome by drying the air before it reaches the leaf chamber (Long and Hallgren 1985). Air is continuously blown into the chamber, which has a separate inlet and outlet. Infra-red radiation is emitted through the chamber and analysed by a detector at the other end of the chamber. Any increase or decrease in the concentration of the infra-red absorbing gas (i.e. CO_2) in the chamber will result in a fall or a rise in the detector signal, respectively (Long and Hallgren 1985). These variations are then expressed as amount of CO_2 assimilated per unit leaf area and time (μmol m^{-2} s^{-1}), which is a direct expression of photosynthesis (Long and Hallgren 1985).

Rooting

One week after establishment in the propagators, cuttings were lifted and examined for number of roots (Plate 2.3a,b). Similar assessments were carried out weekly until no further increase in rooting percentage occurred.

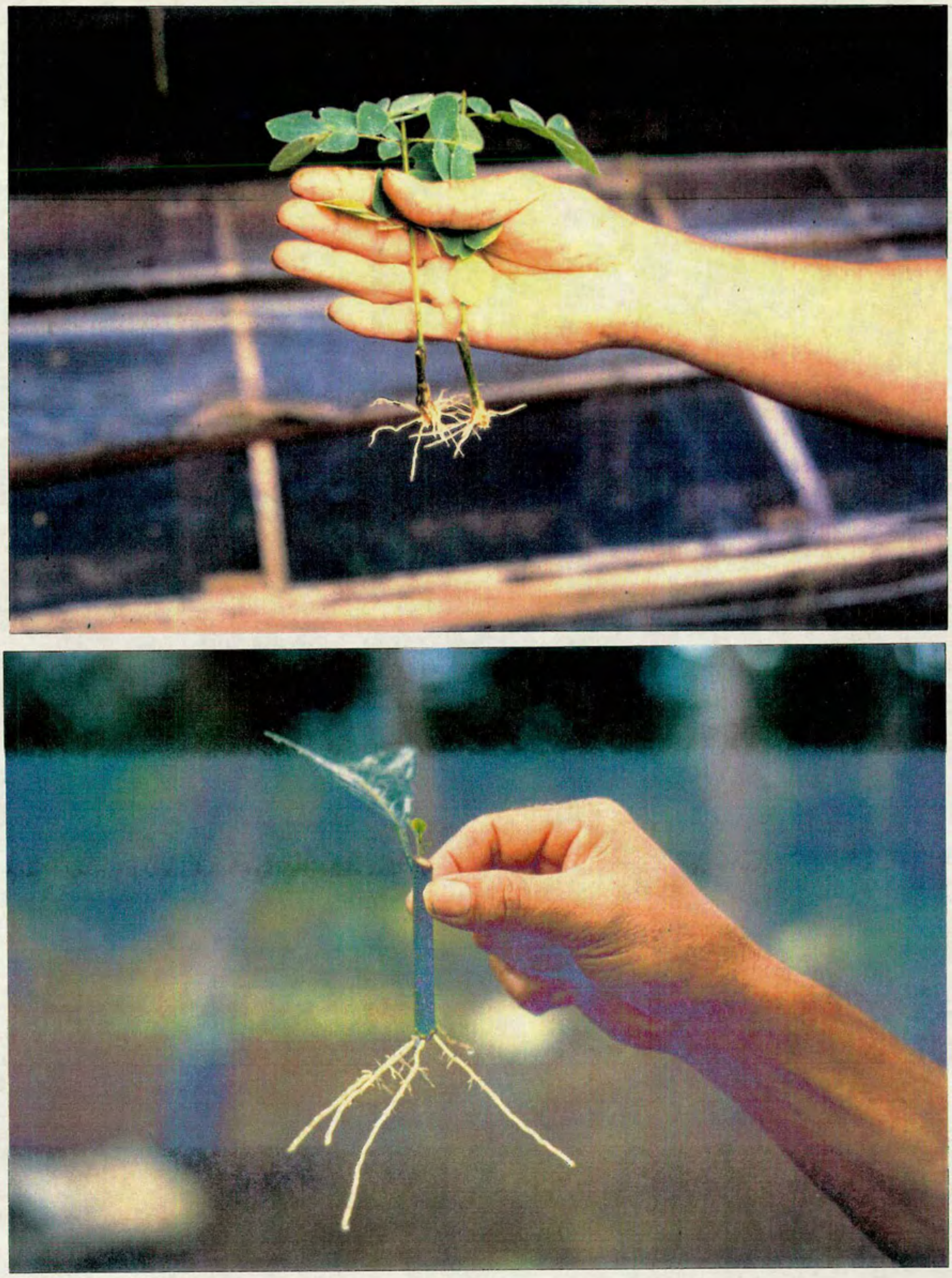


Plate 2.3a,b Rooted cuttings of **(a)** *Albizia guachapele* and **(b)** *Cordia alliodora*.

Propagator microclimate

A data logger (21X Micrologger, Campbell Scientific Ltd., Loughborough, U.K.) was used to characterize propagator microclimate during propagation. Air temperature was measured using thermocouples (Type K chromel-alumel; T.C., Ltd., Uxbridge, U.K.), humidity using a thermistor probe (MP. 100 Rotronic probe, Campbell Scientific Ltd., Loughborough, U.K.), substrate temperature using a 107-thermistor probe (Campbell Scientific Ltd., Loughborough, U.K.) and irradiance using quantum sensors (Skye Instruments Ltd., Llandrindod Wells, U.K., supplied by Campbell Scientific Ltd., Loughborough, U.K.). All sensors of each type were cross-calibrated prior to use. The logger was programmed to record each sensor every 10 s, and to calculate and store mean readings every 15 min.

A tunnel-shaped wooden box was used to shade the thermistor probe and prevent water from the hand-held spray or water condensed in the propagator lid to reach the sensor. The 107-thermistor probes were inserted in the rooting medium to a depth of 2-3 cm. For measurements of leaf temperature, the thermocouples were attached to a plastic label inserted in the medium below a leaf, with the sensor pointing upwards, and the leaf carefully positioned over it to make sure it was in permanent contact with the sensor.

Usually, one of each of the above sensors was placed in a central position inside the propagator, except in experiments involving different rooting media, in which instance a set of thermocouples and 107-thermistor probes was placed centrally in each block of medium.

EXPERIMENTAL DESIGN AND GENERAL STATISTICAL ANALYSIS

Generally, a fully replicated randomized block design was used for all rooting experiments, keeping record of clone and cutting position within the stem. A different number of blocks was used, to achieve at least 35 cuttings per clone/treatment combination. Full details of the experimental designs used are given in the description of each experiment.

Significance of treatment effects was determined by analysis of variance (ANOVAR), using SAS (1980). For data with binomial distribution (e.g. rooting), the arcsin $\sqrt{\%}$ transformation was used. Treatment differences were determined through *t* tests, equivalent to Fisher's least-significant-difference tests, at 5% probability level (Snedecor and Cochran 1980).

Analyses of deviance for stepwise regression were performed using GENSTAT 5 (Payne *et al.* 1987) to determine which of the recorded variables were significantly associated with rooting and the influence of the recorded variables on the photosynthetic rate and stomatal conductance of the cuttings.

Regression coefficients were calculated on a least-squared basis using Sigma Plot 4 (Jandell Scientific, 1989).

CHAPTER 3

The effects of different concentrations of Indole-3-Butyric Acid (IBA) on water relations, dry mass accumulation and rooting ability of leafy, stem cuttings of *Cordia alliodora*

This experiment investigated the effects on rooting ability of a range of concentrations of indole-3-butyric acid (0, 0.2, 0.4, 0.8 and 1.6%) applied to single-node cuttings of *Cordia alliodora*. This involved determination of water relations and changes in dry mass in the cuttings during the propagation period.

MATERIALS AND METHODS

Plant material and experimental design

This experiment was carried out at the CATIE nursery, Turrialba, Costa Rica. In May 1990, the stockplants were cut to a height of 20 cm. By August 1990, a variable number of shoots had grown from each stockplant to heights of 30-40 cm, each one providing five to six, 5 cm-long cuttings. Clones 2, 4 and 8 provided the largest number of cuttings, so these were selected for the present experiment.

As the length of the shoots was variable and the amount of material was limited, it was decided to use every cutting and randomize node position within the experiment, discarding only the uppermost node of each shoot. This was based on the findings of previous preliminary experiments (Mesén, unpublished data), where cuttings from nodes 2 (top) to 8 (basal) rooted equally well, but significantly better than cuttings from node 1 (apical). From these experiments it is also known that survival of cuttings shorter than 5 cm is poor. Consequently, cutting length was standardized to 5 cm, with resulting diameters of 4.19 ± 0.09 mm. Two hundred and fifty cuttings were collected from each clone, after trimming their leaf area to approximately 30 cm^2 and treated immediately with one of five indole-3-butyric acid (IBA) concentrations: 0%, 0.2%, 0.4%, 0.8% and 1.6 %. The appropriate concentration of IBA was applied to the clean-cut base of the cuttings as described in Chapter 2. Cuttings chosen to receive 0% IBA were treated with 10 μl of methanol. Cuttings were inserted in ten randomized blocks in fine sand in two non-mist propagation units. During the course of the experiment, two fine spray waterings were given daily to the cuttings to keep the leaves moist, at 07:00 and 15:00 h.

Assessments

At day 1, ten cuttings from each clone were harvested for assessments of initial stem and leaf fresh and dry mass, and then destructive samples were taken at weeks two, four and six, harvesting a complete block each time. At each harvest, fresh and dry mass of leaves, stems and new shoots were recorded separately, in addition to leaf turgor weight and relative water content. Each clone/treatment combination was represented by five cuttings at each harvest.

After two weeks the cutting diameters were measured at a mid point, i.e., 2.5 cm from the base, and each cutting assessed for number of roots, presence of shoots and leaf shedding. Similar assessments were carried out for the next seven consecutive weeks.

For the determination of leaf relative water content, the method described by Beadle *et al.* (1985) was used. Measurements of fresh (FM), turgor (TM) and dry (DM) mass were obtained, and then the relative water content calculated as ((FM-DM)/(TM-DM)) x 100. Turgor mass was obtained by floating the leaves in distilled water in Petri dishes for 24 h, and then re-weighing the leaves after drying the surface water with a paper towel. To determine dry mass, the cuttings were oven dried at 80°C for 24 hours prior to weighing.

Analysis

Analyses of variance were carried out of the number of roots per rooted cutting, relative water content, production of shoots from the cuttings and variation in foliar, stem and total dry mass, followed by t tests (LSD). Percentage rooting and confidence limits (95%) were calculated by clone and IBA treatment. Estimates of leaf and stem dry mass at day 1 were obtained by multiplying the total fresh mass of the cuttings at day 1 by a reduction factor obtained from a sample of 30 cuttings from the three clones, destructively harvested at day 1. Corrected changes in dry mass were calculated by obtaining the percentage variation in respect to the estimated initial dry mass, and then expressed as mean changes (g) from the initial value (100% = 0 g). Correlation coefficients were calculated on relationships between the percentage of cuttings rooted and i) percentage of cuttings with actively growing shoots, and ii) relative water content.

RESULTS

Rooting ability

Rooting percentage increased with increasing IBA concentration and for all the IBA concentrations showed a steady increase from week 3 to week 9. At the end of nine weeks highly significant differences in rooting percentage were found between IBA concentrations (Table A1), when rooting reached 70% in cuttings under 1.6% IBA, compared with only 10% for the control cuttings (Fig. 3.1). Highly significant differences ($p < 0.01$) were also found between IBA concentrations for number of roots per rooted cutting (Table A2). This trait showed a similar trend to that of rooting percentage, being greater with successive increases in IBA concentrations (Fig. 3.1b).

Cuttings of clone 2 rooted significantly better than clones 4 and 8 after week 5, but these differences became non-significant at week 9 (Fig. 3.2a). In terms of number of roots per rooted cutting, clone 2 produced a significantly larger number of roots than all other clones (Fig. 3.2b).

To investigate the relative influence of treatments, clones and morphological characteristics of the cuttings on their rooting ability, the results at week 9 were analysed by stepwise regression, including data of cutting diameter and leaf shedding. The analysis showed that rooting was highly dependent on the IBA concentration, the presence or absence of shoots and leaves in the cuttings, clone and cutting diameter. Rooting was not affected by the node position within the shoot (Table A3).

During the first 6 weeks, the percentage of cuttings with growing shoots was greater with successive decreases in IBA concentration. After seven weeks there were no further increases in this trait, but shoot production was significantly higher for the concentrations 0% IBA and 0.2% IBA (Fig. 3.3). No significant differences were found between clones for this trait. When the percentage of cuttings with growing shoots was correlated with final rooting percentage, a strong negative relationship was found between this variable at week 4 and final percentage of cuttings rooted (Fig. 3.4).

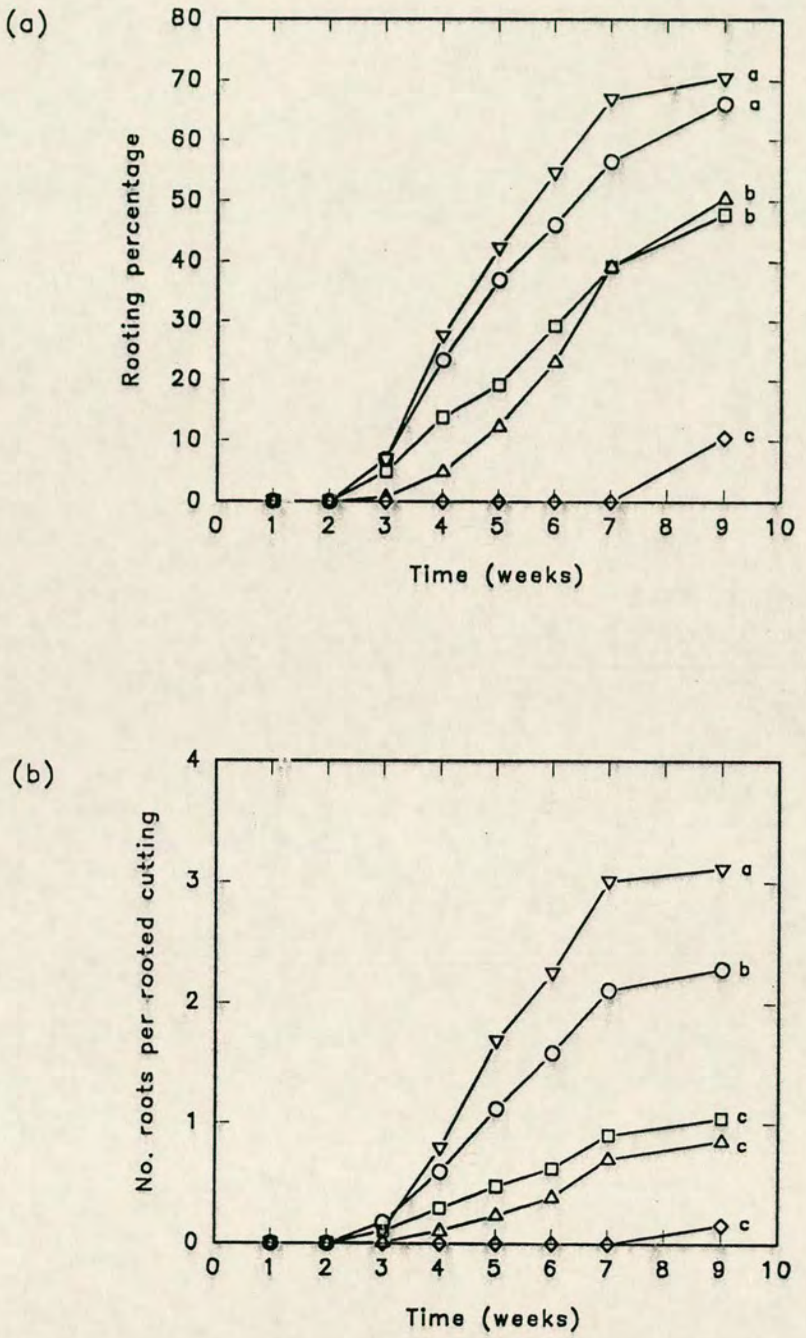


Fig. 3.1a,b The effects of five different concentrations of IBA (0% - diamonds; 0.2% - upright triangles; 0.4% - squares; 0.8% - circles; 1.6% - inverted triangles) on **(a)** the rooting percentage and **(b)** the number of roots per rooted cuttings of single-node, leafy stem cuttings of *Cordia alliodora* during nine weeks in non-mist propagators. Values are means (n = 115) of three clones; means grouped by the same letter are not significantly different ($t_{0.05}$).

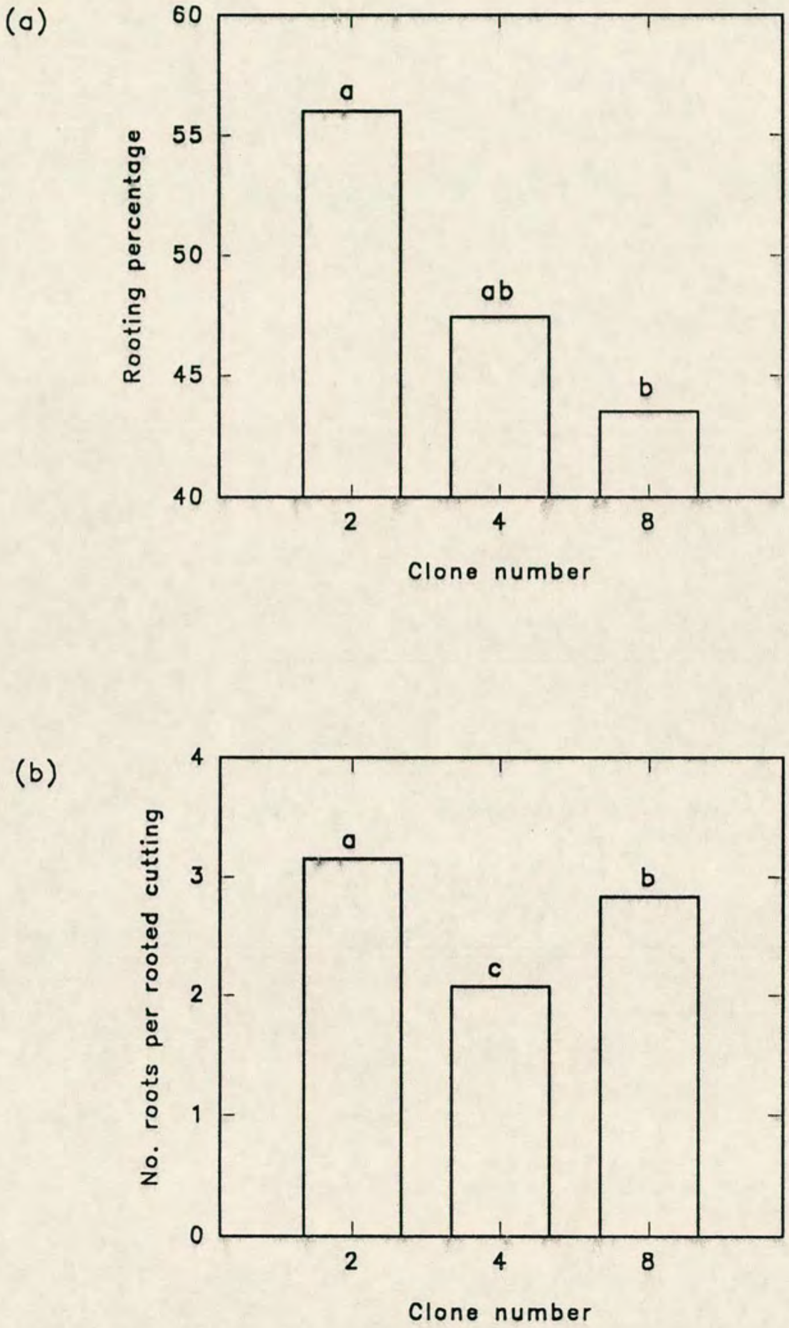


Fig. 3.2a,b Clonal variation in **(a)** the rooting percentage and **(b)** the number of roots per rooted cuttings of single-node, leafy stem cuttings of *Cordia alliodora* during nine weeks in non-mist propagators. Values are means ($n = 200$) of five IBA concentrations; means grouped by the same letter are not significantly different ($t_{0.05}$).

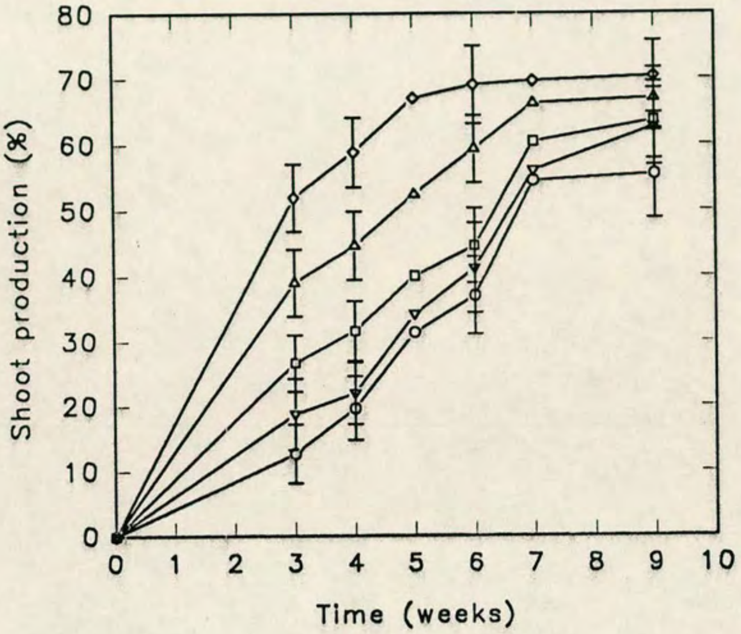
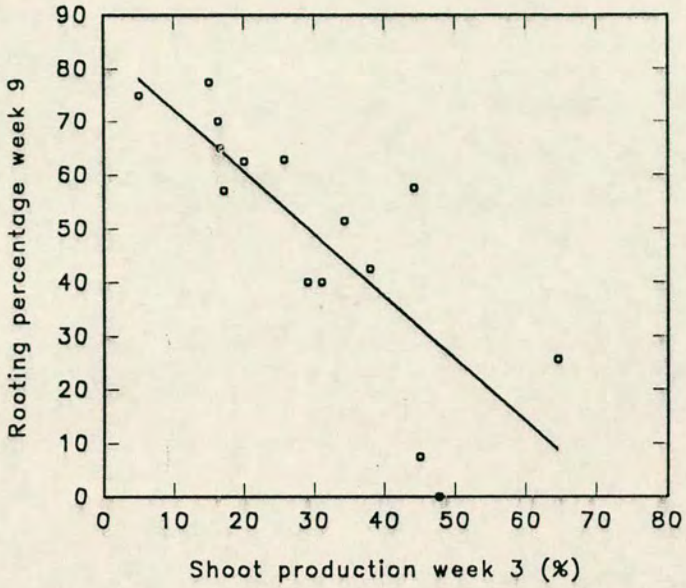


Fig. 3.3 The effects of five different concentrations of IBA (0% - diamonds; 0.2% - upright triangles; 0.4% - squares; 0.8% - circles; 1.6% - inverted triangles) on the percentage of cuttings with growing shoots on single-node, leafy stem cuttings of *Cordia alliodora* during nine weeks in non-mist propagators. Values are means (n = 115) of three clones; bars = ± standard error of the means.

(a)



(b)

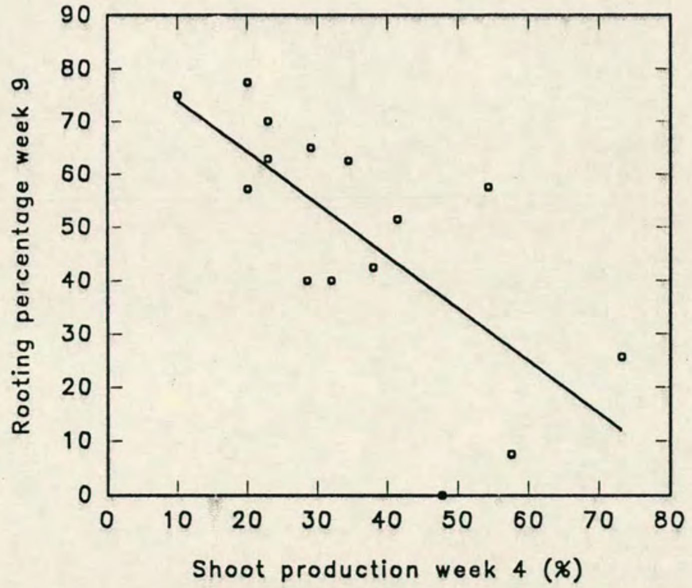


Fig. 3.4a,b The relationship between the percentage of *Cordia alliodora* cuttings rooted after nine weeks in non-mist propagators and the percentage of cuttings with actively growing shoots at (a) week 3 and (b) week 4. Each value is the mean of 40 cuttings from three clones and five different IBA concentrations.

Relative water content

Highly significant differences in relative water content were found between clones but not between treatments. At week 2, the relative water content of all the cuttings was almost 100%, showing that all of them were almost fully turgid (Fig. 3.5). By week 4, relative water content had declined to around 75%, only to increase again by week 6, to levels slightly lower than those of week 2. The relative water content of clone 8 was significantly lower at week 6 than that of clones 2 and 4.

Dry mass

No significant differences in dry mass were found between IBA concentrations during six weeks in the non-mist propagators. The foliar dry mass of all treatments decreased by week 2, and for most of the treatments showed an increase by week 6, but only to levels similar to the initial values (Fig. 3.6a). In terms of total dry mass, most IBA concentrations showed a steady increase during the six weeks in the propagator. Treatment 0.4% IBA showed a slight decrease during the first four weeks, but increased at week 6 to levels similar to the rest of the treatments (Fig. 3.6b).

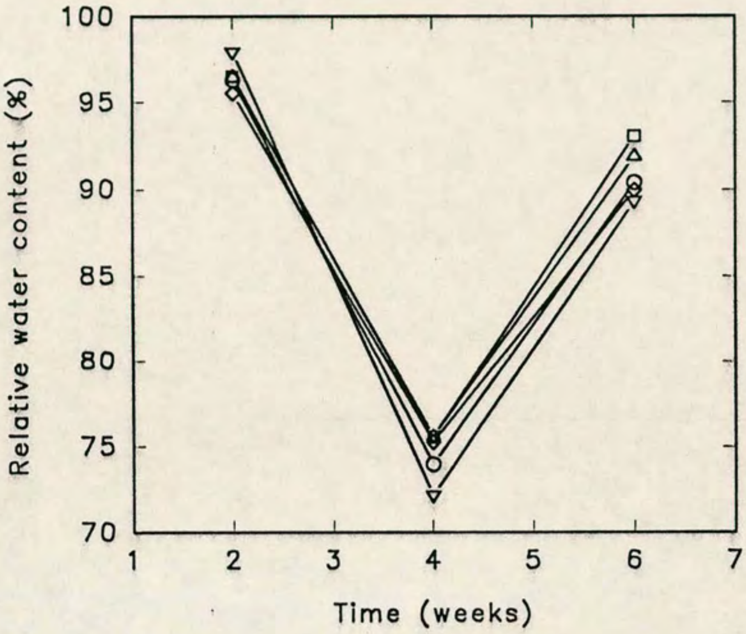


Fig. 3.5 The effects of five different concentrations of IBA (0% - diamonds; 0.2% - upright triangles; 0.4% - squares; 0.8% - circles; 1.6% - inverted triangles) on the foliar relative water content of leafy stem cuttings of *Cordia alliodora* during the first six weeks in non-mist propagators. Values are means (n = 115) of three clones.

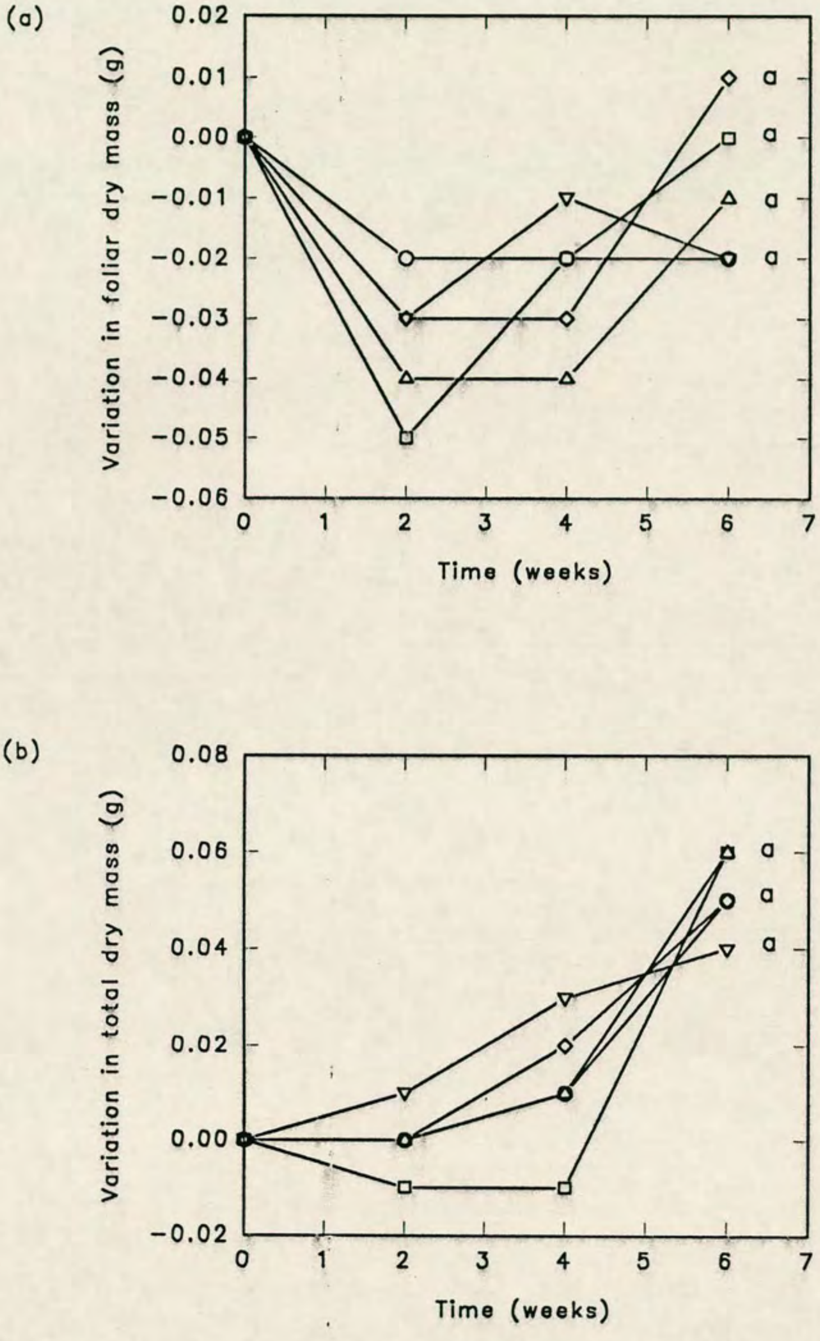


Fig. 3.6a,b The effect of five different concentrations of IBA (0% - diamonds; 0.2% - upright triangles; 0.4% - squares; 0.8% - circles; 1.6% - inverted triangles) on **(a)** the variation in foliar dry mass and **(b)** the variation in total dry mass of single-node, leafy stem cuttings of *Cordia alliodora* during the first six weeks in non-mist propagators. Values are means ($n = 25$) of three clones; means grouped by the same letter are not significantly different ($t_{0.05}$).

DISCUSSION

In this experiment, rooting was considerably enhanced by increasing concentrations of IBA, as has been found for many other tropical forest species (Leakey *et al.* 1990; Leakey *et al.* 1982a). The concentration that was associated with the highest rooting percentage (1.6% IBA) in this experiment is higher than the optimal concentration (0.2% IBA) reported in previous studies with this species (Leakey *et al.* 1990). In previous experiments, cuttings were dipped in a solution of IBA and methanol, whilst in the present experiment the cuttings were applied with a constant amount of 10 μl of solution. The latter technique is obviously preferable for research purposes, since the exact quantity of hormone applied to each cutting is known, whilst with the quick dip method, the cuttings may receive different quantities depending on their diameter, the rate of transpiration, the hairiness of the stem and other factors (Leakey 1988). With the method of application used in this experiment no evidence of toxicity was observed in the cuttings. In addition, the response of the cuttings to increasing concentrations of IBA suggests that the optimal concentration of IBA for the rooting of cuttings of *C. alliodora* may be even higher than those tested in this experiment.

Different clones showed a similar trend in rooting with increasing IBA concentrations, but varied in terms of final rooting percentage, with that of clone 2 being significantly higher than other clones from week 5. The reasons for clonal variation are not fully understood (Leakey *et al.* 1982a), but they may be attributable to the clones differing in their concentrations of endogenous auxins, rooting co-factors or inhibitors; anatomy, leaf retention and many other interacting factors that may influence rooting. Bearing the above in mind, it was perhaps not surprising to find clonal differences in terms of rooting ability.

The water relations in the cuttings were unaffected by variation in concentration of IBA. It is likely that the observed changes were associated primarily with environmental changes and not with water absorption by the newly formed roots, as one should expect, since the same trend was shown by all the cuttings, despite the fact that cuttings under treatment 0% IBA lacked roots after four weeks.

Clones did show significant differences in their relative water content after six weeks in the propagator, and displayed an opposite ranking from that of rooting percentage at the same period. This probably reflects an improvement in the water relations of the cuttings as roots formed. The same relationship was found at week 4, but clonal differences at this time were not significant. However, it is likely that even these minor differences in relative water content had an effect on the final rooting percentage of the cuttings. It is known that the water status of the cuttings may be a prime determinant of successful rooting (Loach 1988b), and even a slight water deficit may result in considerable delay or reduction in the rooting process (Evans 1952; Loach 1977).

The non-mist propagators used in this experiment are known to maintain a very high relative humidity (>90%) when the system is closed (Leakey *et al.* 1990). The absence of cyclical drops in humidity within the propagator, as usually occur in mist systems, is probably the reason for the successful propagation of *C. alliodora* in this experiment. *C. alliodora* appears to be highly susceptible to water stress, and this was thought to be the major cause of failure in earlier attempts to root juvenile cuttings of this species under mist (Dyson 1981; Mesén, unpublished data).

A strong negative relationship was found between the percentage of cuttings with actively growing shoots at week 4 and final rooting. It is known that growing shoots may act as a sink for assimilates in competition with the base of the cutting. Hence, under conditions of rapid shoot growth, the supply of current photosynthates may limit rooting (Davis 1988). In cuttings of *Populus tremula*, for instance, Eliasson (1971) found that root growth was significantly reduced by shoot growth. He attributed this to competition for assimilates and possibly other factors formed during photosynthesis. Under natural conditions, growing shoots have a somewhat stronger ability than roots to compete for carbohydrates for their growth (Eliasson 1968).

The fact that increasing concentrations of IBA clearly inhibited bud growth in *C. alliodora* cuttings may be interpreted as enhanced competition for assimilates by the cutting base, as a result of an IBA-induced downward transport of available assimilates to the cutting base, with sink strength successively enhanced by each increase in IBA concentration. Once the roots are formed, the improved water balance and photosynthetic reactions in the plant may restore the balance of shoot and root growth. This could explain the lack of differences between treatments for this trait at the end of the experiment.

An alternative but perhaps less likely hypothesis is that applied auxins, by upward transport through the xylem, may inhibit bud growth, so increasing the availability of current assimilates and reserves for root formation and growth. This hypothesis has not been critically tested, but some evidence for it has been provided by the delayed sprouting of cuttings from stockplants previously injected with auxins (Leakey 1990).

Current photosynthesis, as defined for use in vegetative propagation by Davis (1988) as the photosynthesis that occurs after excision, is generally thought to positively influence root formation on most types of leafy cuttings (Davis 1988). In some cases, increases in the dry mass of the cuttings have been correlated with the rooting ability of cuttings (Leakey and Coutts 1989), while rooting also seems to be related to net photosynthesis of individual cutting positions on shoots prior to severance (Leakey and Storeton-West 1992). However, in leafless hardwood cuttings and leafy cuttings of other species, root formation has been associated with redistribution of carbohydrates within the cutting (Okoro and Grace 1976). In fact, rooting with some species occurs when current photosynthesis is impaired or minimal, suggesting that in some circumstances, root formation can depend upon carbohydrate reserves rather than current photosynthesis (Davis 1988).

From the findings of the present experiment, it was not possible to establish a clear relationship between variations in dry mass of the cuttings and their rooting ability. Clones showed significant differences in terms of stem and total dry mass, but no relationships were found between these traits and rooting percentage. Clone 2, which displayed the highest rooting percentage, did not show any significant increase in foliar, stem or total dry mass during the course of the experiment. It is known that only moderate photosynthetic active radiation is needed to saturate net photosynthesis of unrooted cuttings (Davis 1988), and even low rates can contribute significantly to the carbon budget of a given plant part (Okoro and Grace 1976). Under the conditions of this experiment, it is likely that photosynthesis did occur in the cuttings, but that assimilate gains only kept pace with respirational losses and dry mass partitioned to roots.

It must be taken into account, however, that dry mass is only a rough indicator of photosynthetic activity (Davis 1988); there is also the problem, which is impossible to avoid experimentally, of calculating gains or losses over time based on different samples. The samples used for assessments of dry mass over time may vary in their relative proportion of leaf, petiole and stem and in their initial dry mass to the cuttings used for the estimations. The corrections used in this study attempted to overcome this problem, at least to some extent. Thus the results presented here perhaps reflect a more accurate description of the variation with time than previously reported (e.g. Leakey and Coutts 1989). It is clear that other systems for measuring current photosynthesis, such as those based on measurements of gas analysis, must be used if more accurate measures are to be obtained.

CHAPTER 4

The effects of different propagation environments and foliar areas on the physiology of rooting of leafy stem cuttings of *Cordia alliodora*

These experiments investigated the effects on rooting ability of the interactions between light environment during propagation and foliar area of single-node cuttings. These involved the determination of the gas exchange of the cuttings. Experiment 1 investigated in detail the photosynthetic activity and stomatal conductance of cuttings during the first days after severance, using a small sample of cuttings. Experiments 2 and 3 involved a larger number of cuttings over a longer period to evaluate the effects of the treatments on the rooting ability of the cuttings.

MATERIALS AND METHODS

Experiment 1 — The physiology of single-node, leafy stem cuttings of *Cordia alliodora* during the first eight days in non-mist propagators with or without shade

Plant material and experimental design

This experiment was carried out at the CATIE nursery, Turrialba, Costa Rica, using the set of stockplants as described in Chapter 2. In February 1992, the stockplants of clone 53 were cut to a height of 20 cm. Two weeks later, the three most vigorous shoots were selected from each stockplant and any other shoots were removed. By late March, 1992, the selected shoots had grown to heights of 30-40 cm. The soft apical nodes were discarded and the leaves at all other nodes trimmed to 30 cm^2, using paper templates. Twenty single-node cuttings were collected, five from each shoot The cutting base was treated with 10 μl indole-3-butyric acid at a concentration of 1.6% before setting the cuttings in sand in non-mist propagators; the cuttings were then assigned randomly to one of two propagators with or without shade. The shade treatment was produced by covering one of the propagators with a single layer of black plastic netting. This was in addition to the layer of netting standardly located two meters above the propagators (see chapter 2). The unshaded propagator was placed under full sunlight, without either of the two layers of netting.

Assessments

Measurements of net photosynthetic rate (P_n), stomatal conductance (g_s) and chlorophyll fluorescence were taken daily in all ten cuttings from each propagator, between 09:00 h and 12:00 h. For assessments of P_n and g_s an infrared gas analyser was used (LCA-3, Analytical Development Co. Ltd., Hoddesdon, U.K.). Measurements of chlorophyll fluorescence were taken using a fluorescence meter (PSM Mark II, Bio Monitor S.C.I. AB, Sweden), set at a light level of 400 μmol photons m^{-2} s^{-1}, a run-time of 25 seconds and a dark-adaptation period of 20 minutes). Within the propagators, a section next to the cuttings was not filled with substrate, so that the gas analyser leaf chamber was placed at a lower level, allowing readings without the removal of the cuttings from the medium.

Measurements of leaf, air and substrate temperatures, relative humidity and irradiance inside the propagators were recorded for the duration of the experiment using a 21X Micrologger (Campbell Scientific Ltd., Loughborough, England) and appropriate sensors. During the course of the experiment, cuttings were finely sprayed twice a day to keep the leaves moist, at 07:00 h and 15:00 h.

Experiment 2 **The effects of two propagation environments and three foliar areas on the rooting of leafy stem cuttings of *Cordia alliodora***

Plant material and experimental design

This experiment was carried out at the CATIE nursery, Turrialba, Costa Rica, using the set of stockplants as described in Chapter 2. In April 1991, the stockplants were cut to a height of 20 cm and three weeks later, the three most vigorous shoots were selected in each stockplant, removing the rest as in experiment 1. By July 1991, the shoots had grown to heights of 30-40 cm, each one providing five to six cuttings. Clones 22, 37 and 38 provided the largest number of cuttings, so these were selected for the present experiment.

The soft apical tip of each shoot was discarded, and six single-node cuttings were taken sequentially down the stem. A total of 864 cuttings were collected, 288 from each clone, their leaf areas were trimmed to 10, 20 or 30 cm^2 using paper templates and then they were treated immediately with indole-3-butyric acid (IBA) at a concentration of 1.6 %. The cuttings were then set to root in sand in non-mist propagators, and were allocated to one of two propagators in node order with or without shade in eight randomized blocks. The shaded and unshaded treatments were as in experiment 1 above. During the course of the experiment, cuttings were finely sprayed twice a day to keep the leaves moist, at 07:00 h and 15:00 h.

Assessments

After two weeks the cutting length and midpoint diameter were measured, and each cutting lifted and assessed for number of roots formed. Similar assessments were carried out for the next seven consecutive weeks.

The environmental conditions inside the propagators were monitored throughout the duration of the experiment using a 21X Micrologger (Campbell Scientific Ltd., Loughborough, England) and associated sensors.

Experiment 3 The effects of two propagation environments and three foliar areas on the physiology of rooting of single-node, leafy stem cuttings of *Cordia alliodora*

Since the infra-red gas analyser and the fluorescence meter were not available during the course of the previous experiment, the experiment was repeated using clones 2, 6 and 37, clone 37 being used in both experiments. In this case, a total of 648 cuttings were collected, 216 from each clone, from stockplants managed and grown under the same conditions as for experiment 2 above. The cuttings were given the same treatments as above and similarly, were set to root in sand in two non-mist propagators, with or without shade, in six randomized blocks. The shaded and unshaded treatments were as described in experiment 1 above.

Assessments

After two weeks the cutting length and midpoint diameter were measured, and each cutting lifted and assessed for number of roots formed. Similar assessments were carried out for the next seven consecutive weeks.

Measurements of chlorophyll fluorescence were taken at weeks 1, 2 and 3 on 36 randomly-selected cuttings, six from each irradiance-foliar area combination, using a fluorescence meter (PSM, Biomonitor S.C.I. AB, Sweden). Measurements of net photosynthetic rate (P_n) and stomatal conductance (g_s) were taken at weeks 2, 3, 4 and 5 on the same cuttings, using an infra-red gas analyser attached to a Parkinson leaf chamber (Analytical Development Co. Ltd., Hoddesdon, U.K.). These measurements were taken between 09:00 h and 12:00 h.

The environmental conditions inside the propagators were monitored throughout the duration of the experiment using a 21X Micrologger (Campbell Scientific Ltd., Loughborough, England) and associated sensors.

Analysis

Given the nature of the treatments, each shade treatment had to be assigned to different propagators, which poses a limitation for the statistical analysis, since the replications of each treatment were not independent, i.e. they were all in the same propagator. This problem, however, is difficult to overcome practically. One possible solution is to replicate using many propagators, but in addition to practical restrictions, this design would bring about other problems, mainly those associated with variation between propagators due to their location within the propagation area. Therefore, the selected design was considered the best compromise between practical restrictions and statistical efficiency. In the analysis of variance, percentages were calculated by environment-clone-area combination, based on 48 cuttings in experiment 1 and 36 cuttings in experiment 2, followed by Fisher's t tests (LSD). Data of percentage rooting used in the analysis were transformed by the formula arcsin $\sqrt{\%}$.

Analyses of deviance for stepwise regression and analyses of variance in Genstat 5 (Payne *et al.* 1987) were utilized to determine the influence of treatment, node position and morphological characteristics of the cuttings on their rooting ability and the influence of treatment and node position on the photosynthetic rate and stomatal conductance of the cuttings. To determine differences between treatment means, analyses of variance were used, followed by Fisher's *t* tests. Similar analyses were carried out on data of chlorophyll fluorescence.

RESULTS

Experiment 1

The propagation microclimate

Large differences in the propagation microclimate were recorded between the two propagators during the period of the experiment (Table 4.1). Irradiance showed maximum values of 638 μmol m^{-2} s^{-1} in the shaded propagator, and of 1639 μmol m^{-2} s^{-1} in the unshaded propagator (Fig. 4.1a). Air, substrate and leaf temperatures also varied with irradiance in both propagators. Air temperature varied between 19.2 °C and 33.6 °C for the shaded propagator and between 17.8 °C and 41.9 °C for the unshaded propagator (Fig. 4.2a). Substrate temperatures were 21.6°C-28.8°C and 21.4 °C-33.1 °C, (Fig. 4.2b), and leaf temperatures were 19.9 °C-39.4 °C and 19.2 °C-48.0 °C, for the shaded and unshaded propagators, respectively (Fig. 4.3a). Relative humidity reached minimum values of 72.9% (mean 88.3%) and 51.4% (mean 92.7%) for the shaded and unshaded propagators, respectively (Fig. 4.1b). Leaf-to-air vapour pressure deficits showed a range of between 0.19 and 5.92 kPa (mean 1.01 kPa) in the shaded propagator, with a larger range (0.35-9.85 kPa) and a larger mean (1.24 kPa) in the unshaded propagator (Fig. 4.3b).

When values of PAR were correlated with VPD, strong positive correlations were found in both propagators (Fig. 4.4a,b).

Table 4.1 The propagator microclimate in the seven days after insertion of *Cordia alliodora* cuttings in non-mist propagators with or without shade.

	Shaded propagator		Non-shaded propagator	
	Mean	Range	Mean	Range
Relative humidity (%)	92.0	72.9-100	87.5	51.4-100
Air temperature (°C)	24.5	19.2-33.6	26.5	17.8-41.9
Substrate temperature (°C)	24.7	21.6-28.8	26.5	21.4-33.1
PAR (μmol m^{-2} s^{-1})	53	0-638	159	0-1638
Leaf temperature (°C)	26.2	19.9-39.4	27.3	19.2-48.0
VPD (kPa)	1.01	0.19-5.92	1.24	0.35-9.85

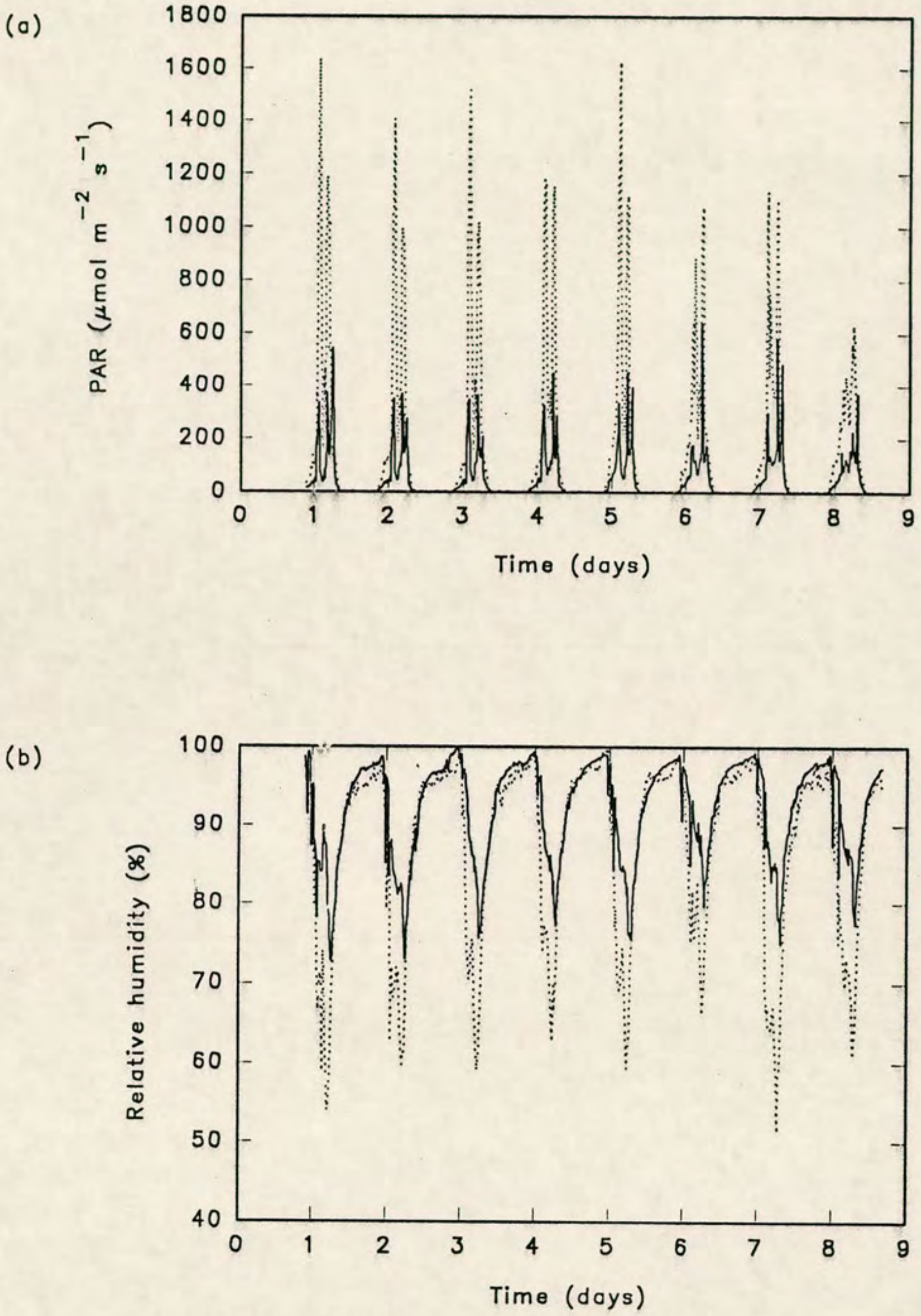


Fig. 4.1a,b Experiment 1. Variations over an eight-day period in (a) photosynthetic active radiation (PAR) and (b) relative humidity inside non-mist propagators with shade (solid line) or without shade (dotted line).

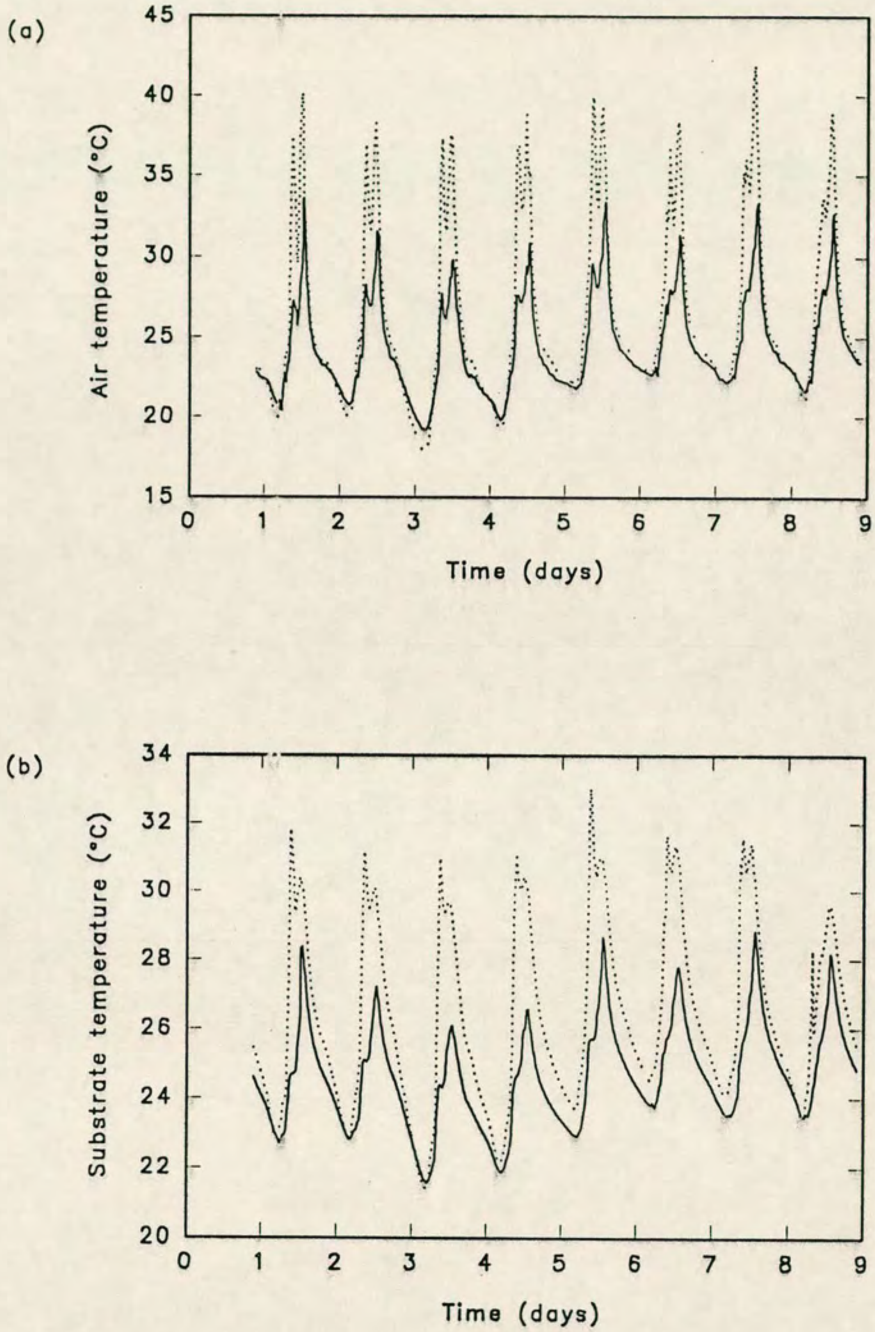


Fig. 4.2a,b Experiment 1. Variations over an eight-day period in **(a)** air temperature and **(b)** substrate temperature inside non-mist propagators with shade (solid line) or without shade (dotted line).

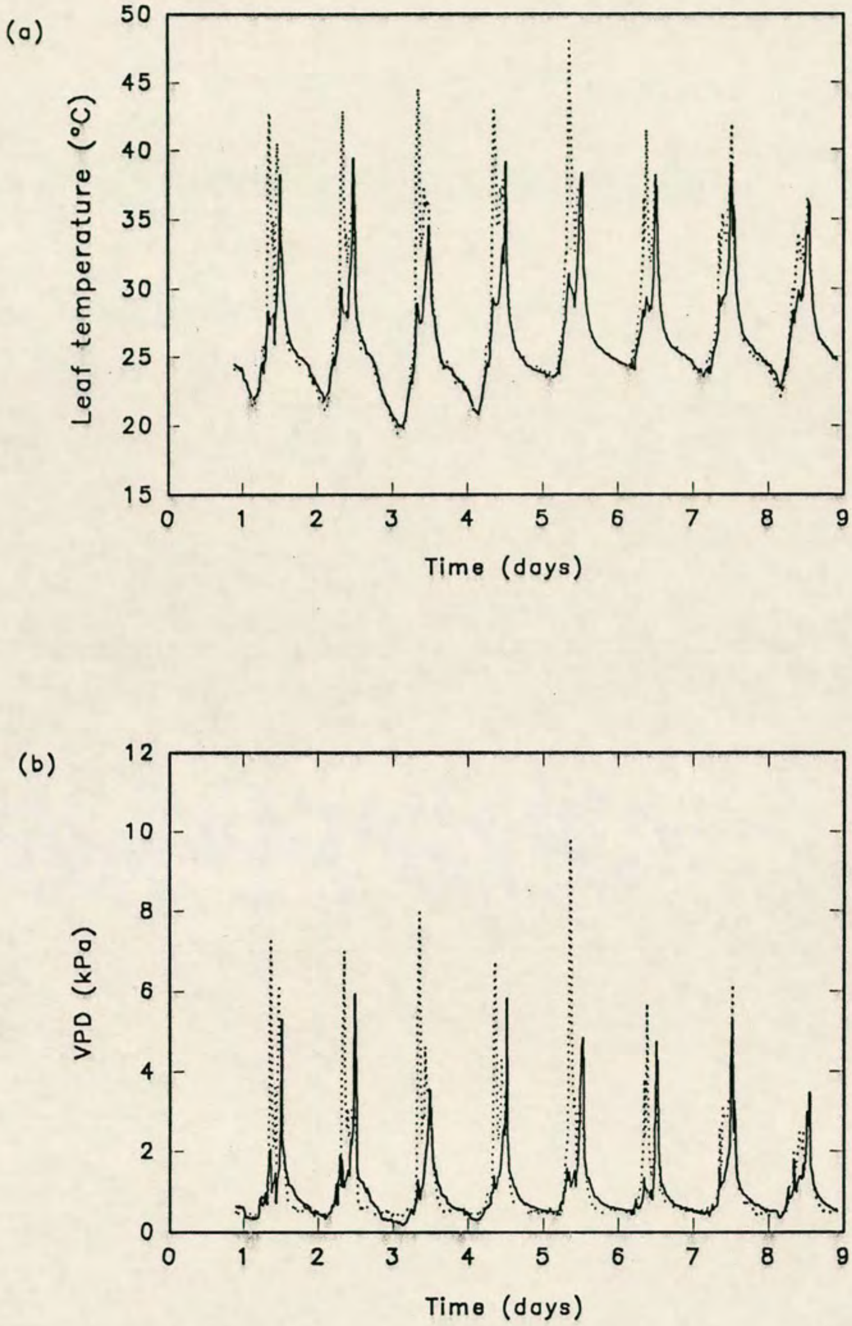


Fig. 4.3a,b Experiment 1. Variations over an eight-day period in **(a)** leaf temperature and **(b)** leaf-to-air vapour pressure deficit (VPD) of single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators with shade (solid line) or without shade (dotted line).

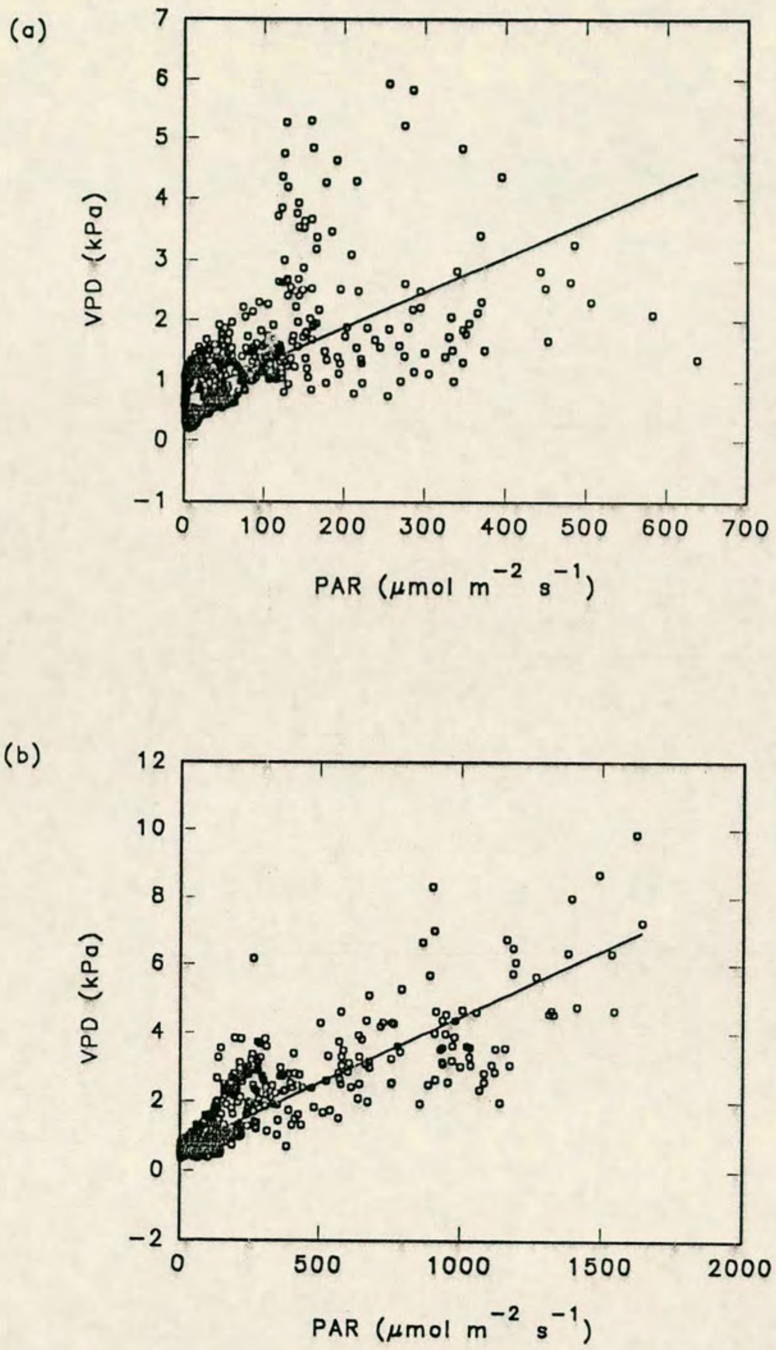


Fig. 4.4a,b Experiment 1. The relationship between leaf-to-air vapour pressure deficit (VPD) and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators **(a)** with shade ($y = 0.722 + 0.006 x$; $r^2 = 0.64$) and **(b)** without shade ($y = 0.628 + 0.004 x$; $r^2 = 0.88$). cf Figs. 4.8 and 4.14 for results of Exps. 2 and 3 respectively.

Net photosynthetic rate (P_n) and stomatal conductance (g_s)

Photosynthetic rates of 2.21-4.96 μmol CO_2 m^{-2} s^{-1} and 0.47-2.54 μmol CO_2 m^{-2} s^{-1} were recorded in the cuttings in the shaded and unshaded propagators respectively. Values were generally higher in the shaded propagator, except at day 5 when P_n was similar in both propagators (Fig. 4.5a). Stomatal conductance varied little from day to day in the unshaded propagator, with values of between 87 mmol H_2O m^{-2} s^{-1} and 139 mmol H_2O m^{-2} s^{-1}. Higher values of g_s were recorded in the shaded propagator (275-636 mmol H_2O m^{-2} s^{-1}), following a similar pattern to that of P_n (Fig. 4.5b).

When values of PAR were correlated with P_n, clear differences were found between both propagators. A strong positive correlation was found in the shaded propagator, while a negative correlation was found in the unshaded propagator (Fig. 4.6).

Chlorophyll fluorescence

The chlorophyll fluorescence ratio (*Fv/Fm*) of the cuttings was generally higher in the shaded propagator, and varied little from day to day (0.78-0.81). In the unshaded propagator, *Fv/Fm* decreased to the lowest values recorded (0.72) two days after insertion of the cuttings in the propagator, but the values increased by day 5 and remained high until the end of the experiment, when there were no significant differences in *Fv/Fm* between both propagators (Fig. 4.7).

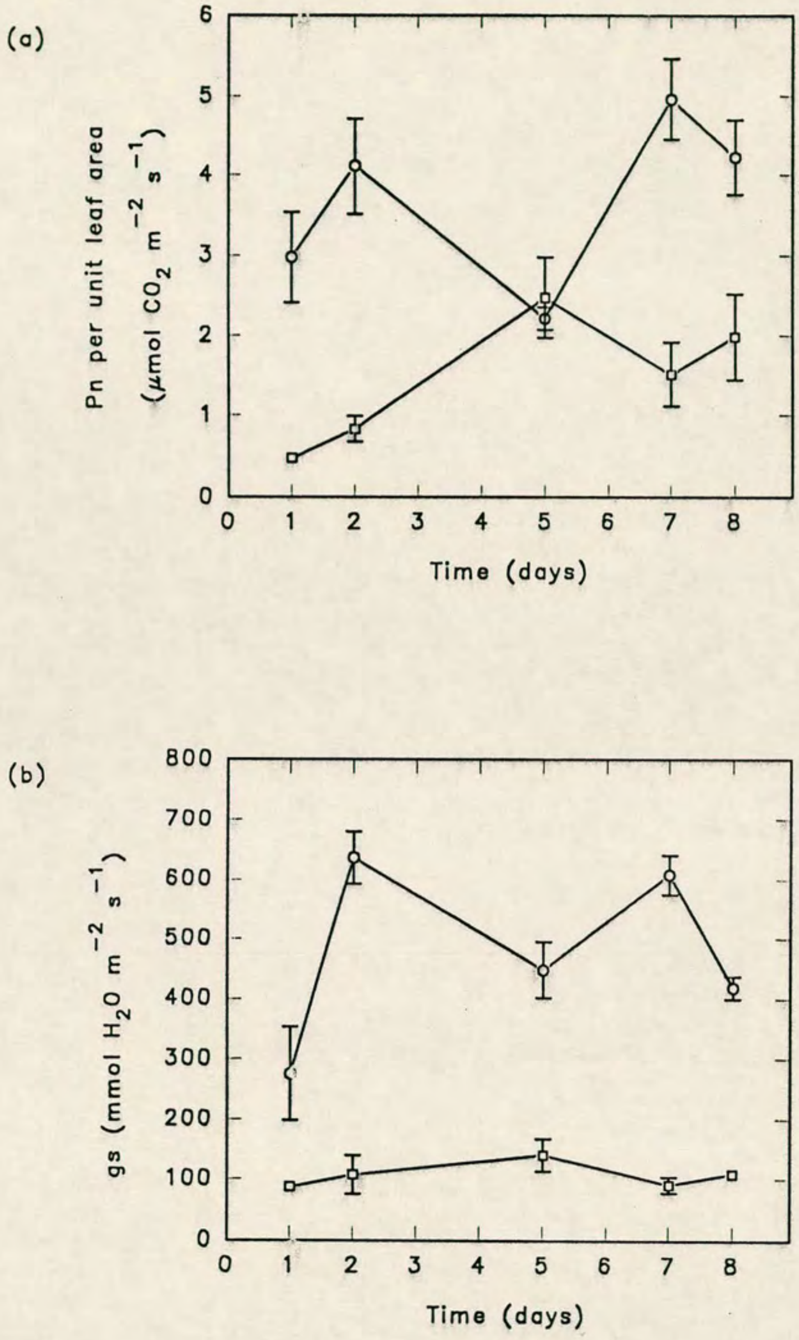


Fig. 4.5a,b Experiment 1. The effect of two propagation environments on (a) the net photosynthetic rate (P_n) and (b) the stomatal conductance (g_s) of single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators with shade (circles) or without shade (squares). Vertical bars = standard error of the mean.

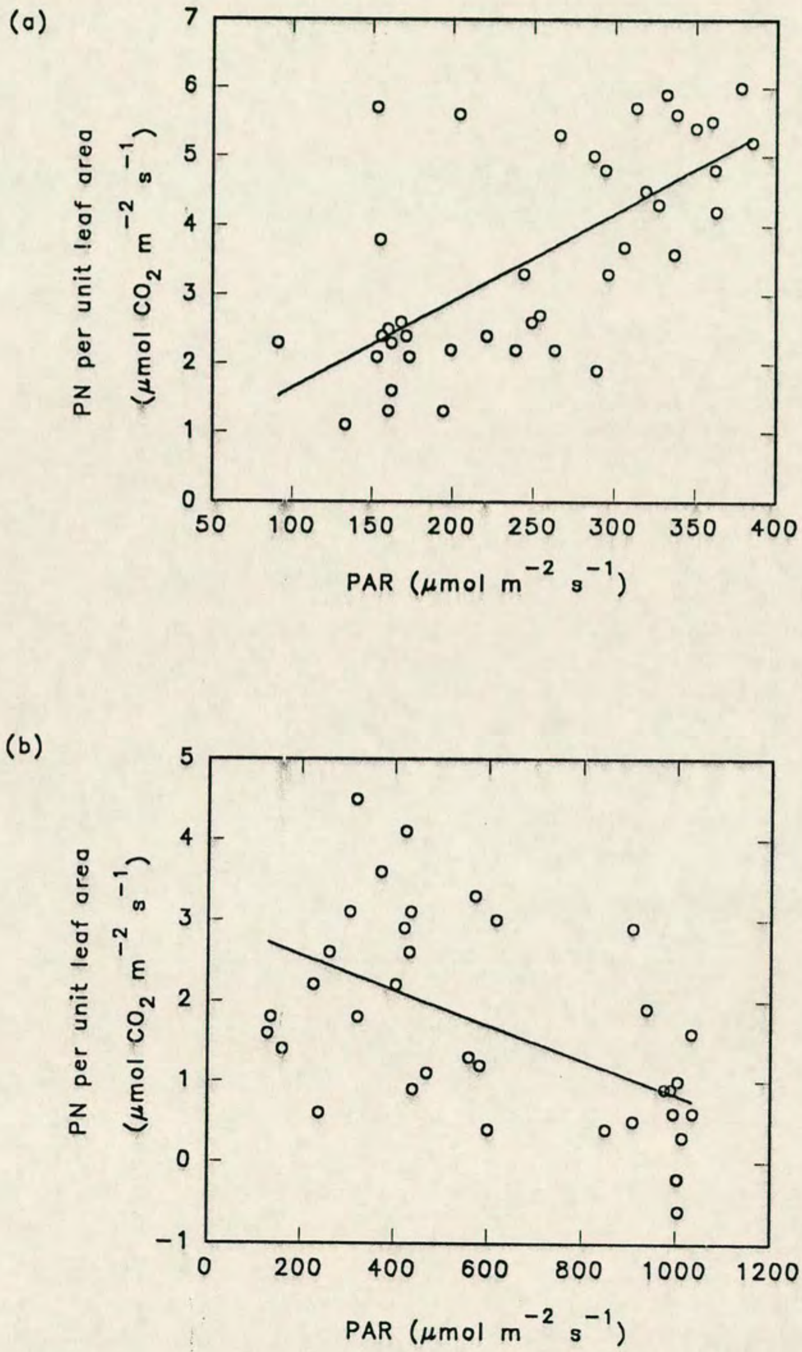


Fig. 4.6a,b Experiment 1. The relationship between net photosynthesis per unit leaf area and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* during the first eight days in non-mist propagators **(a)** with shade ($y = 0.3814 + 0.0127\ x$; $r^2 = 0.68$) and **(b)** without shade ($y = 2.9988 - 0.0002\ x$; $r^2 = 0.54$). cf Fig. 4.21 for results of Exp. 3.

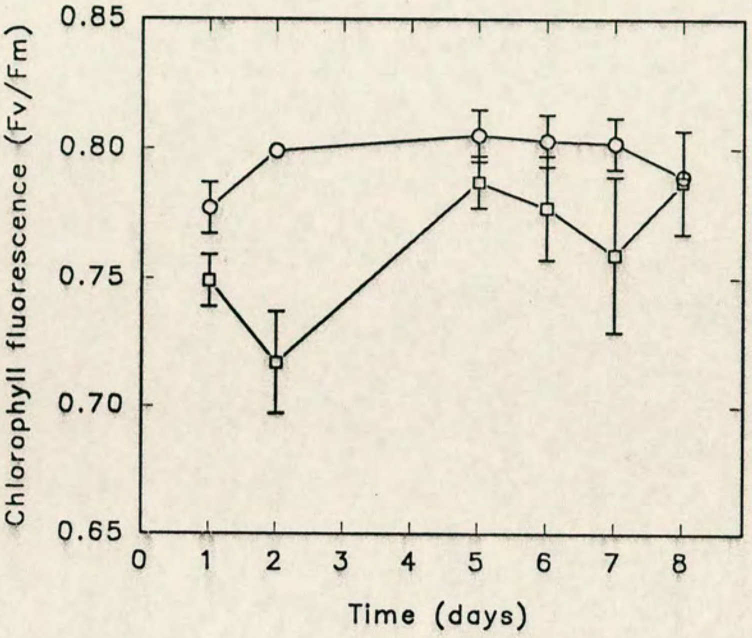


Fig. 4.7 Experiment 1. The effect of two propagation environments on the chlorophyll fluorescence ratio (*Fv/Fm*) of single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators with shade (circles) or without shade (squares).

Experiment 2

The propagation microclimate

The use of shade in one of the propagators produced clear differences between the two propagation environments (PE), with large reductions in irradiance, VPD and air, foliar and substrate temperatures, and an increase in relative humidity in the shaded propagator compared to the unshaded propagator (Table 4.2). Strong positive correlations were found between PAR and VPD in both propagators (Fig. 4.8a,b).

Table 4.2 The propagator microclimate after the insertion of *Cordia alliodora* cuttings in non-mist propagators with or without shade.

	Shaded propagator		Non-shaded propagator	
	Mean	Range	Mean	Range
Relative humidity (%)	98.9	96.0-100	94.7	71.0-100
Air temperature (°C)	21.9	19.9-26.3	24.3	20.4-37.0
Substrate temperature (°C)	22.3	21.1-23.9	23.9	20.8-34.2
PAR (μmol m^{-2} s^{-1})	6	0-88	92	0-1278
Leaf temperature (°C)	23.5	21.2-27.9	25.1	21.2-41.0
VPD (kPa)	0.49	0.30-0.95	0.60	0.18-4.91

Rooting ability

Rooting percentage was significantly ($p < 0.001$) affected by the interaction between PE and leaf area, but not for these variables independently (Table A4). In the shaded propagator rooting was significantly lower for the 10 cm^2 treatment (58.3%), while cuttings with leaf areas of 20 cm^2 and 30 cm^2 rooted equally well (77.8% and 79.2%, respectively). In the unshaded propagator, on the other hand, the highest rooting percentage (78.5%) was obtained with the 10 cm^2 treatment, significantly different from the 20 cm^2 treatment (68.1%) (Fig. 4.9a). There were less variation between treatments for mean number of roots per rooted cutting, which varied from 3.3 to 4.1 (Fig. 4.9b).

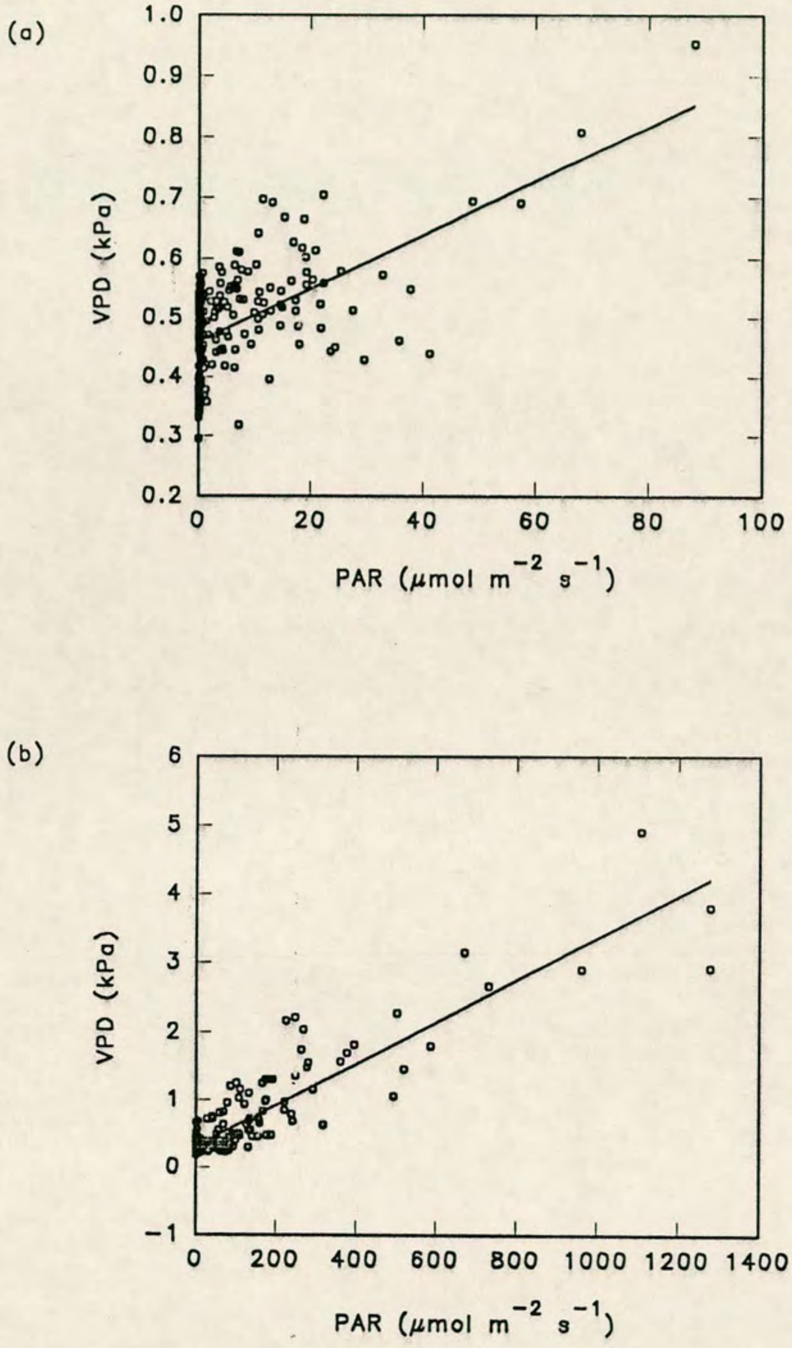


Fig. 4.8a,b Experiment 2. The relationship between leaf-to-air vapour pressure deficit (VPD) and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators **(a)** with shade ($y = 0.460 + 0.004\,x$; $r^2 = 0.59$) and **(b)** without shade ($y = 0.323 + 0.003\,x$; $r^2 = 0.91$). cf Figs. 4.4 and 4.14 for results of Exps. 1 and 3 respectively.

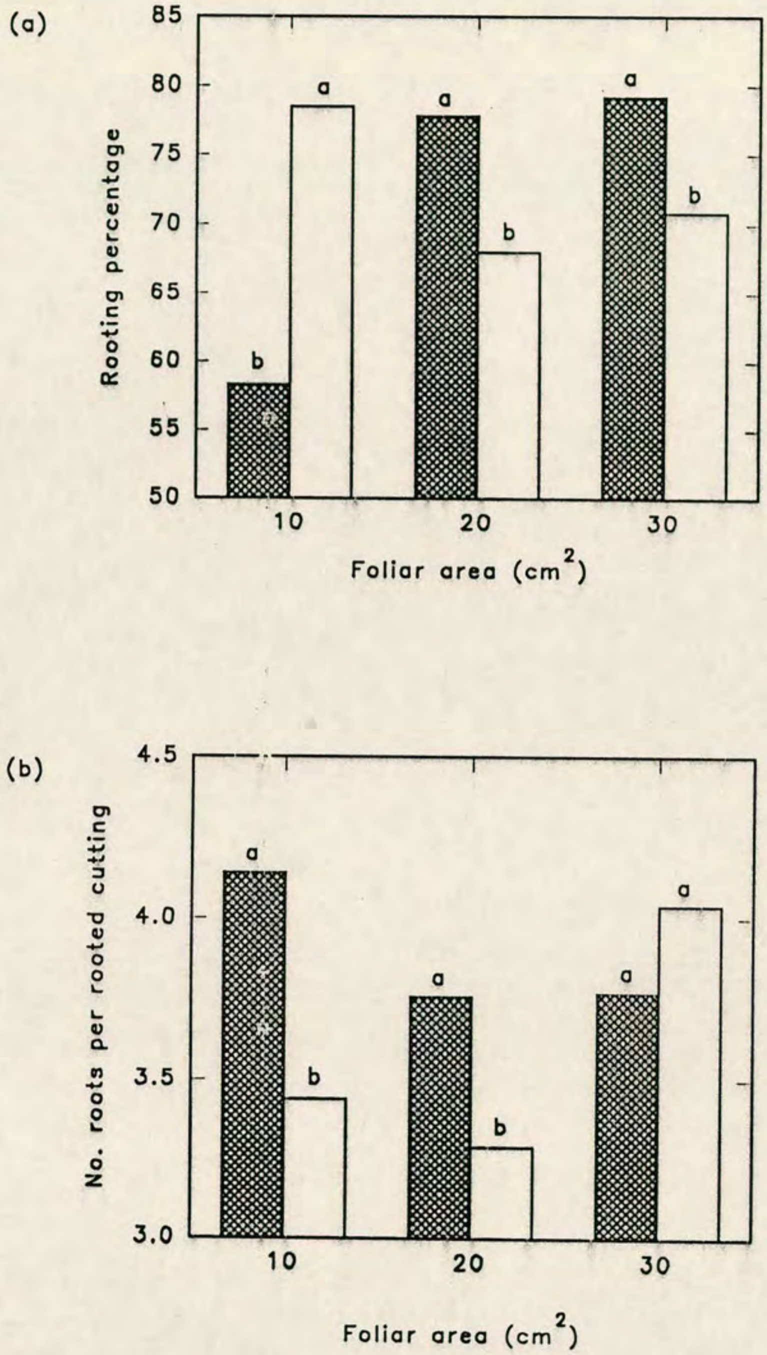


Fig. 4.9a,b Experiment 2. The influence of propagation environment and cutting leaf area (10 cm^2, 20 cm^2, 30 cm^2) on **(a)** subsequent rooting percentage and **(b)** number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* after seven weeks in non-mist propagators with shade (shaded bars) or without shade (open bars). N = 144, means with the same letter are not significantly different ($t_{0.05}$). cf Fig. 4.15 for results of Exp. 3.

Clones also displayed highly significant ($p < 0.001$) differences both in rooting percentage (Table A4) and in number of roots per rooted cutting (Table A5) at week 7. Clone 22 displayed the highest mean rooting percentage (91.7%), followed by clone 38 (77.8%) and clone 37 (46.9%) (Fig. 4.10a). The latter also showed a significantly lower number of roots per rooted cutting (1.8), compared to 4.2 for clone 22 and 4.5 for clone 38 (Fig. 4.10b).

There were highly significant ($p < 0.001$) differences both in rooting percentage (Table A4) and in number of roots per rooted cutting (Table A5) between node positions, with the apical node showing the lowest values for both variables. The number of roots per rooted cutting showed a progressive increase with successive node positions down the stem (Fig. 4.11a,b)

To account for the relative importance of these factors and the morphological characteristics of the cuttings on their rooting ability, the results were analysed by stepwise regression. Rooting was found to be significantly affected by clone, block, node position and cutting diameter, but was not significantly affected by PE and the cutting length (Table A6). Cuttings showed a progressive increase in diameter from the apical to the basal nodes (Fig. 4.12a), while cutting length decreased from node 1 to node 2, to increase progressively again up to node 6 (Fig. 4.12b). When these values of cutting diameter and cutting length were correlated with rooting and mean number of roots per rooted cutting, a strong positive correlation ($r^2 = 0.979$; $p < 0.05$) was found between cutting diameter and mean number of roots per rooted cutting (Fig. 4.13).

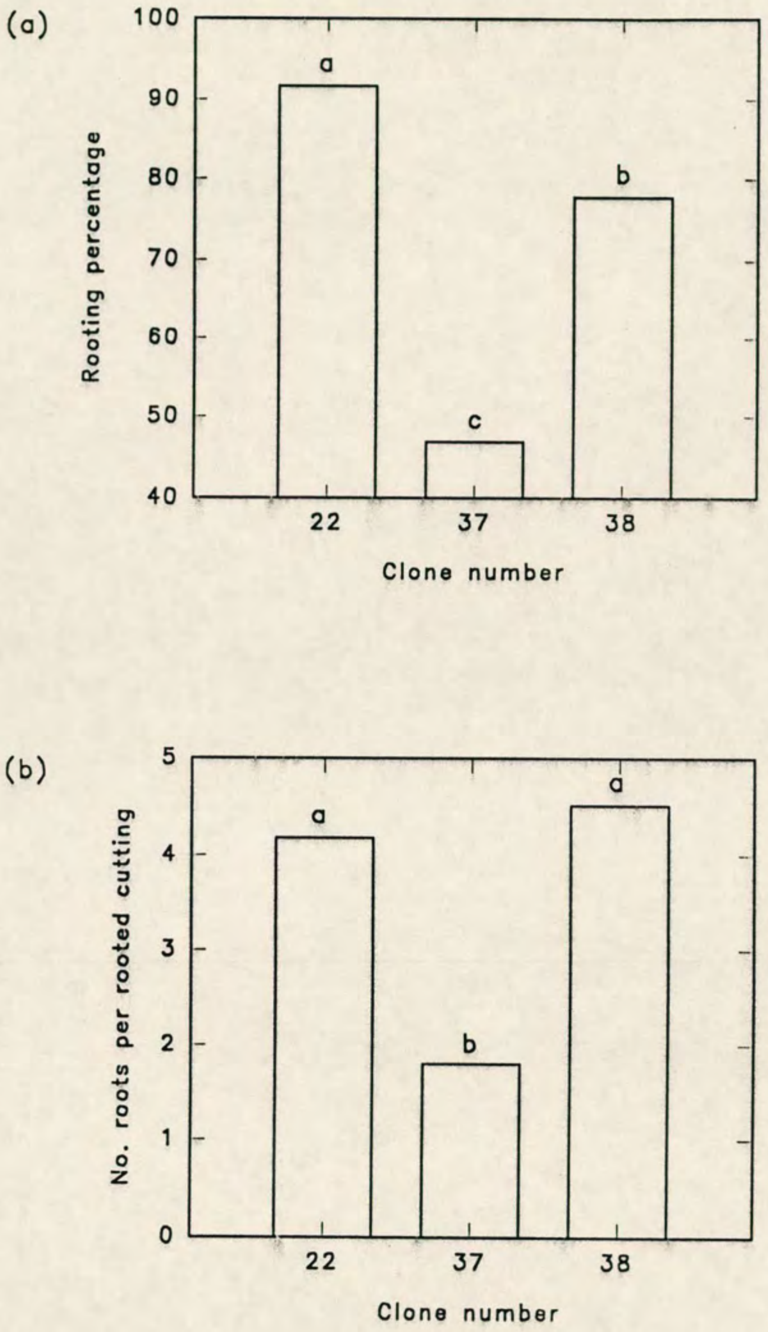


Fig. 4.10a,b Experiment 2. Clonal variation in (a) the rooting percentage and (b) the number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* with leaf areas of 10 cm^2, 20 cm^2 or 30 cm^2, after seven weeks in non-mist propagators with or without shade. N = 144. Means grouped by the same letter are not significantly different ($t_{0.05}$)

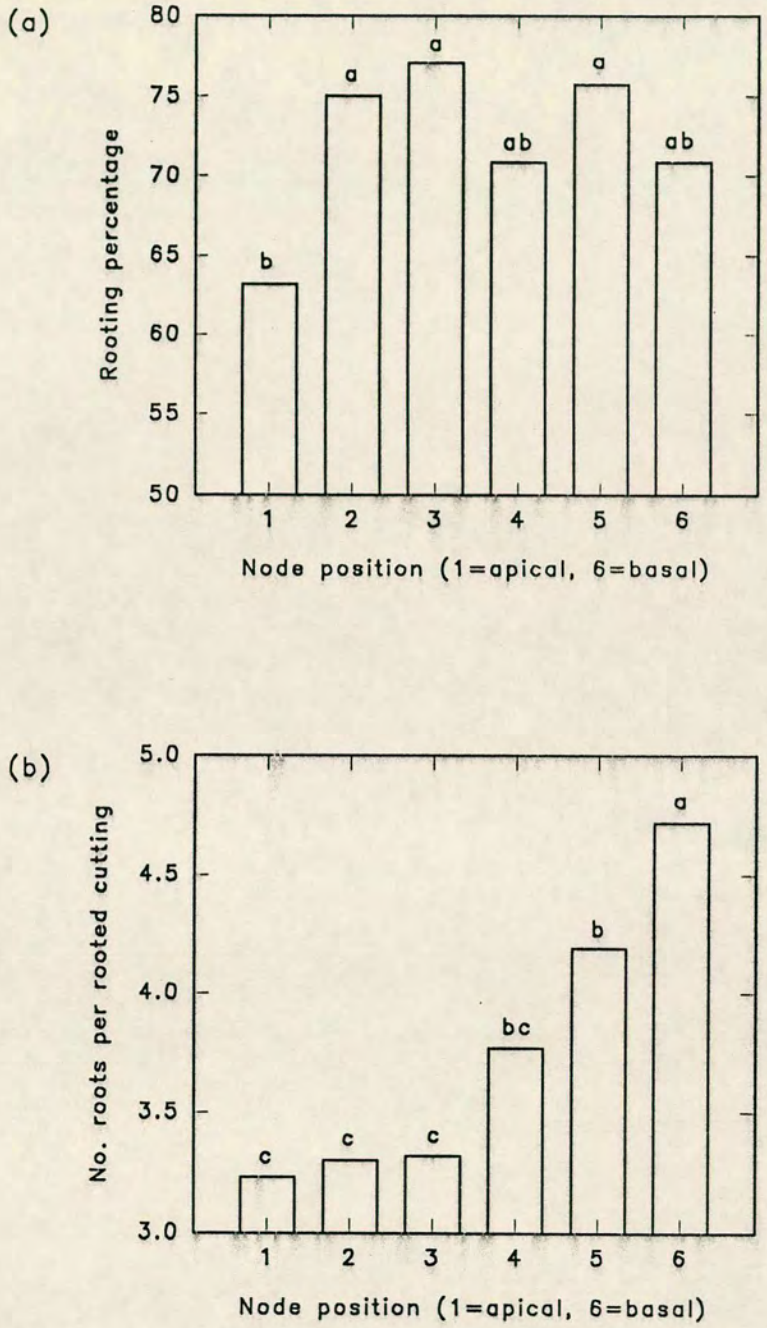


Fig. 4.11a,b Experiment 2. The effect of node position on (a) the rooting percentage and (b) the number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* with three leaf areas (10 cm^2, 20 cm^2 and 30 cm^2) after seven weeks in non-mist propagators with or without shade. N = 144; means grouped by the same letter are not significantly different ($t_{0.05}$). cf Fig. 4.17 for results of Exp. 3.

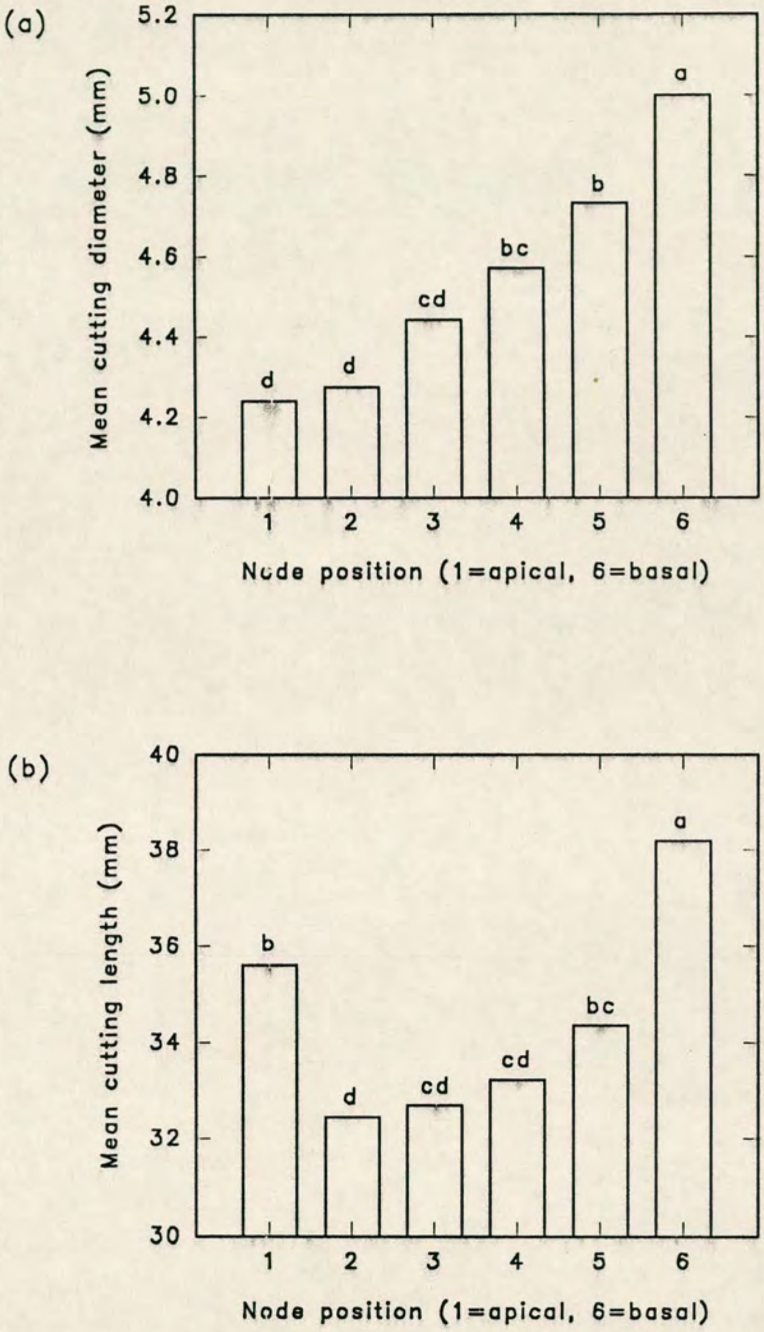


Fig. 4.12a,b Experiment 2. The effect of node position on (a) mean cutting diameter and (b) mean cutting length of single-node, leafy stem cuttings of *Cordia alliodora*, set to root in non-mist propagators with or without shade. N = 144; means grouped by the same letter are not significantly different ($t_{0.05}$). cf Fig 4.18 for results of Exp. 3.

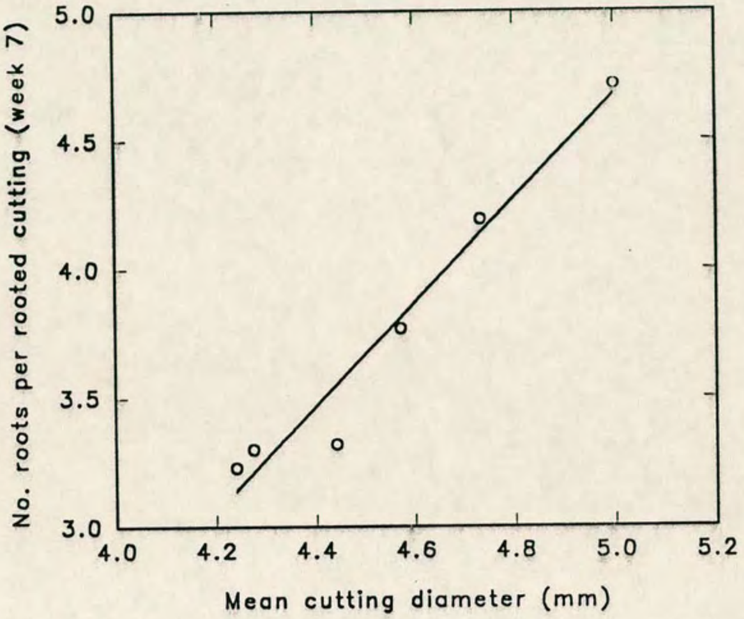


Fig. 4.13 Experiment 2. The relationship between mean cutting diameter and rooting percentage of single-node, leafy stem cuttings of *Cordia alliodora* with three leaf areas, after seven weeks in non-mist propagators with or without shade. Each value represents the mean of 144 cuttings ($y = -5.435 + 2.023 x$; $r^2 = 0.98$). cf Fig. 4.19 for results of Exp. 3.

Experiment 3

The propagation microclimate

As in the first and second experiments, large differences were recorded between propagators for irradiance, leaf, air and foliar temperatures, relative humidity and VPD (Table 4.3). However, higher values of irradiance, temperatures and VPD, and lower values of relative humidity were recorded in both propagators with respect to the second experiment. When values of PAR were correlated with VPD, strong positive correlations were found in both propagators (Fig. 4.14a,b).

Table 4.3 The propagator microclimate after the insertion of *Cordia alliodora* cuttings in non-mist propagators with or without shade.

	Shaded propagator		Non-shaded propagator	
	Mean	Range	Mean	Range
Relative humidity (%)	99.9	92.4-100	94.8	60.0-100
Air temperature (°C)	23.4	19.0-31.8	25.3	18.5-43.4
Substrate temperature (°C)	23.4	20.4-29.0	25.6	20.9-38.9
PAR (μmol m^{-2} s^{-1})	24	0-339	106	0-1460
Leaf temperature (°C)	24.9	20.2-32.1	26.7	19.6-47.5
VPD (kPa)	0.43	0.01-1.47	0.91	0.14-8.36

Rooting ability

Rooting percentage was significantly higher ($p < 0.001$) in the shaded propagator (Table A7), with no differences between the leaf area treatments (75.0%, 78.7% and 74.1% for areas of 10 cm^2, 20 cm^2 and 30 cm^2, respectively). In the unshaded propagator, rooting percentage decreased with an increase in leaf area, from 71.3% to 66.7% and 55.6% (Fig. 4.15a). In both propagation environments, the number of roots per rooted cutting increased with an increase in leaf area (Fig. 4.15b). Significantly fewer roots per rooted cutting (4.0 *vs.* 5.6) were produced in the shaded propagator averaging over all leaf area treatments ($p < 0.001$; Table A8).

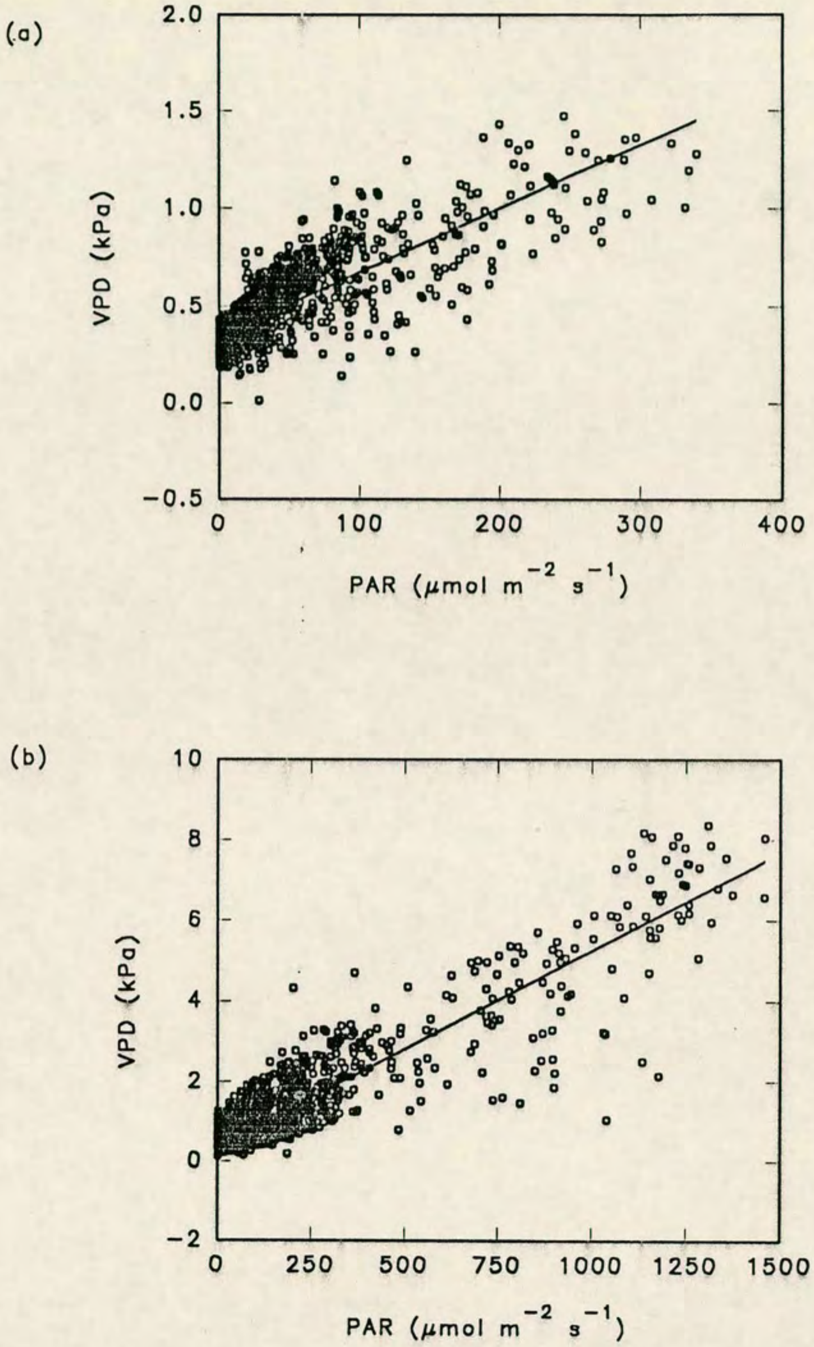


Fig. 4.14a,b Experiment 3. The relationship between leaf-to-air vapour pressure deficit (VPD) and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators **(a)** with shade ($y = 0.356 + 0.003 x$; $r^2 = 0.84$) and **(b)** without shade ($y = 0.396 + 0.005 x$; $r^2 = 0.93$). cf Figs. 4.4 and 4.8 for results of Exps. 1 and 2 respectively.

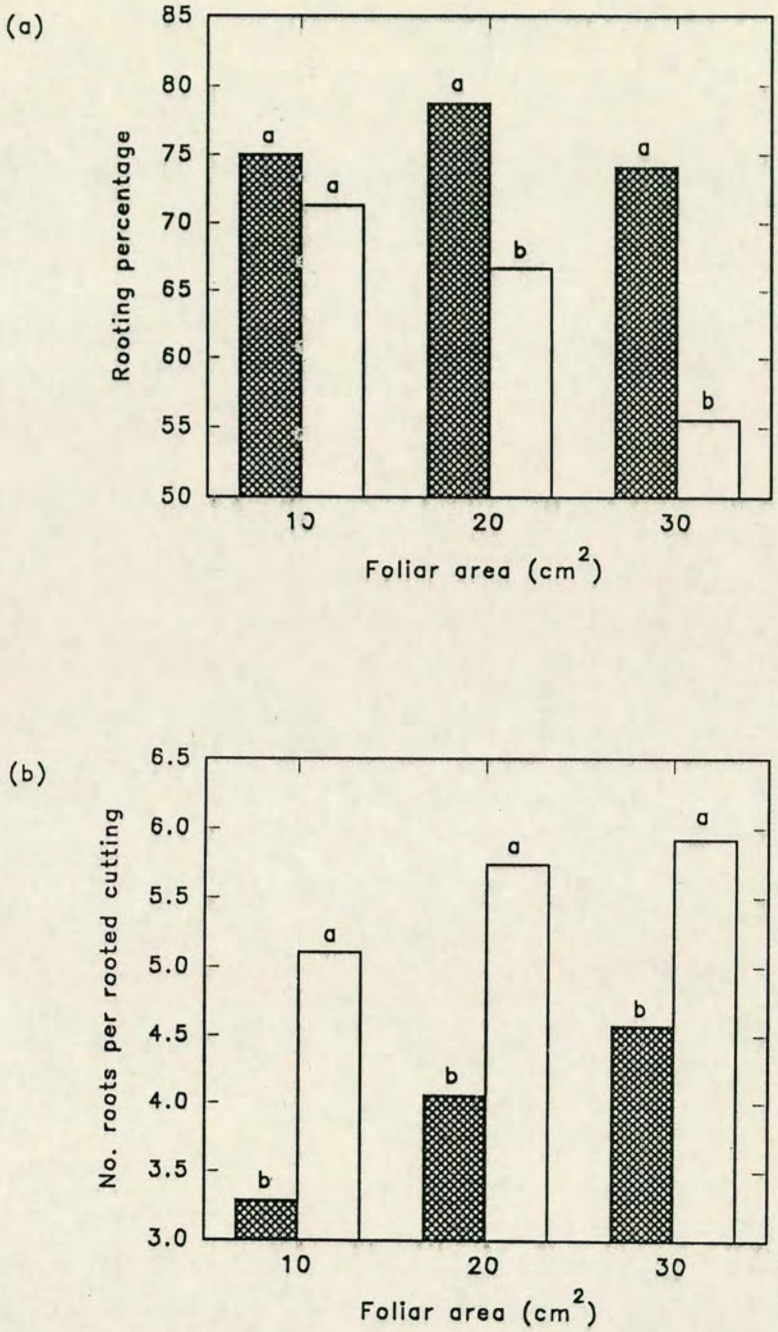


Fig. 4.15a,b Experiment 3. The influence of propagation environment and cutting leaf area (10 cm^2, 20 cm^2, 30 cm^2) on (a) subsequent rooting percentage and (b) number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* after six weeks in non-mist propagators with shade (shaded bars) or without shade (open bars). N = 108; means grouped by the same letter are not significantly different ($t_{0.05}$). cf Fig. 4.9 for results of Exp. 2.

Clones also showed highly significant ($p < 0.001$) differences both in rooting percentage and mean number of roots per rooted cutting, with a similar ranking (clone 6 > clone 2 > clone 37) for both variables. At the end of seven weeks rooting percentage was 83.8%, 71.3% and 55.6% (Fig. 4.16a), and number of roots per rooted cutting 5.5, 5.2 and 3.0 for clones 6, 2 and 37 respectively. (Fig. 4.16b).

The most apical node showed the lowest values for both rooting percentage (Fig. 4.17a) and number of roots per rooted cutting (Fig. 4.17b), with no significant differences between the other node positions. However, as in experiment 2 above, there was an increase in number of roots per rooted cutting from the apical to the basal nodes.

When the results were analysed by stepwise regression, rooting percentage was found to be highly dependent on PE, clone, the leaf area and the node position within the stem, but was not affected by the mean cutting diameter (Table A9). The number of roots per rooted cutting, on the other hand, was strongly affected by cutting diameter. Cutting diameter showed a progressive increase from the apical to the basal nodes (Fig. 4.18), and when these values were correlated with mean number of roots per rooted cutting, a strong positive correlation ($r^2 = 0.993$; $p < 0.05$) was found (Fig. 4.19).

Net photosynthetic rate

Mean photosynthetic rate (P_n) per unit leaf area was significantly higher ($p < 0.001$) in the unshaded propagator (Table A10). Within each environment, P_n per unit leaf area decreased with an increase in leaf area, from 1.77 μmol CO_2 m^{-2} s^{-1} to 1.56 μmol CO_2 m^{-2} s^{-1} and 1.27 μmol CO_2 m^{-2} s^{-1} in the shaded propagator and from 3.56 μmol CO_2 m^{-2} s^{-1} to 2.94 μmol CO_2 m^{-2} s^{-1} and 2.01 μmol CO_2 m^{-2} s^{-1} in the unshaded propagator (Fig. 4.20a). When the results were expressed as μmol CO_2 $leaf^{-1}$, mean photosynthetic rate in both environments increased with successive increases in leaf area, although the difference between the 20 cm^2 and 30 cm^2 treatments was non significant (Fig. 4.20b). When values of PAR were correlated with P_n, a strong positive relationship was found between these variables in the shaded propagator, while a negative relationship was found in the unshaded propagator (Fig. 4.21).

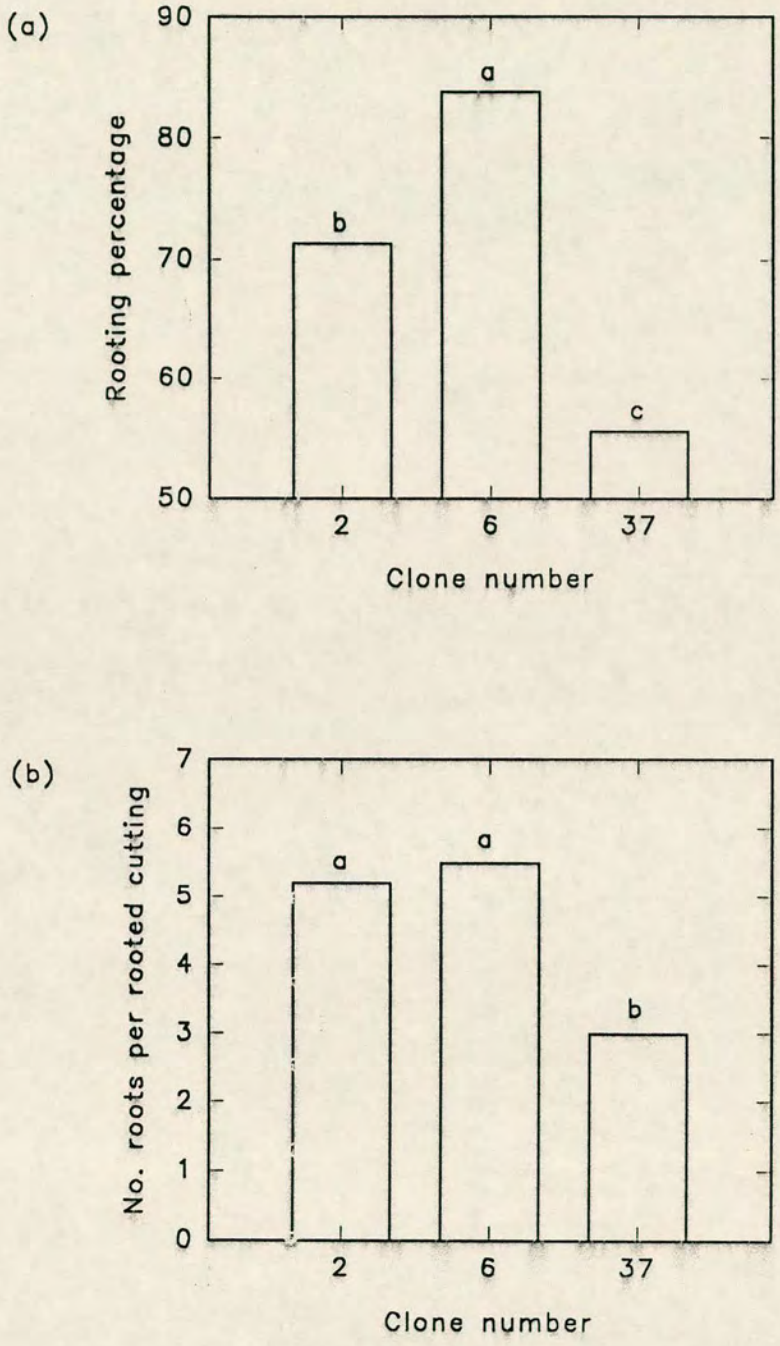


Fig. 4.16a,b Experiment 3. Clonal variation in **(a)** the rooting percentage and **(b)** the number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* with three leaf areas after seven weeks in non-mist propagators with shade or without shade. N = 108; means grouped by the same letter are not significantly different ($t_{0.05}$).

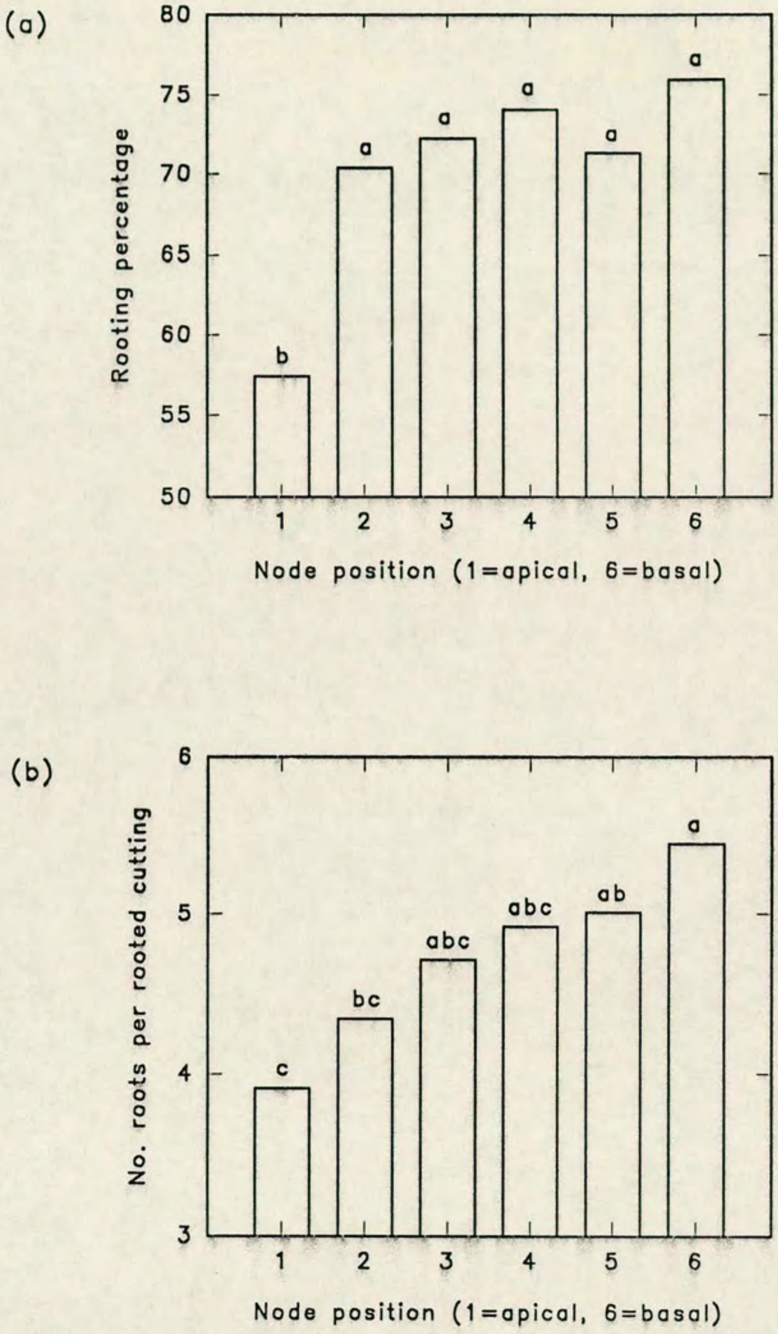


Fig. 4.17a,b Experiment 3. The effect of node position on (a) the rooting percentage and (b) the number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* with three leaf areas after seven weeks in non-mist propagators with shade or without shade. N = 108; means grouped by the same letter are not significantly different ($t_{0.05}$). cf Fig 4.11 for results of Exp. 2.

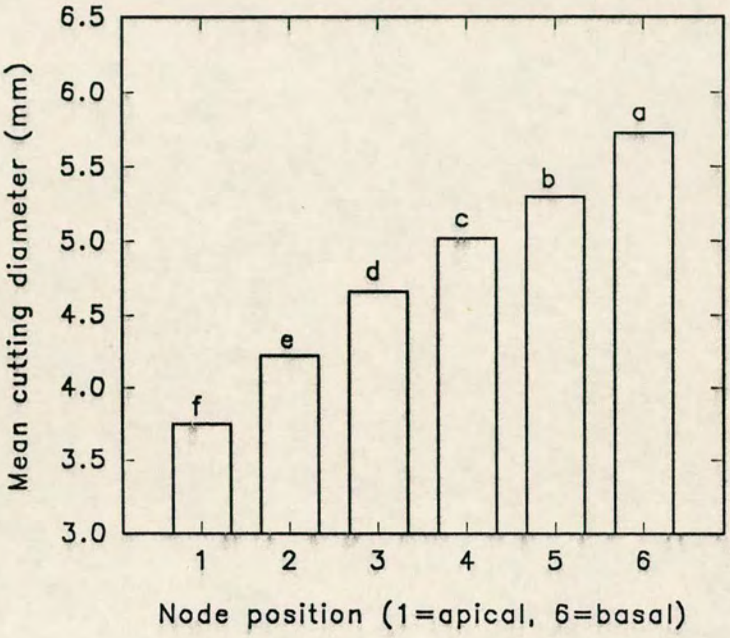


Fig. 4.18 Experiment 3. The effect of node position on the cutting diameter of single-node, leafy stem cuttings of *Cordia alliodora*. N = 108; means grouped by the same letter are not significantly different ($t_{0.05}$). cf Fig 4.12 for results of Exp. 2.

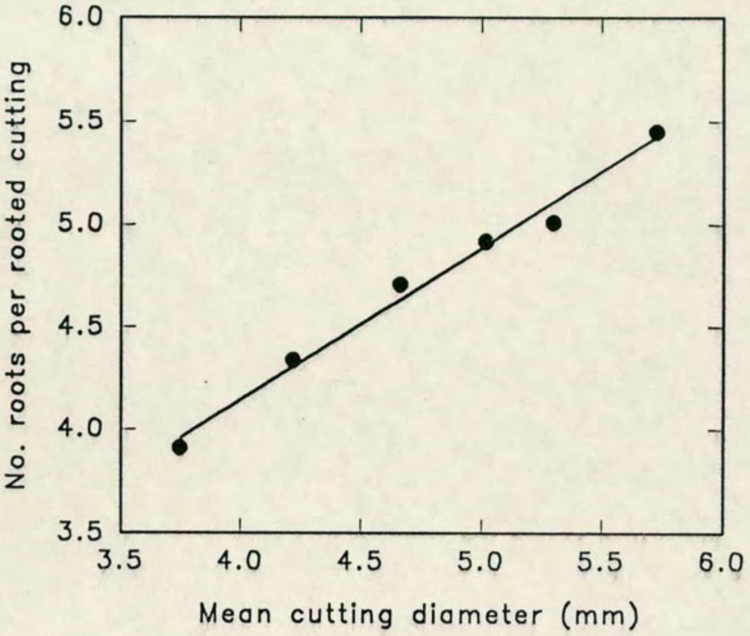


Fig. 4.19 Experiment 3. The relationship between mean cutting diameter and number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* with three leaf areas, after seven weeks in non-mist propagators with or without shade. Each value represents the mean of 108 cuttings ($y = 1.1807 + 0.0741\ x$; $r^2 = 0.99$). cf Fig 4.13 for results of Exp. 2.

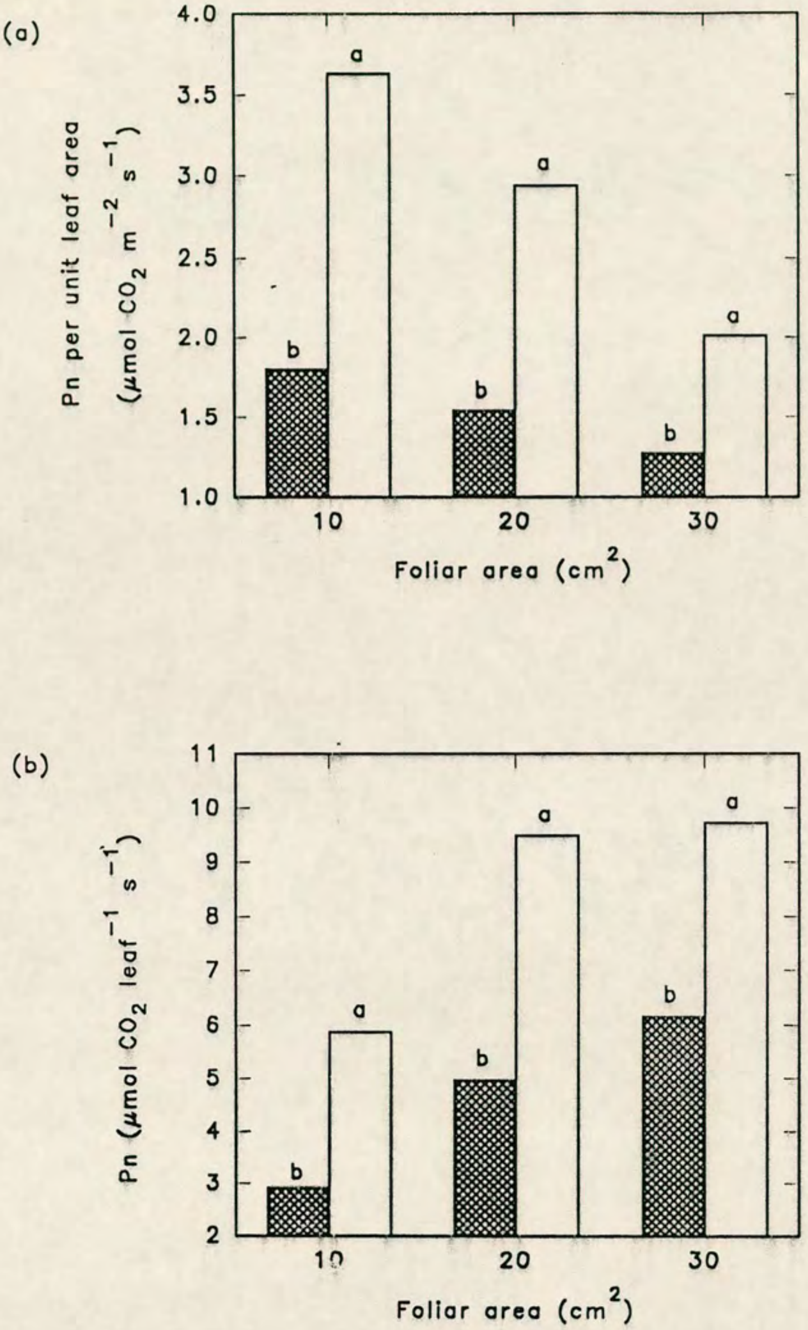


Fig. 4.20a,b Experiment 3. The effect of propagator environment and leaf area treatment (10 cm^2, 20 cm^2 and 30 cm^2) on **(a)** the net photosynthetic rate per unit leaf area and **(b)** the net photosynthetic rate leaf^{-1} of single-node, leafy stem cuttings of *Cordia alliodora*, set to root in non-mist propagators with shade (shade bars) or without shade (open bars). N = 24-26. Values represent the mean of three measurements at weeks 2,3 and 5, those grouped by the same letter are not significantly different.

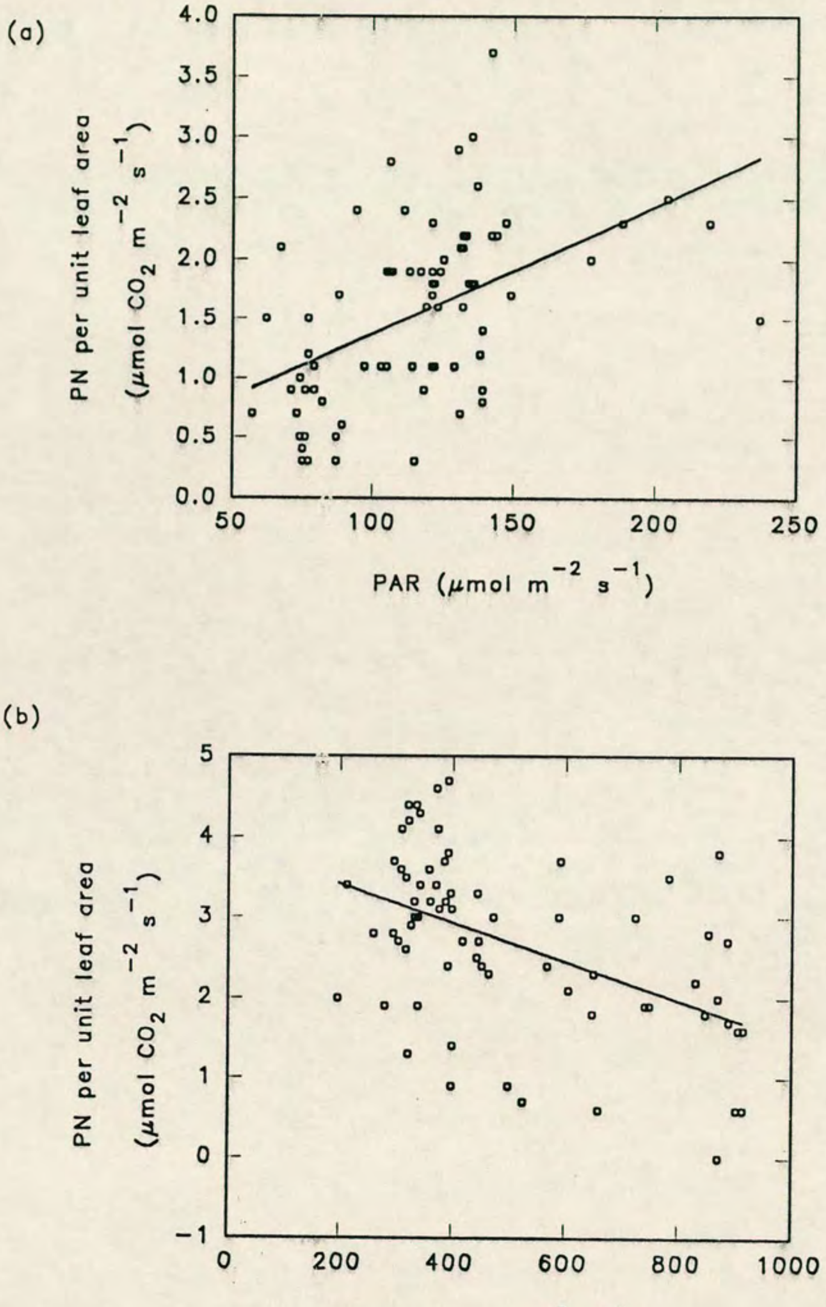


Fig. 4.21a,b Experiment 3. The relationship between net photosynthesis per unit leaf area (P_n) and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* set to root in non-mist propagators **(a)** with shade ($y = 0.3078 + 0.0107\ x$; $r^2 = 0.51$) and **(b)** without shade ($y = 3.8930 - 0.0024\ x$; $r^2 = 0.49$).

No significant differences in g_s were found between treatments, with values between 130 mmol H_2O m^{-2} s^{-1} and 194 mmol H_2O m^{-2} s^{-1} for all treatments.

To determine the relative influence of treatments on the recorded net photosynthetic rates, the results were analysed by stepwise regression and P_n was found to be highly dependent on both PE and leaf area (Table A11).

Chlorophyll fluorescence

There was a highly significant ($p < 0.001$) difference in chlorophyll fluorescence ratios (*Fv/Fm*) between PE, and a highly significant ($p < 0.001$) interaction for PE x leaf area (Table A12). In the shaded propagator, *Fv/Fm* remained roughly constant from week 1 to week 3, with no significant differences between the three leaf area treatments. In the unshaded propagator, the 10 cm^2 treatment showed significantly higher values than the 20 cm^2 and 30 cm^2 treatments, but all the three leaf area treatments showed lower values than those of the shaded propagator. After three weeks there was a marked decrease in *Fv/Fm* in the unshaded propagator for all three leaf area treatments (Fig. 4.22). Mean values over the three weeks for the three leaf areas (10 cm^2, 20 cm^2 and 30 cm^2) were 0.79, 0.79 and 0.76 for the shaded propagator and 0.51, 0.36 and 0.33 for the unshaded propagator. When the results of *Fv/Fm* were correlated with final rooting percentage (week 6), strong positive correlations were found between rooting percentage and mean *Fv/Fm* at weeks 1, 2, 3 (Fig. 4.23a,b,c).

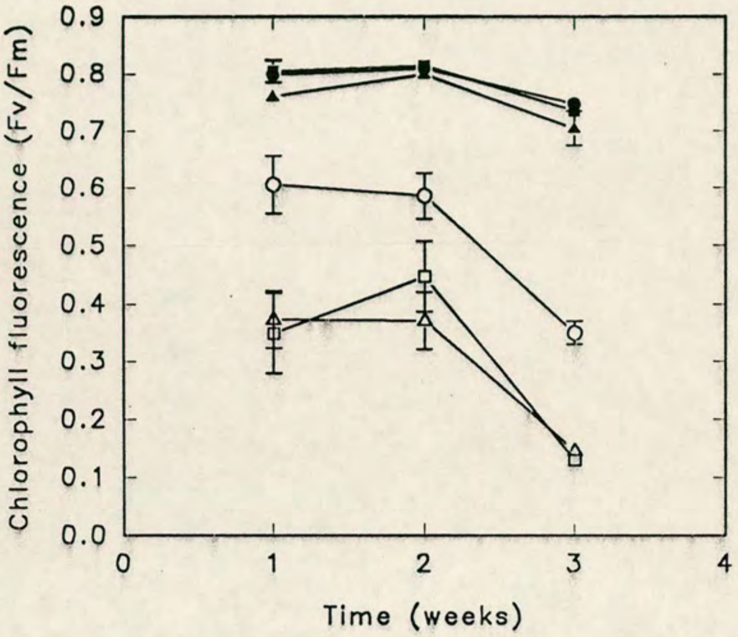


Fig. 4.22 The influence of two propagation environments and leaf area per cutting (10 cm² = circles; 20 cm² = squares; 30 cm² = triangles) on the ratio of the variable fluorescence to maximum fluorescence (*Fv/Fm*) in single-node, leafy stem cuttings of *Cordia alliodora* set to root in non-mist propagators with shade (closed symbols) or without shade (open symbols). N = 6, bars = ± standard error of the means.

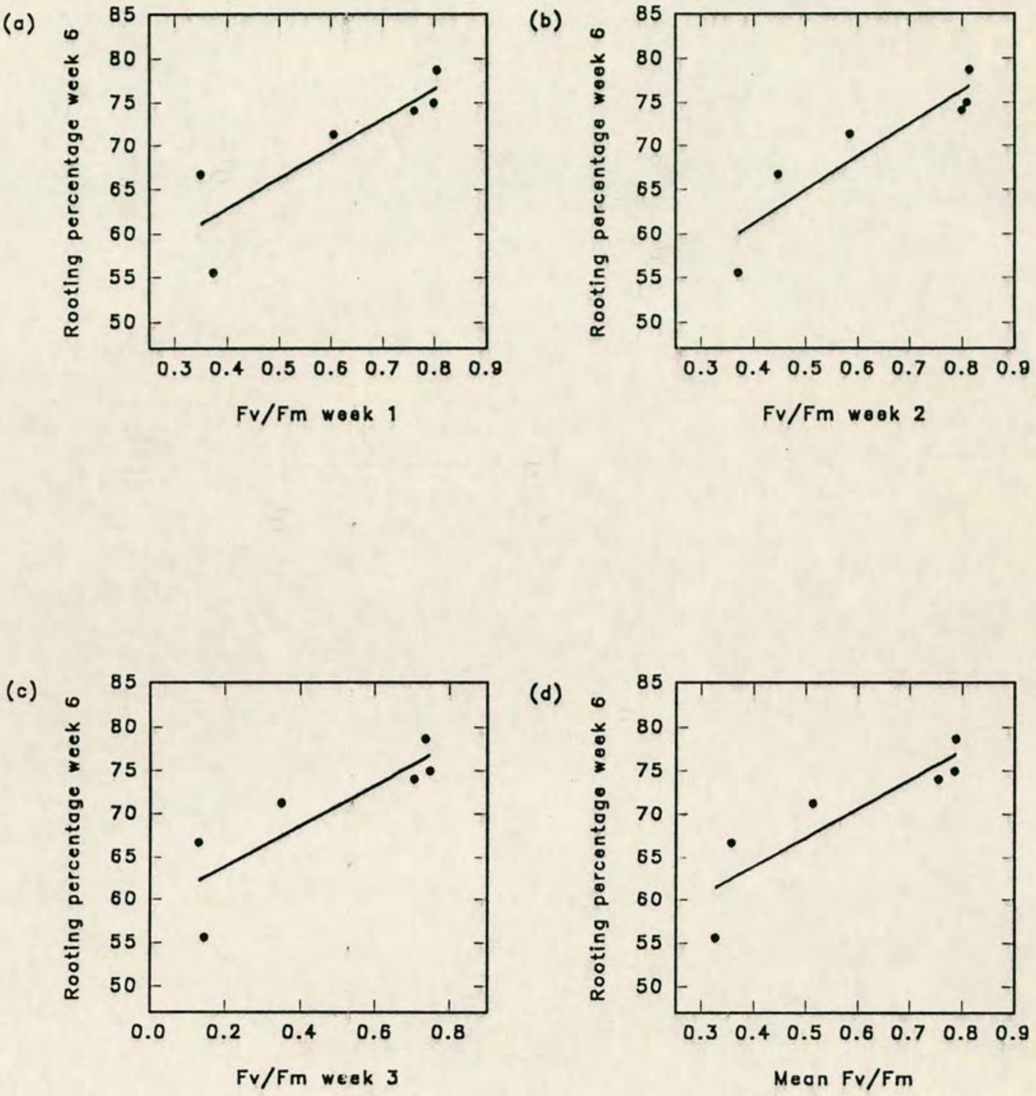


Fig. 4.23a,b,c,d Experiment 3. The relationship between rooting of single-node, leafy stem cuttings of *Cordia alliodora* and the ratio of chlorophyll fluorescence (*Fv/Fm*) at **(a)** week 1 (y = 49.246 + 34.126 x; r^2 = 0.87), **(b)** week 2 (y = 46.080 + 37.888 x; r^2 = 0.92), **(c)** week 3 (y = 59.197 + 23.549 x; r^2 = 0.85) and **(d)** mean *Fv/Fm* (y = 50.565 + 33.469 x; r^2 = 0.88). Cuttings with three leaf areas were set to root in non-mist propagators with or without shade. Each value for rooting is the mean of 108 cuttings.

DISCUSSION

The successful rooting of leafy stem cuttings depends on many environmental and physiological factors, both pre- and post-severance, acting in a complex and interrelated manner (Dick and Dewar 1992; Leakey *et al.* 1992; 1993). Among the pre-severance factors, the position within a stem from which the cutting originates is known to influence the physiology, morphology and subsequent rooting ability of cuttings (Hartmann and Kester 1983; Leakey and Coutts 1989). In *Triplochiton scleroxylon* (Leakey and Mohammed 1985), *Eucalyptus grandis* (Hoad and Leakey 1992) and *Prosopis juliflora* (Wilson *et al.* 1989), cutting stem volume, as determined by its diameter and length, has been considered the most important effect of node position on the rooting ability of cuttings. Based on these results, a hypothesis has been developed that cutting volume determines the capacity of a cutting to store assimilates produced both pre- and post-severance (Leakey *et al.* 1993).

In this study, strong positive correlations were found between cutting diameter and the number of roots produced by the cuttings, but no correlations were found between diameter and rooting percentage. A number of studies have investigated the effects of morphological characteristics of the cuttings, in particular cutting length, on their rooting ability. In *T. scleroxylon*, cutting length was usually more strongly correlated with the rooting percentage of cuttings than with the number of roots per rooted cutting (Leakey and Mohammed 1985). In most studies, however, cutting length has been more commonly correlated with the number of roots produced by the cuttings than with the rooting percentage (Fernquist 1966; Veierskov 1978; Poulsen and Andersen 1980). A relationship between cutting length or cutting diameter with the number of roots produced by the cutting seems to be in agreement with current thinking on the process of adventitious root formation in cuttings. Adventitious root production can be divided into stages; although there is a lack of agreement as to the number and nature of the stages, it is generally accepted that there are at least two stages: root initiation and root growth (Lovell and White 1986). It is not known whether the requirements associated with the creation of root primordia are the same as those associated with root development and growth (Lovell and White 1986). It appears, for instance, that root primordium initiation is hormonally controlled (Moe and Andersen 1988), and not markedly influenced by excesses or deficiencies of particular mineral nutrients (Haissig 1989). This suggests that within limits, the nutritional status of a stockplant or cutting taken from that plant has a greater impact on root growth and development than on root initiation (Blazich 1988). On the other hand, the ability of cuttings to supply carbohydrates, either from stored reserves or through current photosynthesis, to the area where roots appear seems to be very important for root development (Moe and Andersen 1988).

This apparent independence of the process of root initiation on cutting reserves or current assimilates was reflected in the present study by the lack of relationships between cutting diameter and rooting percentage. Once the initial stimulus to initiate root formation occurs, larger cuttings, with a greater carbohydrate storage capacity, are able to support a greater number of roots, a fact which probably explains the strong relationships between cutting diameter and number of roots per rooted cutting found in this study (Fig. 4.13, 4.19). The effect of cutting length on rooting ability was less obvious. In *Cordia alliodora*, cutting length shows an irregular pattern along the stem, with greater length in the apical and basal nodes (as in Experiment 2) or similar length at every position within the stem (as in Experiment 3). Overall, the effect of cutting length on rooting ability was probably overriden by diameter, which shows a systematic increase down the stem. Thus, as with other species mentioned above, cutting volume is likely to be the critical factor.

Once the cutting is taken and set to root, its ability to photosynthesize and to remain turgid has been identified as an important factor determining rooting success. The practice of trimming the leaf aims at minimizing water loss, while allowing some photosynthesis during the propagation period (Okoro and Grace 1976; Eliasson and Brunes 1980; Leakey *et al.* 1982a; Leakey and Coutts 1989; Newton *et al.* 1992a; Leakey *et al.* 1993). These processes are also 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, VPD and hence, stomatal conductance (Loach 1988a).

Experiment 2 was characterized by a relatively low irradiance in both propagators, particularly in the shaded propagator, where maximum irradiance reached only 88 μmol m^{-2} s^{-1} (Table 4.2). In this propagator, cuttings with the smallest leaf area (10 cm^2) showed the lowest rooting percentage (Fig. 4.9a), probably because the rates of current photosynthesis and reserves in the leaf were insufficient to support the cutting long enough to initiate the formation of roots. In the unshaded propagator, on the other hand, the small leaved cuttings showed the highest rooting percentage (Fig. 4.9a). The smaller leaf area probably prevented severe water deficits to occur in such cuttings, as may have happened in the cuttings with greater leaf areas (20 cm^2 and 30 cm^2).

In experiment 3, rooting percentage decreased with an increase in irradiance, probably as a result of the conditions of relatively high irradiance, high temperature and low relative humidity recorded in the unshaded propagator (Table 4.3). This is supported by the lower values of *Fv/Fm* measured in this propagator (Fig. 4.22), and the positive relationships between *Fv/Fm* and final rooting percentage (Fig. 4.23a,b,c). A decrease in *Fv/Fm* is a good indicator of damage to the photosynthetic apparatus and to the physiology of the plant in general, resulting from adverse environmental factors (Bolhar-Nordenkampf *et al.* 1989). Although on average higher photosynthetic rates were recorded in the unshaded propagator (Fig. 4.20a), the lower values of *Fv/Fm* and lower rooting percentage obtained in these cuttings illustrates the importance of achieving a balance between photosynthesis and water loss, and the complex nature of the rooting process. Higher photosynthetic rates did not result in higher rooting percentages when the environmental conditions of the propagation system were not favourable to the general physiological conditions of the cuttings. Irradiance higher than approximately 400 μmol m^{-2} s^{-1}, normally recorded in the unshaded propagator, were associated with a decline in net photosynthetic rates in the cuttings (Fig. 4.6, 4.21).

The effects of leaf area on rooting percentage in the shaded propagator found in experiment 3 differed from that found in experiment 2; few differences were found between leaf area treatments, while a similar response was found in the unshaded propagator (Fig. 4.15a). The irradiance recorded in the shaded propagator in this experiment (339 μmol m^{-2} s^{-1}) was high enough to allow some photosynthetic activity in the cuttings (Fig. 4.20a), but not so excessive as to damage the photosynthetic apparatus and the physiology of the cutting, as indicated by the high values of *Fv/Fm* shown by these cuttings (Fig. 4.22). The maximum irradiance recorded in the unshaded propagator in this experiment is within the range of optimum irradiance (approximately 300 μmol m^{-2} s^{-1} to 400 μmol m^{-2} s^{-1}) for photosynthesis, as shown by the regressions between PAR and P_n in experiments 1 and 3 (Fig. 4.6 and 4.21) and in previous work with *C. alliodora* (Mesén *et al.* 1992). The decrease in rooting percentage associated with increases in leaf area found in the unshaded propagator may be a result of increased water deficit and reduced photosynthetic efficiency, as shown by the reductions in *Fv/Fm* (Fig. 4.22) and in net photosynthetic rates (Fig. 4.20a) shown by these treatments.

It was interesting to find that the results on number of roots per rooted cutting were somehow opposite to those of rooting percentage (Fig. 4.9b and 4.15b). As it was mentioned above, root initiation and root growth are different stages of the rooting process (Lovell and White 1986), and are probably affected by different stimuli. Root initiation was strongly influenced by the physiological condition of the cutting; a decline in *Fv/Fm* was associated with a decline in rooting percentage. However, a high proportion of the cuttings were able to tolerate these conditions and start the process of root formation (Fig. 4.15a). The ability of cuttings from various tropical species to recover from severe water deficits and root successfully has been recently shown by Newton and Jones (1993b). It appears that, if the cutting survives and the initial stimulus for root initiation occurs, other factors associated with the amount of reserves in the cutting and the total rate of net photosynthesis become more important and play a greater role in the number of roots that can be supported by the cutting. When photosynthesis was expressed as net assimilation rate per leaf (Fig. 4.20b), a similar pattern to that of number of roots per rooted cutting was evident (Fig. 4.15b). In this respect, these results support early suggestions that for some species, it may be beneficial to increase the irradiance progressively during the rooting period to improve the rooting systems produced by the cuttings (Leakey personal communication).

High vapour pressure deficits (VPD) are the primary factor influencing the water status of the cuttings (Loach 1988a). The measurements of VPD in shaded and unshaded propagators highlighted the importance of shading to minimize VPDs within the propagator, as has been suggested by Loach (1977) and Grange and Loach (1983a,b). While maximum VPDs of 5.92 kPa, 0.95 kPa and 1.47 kPa were recorded in shaded propagators in experiments 1-3 respectively, this increased respectively to 9.85 kPa, 4.91 kPa and 8.36 kPa in the unshaded propagators (Tables 4.1, 4.2 and 4.3). High values of VPD were clearly associated with increases in irradiance (Fig. 4.4, 4.8 and 4.14), as has been shown previously (Grange and Loach 1983a). For a number of broadleaved species, Grange and Loach (1983b) suggested that values of VPD below 0.5 kPa should be maintained during the propagation period. However, for most species, the degree of water deficit that a cutting can withstand and still root has not yet been defined (Newton and Jones 1993b). In this experiment, higher VPDs were obtained in unshaded propagators; however, a relatively high proportion of these cuttings rooted (> 60%). From these results it appears that relatively high VPDs and marked water deficits can be tolerated by the cuttings and eventually root. This is in agreement with recent work of Newton and Jones (1993a,b), who found similar responses in a number of tropical species, including *Cordia alliodora.*

In this regard, it is interesting to speculate about possible adaptations of the cuttings to their rooting environment. Although this aspect was not investigated in detail, the intensive measurements of gas exchange and chlorophyll fluorescence taken in cuttings with and without shade during the first week after establishment in Experiment 1 showed interesting features. The low initial values of P_n and g_s may represent a shock response to propagation (Gay and Loach 1977), although the cuttings were able to recover within few days, particularly in the shaded propagator. A similar response in water potential has been found by Newton and Jones (1993b) in a number of tropical species. The authors suggested that the water status of the cuttings was more influenced by the prevailing microclimate than by any progressive physiological changes in the cuttings themselves. Overall, cuttings in the unshaded propagator showed a lower rate of net photosynthesis than those in the shaded propagator, but with a trend to increase over time (Fig. 4.5a). Photosynthetic rate of cuttings in the shaded propagator declined dramatically at day 5 (Fig 4.5a), associated with a peak in irradiance and related peaks in temperature and VPD (Fig. 4.1a, 4.2a,b, 4.3a,b). This peak in irradiance had an opposite effect on the cuttings in the unshaded propagator, which suggests a possible adaptation of the cuttings to the high irradiances experienced during the previous days. A similar response was obtained for stomatal conductance (Fig. 4.5b). Similarly, while *Fv/Fm* was lower for the cuttings in the unshaded propagator during the first days, it increased at day 5 and at day 8 the difference in *Fv/Fm* between the cuttings in both propagators became non significant (Fig. 4.7). Little is known about adaptation of cuttings to their light regime during propagation, although in intact seedlings of some species, acclimation has been found to occur over very short periods (Kamaluddin and Grace 1992a,b). It is possible that cuttings respond in a similar way, which explains the relatively high proportion of cuttings which formed roots under the high irradiances and high temperatures measured in the unshaded propagators.

CHAPTER 5

The effects of variation in irradiance and nutrient supply on the physiological condition of *Cordia alliodora* stockplants and subsequent rooting ability of leafy stem cuttings

This experiment investigated the effect of the interactions between the light environment and nutrients (NPK) applied to *Cordia alliodora* stockplants on anatomic characteristics and rooting ability of subsequent cuttings. This involved the determination of the gas exchange of both stockplants and cuttings during the propagation period.

MATERIALS AND METHODS

Growing period

Rooted cuttings from routine propagation work were potted into plastic bags (77 cm^3) containing a soil:sand:compost (1:1:1 by volume) mixture and grown in the CATIE forestry nursery, at a spacing of 20 x 20 cm. When the plants grew to a height of approximately 40 cm, twenty plants from each of clones 2, 4, 8, 19 and 63 were cut back to a standard height of 10 cm. Ten plants from each clone were then allocated randomly to two groups, with and without shade (see below). Within each group, five plants from each clone were selected randomly and given fortnightly soil applications of 7.5 g of a powder fertilizer (FERTICA, Puntarenas, Costa Rica), containing 10% N, 30% P and 10% K. In the shaded treatment the plants were kept under a layer of black plastic netting placed at a height of 1.5 m above the ground, while in the higher irradiance treatment the plants were kept in the open immediately nearby. Three weeks after cutting the stem, the most vigorous shoot was selected in each plant, cutting off the rest.

Such allocation of plants to either shade or unshade treatments poses problems for the analysis, since replicates within each treatment are not independent. This problem, however, is difficult to overcome due to the nature of the treatments. An alternative design with many separate replications would require more plants, space and quantum sensors than were available; therefore, it was decided to accept the limitations of the design but having a more compact design, with less variation between the microenvironment of plants from each treatment.

The irradiance within each treatment was recorded using quantum sensors (Skye Instruments Ltd., Llandrindod Wells, U.K.) attached to a data logger (21 X Micrologger, Campbell Scientific Ltd., Loughborough, U.K.).

Assessments

Growth

The plants were assessed weekly for total height, measured from the base of the shoot to the apex. At the end of 16 weeks, five leaves from each clone/treatment combination were harvested for assessment of specific leaf area. Measurements of leaf area (s) using an area meter (Delta T Devices, Burwell, Cambrdigeshire, U.K.) and dry mass (D, oven dry mass after drying at 80 °C for 24 h) were taken, and SLA calculated as:

$$\text{SLA} = s/D \ (\text{m}^2\ \text{g}^{-1})$$

Net photosynthetic rate and stomatal conductance

At week 16, two measurements of net photosynthetic rate and stomatal conductance were taken on two consecutive days, in a sample of five plants from each treatment, one from each clone, on the first fully developed leaf from the top of each plant. Successive readings were taken across the four treatments, to counteract for fluctuations in irradiance.

For these measurements, a portable gas exchange system with infrared gas analyser was used (LCA-3, Analytical Development Co. Ltd., Hoddesdon, U.K.).

Rooting of stem cuttings

At the end of week 16, the soft apical tip of each plant was discarded and six single-node leafy stem cuttings were collected down the stem, after trimming their leaf areas to 30 cm^2 using paper templates. Indole-3-butyric acid at a concentration of 1.6% was applied to the clean-cut base of the cuttings, as described in Chapter 2. The cuttings were then set to root in sand on non-mist propagator beds, allocated as 5 randomized blocks, keeping record of clone and node position. Each block contained 120 cuttings, six from each clone/irradiance/nutrient combination. The cuttings were sprayed with water twice a day over the entire rooting period.

Assessments

After one week each cutting was lifted and assessed for number of roots. Similar assessments were carried out for the next seven consecutive weeks.

Analysis

For the growing period, analyses of variance were carried out on shoot height and specific leaf area, followed by test of least significant difference, equivalent to Fisher's *t* test. Analyses of variance were conducted on the percentage of cuttings rooted and the number of roots per rooted cutting, by clone and treatment, followed by Fisher's *t* tests (LSD). Data of percentage rooting was transformed by the formula arcsin $\sqrt{\%}$ prior to analysis. Analyses of variance were conducted using SAS (1980). Analyses of deviance by stepwise regression in GENSTAT 5 (Payne *et al.* 1987) were utilized to determine the influence of treatments and node position within the stem on the cuttings' rooting ability. To determine the influence of treatments applied to the stockplants on their net photosynthetic rate and stomatal conductance, analyses of variance for stepwise regression in GENSTAT 5 (Payne *et al.* 1987) were used.

RESULTS

Irradiance

Irradiance varied greatly between the shaded and the unshaded treatments, with means of 80 μmol m^{-2} s^{-1} (range: 0 μmol m^{-2} s^{-1} - 852 μmol m^{-2} s^{-1}) and 236 μmol m^{-2} s^{-1} (range: 0 μmol m^{-2} s^{-1} - 2274 μmol m^{-2} s^{-1}), respectively, during the growing period.

Shoot growth

At the end of sixteen weeks, shoot height was significantly ($p < 0.01$) higher under high light, and the application of NPK did not have a significant effect on shoot growth (Table A13). Plants under treatments high light/low nutrients, high light/high nutrients and low light/low nutrients showed heights of 37.4 cm, 35.3 cm and 31.9 cm respectively, significantly higher than treatment low light/high nutrients, which reached a height of 27.4 cm (Fig. 5.1).

Clones also showed highly significant ($p < 0.001$) differences in terms of shoot growth at the end of sixteen weeks, varying between 41.07 cm for clone 2 to 26.96 cm for clone 8 (Fig. 5.2).

Specific leaf area

Highly significant differences ($p < 0.001$; Table A14) were found for specific leaf area between light treatments, and for the interaction between light and nutrients. Under high light, specific leaf area significantly ($p < 0.05$) increased with the application of NPK, while the difference between nutrient treatments under low light were non significant (Fig. 5.3).

Net photosynthetic rate and stomatal conductance

There were highly significant ($p < 0.01$) differences between treatments for net photosynthetic rate (P_n) (Table A15) and stomatal conductance (g_s) (Table A16). Mean P_n was higher under high irradiance, and within each irradiance treatment the application on NPK did not have any significant effect (Fig. 5.4a). An opposite response to light was found for g_s, but in this case, the application of NPK caused a significant reduction in g_s at the low irradiance (Fig. 5.4b). The same effects of irradiance and nutrient treatments on P_n and g_s were obtained when the results were analysed by stepwise regression (Tables A17 and A18); in addition, P_n was found to be highly dependent on g_s ($p < 0.01$).

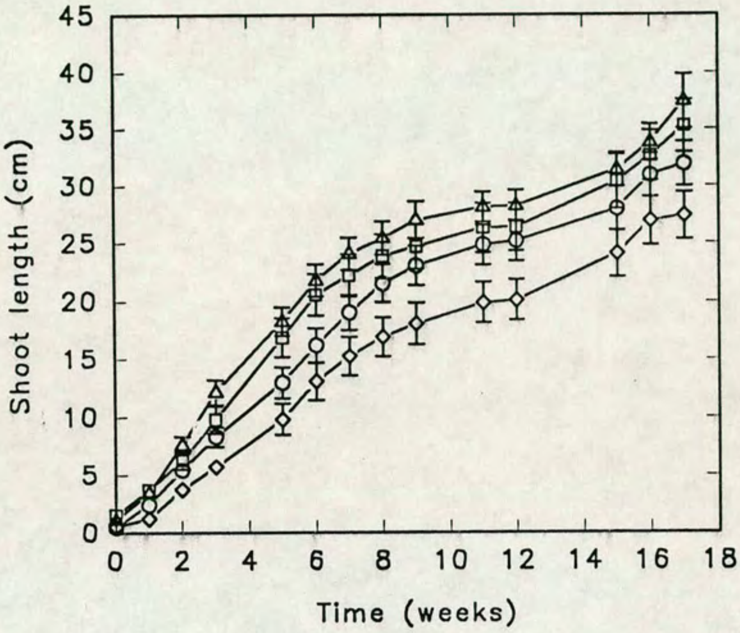


Fig. 5.1 The effect of irradiance and nutrient supply on the mean height of *Cordia alliodora* plants grown under low irradiance without NPK (circles) or with NPK (diamonds) and high irradiance without NPK (triangles) or with NPK (squares). Values are means of five clones (N = 25), bar = ± standard error of the mean.

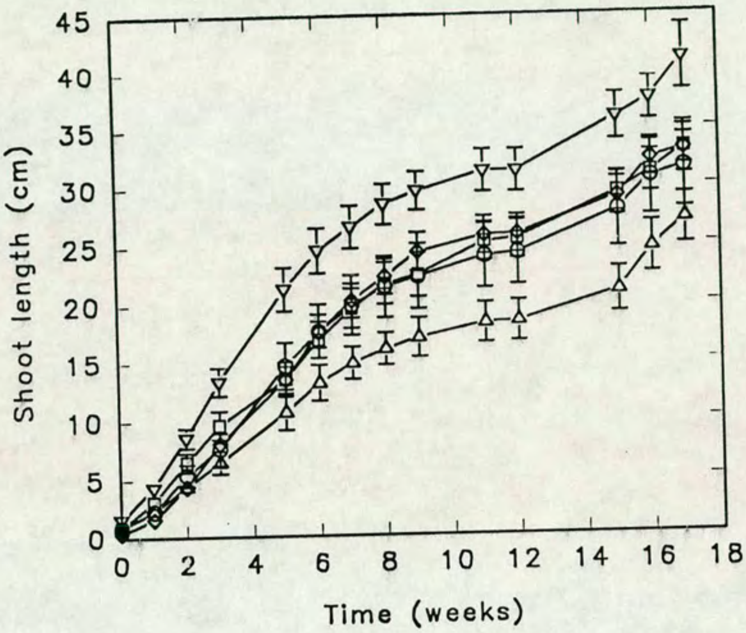


Fig. 5.2 Clonal variation in mean height of five clones of *Cordia alliodora* grown under low or high irradiance, with or without NPK. Clone 2 - inverted triangles; clone 4 - circles; clone 8 - upright triangles; clone 19 - diamonds; clone 63 - squares. Values are means (N = 20) of four irradiance/NPK combinations. Bar = ± standard error of the mean.

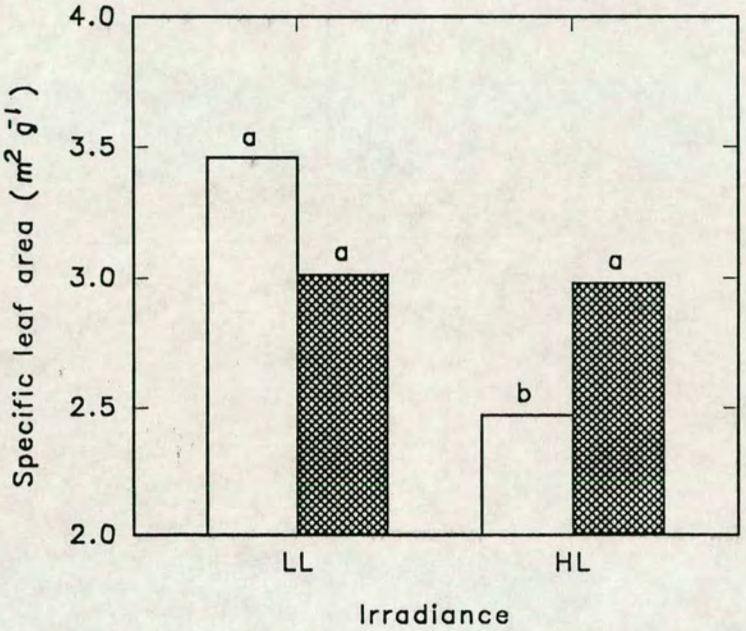


Fig. 5.3 The effect of irradiance and nutrient supply on specific leaf area of *Cordia alliodora* plants grown under low light (LL) or high light (HL), without NPK (open bars) or with NPK (shaded bars). N = 25, means with the same letter are not significantly different ($t_{0.05}$).

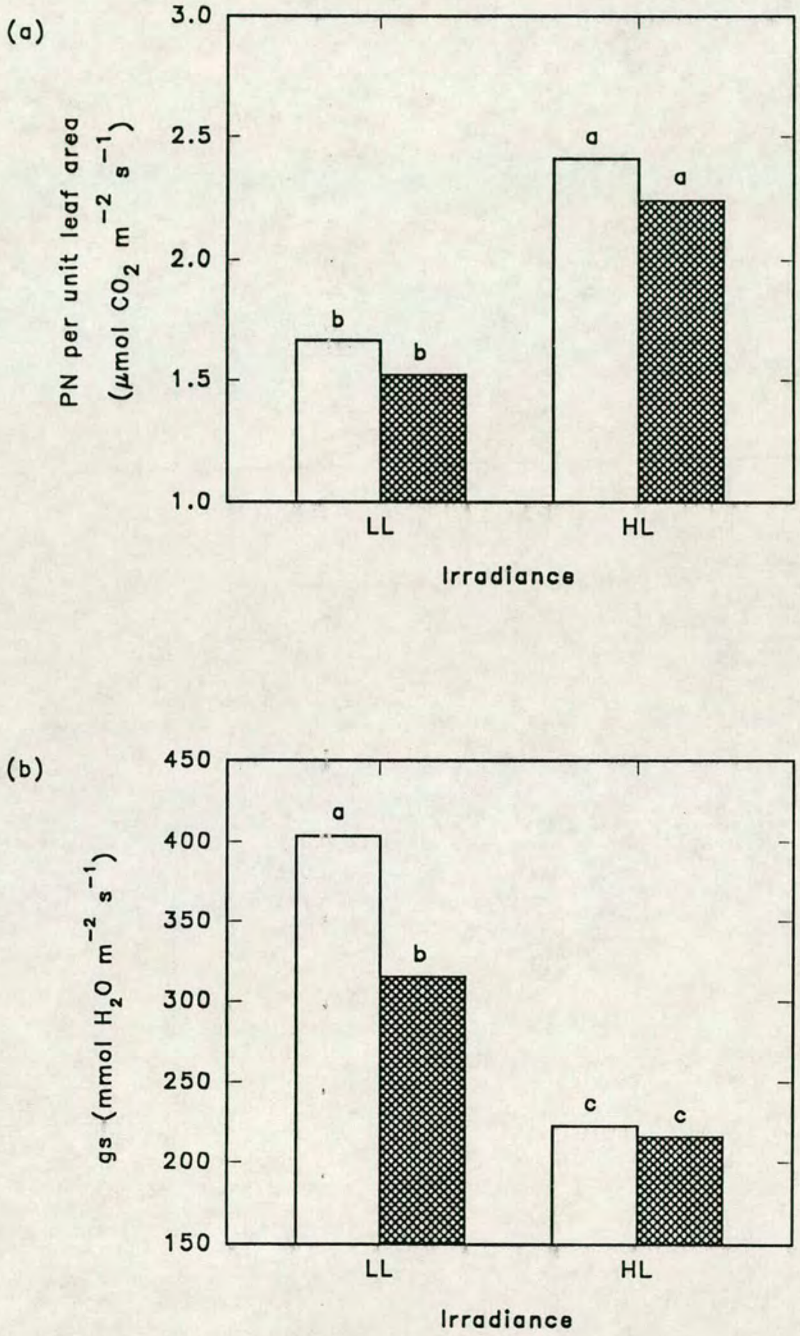


Fig. 5.4a,b The effect of irradiance and nutrient supply on **(a)** net photosynthetic rate per unit leaf area and **(b)** stomatal conductance of *Cordia alliodora* plants grown under low light (LL) or high light (HL), without NPK (open bars) or with NPK (shaded bars). N = 25, means with the same letter are not significantly different ($t_{0.05}$).

Cutting morphology and rooting ability

The irradiance treatment under which the stockplants were grown did not significantly affect the rooting percentage of subsequent cuttings after six weeks (50.0% and 54.0% for low and high light, respectively), but the application of nutrients to stockplants significantly ($p < 0.05$; Table A19) reduced the rooting of subsequent cuttings in both light treatments (Fig. 5.5a). In terms of number of roots per rooted cutting there were no significant effects between nutrient treatments, but the cuttings produced significantly ($p < 0.001$; Table A20) fewer roots when the stockplants were grown under high light (Fig. 5.5b).

Cuttings were shorter and thinner in stockplants grown under low light, and within each light treatment, the application of nutrients caused a reduction in both cutting length and cutting diameter (Fig 5.6a,b). No clear relationships were found between cutting length and node position within the stem, while in every treatment, cutting diameter increased from apical to basal nodes. In this study, no relationships were found between cutting morphology and rooting ability.

There were highly significant differences ($p < 0.001$) between clones for both rooting percentage and number of roots per rooted cutting. Clones 19 and 63 showed the highest rooting percentage (69.2% and 63.3%), clones 2 and 8 were intermediate (50.8% and 47.5%), whilst clone 4 displayed the lowest rooting percentage (29.2%) (Fig. 5.7a). A roughly opposite trend was found for mean number of roots per rooted cutting, with clones 2, 4 and 8 showing more roots (6.8, 7.2 and 7.6, respectively) than the top rooting clones 19 and 63, which produced only 5.8 and 5.1 roots per cutting, respectively (Fig. 5.7b).

When the rooting data were analysed by stepwise regression, rooting was found to be significantly affected by block, clone and application of NPK to the stockplants, but not by the irradiance treatment to the stockplants or the node position (Table A21).

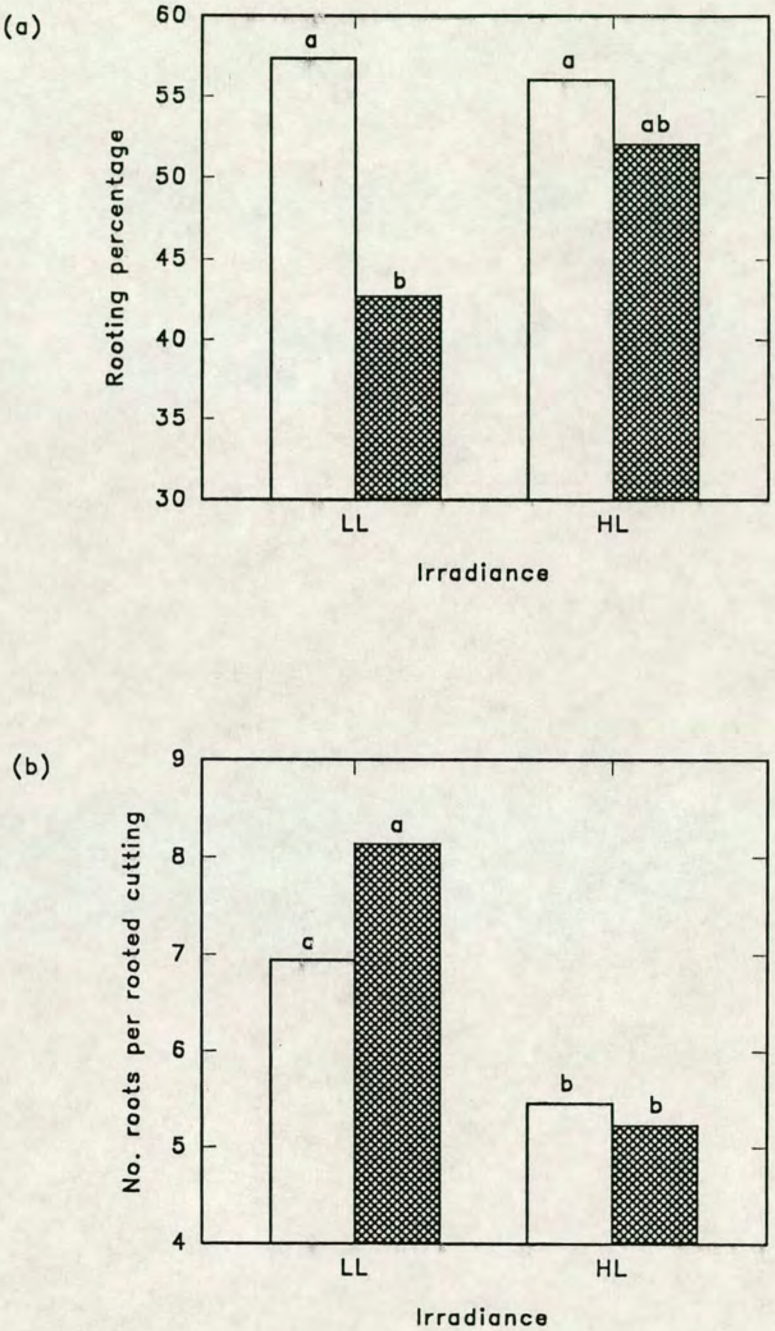


Fig. 5.5a,b The influence of irradiance and nutrients applied to *Cordia alliodora* stockplants on **(a)** the subsequent rooting percentage and **(b)** the number of roots per rooted cutting of single-node, leafy stem cuttings set to root in non-mist propagators. The cuttings were collected from stockplants grown under low light (LL) or high light (HL), without NPK (open bars) or with NPK (shaded bars). N = 150, means with the same letter are not significantly different ($t_{0.05}$).

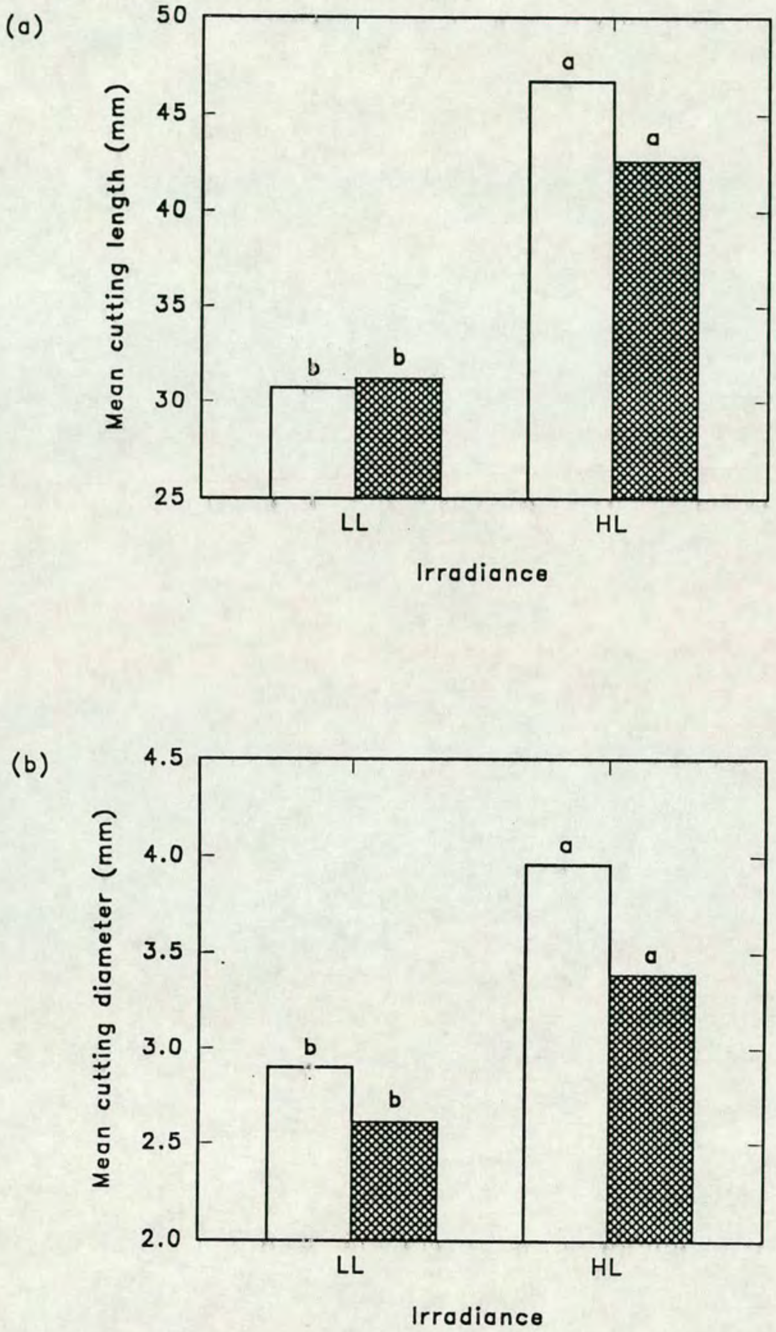


Fig. 5.6a,b The effect of two irradiances (LL - low; HL - high) and two NPK treatments (open bars - without NPK; shaded bars - with NPK) applied to *Cordia alliodora* stockplants on **(a)** the mean cutting length and **(b)** the mean cutting diameter of subsequent single-node cuttings. Means grouped by the same letter are not significantly different ($t_{0.05}$).

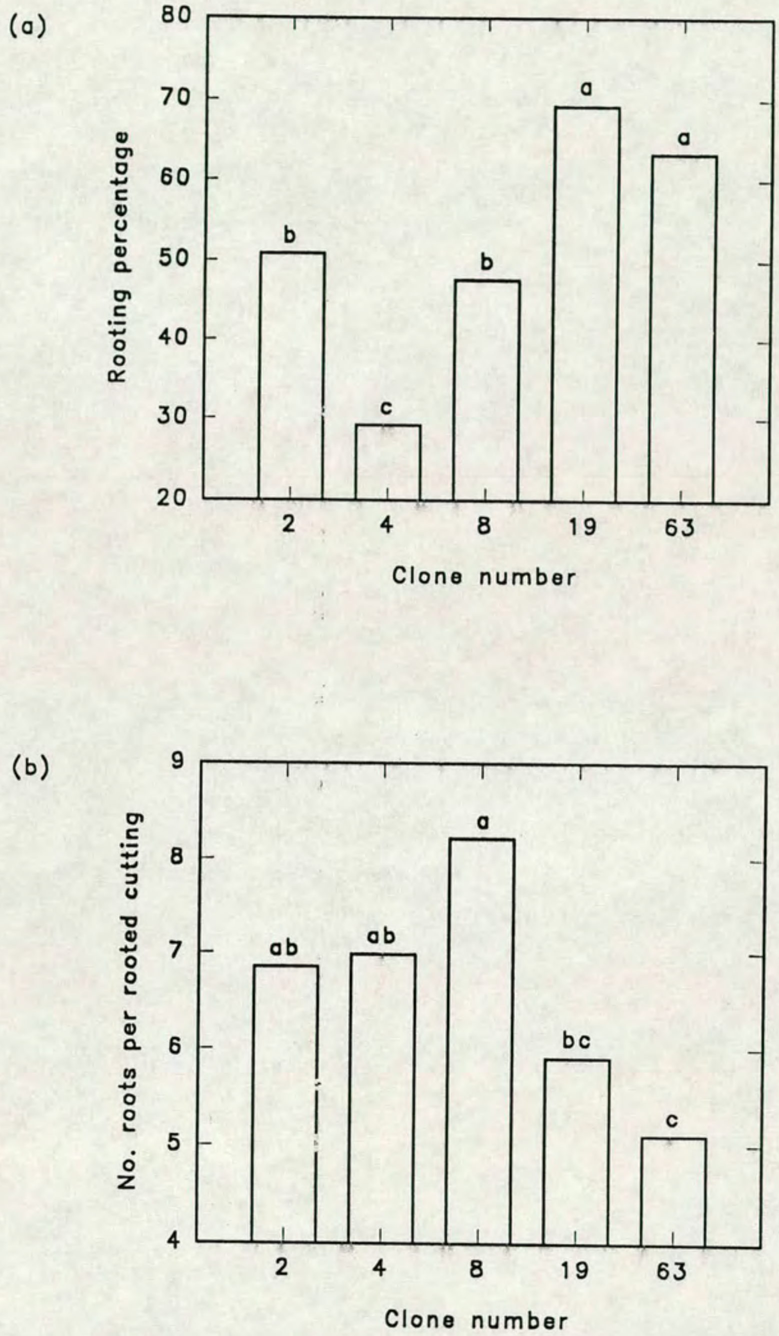


Fig. 5.7a,b Clonal variation in **(a)** the rooting percentage and **(b)** the number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* after six weeks in a non-mist propagator. Values presented are means (n = 120) of four irradiance/NPK treatments applied to the stockplants. Means grouped by the same letter are not significantly different ($t_{0.05}$).

This decline in stomatal conductance has not been fully explained, but may be a result of a rise in leaf temperature, with a consequent increase in leaf-to-air VPD (Ramos and Grace 1990).

Cutting length and diameter are known to affect rooting ability of some species (Fernquist 1966; Veierskov 1978; Poulsen and Andersen 1980; Leakey 1983; Leakey and Mohammed 1985; Wilson *et al.* 1989; Hoad and Leakey 1992; Leakey *et al.* 1993). Growing stockplants under conditions that promote internode elongation has been recommended as a means to increase rooting ability (Hoad and Leakey 1992). Therefore, it would be reasonable to expect a decline in rooting percentage in the shorter cuttings formed under shade. This was not the case in the present study, where cuttings from shade and sun showed a similar rooting percentage. The main differences in rooting were produced by the application of nutrients within each irradiance treatment (Fig. 5.5a). The number of roots produced by the cuttings, on the other hand, was significantly reduced in cuttings from stockplants grown under full sunlight (Fig. 5.5b). This is in agreement with previous results in *Triplochiton scleroxylon* (Leakey and Storeton-West 1992) and *Pisum sativum* (Baadsmand and Andersen 1984).

The results of this study supports once again the view that root initiation and root development may be influenced by a different set of conditions (Lovell and White 1986), as discussed in Chapter 4. Cuttings from stockplants grown in the sun showed higher rates of net photosynthesis (Fig. 5.4a) and lower specific leaf areas (more chlorophyll per unit area) (Fig. 5.3). In this respect, the results shown in this study supports the suggestion of Leakey and Storeton-West (1992) that the low number of roots produced by these cuttings was due to suppressed current photosynthesis resulting from end product inhibition. 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). When these cuttings are set to root at lower irradiances, as in the present study, the cuttings normally root very poorly (Moe and Andersen 1988). Similar results have also been reported by Davis and Potter (1981) and Borowski *et al.* (1981). The lack of differences in rooting percentage between both light treatments applied to the stockplants seems to lie in the nature of the generalized response curve to irradiance on rooting, as illustrated by Moe and Andersen (1988): with increasing irradiance rooting increases and reaches a maximum at the optimal irradiance, to decrease again when irradiance exceeds the optimal level. At stockplant irradiance below optimum, rooting may be limited by a lack of carbohydrates and auxin supplies to the base of the cutting; at supraoptimal irradiance, there is possibly too high a concentration of carbohydrates, photo destruction of auxin and changes in water relations and concentrations of inhibitors and/or promoters (Moe and Andersen 1988). It is likely that the irradiances used in the present experiment occupied positions above and below the optimum, resulting in similar rooting percentages. This trend, together with the fact that stockplants were grown in containers, where there is a greater possibility of water deficits, could explain why the overall rooting percentages in this study were low, as compared with other experiments with this species.

While rooting percentage was unaffected by the light treatment applied to stockplants, it was reduced significantly by the addition of nutrients. Similar responses have been reported by Pearse (1943), Moe and Andersen (1988) and Leakey and Storeton-West (1992), for a range of different species. This result is somehow contradictory to logical thinking, since it would be reasonable to expect that stockplants with a greater availability of nutrients should produce cuttings with a higher carbohydrate storage and hence, a higher rooting ability. However, very low rooting ability occurs when cuttings are harvested from stockplants in which photosynthesis is inhibited by high starch contents (Leakey *et al.* 1993); a high concentration of leaf starch in cuttings may suppress post-severance photosynthesis and their rooting due to end product inhibition, as has been shown by Leakey and Storeton-West (1992) and Hoad and Leakey (1992). Thin leaves may have lower mutual shading of chloroplasts and improve efficiency of gas exchange (Hoad and Leakey 1992). In addition, as described by Hoad and Leakey (1992) in *Eucalyptus grandis*, large but not too concentrated pools of soluble and storage carbohydrates may provide an adequate supply of stored carbohydrates in cuttings without inhibiting their photosynthetic rate. Consequently, a high concentration of nutrients in stockplants may not be advantageous to severed cuttings.

As discussed in previous chapters, the initial stimulus for root formation, and hence, rooting percentage, seems to be little influenced by the nutritional status of a stockplant or cutting, since root primordium initiation appears to be hormonally controlled (Lovell and White 1986; Moe and Andersen 1988; Veierskov *et al.* 1982a,b; Veierskov and Andersen 1982). In this regard, the reduction in rooting percentage as a result of suppressed photosynthesis may be more dependent on hormonal factors than on the actual nutritional status of the cutting. For instance, the reduction in auxin supply to the base of the cuttings under conditions of low photosynthetic activity has been shown by Heide (1968), Hilman and Galston (1961), Scott and Briggs (1963) and Vardar (1968), and discussed by Davis (1988). Preliminary evidence by Kumpula and Potter (1984) tends to support this hypothesis. Suppressed photosynthesis in cuttings may also reduce rooting percentage through reduced transport of other rooting cofactors produced in leaves and buds to the base of the cutting where they promote rooting (Davis 1988). It is also possible that photosynthesis influences the formation of a noncarbohydrate, non-auxin component which may be involved in rooting (Davis 1988). There is evidence that considerable biosynthesis of phenolic compounds, which have been found to act as rooting cofactors, occurs in the chloroplast and hence may be related to photosynthetic activity (Davis 1988). In this study, it is possible that these hormonal, indirect effects of suppressed photosynthesis caused the reduction in rooting percentage in cuttings from stockplants grown under high nutrient supply.

In this experiment no attempt was made to investigate clonal variation in any detail. It was interesting to find, however, that clones showed a roughly opposite trend between rooting percentage and number of roots per rooted cutting. This result tends to support the view, as discussed above and in previous chapters, that root initiation and root development are independent processes, affected by a different set of conditions. It is likely that clones with a high photosynthetic ability and/or a high carbohydrate storage capacity will produce cuttings able to support a high number of roots, but such characteristics are apparently independent of the cuttings' ability to initiate the formation of roots, which is hormonally controlled. Genetic effects are among the least studied factors that control or modify rooting by cuttings (Haissig and Riemenschneider 1988). Evidence exists that rooting by cuttings is genetically controlled, but little is known of the possible modes of action (Haissig and Riemenschneider 1988). It is known that clones vary in their concentrations of endogenous auxin, rooting cofactors or inhibitors, anatomy, leaf retention and many other interacting factors that may affect rooting (Leakey *et al.* 1982a), all of which are likely to produce differences in rooting ability, as observed in the present study.

CHAPTER 6

The effects of cutting origin on the rooting ability and subsequent growth of single-node, leafy stem cuttings of *Cordia alliodora*

This experiment investigated the effects of the cutting position within the shoot on the rooting ability of single-node, leafy stem cuttings of *Cordia alliodora*. This involved the determination of the gas exchange of the cuttings during the propagation period. A second part of the experiment investigated the effects of the cutting position on the subsequent growth of the rooted cuttings.

MATERIALS AND METHODS

Experiment 1 The effects of cutting origin on the physiology of rooting of single-node, leafy stem cuttings of *Cordia alliodora*

Plant material and experimental design

This experiment was carried out at the CATIE nursery, Turrialba, Costa Rica, using the material described in Chapter 2. In July 1991, the stockplants from clones 18, 19, 22, 23, 25, 29, 33, 35, 37 and 38 were cut to a height of 20 cm. Three weeks later, the three most vigorous shoots were selected from each stockplant and any other shoots were removed. By early September 1991, the shoots had grown to heights of 30-40 cm and the eight most vigorous shoots from each clone were selected for the present experiment. The soft apical tip of each shoot was discarded and six single-node, leafy stem cuttings were collected sequentially down the stem, after trimming their leaf areas to 30 cm^2. Indole-3-butyric acid at a concentration of 1.6 % was applied to the base of the cuttings, as described in Chapter 2, before inserting the cuttings in sand in non-mist propagators. The cuttings were allocated in eight randomized blocks, each block containing 60 cuttings (ten clones x six cutting positions in the shoot). The clone number was randomly arranged in each block, but for practical reasons, the cuttings were allocated systematically by node number within each clone. During the course of the experiment, cuttings were finely sprayed with water from a hand-held sprayer twice a day to keep the leaves moist. The propagator was protected from the direct sunlight with a layer of black plastic netting, placed at a height of 2 m above the ground.

Assessments

After one week, the length and midpoint diameter of each cutting were measured, and each cutting lifted and assessed for number of roots. Similar assessments were carried out for the next eight consecutive weeks.

Measurements of leaf, air and substrate temperature, relative humidity and irradiance inside the propagator were recorded for the duration of the experiment using a 21X Micrologger (Campbell Scientific Ltd., Loughborough, England) and appropriate sensors, as described in Chapter 2.

Photosynthetic rates and stomatal conductance were measured in six randomly selected cuttings (including all node positions) from clones 18, 19, 22, 23 and 29 on days 14, 21 and 28 after insertion of the cuttings in the propagator, using an infra red gas analyser attached to a Parkinson leaf chamber (Analytical Development Co. Ltd., Hoddesdon, U.K.).

Experiment 2 The growth characteristics of single-node leafy stem cuttings of *Cordia alliodora* from different node positions within the stem

Plant material and experimental design

The low rooting percentage of most clones from the previous experiment prevented the production of sufficient rooted cuttings to establish the present experiment. Therefore, rooted cuttings from six positions down the stem were obtained from clones 2, 6 and 37, from the experiment described in Chapter 4. After each evaluation of number of roots, cuttings with roots more than 1 cm long were removed from the propagation bed and potted in polythene bags (600 ml) containing a mixture of forest soil, sand and compost (1:1:1 by volume), keeping record of clone and node position. The potted cuttings were kept for three weeks in a greenhouse, where received decreasing watering, from periodical irrigation with an automatic mist system to one daily watering with a hose by the end of the third week. The plants were placed 10 cm apart in open beds in the nursery, allocated as five randomized blocks, each one containing 54 plants, three from each clone/node position. One row of similar-size plants was planted around the experiment. Irrigation and weeding were carried out as necessary.

Assessments

Plants were assessed monthly for basal shoot diameter, total shoot height and height to the first whorl of branches.

RESULTS

Experiment 1

The propagation environment

A maximum irradiance of 556 μmol m^{-2} s^{-1} was recorded during the course of the experiment, which represents approximately 30% of the maximum irradiance recorded in open conditions in Turrialba. However, large variations in air, foliar and substrate temperatures were obtained inside the propagator. Relative humidity was always over 73.7%, with a mean of 94.8%. VPD varied between 0.31 kPa and 2.41 kPa (Table 6.1).

Table 6.1 The propagator microclimate after the insertion of *Cordia alliodora* cuttings in a non-mist propagator system.

Variable	Mean	Range
Relative humidity (%)	94.8	73.7 - 100
Air temperature (°C)	23.9	18.9 - 36.9
Substrate temperature (°C)	21.9	18.1 - 29.2
PAR (μmol m^{-2} s^{-1})	31	0 - 556
Leaf temperature (°C)	25.1	20.3 - 37.0
VPD (kPa)	0.65	0.31 - 2.41

When values of PAR were correlated with VPD, a strong positive correlation was found (Fig. 6.1).

Rooting ability

After nine weeks there were highly significant differences ($p < 0.01$) between node position and clones, and a significant interaction ($p < 0.05$) between clone and node position with respect to rooting percentage (Table A22). Rooting percentage did not show a clear pattern between node positions, varying between 21.3% for node 2 to 46.3% for node 5 (Fig. 6.2a). No significant differences were obtained for number of roots per rooted cutting (ANOVA, $p < 0.05$; Fig. 6.2b). A large variation in rooting was obtained between clones, with a range of between 6.3% (clone 23) and 70.8% (clone 22) (Fig. 6.3).

The step wise regression revealed that the cuttings' ability to form roots was significantly influenced by clone, node position, cutting length and cutting diameter (Table A23). Mean cutting diameter increased steadily from 38.2 mm to 50.4 mm from node 1 to node 6 (Fig. 6.4a), but mean cutting length did not show any systematic relation to node position, varying between 35.4 mm for node 3 to 39.8 mm for node 6 (Fig.6.4b).

Photosynthetic rates and stomatal conductance

Photosynthetic rates of between 0.68 μmol CO_2 m^{-2} s^{-1} and 6.70 μmol CO_2 m^{-2} s^{-1} were recorded from cuttings during propagation. The step wise regression analysis showed that all the variables included had a significant influence on the photosynthetic rate of the cuttings (Table A24). However, PAR and g_s removed the largest proportion of the total variation (58.7% and 14.0%, respectively), while clone (2.6%) and node position (1.5%) had a smaller effect. In average, apical (1 and 2) and basal (6) nodes showed the higher P_n value, while nodes 3-5 showed the lowest values (Fig. 6.5a).

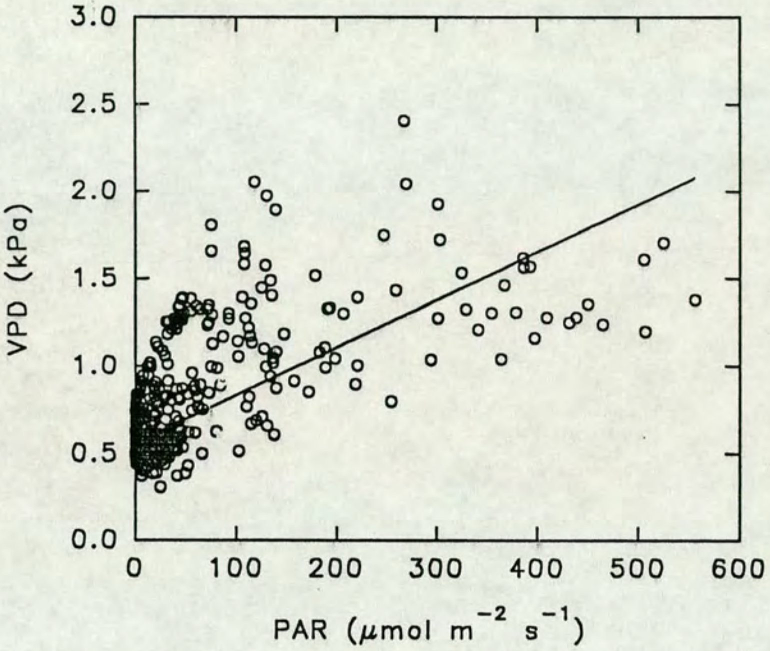


Fig. 6.1 The relationship between leaf-to-air vapour pressure deficit (VPD) and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* set to root in a non-mist propagator. Values are means of ten clones and six node positions within the stem ($y = 0.564 + 0.003\ x$; $r^2 = 0.71$).

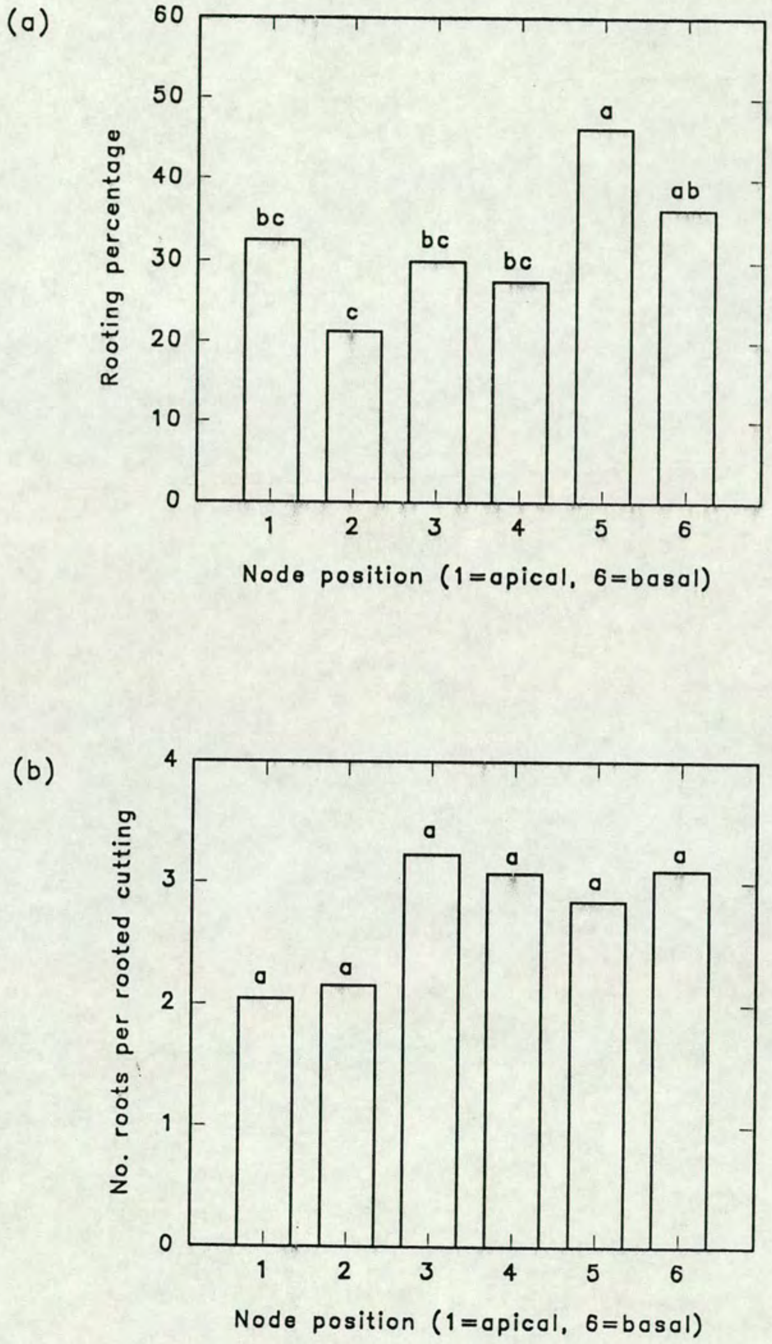


Fig. 6.2a,b The effects of node position within the stem on (a) rooting percentage and (b) number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* after nine weeks in non-mist propagators. N = 80; means grouped by the same letter are not significantly different ($t_{0.05}$).

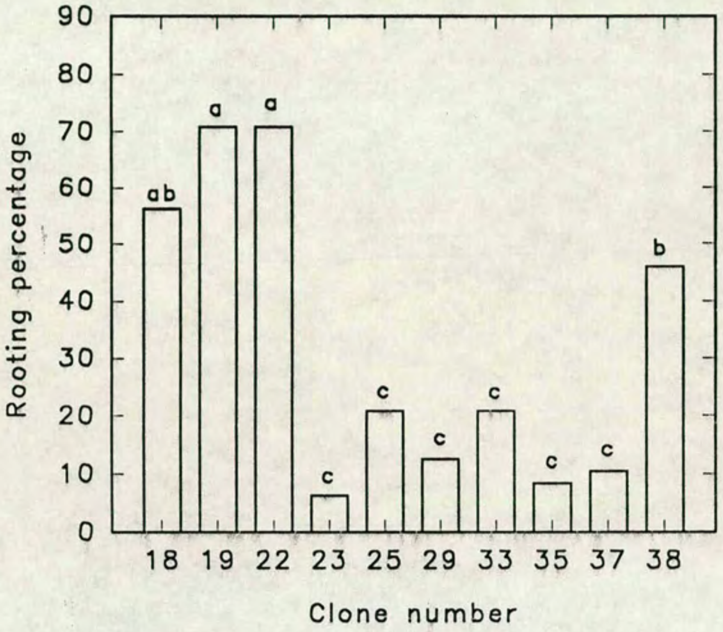


Fig. 6.3 Clonal variation in the rooting percentage of single-node, leafy stem cuttings of *Cordia alliodora* from six positions within the stem (apical to basal), after nine weeks in non-mist propagators. N = 48; means grouped by the same letter are not significantly different ($t_{0.05}$).

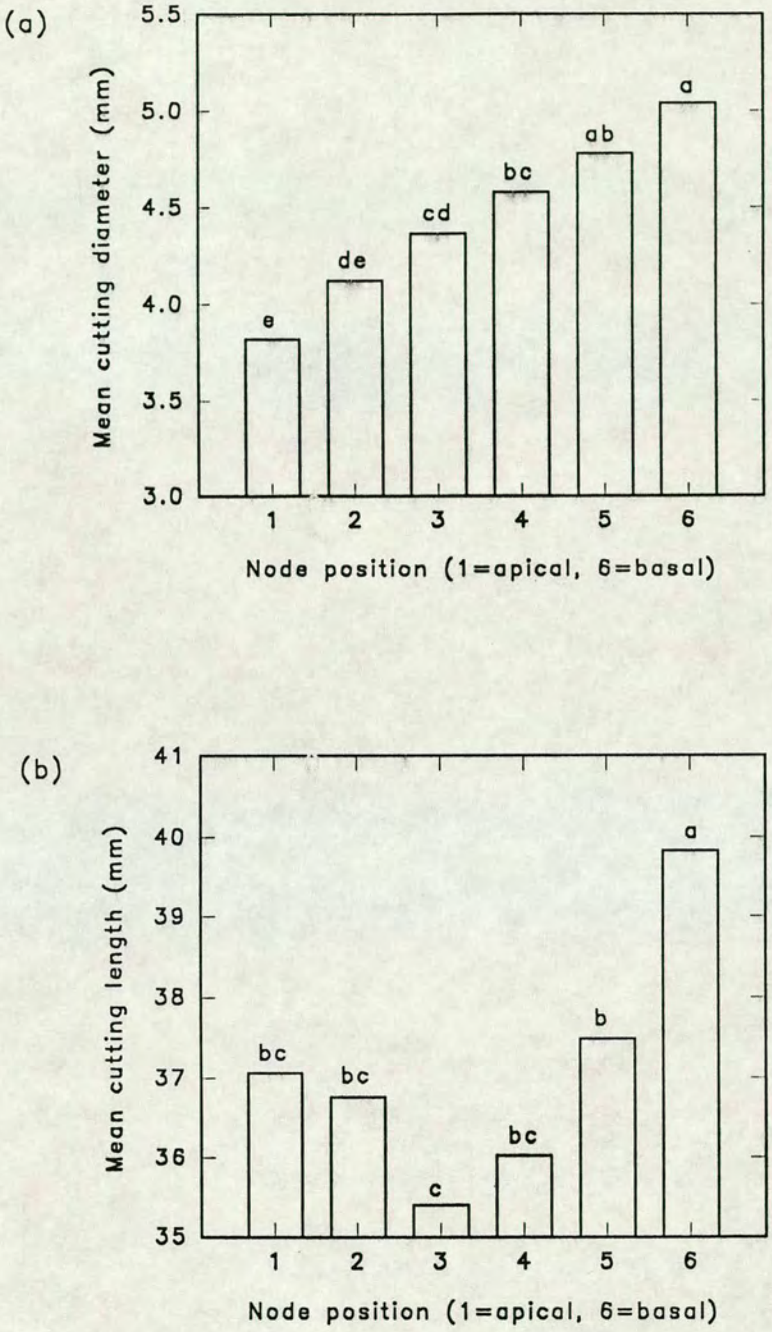


Fig. 6.4a,b The effect of node position within the stem on (a) mean cutting diameter and (b) mean cutting length of single-node, leafy stem cuttings of *Cordia alliodora* from three different clones. N = 48; means grouped by the same letter are not significantly different ($t_{0.05}$)

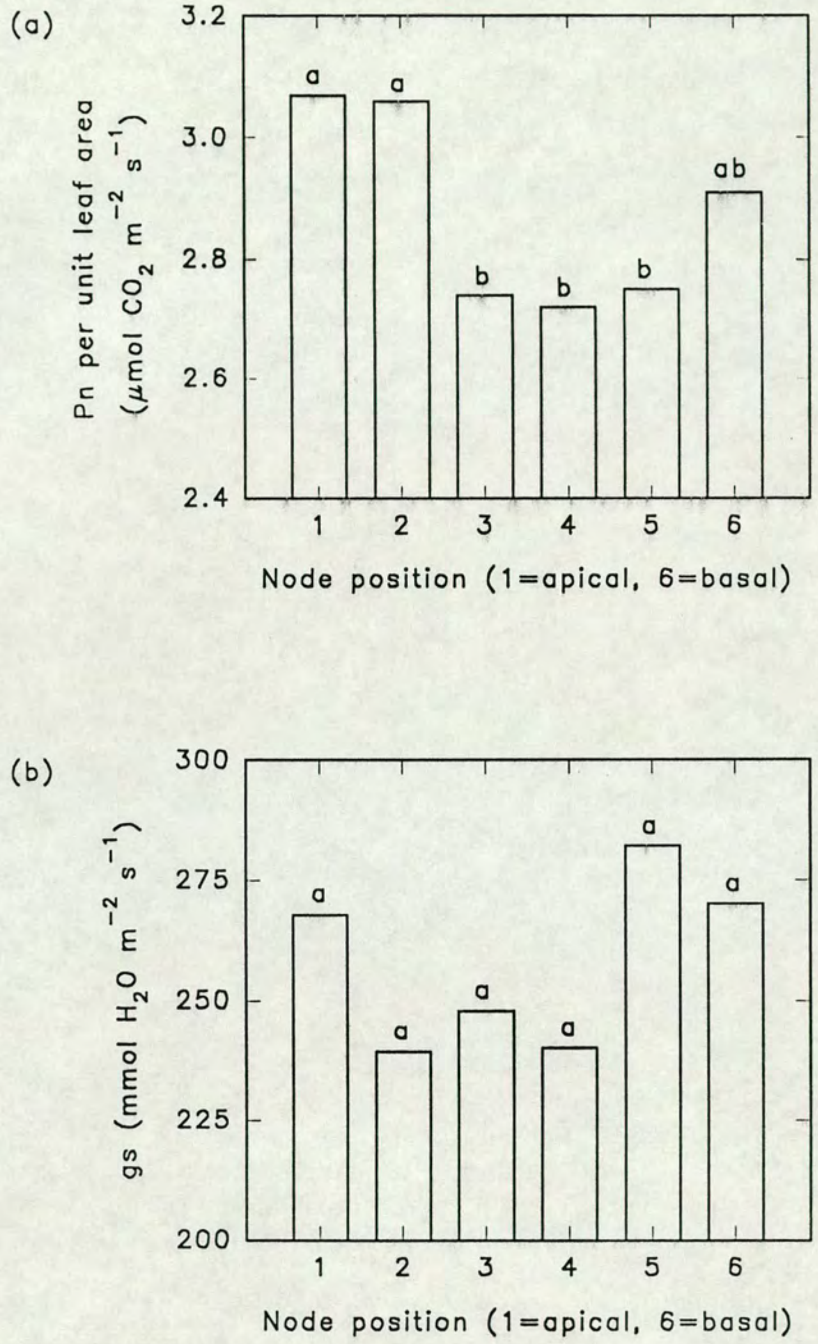


Fig. 6.5a,b The effects of node position within the stem on (a) net photosynthetic rate per unit leaf area (P_n) and (b) stomatal conductance (g_s) of single-node, leafy stem cuttings of *Cordia alliodora*. Values are means of three measurements at weeks 1, 2, 3 and 4, on ten cuttings from each node position. Means with the same letter are not significantly different ($t_{0.05}$).

Stomatal conductances varied between 18.0 mmol H_2O m^{-2} s^{-1} and 680.0 mmol H_2O m^{-2} s^{-1} during propagation. The stepwise regression revealed that g_s was significantly influenced by PAR and clone, but not by node position (Table A25), with values of around 250 mmol H_2O m^{-2} s^{-1} for all node positions (Fig. 6.5b).

When values of PAR were correlated with P_n, a strong positive correlation was found (Fig. 6.6).

Experiment 2

At the end of three months growth in the nursery there were highly significant ($p < 0.001$) differences between node positions in shoot basal diameter (Table A26), and significant ($p < 0.05$) differences in height to the first whorl of branches (Table A28). The analysis of variance did not show significant differences for total shoot height.

Node positions showed values between 34.4 cm for node 1 to 38.9 cm for node 6 in total shoot height (Fig. 6.7a), between 4.4 mm (node 1- apical) to 5.3 mm (node 6-basal) in basal diameter (Fig. 6.7b), and between 29.6 cm for node 1 to 34.3 cm for node 6 in height to the first whorl of branches (Fig. 6.7c). It was interesting to find that nodes 6 and 5, in that order, showed the largest values for all three variables, while node 1 showed the smallest values. Nodes 2, 3 and 4 interchanged positions without any apparent trend. When these values were correlated with the mean cutting diameter, strong positive correlations were found (Fig. 6.8a,b,c).

After six months growth in the nursery, plants showed a similar trend to that at three months for shoot basal diameter and height to the first whorl of branches, but not for shoot height, which did not show any particular trend (Fig. 6.9a,b,c). When these values at six months were correlated with cutting diameter, the correlations became weaker for basal diameter and height to the first whorl, and no correlation was found for total shoot height (Fig. 6.10a,b,c).

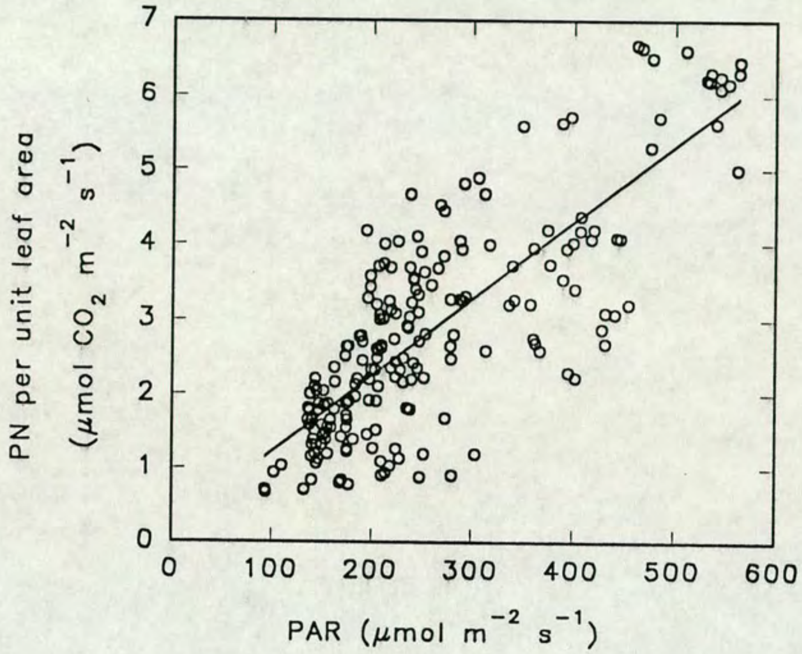


Fig. 6.6 The relationship between net photosynthesis per unit leaf area (P_n) and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* from 10 clones and six different positions within the stem. ($y = 0.205 + 0.010\ x$; $r^2 = 0.80$)

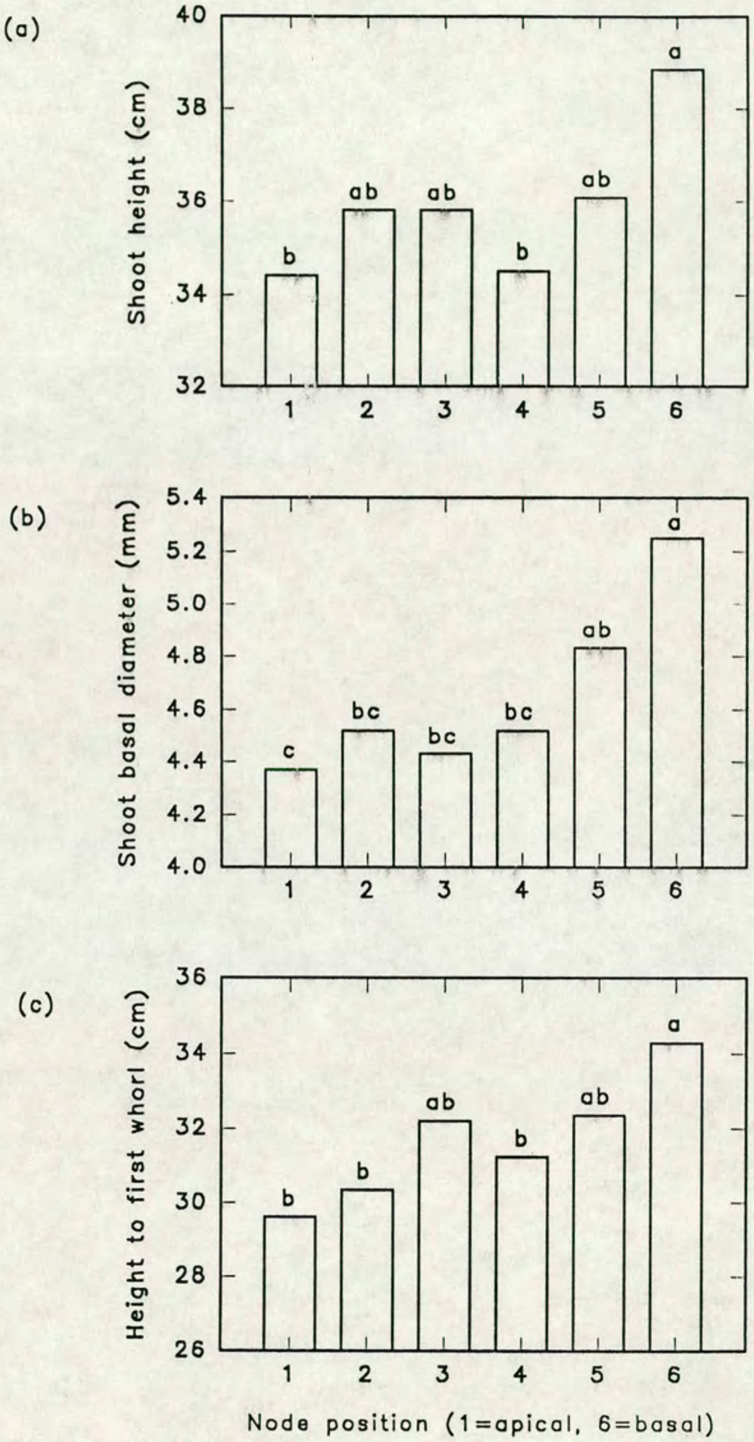


Fig. 6.7a,b,c The effect of node position within the stem - apical (1) to basal (6) - on **(a)** the total height, **(b)** the basal diameter and **(c)** the height to the first whorl of branches produced by the shoot of rooted cuttings of *Cordia alliodora* from three clones, after three months growth in the nursery. N = 45; means grouped by the same letter are not significantly different ($t_{0.05}$).

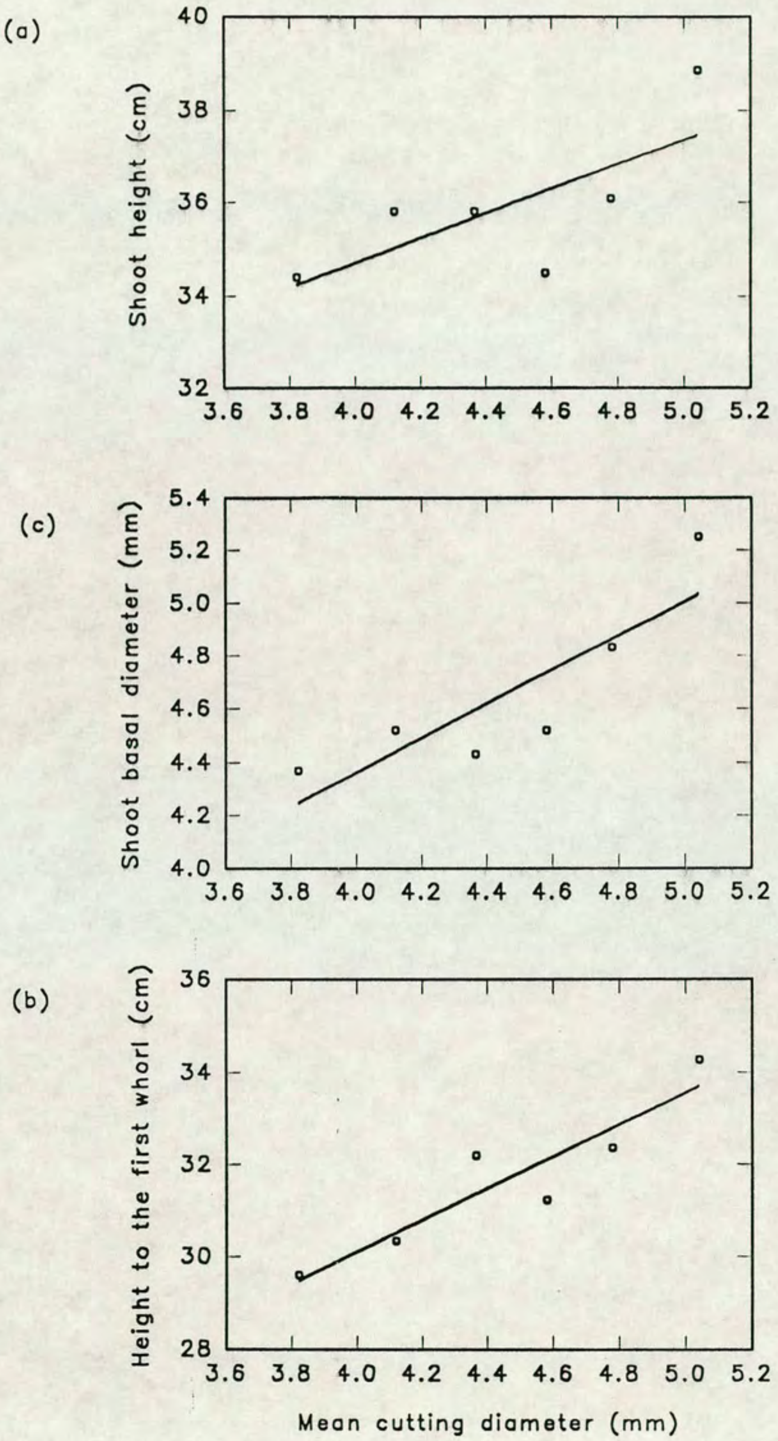


Fig. 6.8a,b,c The relationships between mean cutting diameter and **(a)** shoot height ($y = 24.160 + 2.640\ x$; $r^2 = 0.73$), **(b)** shoot basal diameter ($y = 1.787 + 0.644\ x$; $r^2 = 0.86$) and **(c)** height to the first whorl of branches ($y = 16.311 + 3.451\ x$; $r^2 = 0.92$) of plants of *Cordia alliodora* from rooted cuttings after three months growth in the nursery.

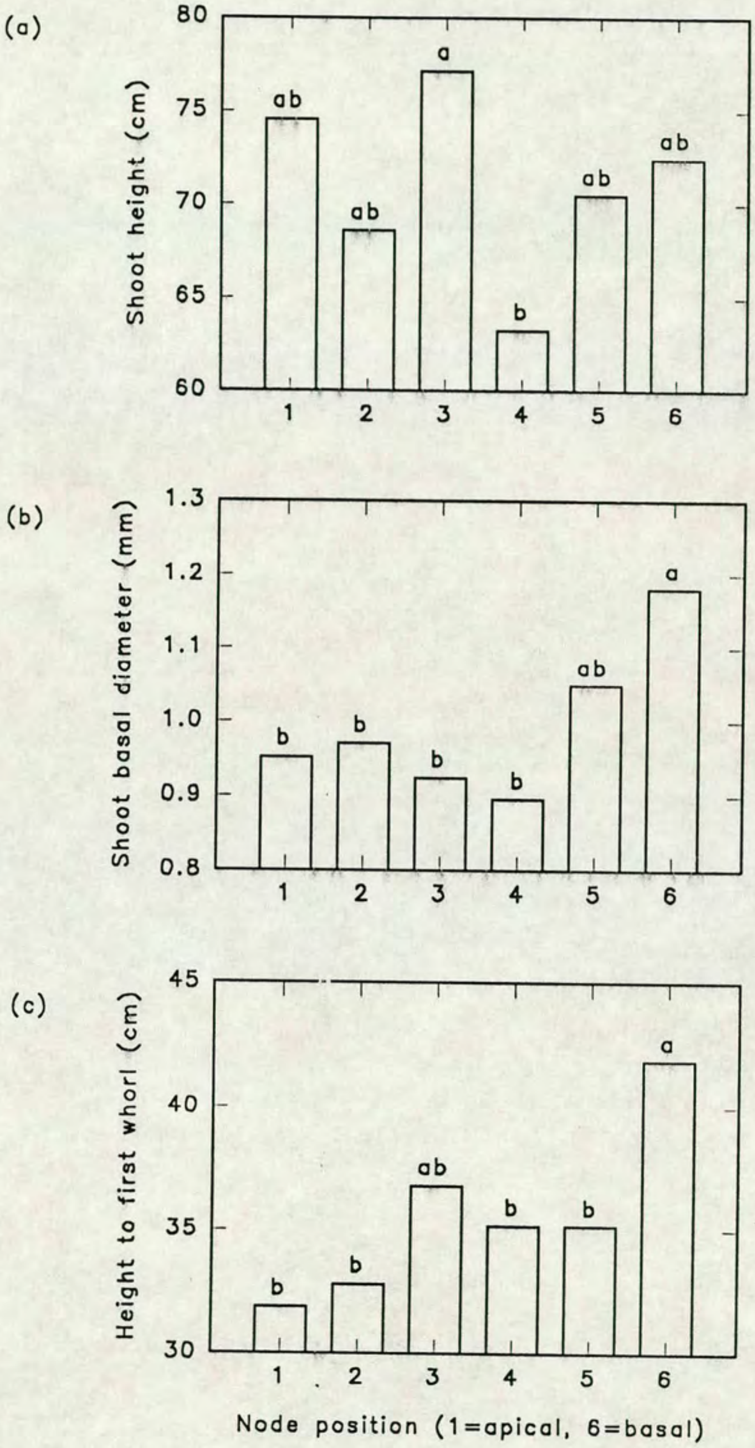


Fig. 6.9a,b,c The effect of node position within the stem - apical (1) to basal (6) - on **(a)** the total height, **(b)** the basal diameter and **(c)** the height to the first whorl of branches produced by the shoot of rooted cuttings of *Cordia alliodora* from three clones, after six months growth in the nursery. N = 45; means grouped by the same letter are not significantly different ($t_{0.05}$).

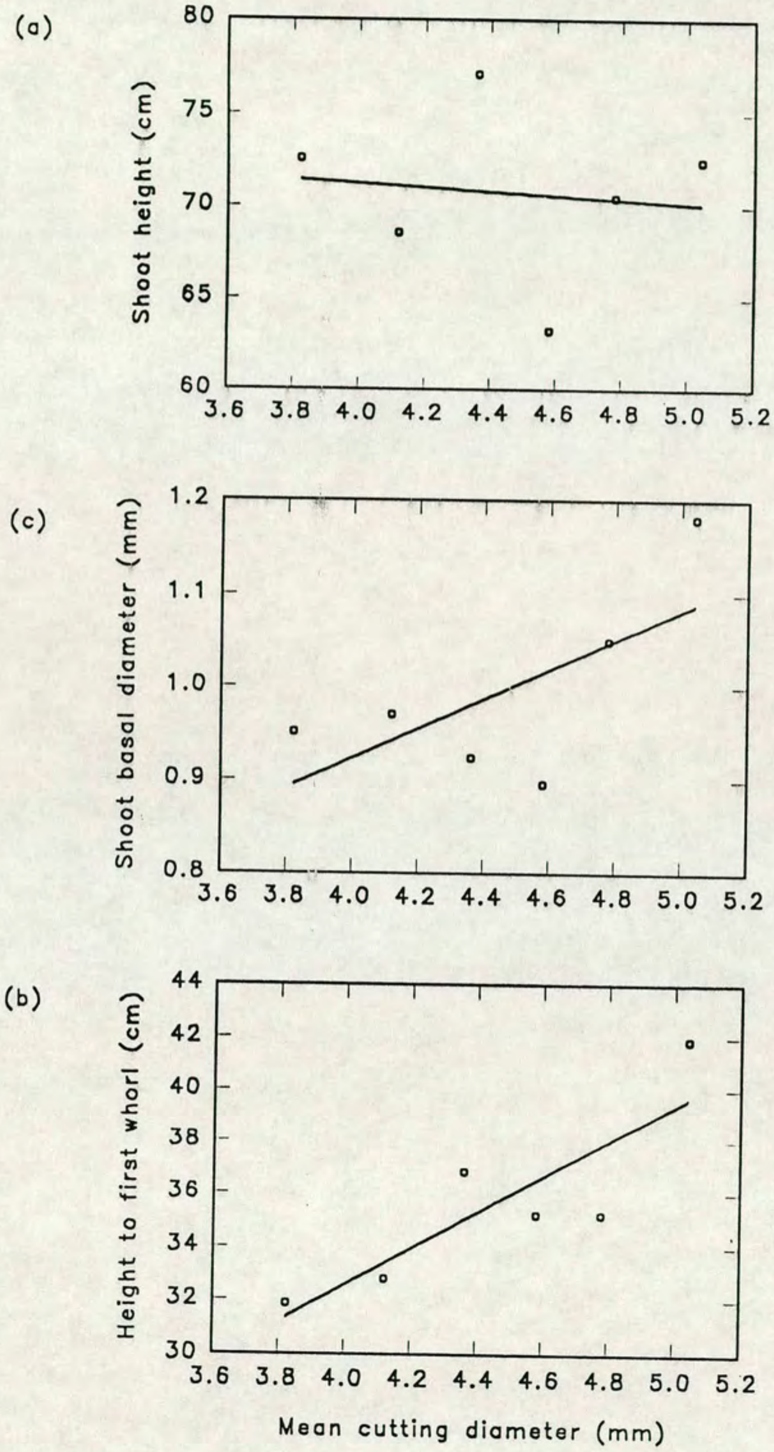


Fig. 6.10a,b,c The relationships between mean cutting diameter and **(a)** shoot height ($y = 75.402 - 1.052\ x$; $r^2 = 0.10$), **(b)** shoot basal diameter ($y = 0.289 + 0.159\ x$; $r^2 = 0.68$) and **(c)** height to the first whorl of branches ($y = 5.392 + 6.796\ x$; $r^2 = 0.85$) of plants of *Cordia alliodora* from rooted cuttings after six months growth in the nursery.

Larger variations were found between clones for the three variables evaluated (Tables 26, 27 and 28). Clone 2 showed a mean height of 42.5 cm, followed by clone 6 (33.6 cm) and clone 37 (30.1 cm) (Fig. 6.11a). Clone 6 showed the largest basal diameter (5.0 mm), clone 2 was intermediate (4.7 mm), and clone 37 again showed the smallest value (4.2 mm) (Fig. 6.11b). There were no significant differences between clones 6 and 37 for height to the first whorl of branches (28.9 cm and 28.0 cm, respectively), while clone 2 showed a significantly ($p < 0.001$) higher value (37.4 cm) for this variable (Fig. 6.11c).

After 10 months growth in the nursery, no particular trends were found between total height and basal diameter in relation to node position (Fig. 6.12a,b), and the correlations with cutting diameter disappeared ($r^2 = 0.05$ and 0.35 respectively). The effect of node position on height to the first whorl remained constant, as expected (Fig. 6.12c), with a similar correlation with cutting diameter ($y = -13.66 + 12.00\ x$; $r^2 = 0.92$ - not shown).

Clones showed a similar trend to that at six months for total shoot height (Fig. 6.13a) and height to the first whorl of branches (Fig. 6.13c), with clone 2 showing significantly larger values for these variables. There was a change in ranking for basal diameter, with clone 2 again showing the largest value (Fig. 6.13b).

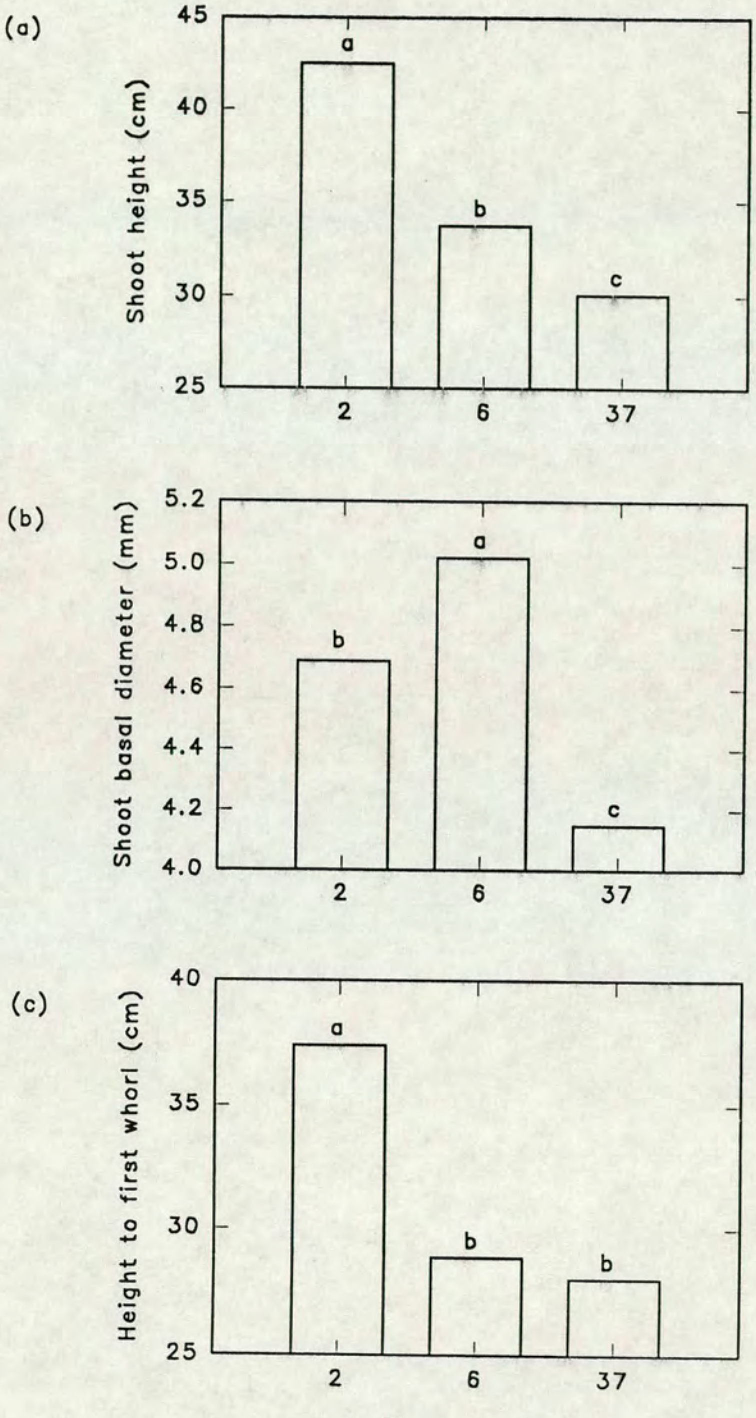


Fig. 6.11a,b,c Clonal variation in **(a)** the total height **(b)** the basal diameter and **(c)** the height to the first whorl of branches of shoots produced by rooted cuttings of *Cordia alliodora* from six node positions within the stem, after three months growth in the nursery. N = 90; means grouped by the same letter are not significantly different ($t_{0.05}$).

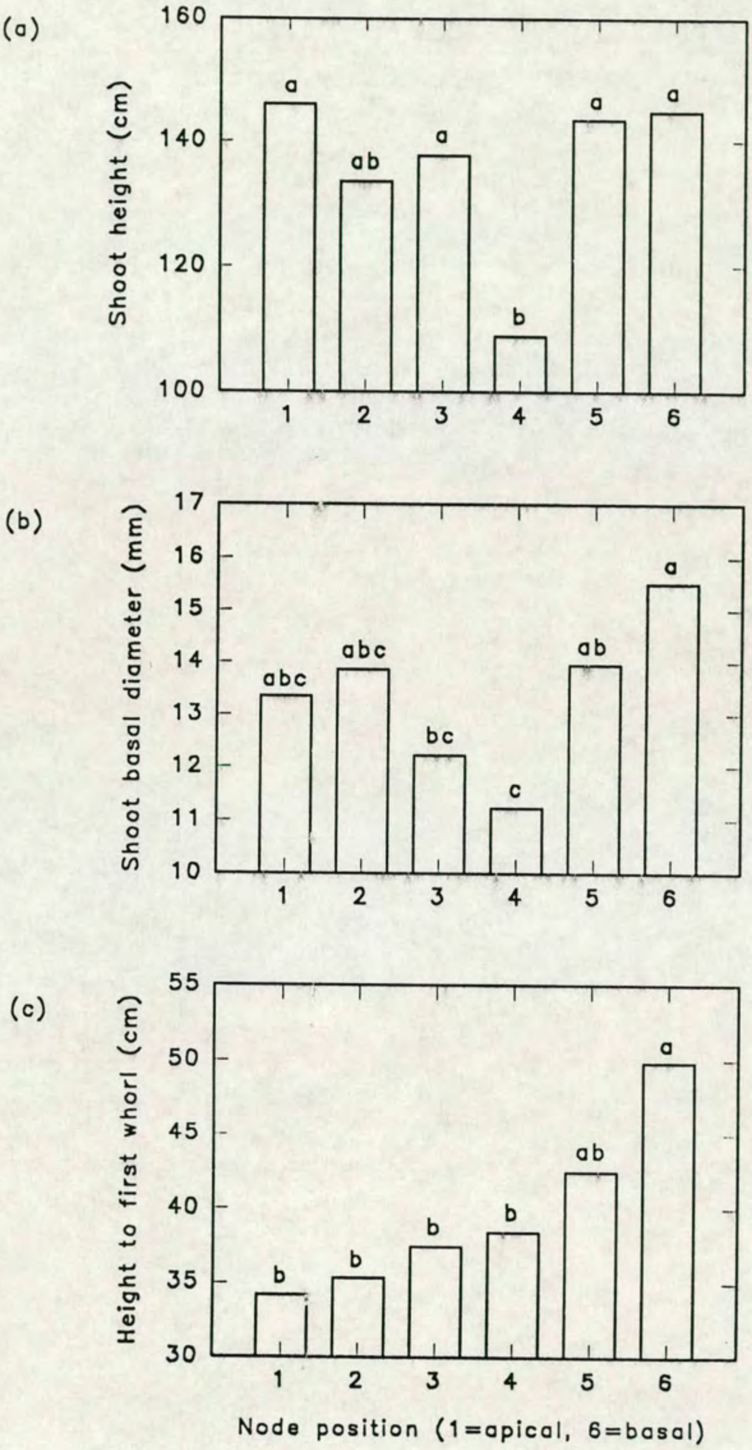


Fig. 6.12a,b,c The effect of node position within the stem - apical (1) to basal (6) - on (a) the total height, (b) the basal diameter and (c) the height to the first whorl of branches produced by the shoot of rooted cuttings of *Cordia alliodora* from three clones, after 10 months growth in the nursery. N = 45; means grouped by the same letter are not significantly different ($t_{0.05}$).

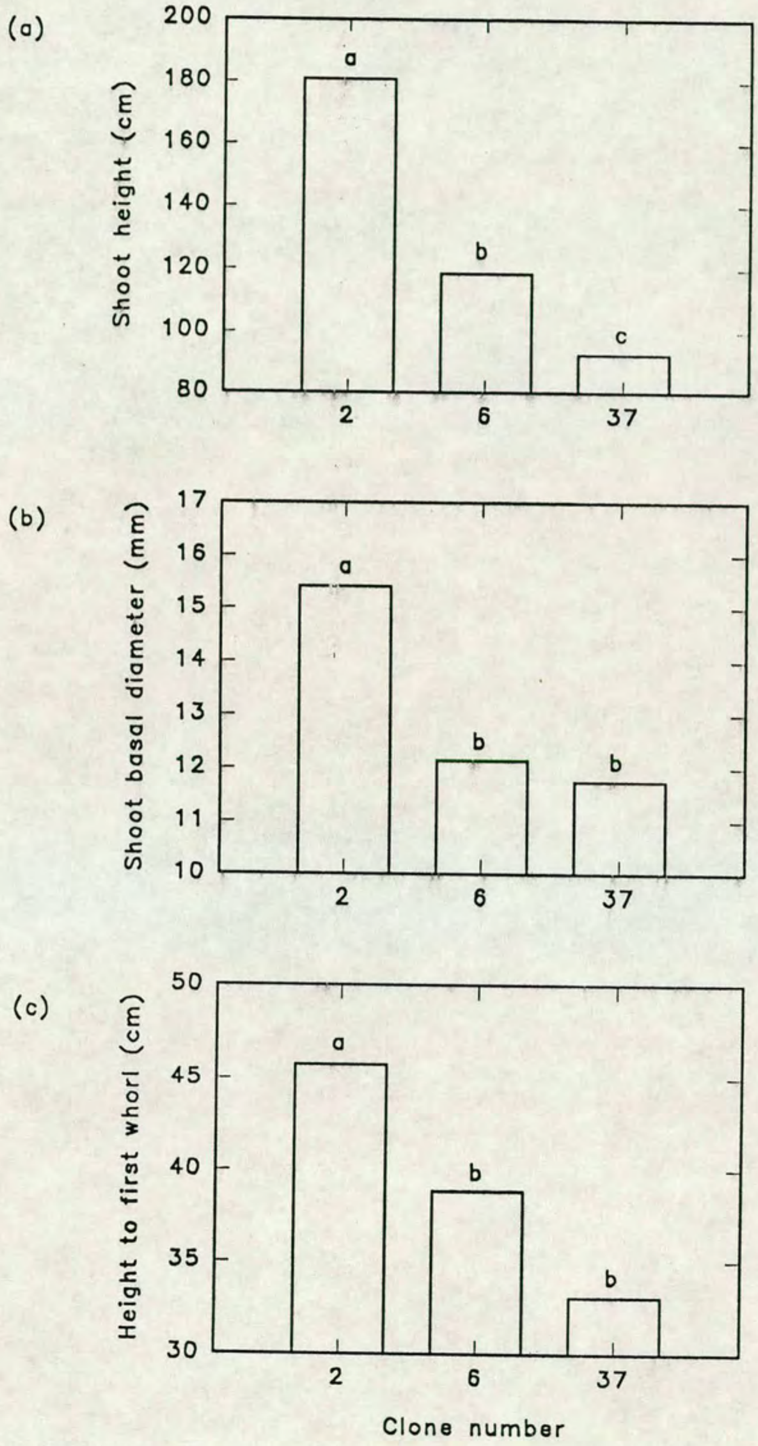


Fig. 6.13a,b,c Clonal variation in **(a)** the total height; **(b)** the basal diameter; and **(c)** the height to the first whorl of branches of shoots produced by rooted cuttings of *Cordia alliodora* from six node positions within the stem, after 10 months growth in the nursery. N = 90; means grouped by the same letter are not significantly different ($t_{0.05}$).

DISCUSSION

It has been known for many years that rooting ability may vary between cuttings from different parts of the same plant (Hartmann and Kester 1983; Leakey 1983; Leakey and Mohammed 1985; Lo 1985). This has been attributed to gradients in chemical composition along the shoot, including plant growth regulators and rooting co-factors (Hartmann and Kester 1983; Leakey *et al.* 1993); shoot morphology (Leakey 1983; Leakey and Mohammed 1985; Leakey *et al.* 1993); starch and sugar content (Leakey and Coutts 1989) and nutrient content (Tchoundjeu 1989). There are also gradients in leaf water potential (Leakey 1983), photosynthetic rate and stomatal conductance (Wilson *et al.* 1989) and mutual leaf shedding, affecting the amount and quality of light received by the leaves (Leakey and Storeton-West 1992). In woody cuttings, normally the basal portions of the shoot root more readily than terminal sections. This has been attributed to the accumulation of carbohydrates at the base of the shoot and to the increasing number of preformed root initials from the tip to the base of the shoot (Hartmann and Kester 1983). In succulent cuttings the situation is normally the reverse, with apical cuttings rooting better than basal ones (Hartmann and Kester 1983; Leakey 1983). In this case, the higher rooting of these cuttings has been explained by the possibility of a higher concentration of endogenous root-promoting substances arising in the terminal sections; and also to the presence of a larger number of cells capable of becoming meristematic (Hartmann and Kester 1983). In *Triplochiton scleroxylon*, Leakey (1983) suggested that the low rooting of lower nodes was partly the result of cutting mortality due to leaf shedding in such cuttings, reflecting the greater internal water deficits and perhaps the lower photosynthetic efficiency of the older leaves at lower nodes. Additionally, cuttings taken from the upper parts of the stem usually had longer internodes, which has been positively related to rooting ability of many species (Hoad and Leakey 1992; Leakey 1983; Leakey *et al.* 1992; 1993; Poulsen and Andersen 1980; Veierskov 1978). In many species it has been found that the effect of cutting position within the shoot on internode length and diameter is probably the most important factor influencing rooting ability (Leakey *et al.* 1993) and the number of roots produced by the cuttings in particular (Fernquist 1966; Poulsen and Andersen 1980; Veierskov 1978). In fact, numbers of roots was found to be strongly correlated with cutting diameter in previous experiments with *Cordia alliodora* (see Chapter 4). In the present experiment, however, no clear patterns were found between node positions in terms of rooting percentage and number of roots produced by the cuttings, nor in their rates of net photosynthesis and stomatal conductance.

Lack of differences in rooting ability between node positions has been found in softwood cuttings of many species, in which satisfactory rooting has usually been obtained regardless of the position of the cutting on the shoot, especially when internode lengths are fairly constant (Hartmann and Kester 1983). In *T. scleroxylon*, when stockplants were decapitated and the shoots formed were grown under the same light environment, the differences in rooting ability between node positions were eliminated, and in fact, lower cuttings showed higher rooting (Leakey 1983). In the same study, the percentage rooting of cuttings from the lateral shoots of decapitated plants was significantly greater than that of mainstem cuttings of undecapitated plants, and on average, cuttings from twin-shooted plants showed a higher rooting than those from single-shooted stockplants. The effect of cutting position on rooting was also reduced when similar length cuttings were used (Leakey and Mohammed 1985).

A combination of all these factors may be the reason for the lack of differences between node positions found in this study. As explained above, the stockplants used in this study were cut to a standard length and only three shoots were allowed to develop, a situation resembling that described above for *T. scleroxylon*. Cuttings were collected when the shoots had reached a height of approximately 25 cm, probably before the development of any significant variation in the light environment of the cuttings. This uniformity in growing environment probably contributed to the lack of systematic differences in rooting ability of cuttings from different positions on the stem. The reported effects of node position on leaf shedding and on gradients of lignification, nutrient content, growth regulators, etc., were probably minimal in the relatively short shoots used in this study. The absence of correlations between cutting diameter and number of roots, as found in other experiments with this species, was probably due to the lack of a systematic variation in cutting length, which may have cancelled out any possible trend in cutting volume. This was probably enhanced by the low rooting observed in most clones, which showed an interaction with node position but again, without any specific trend.

An interesting situation was found when cuttings from different positions were potted and evaluated for total height, basal diameter and height to the first whorl of branches. After three months growth, all three variables roughly increased from the apical to the basal nodes, showing a positive correlation with cutting diameter. The effects of these nongenetic factors, called *C*-effects (Lerner 1958) or more specifically *m*-effects, when referring to characteristics of individual propagules (Burdon and Shelbourne 1974), have been shown to influence the rooting ability of cuttings, but there is little information concerning their effects on the subsequent development of the ramet. In this study, node position, presumably through its effects on cutting diameter and nutrient storage, affected the initial height and diameter of shoots originated from cuttings. It is not clear why the height to the first whorl was also affected. In a given organism, although all cells share the same set of genes, not all are active at the same time, which leads to the formation of different organs and functions (Zobel and Talbert 1984). This is shown, for instance, when propagules from lateral shoots of some species produce only branches spreading along the ground, while cuttings from upright-growing shoots produce the desired vertical plants (Hartmann and Kester 1983). In this regard, it is interesting to speculate about the possibility that each individual internode maintains the same role previously defined within the whole plant, perhaps due in some way to endogenous levels of growth regulators along a gradient related to correlative inhibition. *C. alliodora* produces branches in tiers along the stem, and cuttings were always collected before the formation of the first tier. Therefore, cuttings from apical internodes, being closer to the first tier of branches within the plant, would regenerate such structure at a lower height than cuttings from basal nodes.

After six months growth of the plants, the initial effect of node position on total height and basal diameter was eliminated, as more factors which normally influence growth, such as soil fertility, type of root system, biomass production, availability of water, etc., started to play a greater role. This was reflected by the relationship between these two variables and cutting diameter, which became weaker after six months and disappeared after ten months. The height to the first whorl of branches, being a fixed characteristic, remained constant. This loss of significant growth differences attributable to C-effects, after a period of field growth has also been reported for *Triplochiton scleroxylon* (Howland and Bowen 1977).

It will be interesting to continue the study to the adult stage of the tree, since the formation of more branches per meter of mainstem could have a negative effect on growth, i.e. more energy is used for production of branches at the expense of mainstem growth. This was the case for instance in *T. scleroxylon*, in which slow growing clones often had a high branching frequency (Ladipo 1981; Ladipo *et al.* 1983; 1991a,b; 1992; Leakey 1986; Leakey and Ladipo 1987). If similar relationships exist for *C. alliodora*, predictive tests could be developed to allow the selection of highly productive clones at a very early age.

CHAPTER 7

The effects of rooting media on the physiology of rooting of single-node, leafy stem cuttings of *Cordia alliodora*

MATERIALS AND METHODS

Plant material and experimental design

This experiment was carried out at the CATIE nursery, Turrialba, Costa Rica, using stockplants of clones 19, 22 and 35. The bed of a non-mist propagator was subdivided in 12 compartments, and each one assigned randomly to one of three rooting media - fine sand, gravel or sawdust. This poses restrictions to the anlaysis, since there are no true blocks, but this problem is difficult to overcome for practical reasons. The random location of rooting media to four different compartments at least prevents a single medium to be associated with a particular section of the propagator.

When the shoots had grown to a height of 30-40 cm, the soft apical tip of each shoot was discarded, and 360 cuttings were taken down the stem, 120 from each clone, to a standard length of 5 cm. Leaves were trimmed to 30 cm^2 using paper templates and the cutting base treated with indole-3-butyric acid at a concentration of 1.6%. Cuttings from each clone were mixed and then assigned randomly to each compartment in the propagator, so that each compartment had 30 cuttings, 10 from each clone. During the course of the experiment, cuttings were sprayed with water from a hand-held sprayer to keep the leaves moist.

Assessments

The propagation microclimate

The environmental conditions inside the propagators were monitored during the duration of the experiment using a 21X Micrologger (Campbell Scientific Ltd., Loughborough, England) and associated sensors, as described in Chapter 2. Single relative humidity, air temperature and irradiance sensors were placed in a central position inside the propagator, while different substrate temperature and leaf temperature sensors were used for each medium.

Composition of rooting media

To determine the relative proportion of solids, air and water in the rooting media, three 100 cc samples of each medium were taken. The air volume in the media was determined by adding water to the sample and measuring the amount of water required to saturate the air spaces. The water content was determined by the difference between the mass of wet media and their dry mass, after drying in an oven for 24 hours. The results were expressed as percentage of each component by volume.

Rooting ability

After one week the cutting diameter at a midpoint was measured, and each cutting lifted and assessed for number of roots. Similar assessments were carried out for the next five consecutive weeks.

Chlorophyll fluorescence

Measurements of chlorophyll fluorescence were taken at weeks 1, 2 and 3, in a sample of six cuttings from each medium, using a Plant Stress Meter (PSM Mark II, Bio Monitor S.C.I. AB, Sweden), pre-set at a light level of 400 μmol photons m^{-2} s^{-1} and a run-time of 10 s; the dark adaptation period of the leaves was 15 min.

Net photosynthetic rate and stomatal conductance

Measurements of net photosynthetic rate (P_n), stomatal conductance (g_s) and chlorophyll fluorescence were taken at week 1, 2 and 3 in a randomly selected sample of six cuttings from each medium. For these assessments, an infra-red gas analyser was used (LCA-3, Analytical Development Co. Ltd., Hoddesdon, U.K.). Within the propagators, a subdivision next to the cuttings was not filled with substrate, so that the gas analyser leaf chamber was placed at a lower level and allowed readings without removing the cuttings from their medium.

Analysis

In this experiment there are no true blocks, which poses a limitation for the statistical analysis. This problem, however, is difficult to overcome practically. One possible solution is to replicate using many propagators, but in addition to practical restrictions, this design would create other problems, mainly those associated with variation between propagators due to their location within the propagation area. Therefore, the selected design was considered the best compromise between practical restrictions and statistical efficiency. In the analysis of variance, percentages were calculated by rooting medium-clone combination, based on 40 cuttings, followed by Fisher's t tests (LSD). Data of percentage rooting used in the analysis were transformed by the formula arcsin $\sqrt{\%}$.

Analyses of deviance for stepwise regression and analyses of variance in Genstat 5 (Payne *et al.* 1987) were utilized to determine the influence of medium and morphological characteristics of the cuttings on their rooting ability. To determine differences between treatment means, analyses of variance were used, followed by Fisher's *t* tests. Similar analyses were carried out on data of chlorophyll fluorescence.

RESULTS

The propagation microclimate

Irradiance showed a maximum value of 335 μmol m^{-2} s^{-1}, with a mean of 22 μmol m^{-2} s^{-1}; relative humidity varied between 78 and 100%, with a mean of 97%, and air temperature varied between 20.7 °C and 37.7 C, with a mean of 24.2 °C (Table 7.1). Small differences were found between substrate temperatures; gravel showed the lowest mean temperature (22.9 °C), followed by sawdust (23.2 °C) and sand (24.3 °C). Leaf temperatures were slightly higher, from 24.3 °C (sawdust) to 25.6 °C (sand) and 25.8 °C (gravel). The largest differences were found in leaf-to-air vapour pressure deficits (VPD), with values of -2.06 kPa to 0.61 kPa for sawdust (mean 0.15 kPa), 0.34 kPa to 2.21 kPa for sand (mean 0.57 kPa) and -0.66 kPa to 6.58 kPa for gravel (mean 0.66 kPa) (Table 7.1).

When values of PAR were correlated with VPD, strong correlations were found (Fig. 7.1).

Table 7.1 The propagator microclimate after insertion of *Cordia alliodora* cuttings in three different rotting media in non-mist propagators.

	Sawdust		Sand		Gravel	
	Mean	Range	Mean	Range	Mean	Range
Relative humidity (%)	97	78-100	97	78-100	97	78-100
Air temperature (°C)	24.2	20.7-37.7	24.2	20.7-37.7	24.2	20.7-37.7
Substrate temperature (°C)	23.2	18.3-32.1	24.3	21.7-31.7	22.9	21.2-26.5
PAR (μmol m^{-2} s^{-1})	22	0-335	22	0-335	22	0-335
Leaf temperature (°C)	24.3	22.0-31.4	25.6	22.2-43.1	25.8	22.2-43.3
VPD (kPa)	0.15	-2.06-0.61	0.57	0.34-2.21	0.66	-0.66-6.58

Composition of the rooting media

The three rooting media showed clear differences in their relative proportions of solids, water and air. The water component was higher in sawdust (53.8 %), to decrease drastically in sand (17.6 %) and gravel (4.5 %). The air content was similar in sawdust (30.3 %) and gravel (30.5 %), and relatively low (5.5 %) in sand (Fig. 7.2).

Net photosynthetic rate (P_n) and stomatal conductance (g_s)

Photosynthetic rates of 1.67 and 3.41 μmol CO_2 m^{-2} s^{-1} were recorded in the cuttings during the first three weeks in the propagators. On average, values were higher in sand (2.69 μmol CO_2 m^{-2} s^{-1}), followed by gravel (2.48 μmol CO_2 m^{-2} s^{-1}) and sawdust (2.41 μmol CO_2 m^{-2} s^{-1}) (Fig. 7.3a).

Similar values of mean stomatal conductance were recorded in cuttings in sand (127.03 mmol H_2O m^{-2} s^{-1}) and gravel (127.92 mmol H_2O m^{-2} s^{-1}), significantly higher than those in sawdust (100.62 mmol H_2O m^{-2} s^{-1}) (Fig. 7.3b).

When values of PAR were correlated with P_n, a similar pattern was found in all rooting media (Fig. 7.4a,b,c).

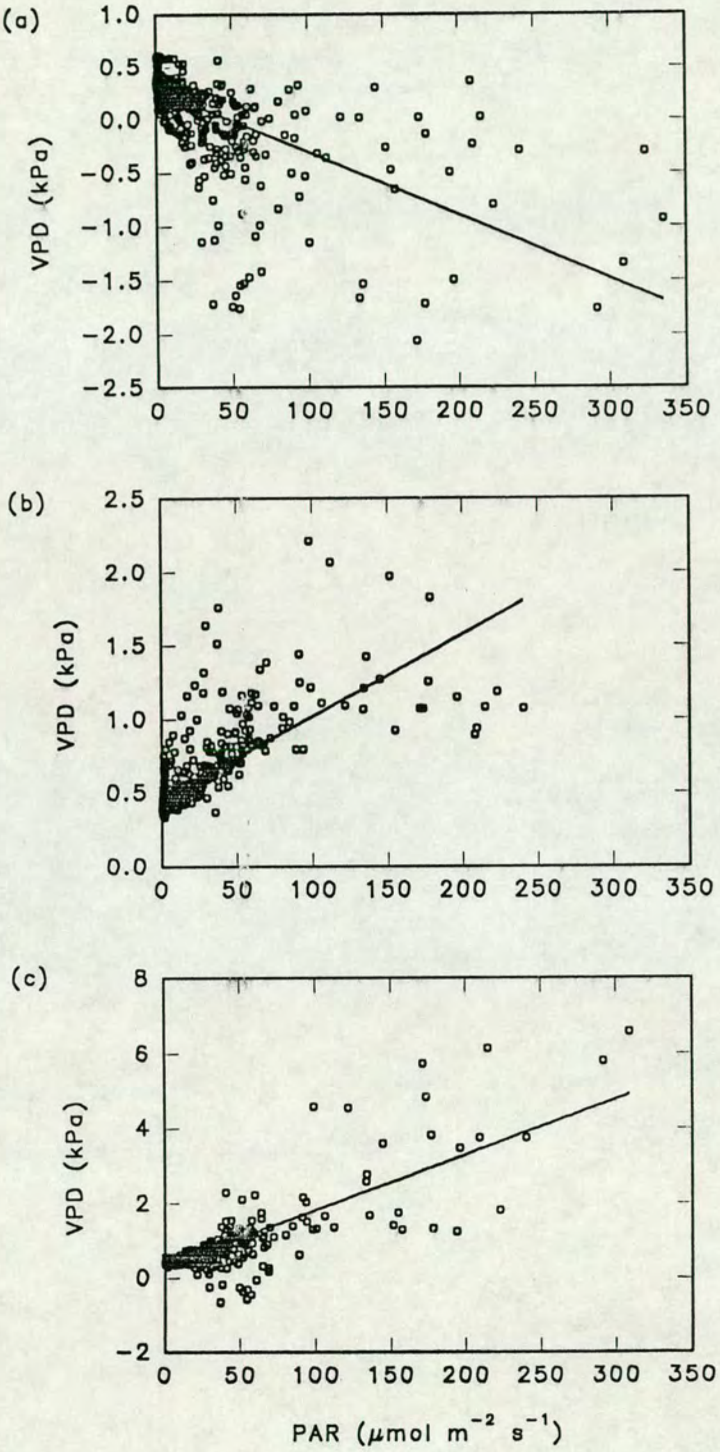


Fig. 7.1a,b,c The relationship between leaf-to-air vapour pressure deficit (VPD) and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators in **(a)** sawdust ($y = 0.280 - 0.006\ x$; $r^2 = 0.63$), **(b)** sand ($y = 0.464 + 0.006\ x$; $r^2 = 0.75$) and **(c)** gravel ($y = 0.353 + 0.015\ x$; $r^2 = 0.82$).

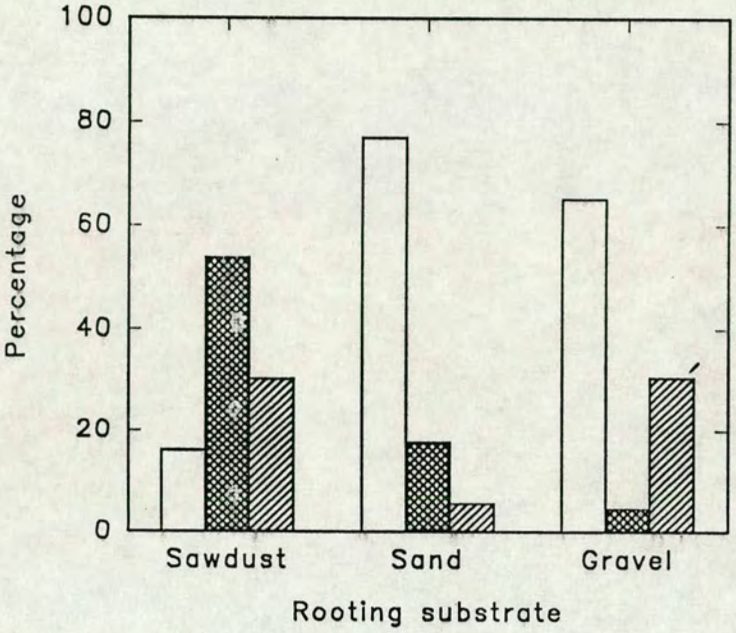


Fig. 7.2 The relative percentage by volume of solids (open bars), water (crosshatched bars) and air (simple hatched bars) in three different rooting substrates.

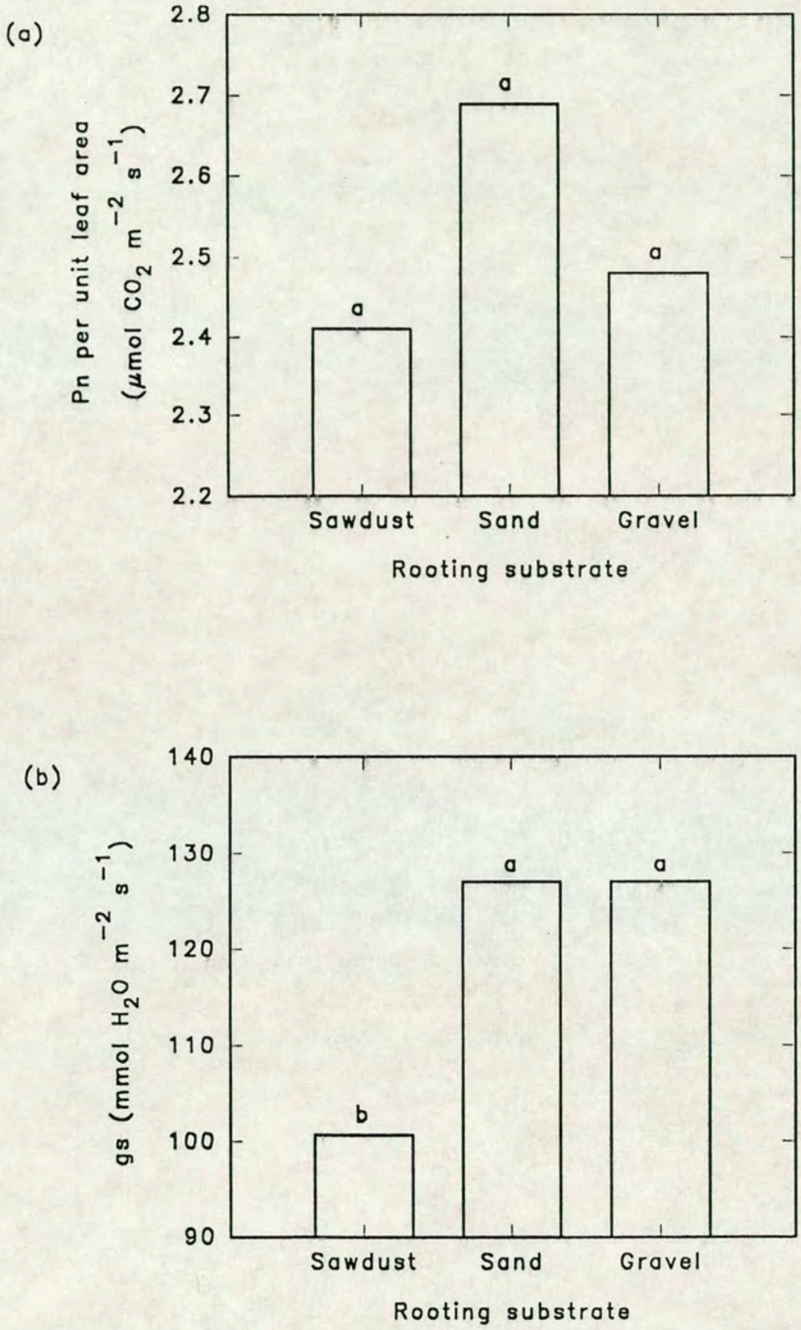


Fig. 7.3a,b The effect of rooting substrate on **(a)** the mean net photosynthetic rate (P_n) and **(b)** the mean stomatal conductance (g_s) of single-node, leafy stem cuttings of *Cordia alliodora* set in non-mist propagators.

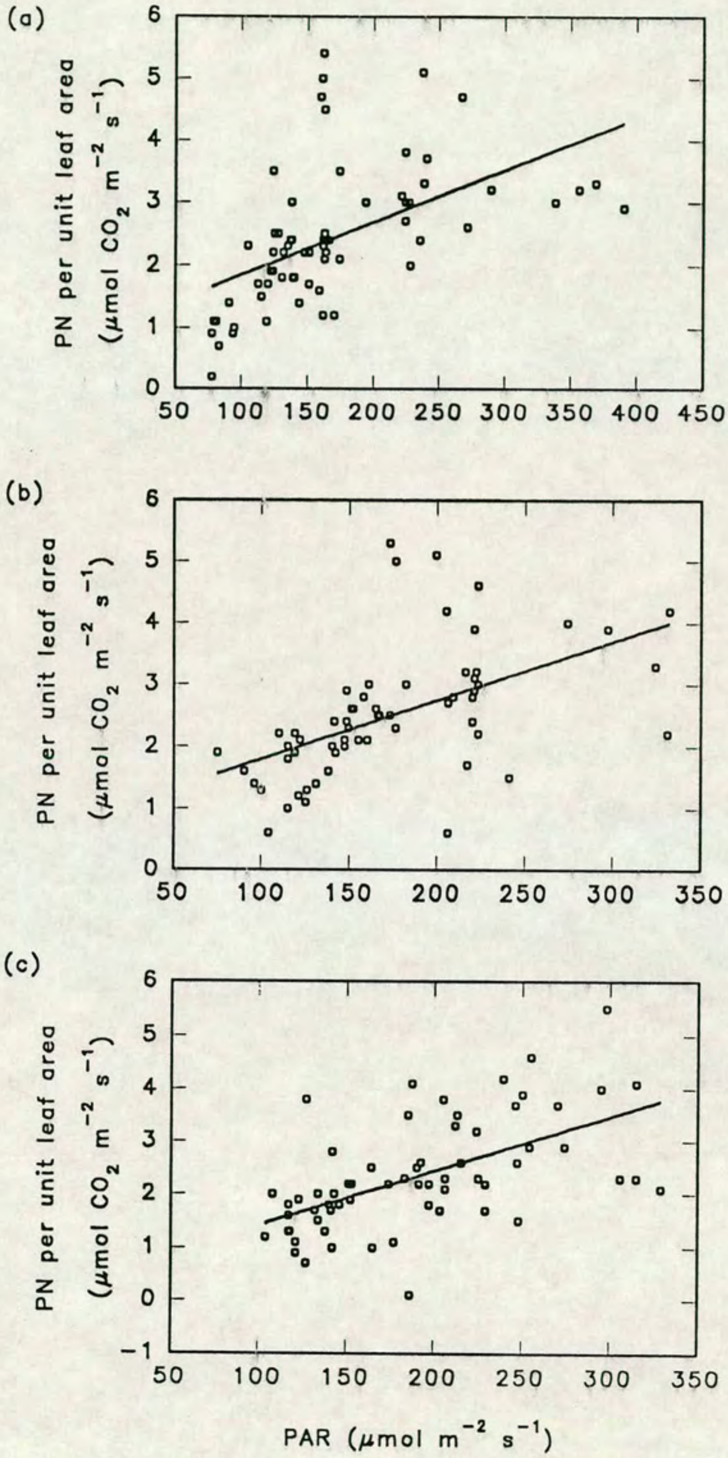


Fig. 7.4a,b,c The relationship between net photosynthesis per unit leaf area and photosynthetic active radiation (PAR) in single-node, leafy stem cuttings of *Cordia alliodora* set to root in non-mist propagators with **(a)** sawdust ($y = 1.009 + 0.008\,x$; $r^2 = 0.54$), **(b)** sand ($y = 0.851 + 0.010\,x$; $r^2 = 0.53$) and **(c)** gravel ($y = 0.392 + 0.010\,x$; $r^2 = 0.58$).

Rooting ability

Rooting percentages were generally high in this experiment, with values over 75% for all media. However, highly significant differences ($p < 0.01$) were found between rooting media at the end of six weeks (Table A29). Rooting percentage was similar in both gravel (89.2%) and sand (87.5%), significantly higher than sawdust (75.8%) (Fig. 7.5a). A similar pattern was found for number of roots per rooted cutting (Table A30), with means of 3.3, 7.5 and 6.8 for sawdust, sand and gravel respectively (Fig. 7.5b).

Clones also showed significant differences in rooting percentage; clones 19 and 22 rooted equally well (97.5% and 90.8%), while clone 35 showed the lowest rooting percentage (64.2%) (Fig. 7.6a). A similar ranking was found for number of roots per rooted cutting, with means of 5.8, 7.3 and 4.6 for clones 19, 22 and 35 respectively (Fig. 7.6b).

When the results were analysed by stepwise regression to include values of cutting diameter, rooting was found to be highly dependent on clone and rooting medium, as shown in the analysis of variance, but it was not significantly affected by the cutting diameter (Table A31).

Chlorophyll fluorescence

Cuttings in the three media showed little variation in the chlorophyll fluorescence ratio (*Fv/Fm*) during the course of the experiment, with mean values of between 0.740 and 0.82. *Fv/Fm* was always significantly higher in sawdust, with no significant differences between gravel and sand (Fig. 7.7).

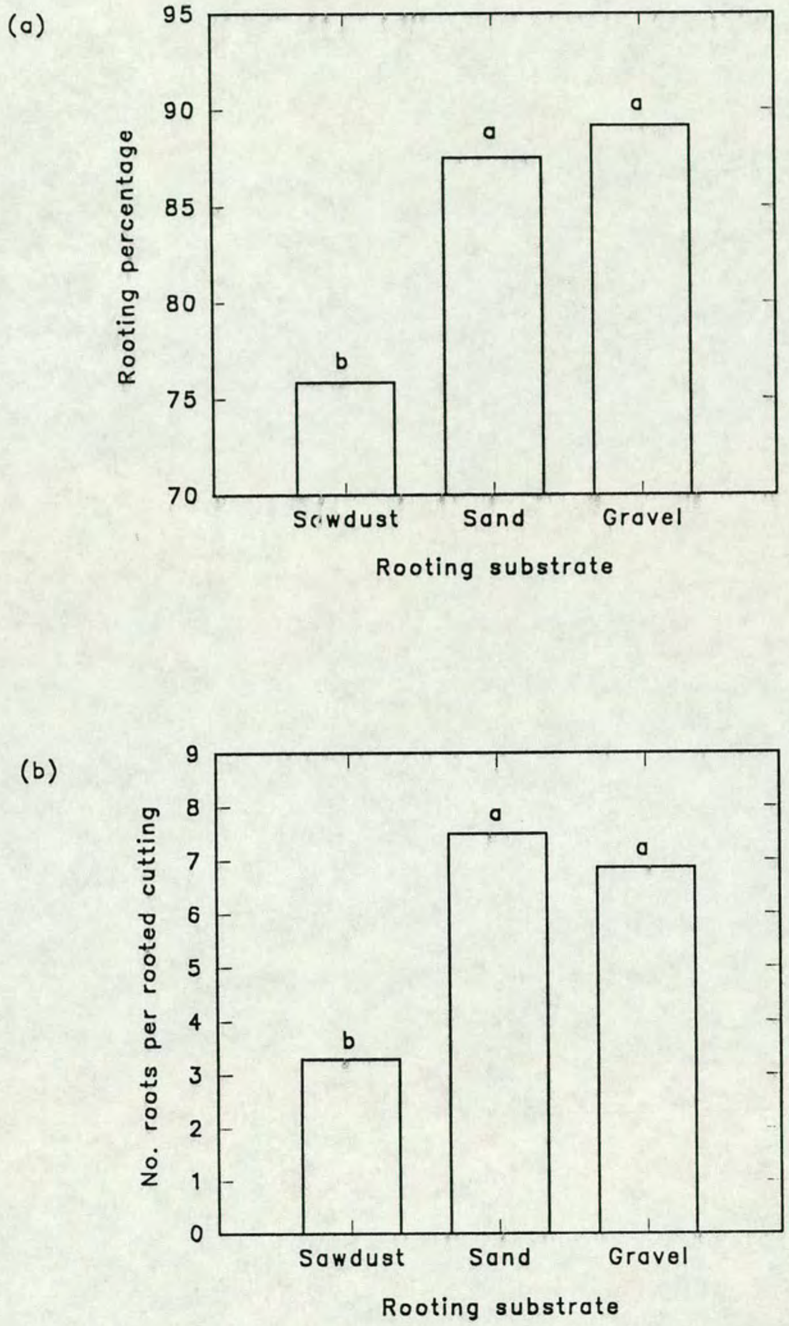


Fig. 7.5a,b The effects of three different rooting media on **(a)** the rooting percentage and **(b)** the number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora*, after six weeks in a non-mist propagator. N = 120; means grouped by the same letter are not significantly different ($t_{0.05}$).

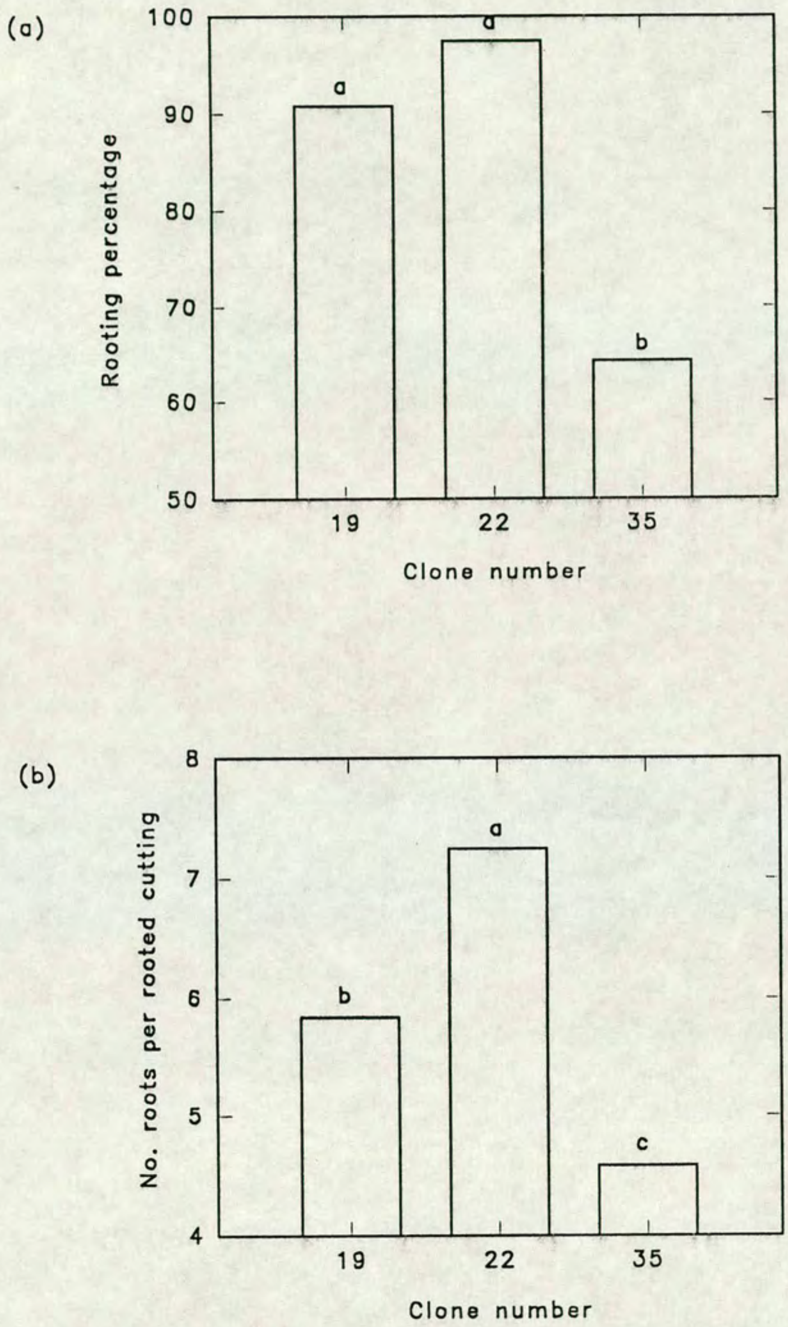


Fig. 7.6a,b Clonal variation in (a) the rooting percentage and (b) the number of roots per rooted cutting of single-node, leafy stem cuttings of *Cordia alliodora* after six weeks on three different rooting media in a non-mist propagator. N = 120; means grouped by the same letter are not significantly different ($t_{0.05}$).

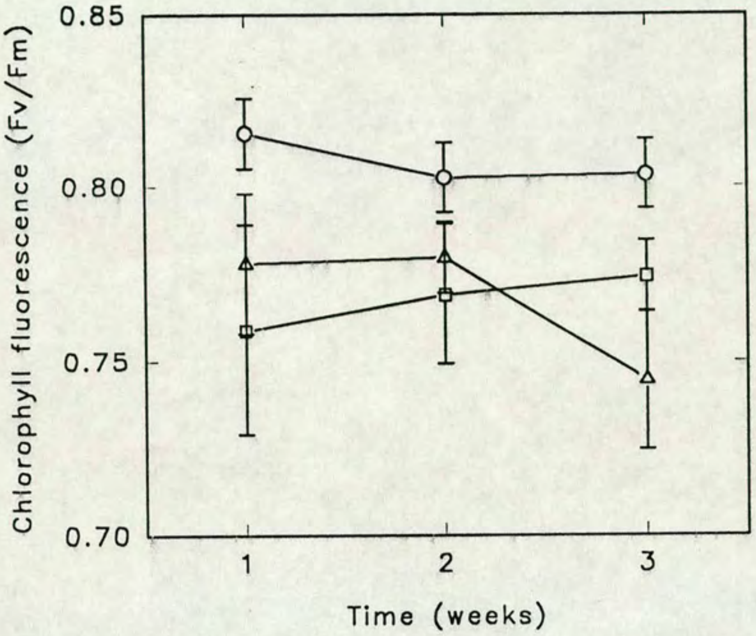


Fig. 7.7 The effects of three different rooting media - sawdust (circles), sand (triangles) and gravel (squares) on the chlorophyll fluorescence ratio (*Fv/Fm*) of single-node, leafy stem cuttings of *Cordia alliodora* during the first three weeks in a non-mist propagator. N = 18; bars = ± standard error of the means.

DISCUSSION

The importance of the rooting medium in propagation has been recognized for a long time (Andersen 1986; Hartmann and Kester 1983). In general, an appropriate substrate is described as one with an optimal volume of gas-filled porespace, an oxygen diffusion rate adequate for the needs of respiration, and a reasonable water retention capacity (Andersen 1986). Early experiments with *Hedera helix* showed that available oxygen in the rooting substrate is essential for root development (Hartmann and Kester 1983). It is also clear that the provision of sufficient water to prevent wilting is a prime requirement (Loach 1986). It is only occasionally, however, that correlations between air or water content and rooting have been found, and in most cases, they have not been reproducible in repeated propagations (Loach 1986). In any case, since the percent air and water are inversely correlated for any one medium, it is not possible to say whether high air content or low water content promoted better rooting (Loach 1986).

In the present study, rooting percentage was lower when sawdust was used, while a significantly higher rooting percentage was found in both sand and gravel. It is interesting that the main difference in composition between sawdust and gravel was the higher proportion of water in the sawdust (at the expense of solids), while air content was almost identical in both media (Fig. 7.2). On the other hand, sand and gravel showed a lower water content (less than 18%) than sawdust (more than 50%). The fact that rooting in sand and gravel was not significantly different seems to indicate that the proportion of water in the media was the main substrate-related factor determining rooting ability of cuttings in this study. The air:water ratio did not seem to have any relationship with rooting ability; while rooting was similar in sand and gravel, the air:water ratio was more similar between sand and sawdust (0.31 and 0.56 respectivley) than between sand and gravel (0.31 and 6.78 respectively).

Measurements of the rates of water uptake by cuttings placed in media with increasing volumetric water content have shown a simple linear relationship (Loach 1986). The same relationship has been found in *C. alliodora* (Newton *et al.* 1991). This indicates that water uptake of cuttings is restricted in media with a low water content, and therefore, cuttings would be more subject to drought (Newton *et al.* 1991). However, the high rooting percentages obtained in sand and gravel in this study suggests that excess water in the medium is not always beneficial. Poor rooting has been often associated with a large gain of water by the cuttings themselves, most notably in the basal portion; this was evident in an experiment with *Chamaecyparis lawsoniana* cuttings, in which rooting decreased from 70% to 30% with increases in water content of basal portion of the cuttings from 3 mg g^{-1} day^{-1} to 9 mg g^{-1} day^{-1} (Loach 1986; 1988b). This was explained by the fact that water, in both the rooting medium and within the base of the cutting, can present a major diffusion barrier, since diffusion of oxygen through water is 10.000 times slower than in air (Loach 1986). This may result in anoxia within the tissues, which may cause damage to waterlogged cutting bases (Loach 1986). Rotting of cutting bases in sawdust was often observed in the present study. The reduced water absorption through the cutting base may also explain the reduced transpiration rate showed by cuttings in sawdust (Fig. 7.3b). It is known that cuttings respond very rapidly to water deficits by closing stomata (Loach 1988b). The importance of water absorption through the cutting base to keep pace with transpirational losses has been shown in a range of species by Grange and Loach (1983b).

Cuttings in sawdust also produced significantly fewer roots per rooted cutting than cuttings in sand and gravel. This was also probably related to the lack of available oxygen at the cutting bases. The effect of oxygen has been well illustrated in hydroponic systems; with *Chrysanthemum* cuttings, for instance, root production increased markedly as the water in which they were rooted was aerated with increasing amounts of oxygen, from 0 to 21 percent (Hartmann and Kester 1983). A similar response was found with *Cupressocyparis leylandii* cuttings in a 1:1 peat/perlite medium, with an increase in number of roots as the volumetric air content of the medium increased from 25% to 40% (Loach 1986).

In organic compounds, such as some sources of bark, a negative effect on rooting has been observed due to the presence of toxins (Loach 1988a). Although this effect was not investigated in the present study, it is unlikely that such effect was the responsible for the lower rooting found in sawdust, since the same source of sawdust has been used in many other species (e.g. *Bombacopsis quinata*, *Eucalyptus* spp.) with good results (Mesén *et al.* 1992).

When analysing photosynthetic relations and chlorophyll fluorescence in cuttings, it became evident once again the complex nature of the rooting process and the importance of optimizing all factors involved, as described by Leakey *et al.* (1993). While some factors operate primarily in the leaf (e.g. photosynthesis, transpiration), others are generally of greatest importance in the cutting base. The results obtained in this study indicate clearly that optimizing the processes that occur in the leaf does not necessarily result in high rooting percentages, if other processes that occur in the stem are limiting. In this regard, while rooting percentage and number of roots per cutting were significantly lower in sawdust, the rooting medium utilized did not affect the water content of basal portion of the cuttings from 3 mg g^{-1} day^{-1} to 9 mg g^{-1} day^{-1} (Loach 1986; 1988b). This was explained by the fact that water, in both the rooting medium and within the base of the cutting, can present a major diffusion barrier, since diffusion of oxygen through water is 10.000 times slower than in air (Loach 1986). This may result in anoxia within the tissues, which may cause damage to waterlogged cutting bases (Loach 1986). Rotting of cutting bases in sawdust was often observed in the present study. The reduced water absorption through the cutting base may also explain the reduced transpiration rate showed by cuttings in sawdust (Fig. 7.3b). It is known that cuttings respond very rapidly to water deficits by closing stomata (Loach 1988b). The importance of water absorption through the cutting base to keep pace with transpirational losses has been shown in a range of species by Grange and Loach (1983b).

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These results suggest that the lower rooting ability of cuttings in sawdust was more related to the excessive water content of the rooting medium, as discussed above, and not to the physiological condition of the aerial part of the cuttings, which was similar in all three media or in fact superior in sawdust.

CHAPTER 8

Rooting of leafy stem cuttings of *Albizia guachapele*

Several experiments, similar to those described for *Cordia alliodora*, were established with *Albizia guachapele*. However, stockplants established at the CATIE nursery were repeatedly infected by *Pseudomonas* spp., causing the rotting of cuttings soon after establishment in the propagators. Control measures were hindered by an incorrect early identification of the organism involved as a fungus, which was later found to be a secondary pathogen. Repeated attempts to grow bacteria-free plants failed, as the infecting organism was widely distributed in the nursery. However, before establishing the stockplant area, two preliminary studies were conducted at CATIE, using seedling material from a different nursery. A larger experiment, using clonal material, was conducted at the Institute of Terrestrial Ecology, based on the preliminary results at CATIE.

Experiment 1. The effects of different concentrations of indole-3-butyric acid and four rooting substrates on the rooting ability of single-node, leafy stem cuttings of *Albizia guachapele*

MATERIALS AND METHODS

Plant material and experimental design

This experiment was carried out at the CATIE nursery, Turrialba, Costa Rica, using seedling material from a progeny test. The seedlings were derived from the open pollinated progeny of plus trees selected in Costa Rica. One hundred and sixty eight cuttings were collected down the stem, discarding the uppermost node of each shoot, and their leaf area trimmed to two pairs of pinnae (approximately 115 cm^2). The base of the cutting was treated immediately with one of six concentrations of indole-3-butyric acid (IBA): 0%, 0.05%, 0.1%, 0.2%, 0.4% and 0.8%. The appropriate concentration of IBA was applied to the clean cut base of the cuttings as described in Chapter 2. Cuttings under treatment 0% IBA were applied with only 10 μl of methanol. Cuttings were established in one of four rooting substrates (gravel, sand, and mixtures with sawdust 50:50 p/v) in a non-mist propagator. During the course of the experiment, cuttings were finely sprayed twice a day to keep the leaves moist.

Assessments

After one week the cutting length was measured and each of the cuttings assessed for number of roots. Similar assessments were carried out after 15 and 24 days. Samples from each medium were collected on day 0 for determination of their wet and dry mass, together with a further set for determination of the volume of air spaces in the wet media.

Results

Cuttings length did not show significant differences between treatments, with a mean of 59.3 mm ± 0.9.

Rooting percentage significantly increased with all auxin treatments, while only 17.9% of the cuttings formed roots when no IBA was applied. The highest rooting percentage (96.4%) was obtained with 0.1% IBA, with a progressive decrease below and above this concentration (Fig. 8.1a). The mean number of roots per rooted cutting showed a progressive increase from 0% IBA to 0.4% IBA, to decrease again with 0.8% IBA, although this decline was not significant (Fig. 8.1b).

No significant differences were found for rooting percentage between rooting media, although rooting was lower when fine sand was used (Fig. 8.2a). The mean number of roots per rooted cutting was similar for gravel and sand, and showed a significant increase with the addition of sawdust to either medium (Fig. 8.2b).

This experiment was repeated using a larger number of cuttings (720), to obtain 30 cuttings per IBA/rooting substrate combination, and using standard length cuttings (60 mm). Assessments for number of roots were carried out at weeks 3 and 4 after establishment. Again, rooting percentage significantly increased when IBA was applied, but overall rooting percentages were about half of those obtained in the previous experiment (Fig. 8.3a). The mean number of roots per rooted cuttings increased with successive increases in the IBA concentration (Fig. 8.3b).

With regard to the rooting medium, the use of sand significantly reduced the rooting percentage of cuttings, in agreement with the previous experiment (Fig. 8.4a), and the mean number of roots produced by the cuttings (Fig. 8.4b).

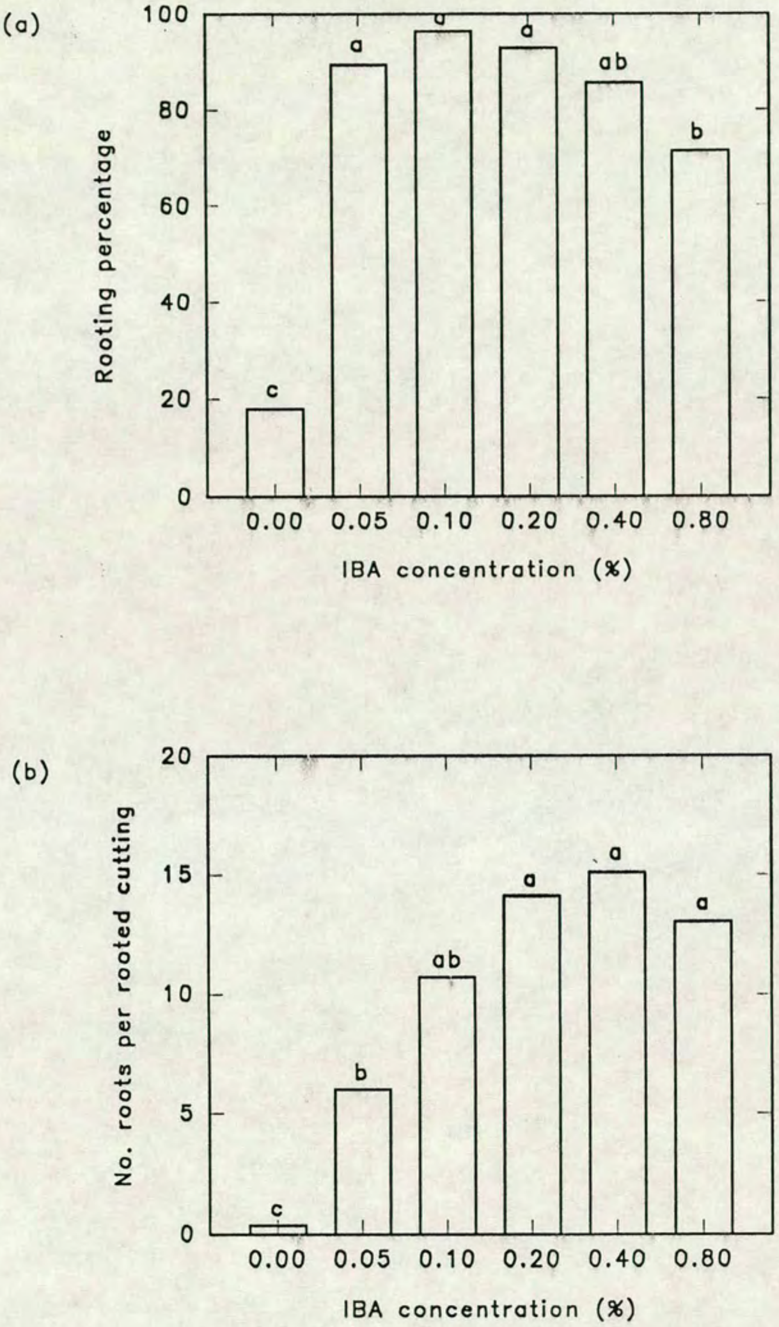
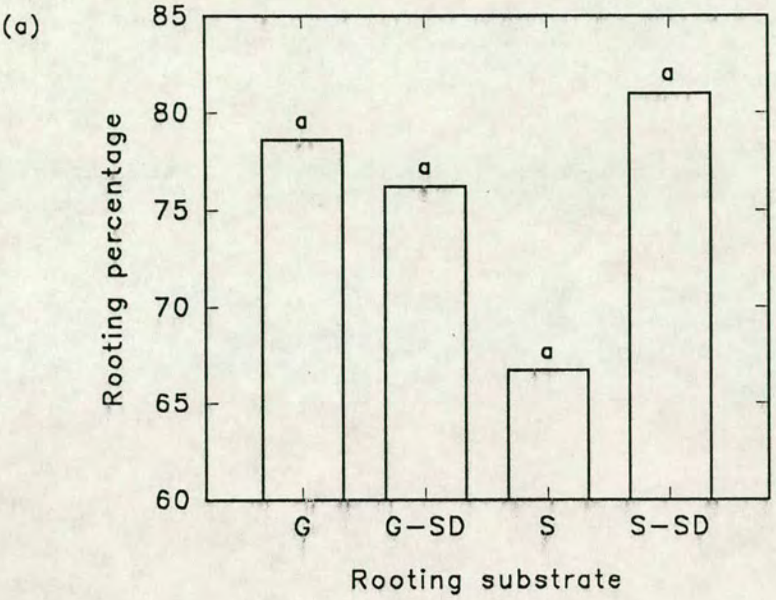


Fig. 8.1a,b The effects of five different concentrations of IBA on **(a)** the rooting percentage and **(b)** the mean number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after 24 days in a non-mist propagator. Values are means (n = 28) of four rooting substrates; means grouped by the same letter are not significantly different ($t_{0.05}$).



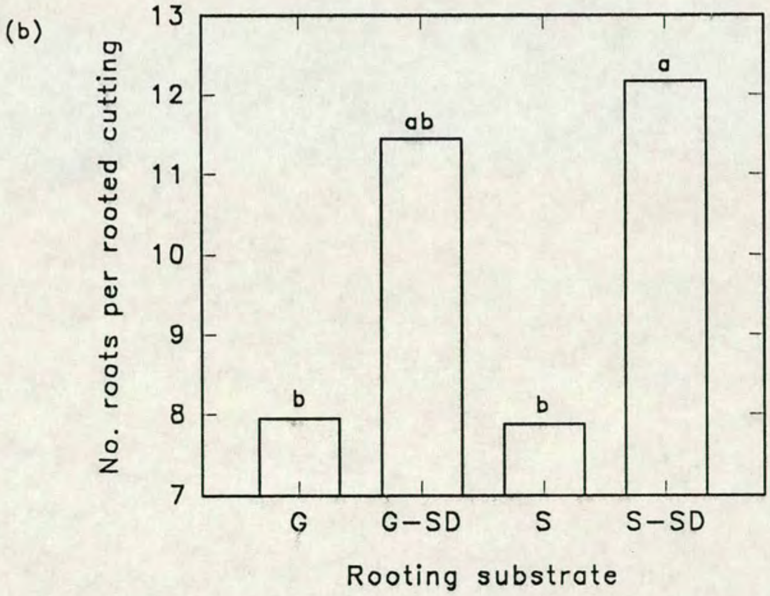


Fig. 8.2a,b The effects of four rooting substrates (G = gravel; G-SD = gravel + sawdust; S = sand; S-SD = sand + sawdust) on **(a)** the rooting percentage and **(b)** the mean number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after 24 days in a non-mist propagator. Values are means (n = 42) of five IBA concentrations; means grouped by the same letter are not significantly different ($t_{0.05}$).

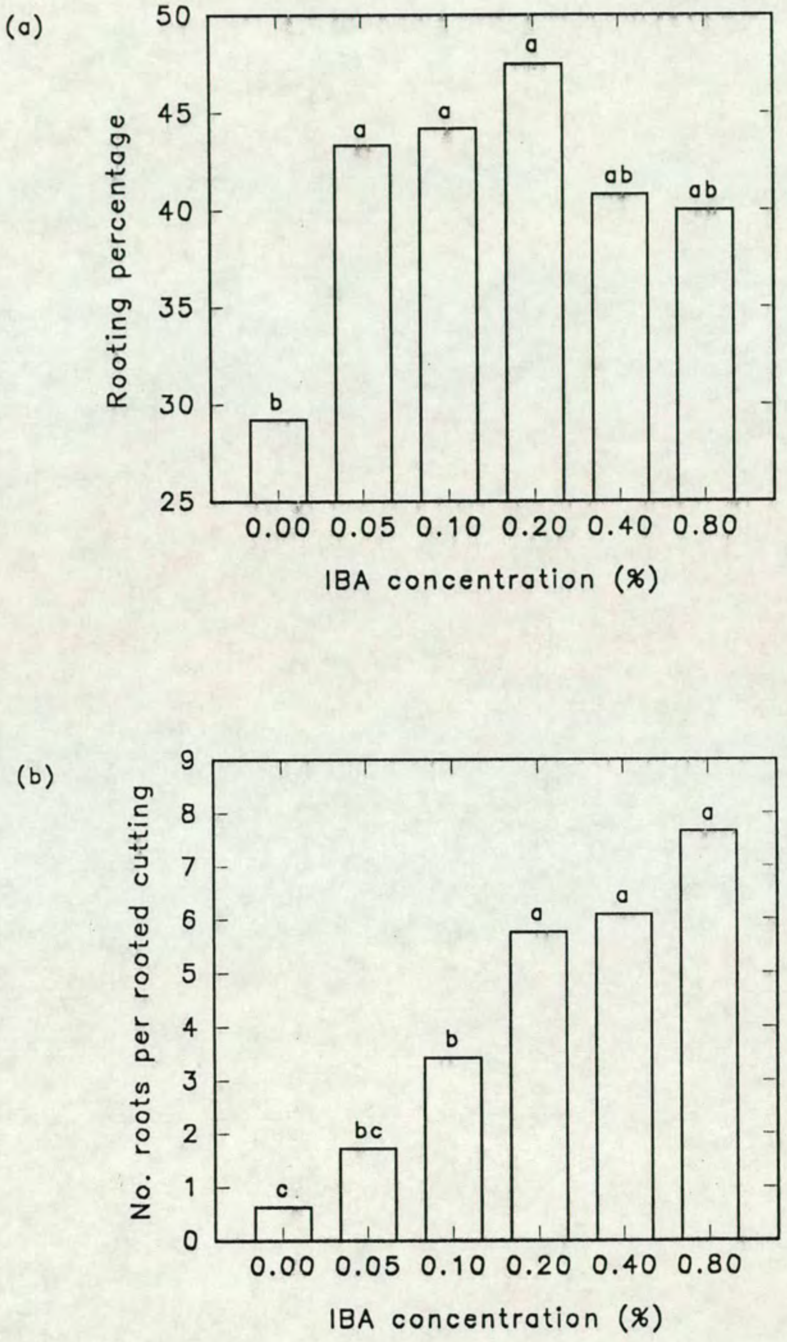


Fig. 8.3a,b The effects of five different concentrations of IBA on **(a)** the rooting percentage and **(b)** the mean number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after 28 days in a non-mist propagator. Values are means (n = 120) of four rooting substrates; means grouped by the same letter are not significantly different ($t_{0.05}$).

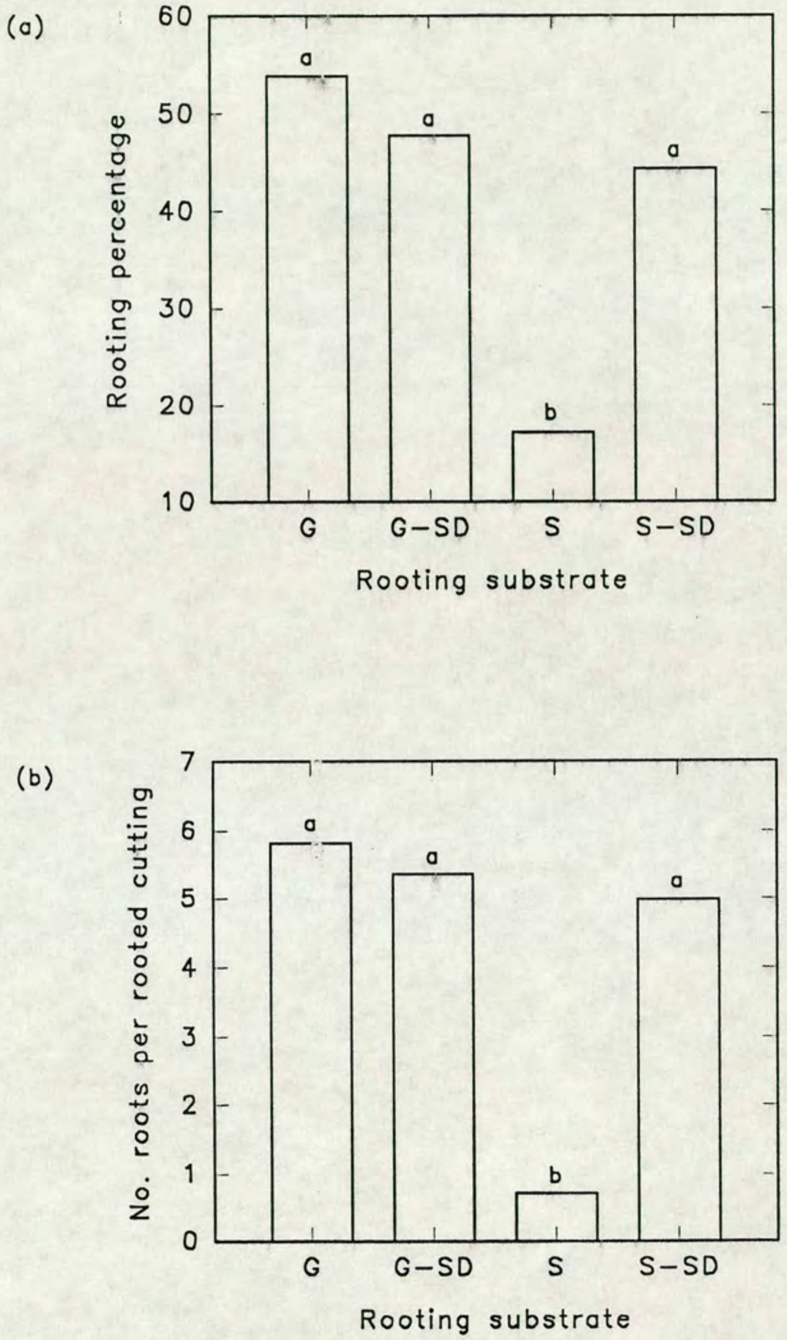


Fig. 8.4a,b The effects of four rooting substrates (G = gravel; G-SD = gravel + sawdust; S = sand; S-SD = sand + sawdust) on **(a)** the rooting percentage and **(b)** the mean number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after 28 days in a non-mist propagator. Values are means (n = 180) of five IBA concentrations; means grouped by the same letter are not significantly different ($t_{0.05}$).

Experiment 2. The effects of different concentrations of indole-3-butyric acid and two rooting substrates on the rooting ability of single-node, leafy stem cuttings of *Albizia guachapele*

MATERIALS AND METHODS

Plant material and experimental design

This experiment was carried out at the CATIE nursery, Turrialba, Costa Rica, using seedling material from a progeny test, to investigate the possible interaction between auxin concentration and the presence/abscence of sawdust in the rooting substrate. Three hundred and thirty six cuttings were collected down the stem, discarding the uppermost node of each shoot, and their leaf area trimmed to two pairs of pinnae (approximately 115 cm^2). The base of the cutting was treated immediately with one of six concentrations of indole-3-butyric acid (IBA): 0%, 0.05%, 0.1%, 0.2%, 0.4% and 0.8%. The appropriate concentration of IBA was applied to the clean cut base of the cuttings as described in Chapter 2. Cuttings under treatment 0% IBA were applied with only 10 μl of methanol. Cuttings were established in gravel or gravel + sawdust (50:50 p/v) in four randomized blocks in a non-mist propagator, each block containing 84 cuttings, seven from each IBA concentration/rooting substrate combination. During the course of the experiment, cuttings were finely sprayed twice a day to keep the leaves moist.

Assessments

The cutting length was measured at the time of establishment, and after two weeks, each cutting assessed for number of roots. Similar assessments were carried out after 20 and 27 days. A sample of 16 cuttings were harvested for determination of fresh and dry mass on day 0 and a further 16 per auxin treatment for fresh and dry mass on day 7. These cuttings were also subdivided into the leaf and the top and bottom half of the stem. Samples of the medium were collected on day 0 for wet and dry mass, together with a further set for determination of the volume of air spaces in the wet media.

Results

Cutting length showed little variation between treatments, with a mean of 61.7 mm ± 0.6

Rooting percentage increased with IBA concentrations of 0.05-0.4%, significantly higher than the concentration of 0.8% IBA. Contrary to previous experiments, 56% rooting was obtained in cuttings without application of IBA (Fig. 8.5a). The mean number of roots per rooted cutting increased with successive increases in the concentration of IBA from 0% to 0.4% and decreased at 0.8% IBA, although this decline was not significant (Fig. 8.5b).

Rooting percentage was unaffected by the substrate used (Fig. 8.6a), but the mean number of roots per rooted cutting was significantly increased by the addition of sawdust (Fig. 8.6b).

No significant changes with time were found between fresh and dry mass of the stem. The fresh and dry mass of the leaf decreased after seven days, but this was due to some shedding of their leaflets.

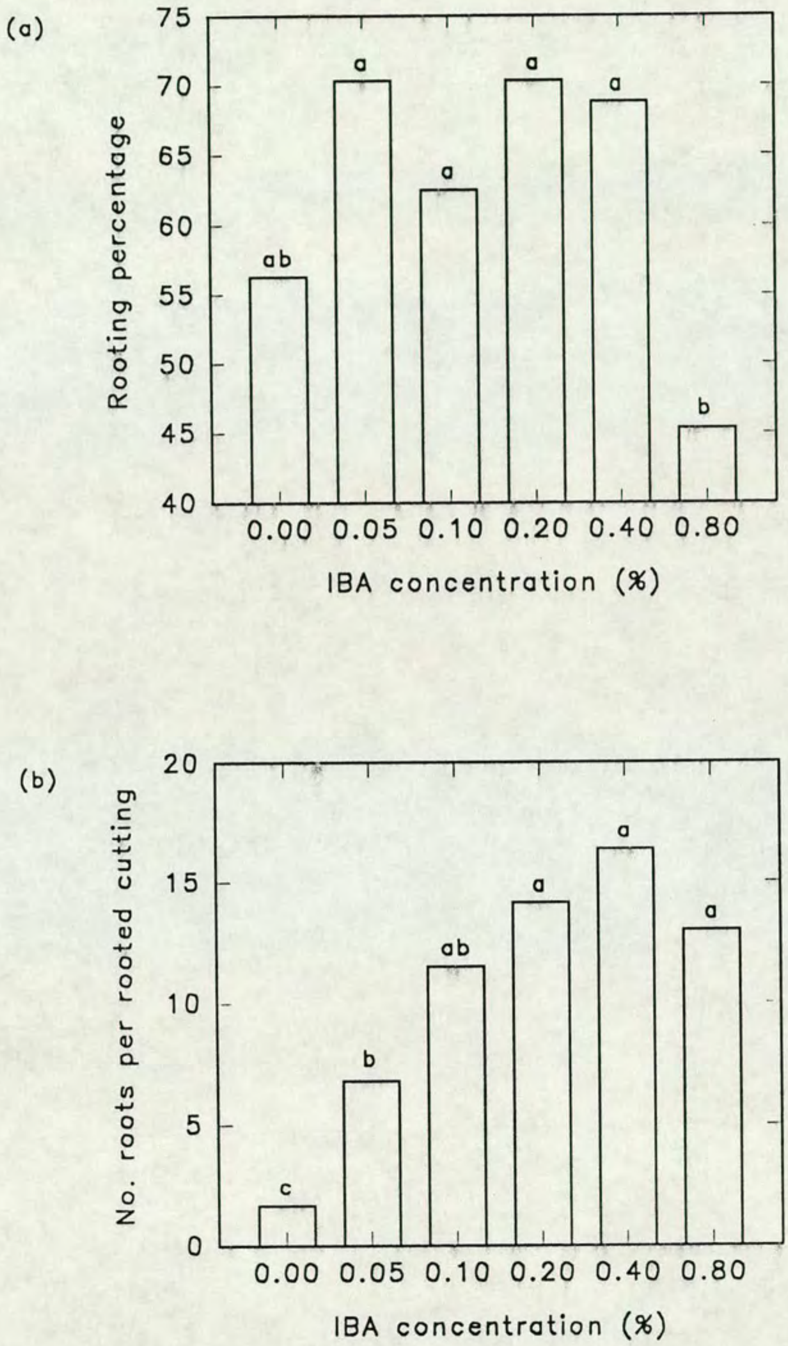


Fig. 8.5a,b The effects of five different concentrations of IBA on **(a)** the rooting percentage and **(b)** the mean number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after 27 days in a non-mist propagator. Values are means (n = 48) of two rooting substrates; means grouped by the same letter are not significantly different ($t_{0.05}$).

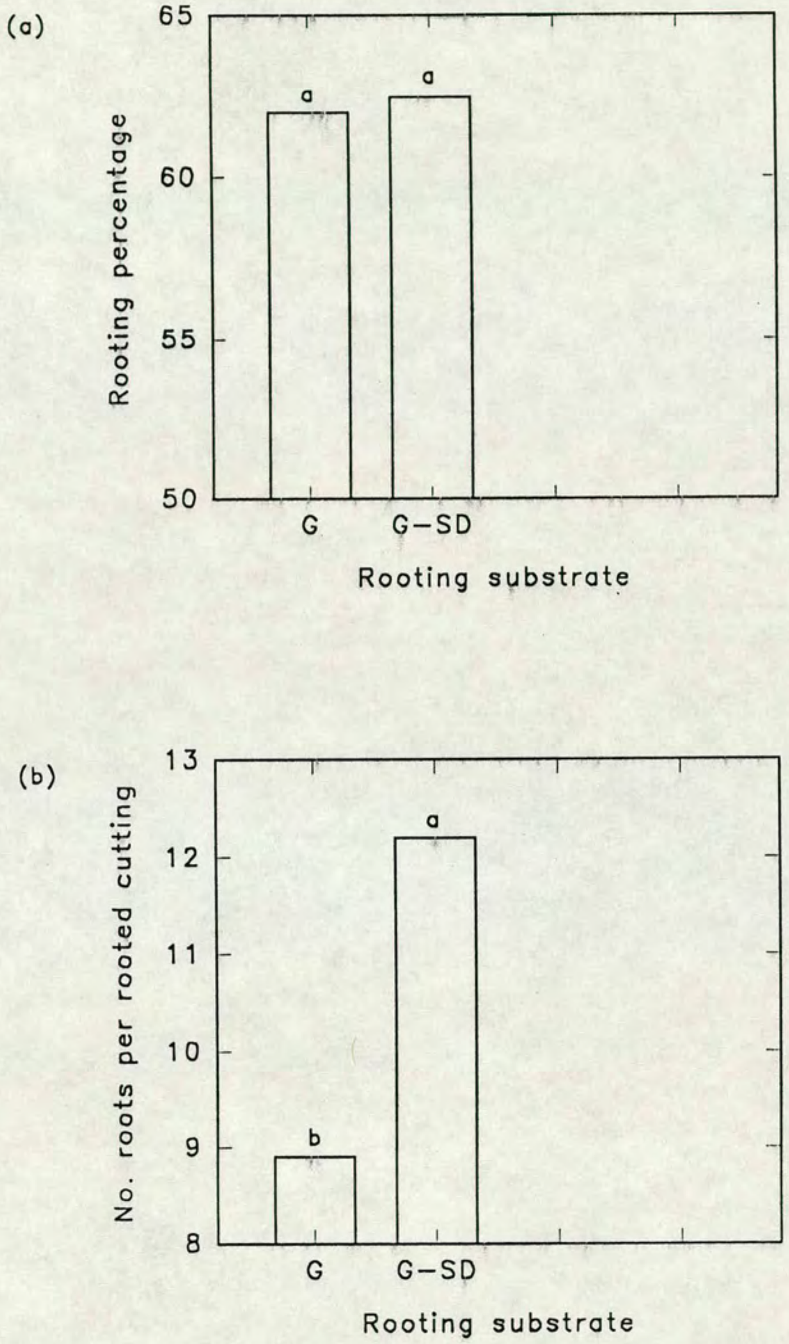


Fig. 8.6a,b The effects of two rooting substrates (G = gravel; G-SD = gravel + sawdust) on **(a)** the rooting percentage and **(b)** the mean number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after 27 days in a non-mist propagator. Values are means (n = 64) of five IBA concentrations; means grouped by the same letter are not significantly different ($t_{0.05}$).

Experiment 3. The effects of variation in irradiance and nutrient supply on the physiological condition of *Albizia guachapele* stockplants and subsequent rooting ability of leafy stem cuttings

This experiment investigated the effect of the interactions between the light environment and nutrients (NPK) applied to *Albizia guachapele* stockplants on anatomic characteristics and rooting ability of subsequent cuttings. This involved the determination of the gas exchange and chlorophyll fluorescence of both stockplants and cuttings during the propagation period.

MATERIALS AND METHODS

Growing period

Plant material

This experiment was carried out at the Institute of Terrestrial Ecology, Edinburgh. All plants were cut back to a height of 10 cm, and after two weeks, twenty plants of clones 5003 and 5005, sixteen plants of clone 5007 and eight plants of clones 5013, 5014 and 5015 were placed into four growth cabinets (model E 15, Controlled Environment Ltd., Winnipeg, Manitoba, Canada). The plants were grouped by clone and allocated randomly to the cabinets, so that the same clonal composition was maintained in each cabinet.

Growing environment

The cabinets were illuminated by 32 fluorescent tubes (160 W, Sylvania, USA) and 12 tungsten bulbs (75 W, Phillips, U.K.). The proportion of tubes and bulbs was varied to adjust the photon flux density within the growth cabinets, while maintaining a constant R:FR ratio of 1.91 ± 0.06. The photon flux density at canopy level was maintained at 200 μmol m^{-2} s^{-1} for the low irradiance treatment in two of the cabinets, and at 500 μmol m^{-2} s^{-1} for the high irradiance treatment in the other two cabinets. Photon flux density and R:FR ratio were determined using a quantum sensor (SKP 2200, Skye Instruments, Llandrindod Wells, Powys, Wales) and a red:far red sensor (SKR 100, Skye Instruments, Wales), respectively.

Photoperiod was kept constant at 19.5 h. Day and night temperatures were maintained at 28 °C and 20 °C, and day and night relative humidity at 70% and 80%, respectively. These temperature and humidity values are similar to those recorded in the Guanacaste region of Costa Rica, where *A. guachapele* grows naturally. The CO_2 concentration inside the cabinets was around 430 μmol mol^{-1}.

The plants were watered daily and received a twice weekly supply of 1.25% or 0.25% liquid fertilizer (SHL "Solinure", 20%N, 20%P, 20%K) depending on the treatment, applied instead of the daily watering.

Both the irradiance and the red:far red ratio were measured weekly, and the lamps were raised to maintain a constant irradiance at the top of the canopy as the plants grew taller. Every week the positions of the plants were rerandomised, to counteract for any possible environmental variation within the cabinets.

Assessments

Growth

All the plants were assessed weekly for total height, from the base of the shoot to the apex. For the determination of specific leaf area (SLA), ten leaflets were harvested at week 8 from the first fully developed leaf of two plants from each treatment, one from each of clones 5003 and 5005. Measurements of leaf area (s) using an area meter (Delta-T Devices, U.K.) and dry mass (D, oven dry mass after drying at 60°C during 24 h) were taken, and the SLA calculated as:

$$\mathrm{SLA} = s/D \ (\mathrm{m}^2\ \mathrm{g}^{-1})$$

Gas exchange

At week 8, measurements of CO_2 and water exchange were taken on four consecutive days, in a sample of six randomly selected plants from each cabinet, three from each of clones 5003 and 5005. For these measurements, a portable gas exchange system with infrared gas analyser was used (LCA-3, Analytical Development Co. Ltd., Hoddesdon, U.K.). The leaf chamber was held in a fixed horizontal position inside the cabinet to maintain a constant photon flux density at the leaf level of 200 or 500 μmol m^{-2} s^{-1} according to treatment. Measurements were taken on the first fully developed leaf from the top of the plant, selecting a fully exposed leaflet. Readings on consecutive days were taken using the same leaflet. The leaf chamber was connected to the LCA through a hole in the cabinet, so that the cabinet door was closed during readings.

Rooting of stem cuttings

At the end of the eighth week, the soft apical tip of each plant was discarded and six single-node leafy stem cuttings were collected sequentially down the stem, after trimming their leaf area to two pairs of pinnae (approximately 115 cm^2). Indole-3-butyric acid at a concentration of 0.2% was applied to the clean-cut base of the cuttings, as described in Chapter 2. The cuttings were set to root in coarse gravel on non-mist propagator beds, allocated as 18 randomized blocks, keeping record of clone and node position within each stem. Each block contained 24 cuttings from a single clone, six from each of four irradiance/nutrient combinations.

The propagation beds were heated to 30 ± 1.5°C at the base of the cuttings and artificial lights provided a photon flux density above 50 μmol m^{-2} s^{-1}, which increased to over 200 μmol m^{-2} s^{-1} during bright weather. Photoperiod was maintained at 19.5 h throughout the experiment. The cuttings were finely sprayed with water twice a day to keep the leaves moist.

Assessments

Dry mass accumulation

For the determination of dry mass accumulation, 12 cuttings from each irradiance-nutrient combination - six from each of clones 5003 and 5005 - were harvested at days 1, 10 and 20 for assessments of leaf, petiole and stem fresh and dry mass and leaf area. Leaf areas were determined using an area meter (Delta-T Devices, U.K.), and dry mass was obtained after oven drying the tissues for 48 h at 60 °C.

Estimates of leaf, stem and total dry mass at day 1 were obtained by multiplying the total fresh mass of the cuttings at day 1 by a reduction factor obtained from the cuttings destructively harvested at day 1. Corrected changes in dry mass were calculated by obtaining the percentage variation with respect to the estimated initial dry mass, and then expressed as the mean changes (g) from the initial value (100% = 0 g dry mass gain).

Chlorophyll fluorescence

Assessments of chlorophyll fluorescence were carried out every three days on a sample of 48 cuttings of clone 5007 - 12 from each irradiance-nutrient combination. For the assessments, a Plant Stress Meter was used (PSM Mark II, Bio Monitor S.C.I. AB, Sweden), pre-set at a light level of 400 μmol photons m^{-2} s^{-1} and a run-time of 10 s; the dark-adaptation period was 15 min. These settings were determined after conducting a preliminary test on a random sample of cuttings. The measurements were taken on the distal leaflet of the largest pinna.

Rooting ability

At day 2, the midpoint diameter and total length of the cuttings were measured. After one week, the cuttings were lifted and examined to assess number of roots and leaf shedding; similar assessments were carried out for the next two consecutive weeks.

Analysis

For the growing period, analyses of variance were carried out on shoot growth and specific leaf area, followed by tests of least significant difference, equivalent to the Fisher's t test. For the rooting period, analyses of variance and Fisher's t tests were carried out on rooting percentage (previous transformation of the data by the formula arcsin $\sqrt{\%}$), number of roots per cutting and dry mass accumulation.

Analyses of deviance for stepwise regression using GENSTAT 5 (Payne *et al.* 1987) were utilized to determine the influence of treatments and morphological characteristics of the cuttings on their rooting ability. To determine the influence of treatments on the net photosynthetic rate and stomatal conductance of the cuttings, analyses of variance for stepwise regression in GENSTAT 5 (Payne *et al.* 1987) were used.

RESULTS

Growing period

The analysis of variance showed highly significant differences ($p < 0.001$) in shoot growth between irradiances and nutrient supply at the end of eight weeks (Table A32). Mean shoot growth decreased with increasing irradiance and with increasing nutrient supply, from 52.7 cm for the combination 200 μmol m^{-2} s^{-1} /0.25% NPK to 27.7 cm for the combination 500 μmol m^{-2} s^{-1}/1.25% NPK (Fig. 8.7).

Clones also showed highly significant differences ($p < 0.001$) in terms of shoot growth, varying between 49.5 cm for clone 15 to 27.2 cm for clone 13 (Fig. 8.8).

There were highly significant differences in specific leaf area (SLA) between irradiances and nutrient treatments and the interactions between these variables (Table A33). Results were consistent with those of shoot growth; at 200 μmol m^{-2} s^{-1}, specific leaf area was significantly lower at the high nutrient supply (5.1 vs. 4.1), while at 500 μmol m^{-2} s^{-1} the difference between nutrient treatments was non-significant (3.0 vs. 2.9 for the low and high nutrient supply, respectively) (Fig. 8.9).

Net photosynthetic rates (P_n) were higher at the high irradiance, and within each irradiance treatment, high nutrients caused a reduction in P_n, although for the high irradiance, this reduction was not significant (Fig. 8.10a). No significant differences were found between irradiance and nutrient treatments for mean stomatal conductance (g_s), which was in the range 118.7 mmol H_2O m^{-2} s^{-1} to 122.7 mmol H_2O m^{-2} s^{-1} (Fig. 8.10b) The analysis of variance for stepwise regression showed that P_n was significantly dependent on g_s ($p < 0.01$) and irradiance ($p < 0.01$), but was not significantly affected by the nutrient treatment (Table A34). When the g_s measurements were analysed in the same way, the analysis showed no relationship between g_s and either irradiance or nutrient treatment (Table A35).

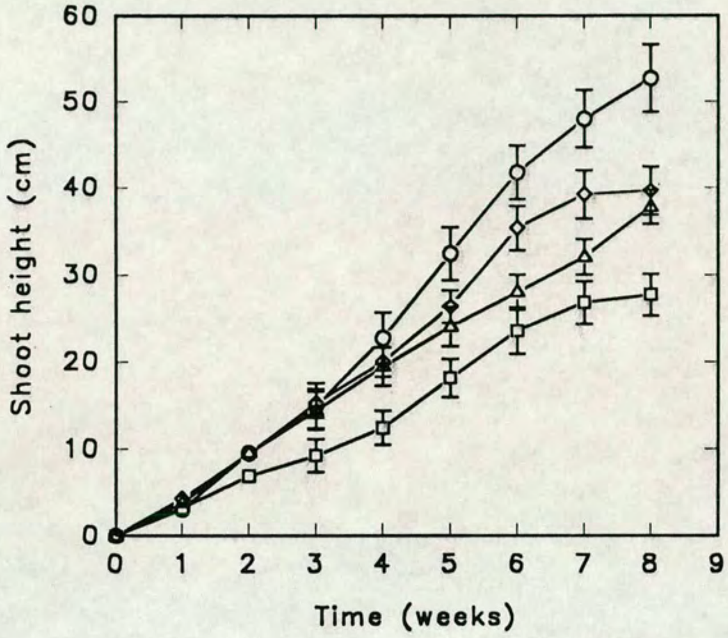


Fig. 8.7 The effect of irradiance and nutrient supply on mean height of *Albizia guachapele* plants grown in controlled-environment cabinets to give four irradiance-nutrient combinations: 200 μmol m^{-2} s^{-1} with 0.25% NPK (circles) or 1.25% NPK (diamonds) and 500 μmol m^{-2} s^{-1} with 0.25% NPK (triangles) or 1.25% NPK (squares). N = 20, bar = ± standard error of the mean.

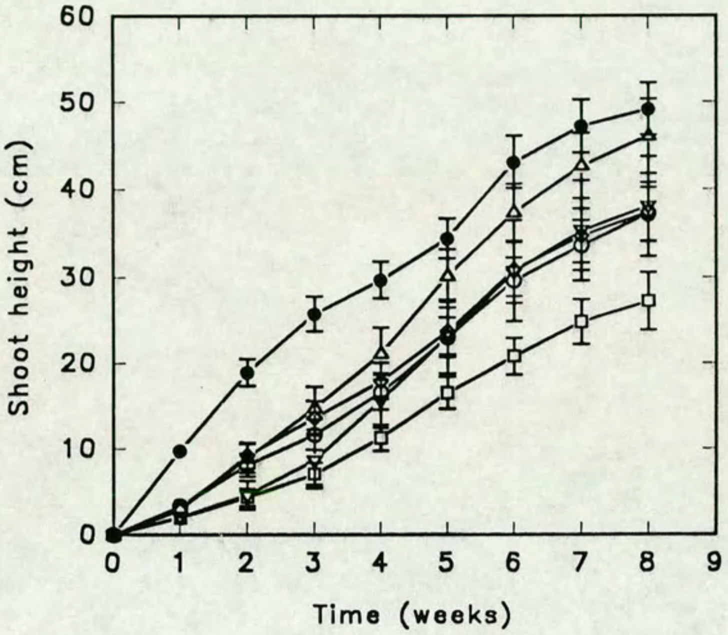


Fig. 8.8 Clonal variation in mean height of six clones of *Albizia guachapele* grown in controlled-environment cabinets. Values presented are means of 4 to 20 plants from four irradiance-nutrient combinations (200 and 500 μmol m^{-2} s^{-1}, 0.25% and 1.25% NPK). Open circles - clone 5003; filled circles - clone 5005; open triangles - clone 5007; filled triangles - clone 5013; filled squares - clone 5015. Bar = ± standard error of the mean.

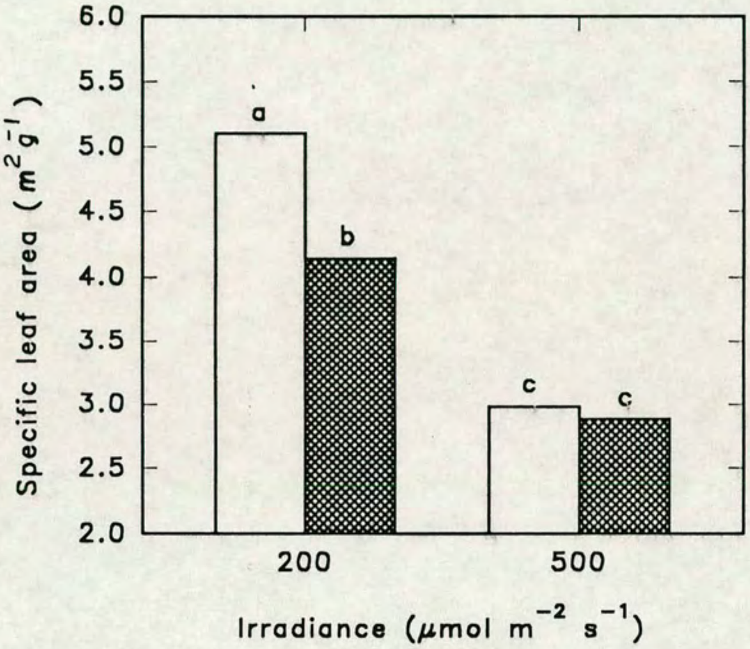


Fig. 8.9 The effect of irradiance and nutrient supply (0.25% NPK - open bars; 1.25% NPK - shaded bars) on the specific leaf area of *Albizia guachapele* plants grown in controlled-environment cabinets. N = 20, means with the same letter are not significantly different ($t_{0.05}$).

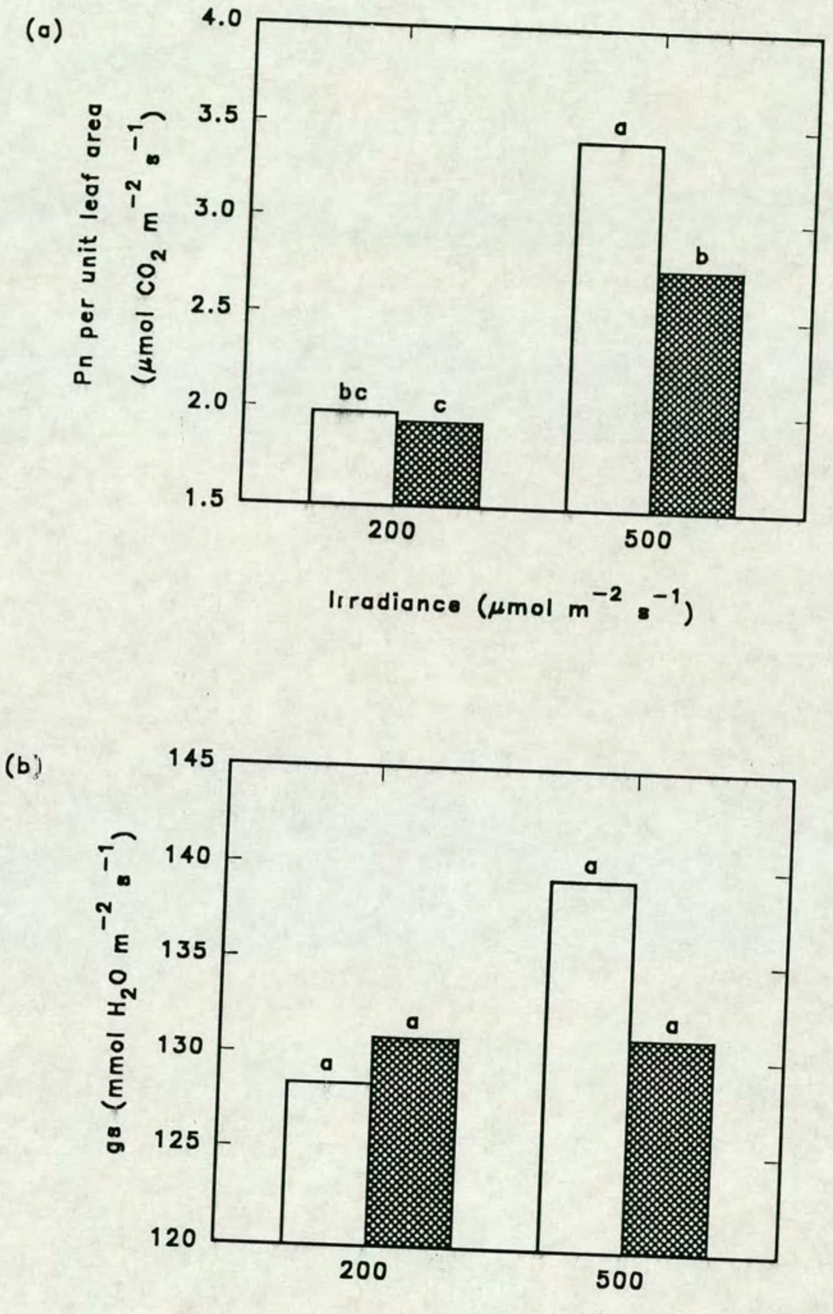


Fig. 8.10a,b The effect of irradiance and nutrient supply (0.25% NPK - open bars; 1.25% NPK - shaded bars) on (a) net photosynthetic rate per unit leaf area and (b) stomatal conductance of *Albizia guachapele* plants grown in controlled-environment cabinets. N = 24, means with the same letter are not significantly different ($t_{0.05}$).

Rooting of stem cuttings

Dry mass accumulation

No significant differences were found between treatments for estimated dry mass accumulation at the end of three weeks. However, the combination 200 μmol m^{-2} s^{-1}/0.25% NPK showed a steady increase from day 0 (standard value: 0 g) to day 10 (0.11 g) and day 20 (0.24 g). The combinations 500 μmol m^{-2} s^{-1}/0.25% NPK and 200 μmol m^{-2} s^{-1}/1.25% NPK showed a slight decline after 10 days (-0.06 and -0.15, respectively), to recover again at day 20 (0.04 and 0.06, respectively) only to levels similar to those at day 0. The treatment 500 m^{-2} s^{-1}/1.25% NPK showed a decrease at day 10 (-0.41) and all cuttings died before the following assessment at day 20 (Fig. 8.11).

Chlorophyll fluorescence

The chlorophyll fluorescence ratio (*Fv/Fm*) showed a similar pattern for all treatments, with significant differences only at days 8 and 21, when the combination 200 μmol m^{-2} s^{-1}/1.25% NPK showed values significantly lower than the rest of the treatments (Fig. 8.12). In general, *Fv/Fm* decreased from day 4 to day 8, and increased again by day 14. After 18 days there was a further reduction in *Fv/Fm* in all treatments and a final increase by day 21, with the exception of treatment 200 μmol m^{-2} s^{-1}/1.25% NPK which showed similar values to those at day 18.

A fault in the filter originally supplied with the fluorescence meter was detected after the measurements. This affected the readings, producing values of *Fv/Fm*, according to the suppliers, approximately 0.1 units lower than the actual value. The values presented here are therefore corrected values.

Rooting ability

There were highly significant differences ($p < 0.001$) in rooting percentage after three weeks between irradiances and nutrient supply, and a significant interaction ($p < 0.05$) between irradiances and nutrients (Table A36). Rooting percentage decreased from 53.8% with low light/low nutrients to 11.2% with high light/high nutrients (Fig. 8.13a).

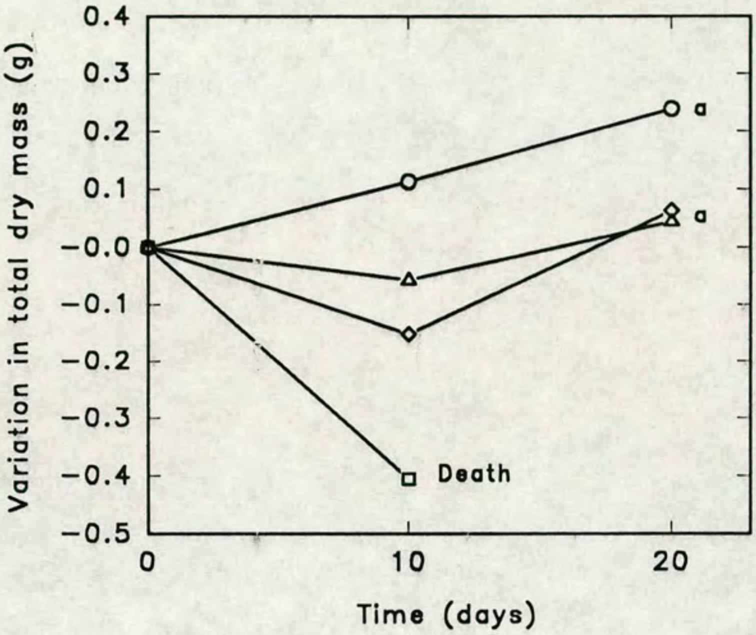


Fig. 8.11 The effect of irradiance and nutrient supply applied to *Albizia guachapele* stockplants on variation in total dry mass of subsequent single node, leafy stem cuttings set to root in non-mist propagators. Values presented are means of 12 cuttings from clones 5003 and clone 5005 destructively harvested 10 and 20 days after severance. Circles - 200 μmol m^{-2} s^{-1}/0.25% NPK; triangles - 200 μmol m^{-2} s^{-1}/1.25% NPK; diamonds - 500 μmol m^{-2} s^{-1}/0.25% NPK; squares - 500 μmol m^{-2} s^{-1}/1.25% NPK. Means with the same letter are not significantly different ($t_{0.05}$)

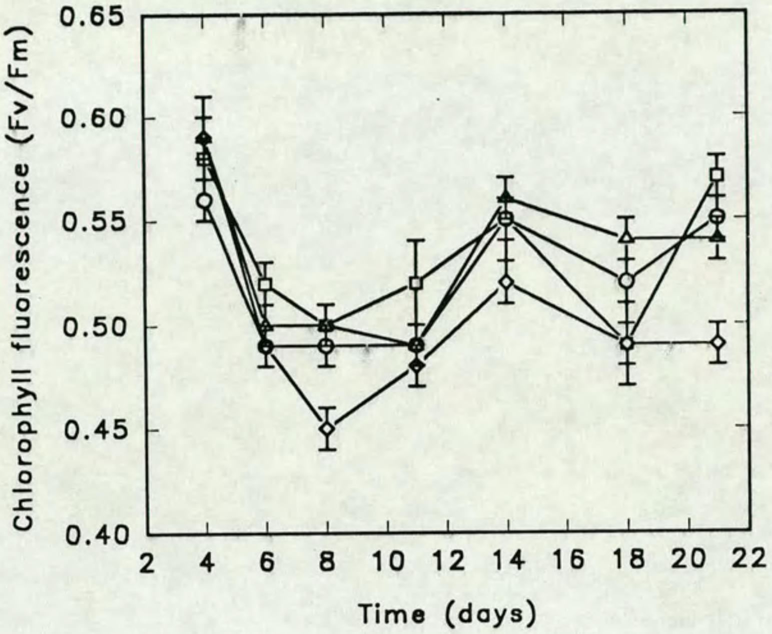


Fig. 8.12 The ratio of the variable fluorescence to maximum fluorescence (*Fv/Fm*) for single-node, leafy stem cuttings of *Albizia guachapele* set to root in non-mist propagators. Cuttings were collected from stockplants grown under four irradiance-nutrient combinations: 200 μmol m^{-2} s^{-1} with 0.25% NPK (circles) or 1.25% NPK (squares) and 500 μmol m^{-2} s^{-1} with 0.25% NPK (triangles) or 1.25% NPK (diamonds). N = 12, bars = ± standard error of the means.

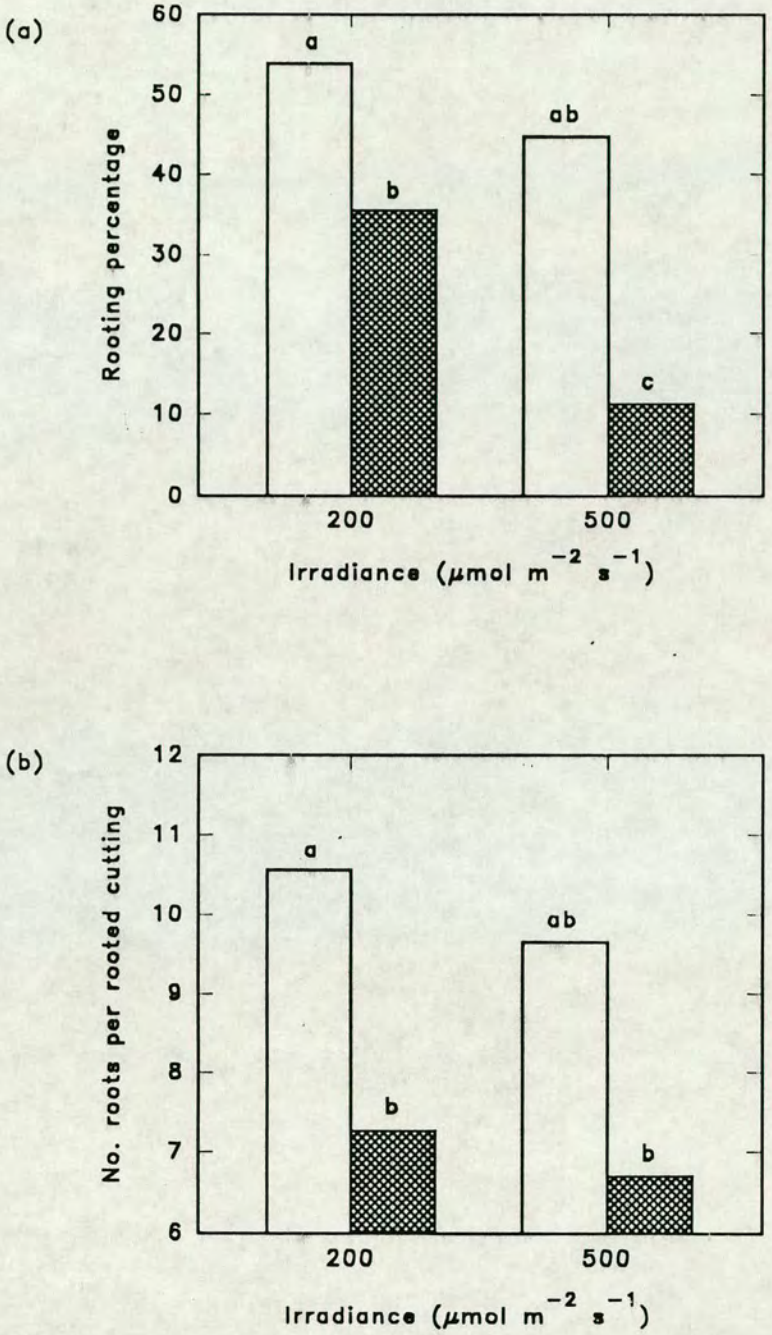


Fig. 8.13a,b The influence of irradiance and nutrients (0.25% NPK - open bars; 1.25% NPK - shaded bars) applied to *Albizia guachapele* stockplants on (a) subsequent rooting percentage and (b) number of roots per rooted cutting of single-node, leafy stem cuttings set to root in non-mist propagators. N = 92-107, means with the same letter are not significantly different ($t_{0.05}$).

The analysis of variance for number of roots per rooted cutting showed highly significant differences between clones ($p < 0.01$) and nutrient treatments ($p < 0.001$) (Table A37). A similar trend to that of rooting percentage was obtained for this variable, which decreased from 10.6 for the combination 200 μmol m^{-2} s^{-1} /0.25% NPK to 6.7 for the combination 500 μmol m^{-2} s^{-1}/1.25% NPK (Fig. 8.13b).

There were also highly significant differences ($p < 0.001$) in rooting percentage between clones. Clones 5007 and 5013 showed the highest rooting percentage (52.5% and 47.2%), clones 5003 and 5005 were intermediate (36.0% and 31.1%), whilst clones 5014 and 5015 displayed the lowest rooting percentage (21.2% and 18.9%) (Fig. 8.14a). There were less variation in terms of mean number of roots per rooted cutting, which varied from 5.0 to 11.4 for clone 5014 and 5013 respectively (Fig. 8.14b).

Rooting percentage decreased progressively from the apical to the basal nodes, with the exception of node 5, which had the lowest rooting percentage (Fig. 8.15a). The mean number of roots per rooted cutting tended to increase from node 1 (apical) to node 4, and to decrease again to node 6 (basal). Differences between nodes 1-5 were not significant, but the low number of roots produced by the most basal node was a significant reduction (Fig. 8.15b).

When the rooting data was analysed by stepwise regression, rooting was found to be significantly affected by cutting diameter, nutrient treatment applied to the stockplants, clone and cutting length, but was not significantly affected by irradiance applied to the stockplants or the node position (Table A38). When the data was analysed in the same way, using values of cutting volume instead of diameter and length, the effect of volume on rooting became non significant (Table A39). Cutting diameter increased progressively from the apical to the basal nodes (Fig. 8.16a), while cutting length showed roughly the opposite trend (Fig. 8.16b). When these results were correlated with rooting percentage, a strong negative relationship was found between cutting diameter and rooting percentage (Fig. 8.17a), and a positive relationship between cutting length and rooting percentage (Fig. 8.17b).

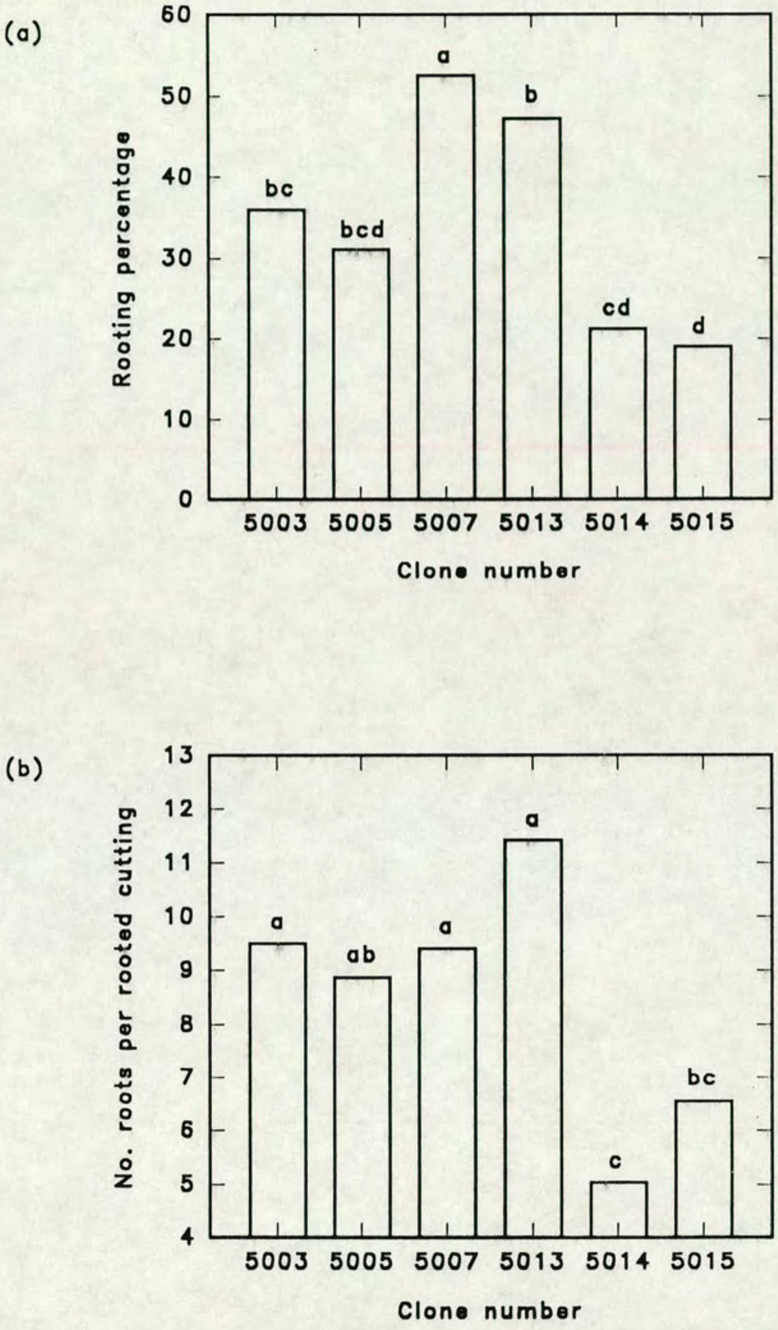


Fig. 8.14a,b Clonal variation in (a) the rooting percentage and (b) the number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after three weeks in non-mist propagators. Values presented are means (n = 42-87) of four irradiance/nutrient treatments applied to the stockplants. Means grouped by the same letter are not significantly different ($t_{0.05}$).

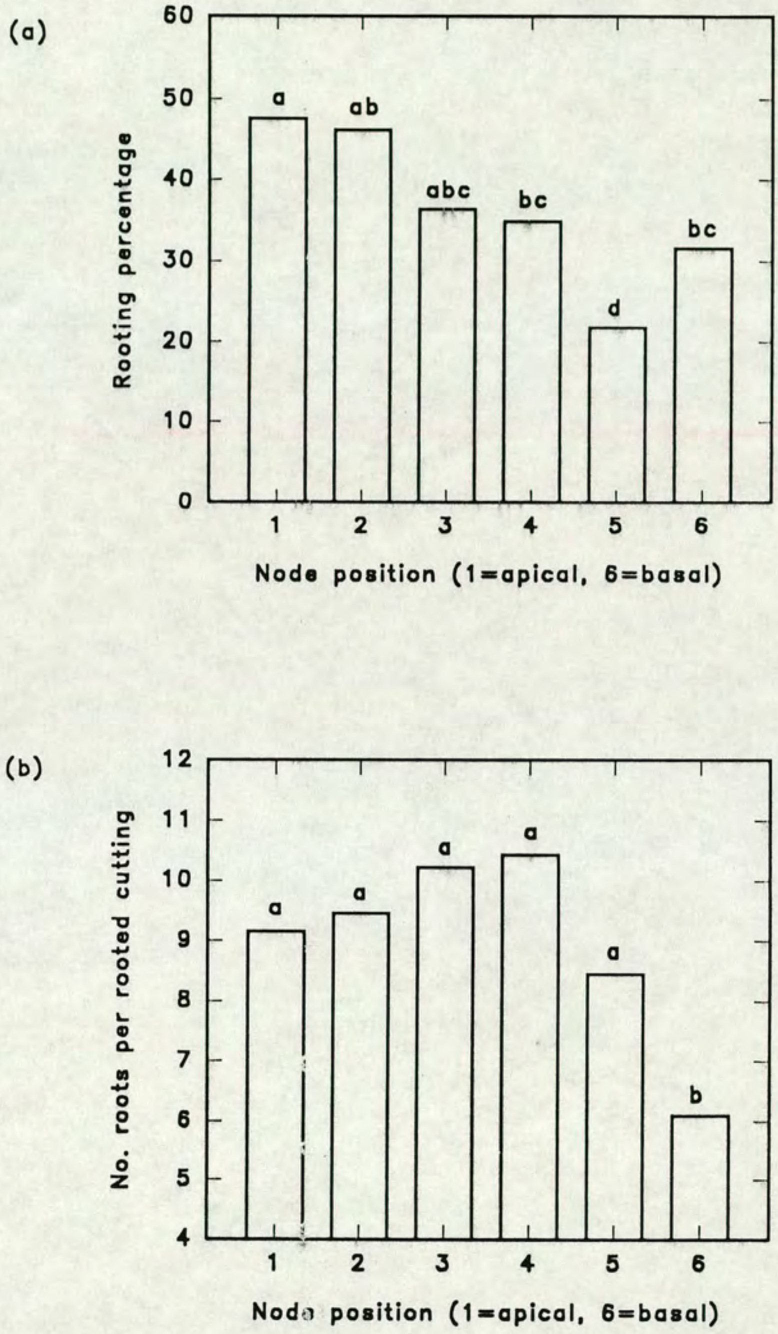


Fig. 8.15a,b The effect of node position on (a) the rooting percentage and (b) the number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* after three weeks in non-mist propagators. Values presented are means ($n = 68$) of six clones and four irradiance/nutrient treatments applied to the stockplants. Means grouped by the same letter are not significantly different ($t_{0.05}$).

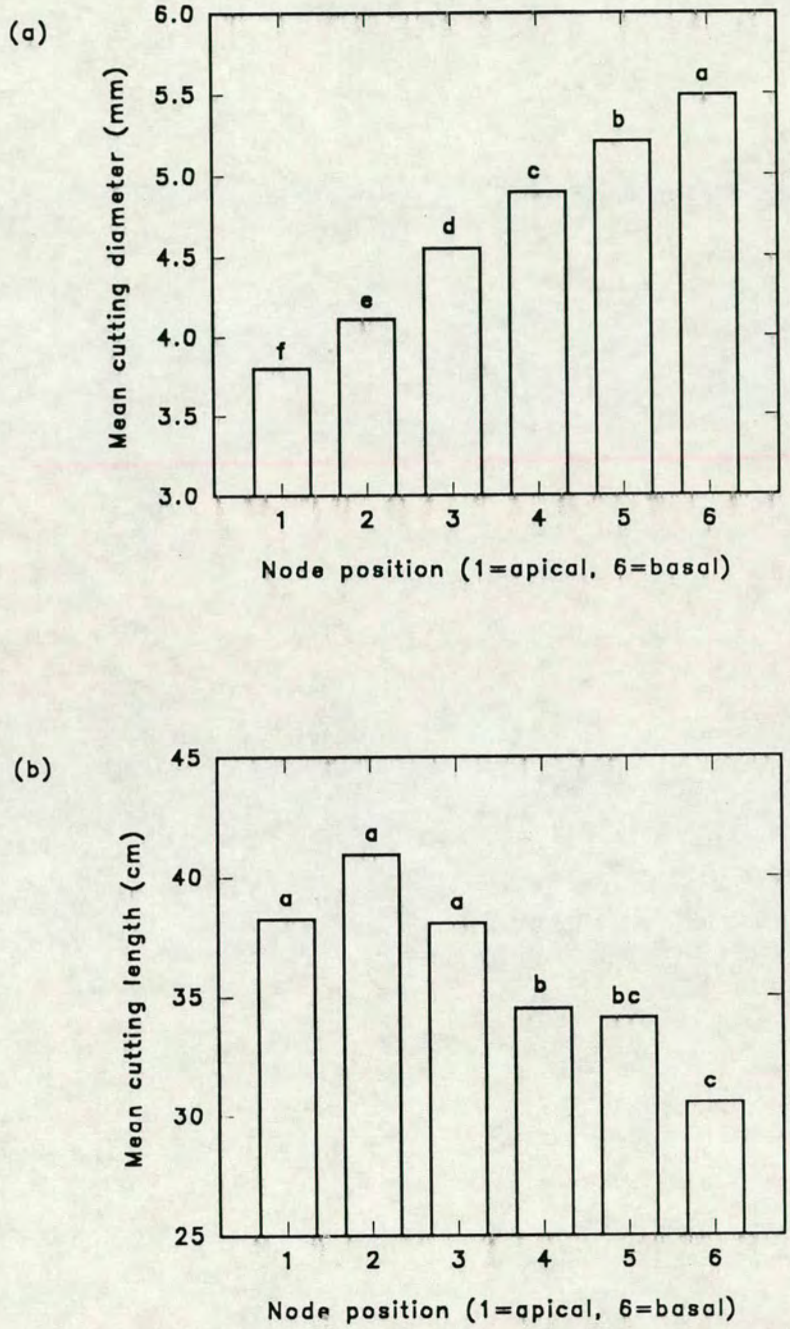


Fig. 8.16a,b The effect of node position on (a) the mean cutting diameter and (b) the mean cutting length of single-node, leafy stem cuttings of *Albizia guachapele*. The cuttings were collected from stockplants grown under four irradiance/nutrient combinations. N = 68; means grouped by the same letter are not significantly different ($t_{0.05}$).

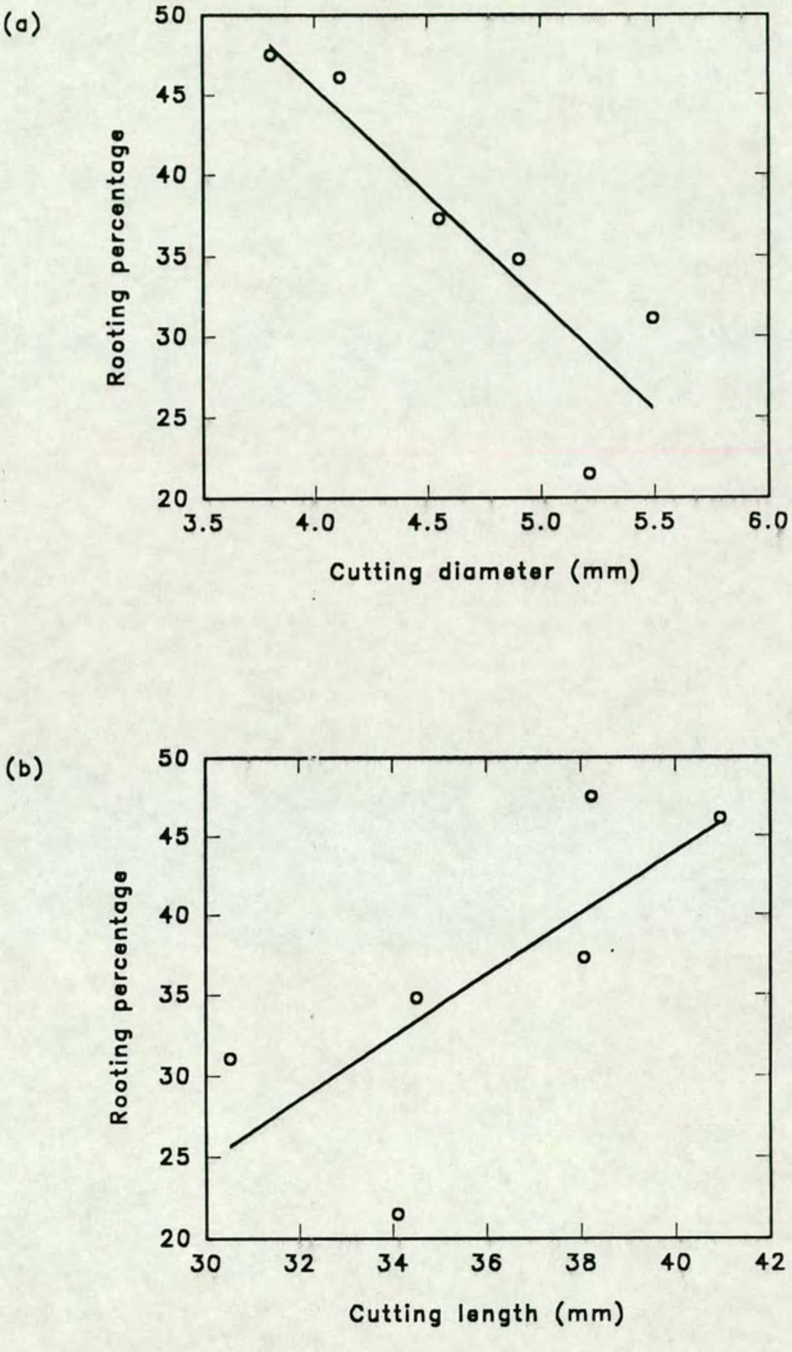


Fig. 8.17a,b The relationship between **(a)** mean cutting diameter and **(b)** mean cutting length and rooting of single-node, leafy stem cuttings of *Albizia guachapele* after three weeks in non-mist propagators. Each value is the mean (n = 68) of six clones and four irradiance/nutrient combinations applied to the stockplants.

DISCUSSION

Under artificial light conditions it is difficult to obtain natural light quality, in particular, natural red:far red ratios (Leakey and Storeton-West 1992). Red:far red ratio for the natural environment is 1.2 (Chazdon and Fletcher 1984); greater ratios, as the ratio of 1.91 recorded in the present experiment, must be considered as a coarse simulation of what might be recorded in the field (Ramos and Grace 1990). The irradiances used, however, can easily be duplicated under natural conditions in the tropics. Moreover, the use of a constant red:far red ratio for all the cabinets allows the interpretation of treatment differences without the possible variations introduced by different light qualities.

Cuttings of *A. guachapele* rooted better from stockplants grown at low irradiance and with a low nutrient supply. Correspondingly, cuttings from stockplants grown under the higher levels of both variables rooted very poorly. There was however an overall interaction between irradiance and nutrients, such that cuttings rooted better when stockplants were grown with low nutrient supply, and within each rate of nutrient supply rooting was higher when a low stockplant irradiance was used.

There has been a great deal of discussion about the effects of irradiance and nutrients on stockplant growth and subsequent rooting and, to date, there does not seem to be a consistent response in all tree species. Perhaps this is not surprising since trees are known to vary in their light requirements and some tree species (e.g. *Albizia guachapele*) are nitrogen fixing, especially at low soil fertility.

The effect on rooting after exposing stockplants to different irradiances is controversial (Andersen 1986); increased irradiance has been shown to inhibit or delay rooting, promote rooting or have no effect (Moe and Andersen 1988). However, in a wide number of plants, low stockplant irradiance has enhanced rooting ability (Eliasson and Brunes 1980; Hansen and Eriksen 1974; Hansen *et al.* 1978; Leakey and Storeton-West 1992; Moe and Andersen 1988). Moe and Andersen (1988) presented a response curve of stockplant irradiance on rooting which appears to be valid for most plant species. The curve indicates that very low stockplant irradiances result in poor rooting, with improved rooting as irradiance increases, until an optimum is reached. Above this optimum, rooting ability again declines.

The effect of stockplant nutrition on rooting is also the subject of controversy. However, results appear to be more consistent than those of irradiance; in general, it appears that stockplants which are fertilized suboptimally for shoot growth also result in cuttings that root best (Moe and Andersen 1988).

The reasons for these responses are certainly complex and are probably the result of interactions between many factors, with optima varying between species. The effects of photosynthesis are obviously important, through its effects on the initial carbohydrate content of the cuttings. It is now widely recognized that the ability of the cuttings to supply carbohydrates, either through stored reserves or through current photosynthesis, to the area where roots appear seem to be very important for root development (Moe and Andersen 1988). In an experiment with *Triplochiton scleroxylon*, Leakey and Storeton-West (1992) attributed the poor rooting found in cuttings from stockplants grown at high irradiances to suppressed photosynthetic ability in the shoots, resulting from end product inhibition. In the same experiment, the addition of fertilizer to stockplants seemed to lower the photosynthetic ability of the shoots and hence the rooting ability of cuttings. Hansen and Ericksen (1974) also suggested that supraoptimal carbohydrate contents in *Pisum* stockplants grown at high irradiance were responsible for the lack of root formation found in subsequent cuttings. In the present experiment, nutrient supply seems to have produced a greater effect than irradiance on rooting. The addition of fertilizer at a higher rate did reduce the photosynthetic rate of stockplants grown at high irradiance and this could be seen as a reason for the poor rooting of these cuttings; however, it does not explain why the cuttings from stockplants grown at low irradiance showed better rooting but even lower photosynthetic rates.

One can thus suggest that photosynthesis was not a limiting factor and that the two irradiances used were probably appropriate for an adequate photosynthetic rate, but other factors exerted a greater influence on the subsequent rooting ability of cuttings. In the present experiment, the effects of irradiance and nutrient supply on stockplant growth were very clear. Plants at low irradiance produced longer shoots, as expected, and within each irradiance, the application of high rates of fertilizer seems to have lowered the elongation capacity of the shoots. Leaves of stockplants grown with a higher nutrient supply had a lower specific area, probably indicating a diversion of nutrients to the leaves at the expense of shoot growth. It is known that leaves are not only importers of photosynthate during much of their period of laminar extension but also retain much of their own photosynthate (Thrower 1962). With a wide range of plants, it has been found that nutrient content, chiefly N, correlates negatively with rooting (Hartmann and Kester 1983; Leakey and Storeton-West 1992; Pearse 1943). In general, it appears that suboptimal fertilization, in some cases to the point when the shoots show deficiency symptoms, results in cuttings that root best (Hartmann and Kester 1983; Moe and Andersen 1988). There are also a considerable number of studies claiming that stems with high C/N ratios root much better than those with low C/N ratios (Hartmann and Kester 1983; Veierskov 1988). Based on the hypothesis of C/N ratio and its effects on rooting, growing stockplants under conditions of limited N supply has been recommended as a way to increase the C/N ratio in the shoots and hence, to stimulate rooting (Hartmann and Kester 1983). It is important to understand, however, that C/N ratio is only one parameter which may affect rooting (Veierskov 1988). All essential elements/compounds are important in the rooting process and to ascribe a more significant role to one or two is probably misleading (Veierskov 1988). The exact role of carbohydrates in rooting remains obscure, partly because altering the carbohydrate status of stockplants is very difficult without interactions with other important developmental parameters (Veierskov 1988).

It is clear, however, that without some threshold level of carbohydrates, growth and development will cease (Veierskov 1988). Therefore, in stockplants with a low carbohydrate content, the energy charge will be too low to support rooting (Veierskov 1988). In this experiment, the greater rooting percentage obtained with cuttings from stockplants grown with low N-fertilizer applications may be a result of their greater carbohydrate content. Within each rate of fertilizer application, cuttings from stockplants grown at low irradiances, as expected, rooted better than those from high irradiance. Promotion of rooting by low irradiances has been associated with an increased auxin content in the shoots, possible changes in rooting inhibitors and/or promoters, with a beneficial change in the internal structure of the stem where roots later will appear (Blazich 1988; Hartmann and Kester 1983; Moe and Andersen 1988), and increasing sensitivity of tissues to auxin (Maynard and Bassuk 1988). Although enhancement of rooting by low stockplant irradiance is common, the exact mechanisms involved are, however, poorly understood.

To evaluate the effect of stockplant irradiance on rooting, the light conditions during rooting also must be considered (Moe and Andersen 1988). The light compensation point and the respiration rate is usually higher in stockplants grown under high irradiance. Cuttings taken from such stockplants and rooted at irradiances below the light compensation point frequently show poor rooting (Moe and Andersen 1988). This did not seem to be the case in the present experiment, according to the better rooting of cuttings from stockplants grown at 500 μmol m^{-2} s^{-1} (and low nutrients) in comparison with cuttings from stockplants grown at 200 μmol m^{-2} s^{-1} (and high nutrients). Also, cuttings from both low and high irradiance stockplants showed an overall increment in total dry mass after 20 days in the propagator. It appears that irradiance during rooting was sufficient to support an adequate rate of current photosynthesis, independently of the previous light conditions of the stockplants. This is supported by the fact that no differences were found between treatments in their F*v*/F*m* ratios during the propagation period. The F*v*/F*m* ratio is a good indicator of photosynthetic efficiency, a decline in the ratio being indicative of a decrease in photosynthetic efficiency and *vice versa* (Bolhar-Nordenkampf *et al.* 1989). The F*v*/F*m* ratios of the cuttings were low compared with those reported as normal by Bolhar-Nordenkampf *et al.* (1989). This may be well due to a inherent characteristic of the species, which has not been studied in any detail, or is probably a normal feature of cutting leaves, which normally show a low photosynthetic rate due primarily to stomatal closure (Davis 1988). It is known that leafy cuttings generally require a lower level of irradiance during propagation than that applied to the stockplants (Moe and Andersen 1988), and that even low rates of current photosynthesis can significantly influence the carbon balance and dry matter accumulation within a cutting (Davis 1988). Also, a number of studies have shown that saturation of current photosynthesis by unrooted leafy cuttings is generally at a much lower PAR than that of intact plants (Davis and Potter 1981; 1987; Machida *et al.* 1977).

Cuttings from high irradiance-high nutrient stockplants were also much shorter than those from any other treatment, which also surely negatively influenced their rooting ability through its effects on the cutting reserves. These cuttings showed a heavy leaf shedding after 10 days in the propagator, a behaviour similar to that reported for *Albizia falcataria*, when cuttings were collected from vigorously growing stockplants (Leakey 1991).

Therefore, it seems that the *A. guachapele* cuttings are not only dependant on current photosynthesis for an adequate rooting, but also on their initial carbohydrate content. Cuttings from the high irradiance-high nutrient stockplants were probably deficient in stored carbohydrates to support rooting, in spite of the fact that light during propagation was sufficient for an adequate rate of current photosynthesis.

CHAPTER 9

CONCLUSIONS

Introduction

Cordia alliodora and *Albizia guachapele* can be easily propagated by leafy, stem cuttings, using low-technology, non-mist propagation systems, regarding that pathogen-free stockplants are used. The process of adventitious root formation, however, was dependant on a large number of genetic, physiological, morphological and environmental factors, operating both pre- and post-severance in a complex and interrelated fashion. In addition, evidence was presented suggesting that treatments which may be optimum during the initial stages of the propagation process, may not be so in later stages. This is in agreement with the generally accepted concept that there is at least two stages involved in adventitious root production, and that the factors which influence root initiation may not be the same as the ones that influence root development (Lovell and White 1986).

C. alliodora and *A. guachapele* varied in their response to similar treatments, sometimes in a clearly opposite pattern, probably as a result of the genetic differences associated with adaptations to the contrasting ecological conditions where each of the species grow (wet and dry forest respectively).

This study focussed on some of the factors, both pre- and post-severance, generally accepted as having a crucial influence in determining the rooting ability of leafy cuttings. The effects of these factors and their influence on the physiological processes known to influence adventitious root formation in cuttings are discussed below.

Stockplant growth environment

The physiology and morphology of stockplants, and their subsequent rooting ability, were modified by variations in irradiance and nutrient supply. The growth of *C. alliodora* stockplants was reduced by low light and by the application of nutrients. High nutrient supply also reduced the growth of *A. guachapele* stockplants, but plants were taller under low light. The negative effect of high nutrients on the growth of both species was probably related to a greater diversion of nutrients to the leaves at the expense of stem growth, as leaves of stockplants grown under high nutrients had a lower specific leaf area, particularly under low light. It is also possible that excess NPK in the soil caused an imbalanced in the general nutritional state of the plants, with negative consequences on growth, but this aspect was not investigated. The lower growth rate of *C. alliodora* plants under low light was unexpected, as most plants respond to low irradiance by etiolation, i.e. internode elongation. Such was the case with *A. guachapele* in this study and with a number of other tropical species (Leakey and Storeton-West 1992; Hoad and Leakey 1992). The effect on stem elongation of the latter studies, however, were associated with the R:FR ratios achieved under the controlled environment conditions where the experiments were carried out. In the *C. alliodora* study, plants were grown under natural R:FR ratios; the higher shoot growth obtained under high irradiance probably resulted from the higher photosynthetic rates shown by these plants. A retardation in growth of plants grown under low irradiance has also been found in a number of other species (Corré 1983a,b,c). According to this author, such retardation occurs before the morphological adaptations to weak light are accomplished; the relative increase in leaf area that normally occurs under low light cannot compensate for the lower productivity any longer, and the relative growth rate will decline. This response of plants to different irradiances is consistent with the results obtained in the present study.

In both species, a high nutrient application to the stockplants reduced the subsequent rooting percentage of cuttings, in agreement with the findings of Pearse (1943), Moe and Andersen (1988) and Leakey and Storeton-West (1992) in a range of different species. It appears that cuttings from stockplants grown with a high nutrient supply may have had a supraoptimal carbohydrate content. A high concentration of leaf starch in cuttings may suppress post-severance photosynthesis and their rooting due to end-product inhibition, as has been shown by Leakey and Storeton-West (1992) and Hoad and Leakey (1992). A reduction in net photosynthetic rate as a result of high nutrient supply was found in *A. guachapele* stockplants grown under high irradiance. Stockplants with a high nutrient supply also had lower specific leaf areas (thicker leaves); this may result in greater mutual shading of chloroplasts and reduced efficiency of gas exchange (Hoad and Leakey 1992). The initial stimulus for root formation, and hence rooting percentage, seems to be little influenced by the nutritional status of a stockplant or cutting, since root primordium initiation appears to be hormonally controlled (Lovell and White 1986; Moe and Andersen 1988; Veierskov *et al.* 1982a,b; Veierskov and Andersen 1982). Therefore, the reduction in rooting percentage as a result of high nutrient supply to the stockplant may be dependent on hormonal factors rather than the nutritional status of the stockplants or cutting. In this regard, low photosynthetic activity, induced by a supraoptimal concentration of nutrients in the cutting, may reduce the auxin supply to the base of the cutting, as has been shown by Heide (1968), Hilman and Gaston (1961), Kumpula and Potter (1984), Scott and Briggs (1963) and Vardar (1968). The transport of other rooting cofactors produced in leaves and buds is also reduced under conditions of low photosynthetic activity (Davis 1988). It is also possible that photosynthesis influences the formation of a non carbohydrate, non-auxin component which may be involved in root initiation (Davis 1988). These indirect effects of reduced photosynthesis on rooting, influenced by high nutrient supply to the stockplants, were probably responsible for the low rooting shown by subsequent cuttings in this study. Indeed, *A. guachapele* cuttings from stockplants grown with high nutrients showed a significant reduction in the chlorophyll fluorescence ratio (*Fv/Fm*) during most part of the propagation period, as well as a reduction in dry mass after 10 days in the propagator. These are good indicators of a reduction in the photosynthetic ability of such cuttings.

The light environment under which the stockplants were grown had a less dramatic effect than the nutrient treatments on subsequent rooting, but some interesting interactions were found. In *A. guachapele*, high stockplant irradiance reduced subsequent rooting, and this effect was greater when high nutrients were applied. In *C. alliodora*, on the other hand, high irradiance increased rooting in cuttings from stockplants grown with a high nutrient supply. There is a similarity in this response, however, in the sense that high nutrients, in both cases, reduced rooting of cuttings from stockplants grown under those light environments which caused a reduction in their growth rate. Therefore, it seems that the negative effect on rooting of short cuttings formed under low (*C. alliodora*) or high light (*A. guachapele*) was enhanced by a high nutrient application, probably as a result of supraoptimal carbohydrate content in these cuttings, as discussed above.

Regarding the number of roots produced by the cuttings, results were consistent with the widely accepted view that root initiation and root development may be influenced by a different set of conditions (Lovell and White 1986). Thus, treatments associated with a high rooting percentage may induce the formation of fewer roots per cutting and *vice versa*. The initial stimulus for root formation, and hence rooting percentage, seems to be little influenced by the nutritional status of a stockplant or a cutting, since root primordium initiation appears to be hormonally controlled (Lovell and White 1986; Moe and Andersen 1988; Veierskov *et al.* 1982a,b; Veierskov and Andersen 1982). The number of roots produced by the 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). Therefore, there was an apparent contradiction that either high light, high nutrients, or both, caused a reduction in root numbers in both species. Similar results have been observed in a range of different species (Hoad and Leakey 1992; Leakey and Storeton-West 1992; Pearse 1943; Moe and Andersen 1988). It appears that a high concentration of leaf starch in cuttings may suppress current photosynthesis due to end-product inhibition. As noted above, indications of a reduction in photosynthetic ability of these cuttings during propagation were observed in *A. guachapele*. This was consistent with a greater pre-severance photosynthetic rate. These results correspond with those of Hoad and Leakey (1992), Leakey and Coutts (1989), Leakey and Storeton-West (1992), Leakey *et al.* (1992; 1993) and Newton *et al.* (1992a), who suggested that root formation in cuttings is dependant not only on their initial carbohydrate content but also on an adequate rate of current photosynthesis during propagation, this being related to photosynthetic capacity prior to severance.

Cutting origin

The physiology, morphology and subsequent rooting potential of a cutting is influenced by the position within a stem from which the cutting originates (Hartmann and Kester 1983; Lo 1985; Leakey and Coutts 1989; Leakey *et al.* 1993). In *Triplochiton scleroxylon*, it has been found that among the large variety of factors affected by cutting origin, the effect on internode length and diameter seems to be the most important, through its influence on the cuttings capacity to store assimilates produced both pre- and post-severance (Leakey et al. 1993). In this species, cutting length was found to be strongly correlated with rooting percentage (Leakey and Mohammed 1985). In most studies, however, cutting morphology has been more commonly correlated with the number of roots produced by the cutting (Poulsen and Andersen 1980; Veierskov 1978). This is in agreement with the view that the storage capacity of cuttings appears to be very important for root development but not for root initiation (Veierskov and Andersen 1982; Veierskov *et al.* 1982a,b). The results of this study seems to support the latter view, since cutting morphology generally affected the number of roots produced by the cuttings of both species but not their rooting percentage.

With *C. alliodora*, an increase in cutting diameter (within the tested range - 3 to 6 mm) generally resulted in a significant increase in number of roots, while no significant correlations were found between number of roots and cutting length. Cutting diameter followed the normal trend of increase from apical to basal nodes but no clear patterns were evident between cutting origin and cutting length.

The lack of relationships between number of roots and cutting length in *C. alliodora* was probably due to the fact that differences in length were usually small or absent, when standard length cuttings were used. The beneficial effect of increases in cutting diameter on the number of roots produced by the cuttings indicated the importance of stored reserves on root development. The rate of current photosynthesis did not show any clear pattern in cuttings from different origins within the stem and no relationships were found between this and number of roots. A great deal of discussion has been devoted to the relative importance of stored reserves and assimilates formed during propagation on rooting, but separating these effects is probably impossible, since induced variations in current photosynthesis would affect many other related processes that may also have a determinant effect on rooting. From this study, it would appear that cutting diameter, and hence stored reserves, had a stronger influence on the number of roots produced by the cuttings than current photosynthesis. However, it is known that very little light is needed to saturate photosynthesis of unrooted cuttings (Davis and Potter 1987) and even low rates of current photosynthesis can contribute significantly to the carbon budget of a given plant part (Okoro and Grace 1976). In this study, cuttings from all node positions actively photosynthesized during the propagation period, with mean rates of between 2.5 μmol m^{-2} s^{-1} and 3.1 μmol m^{-2} s^{-1}. It is possible that even the lowest rate of current photosynthesis was sufficient for an adequate supply of assimilates but cuttings with greater stored reserves had a comparative advantage. Cuttings taken sequentially down a stem are known to vary in morphology, nutrient, starch and sugar contents, water relations, plant growth regulators, rooting cofactors, photosynthetic capacity and probably other factors, but the magnitude and importance of this variation across short stems is unknown. The lack of systematic differences in rates of current photosynthesis and stomatal conductance between cuttings from different origins suggests that these processes were not differentially affected, and that the main effect of node position on root development resulted from differences in cutting diameter and the related differences in stored reserves.

With *A. guachapele*, a positive relationship was found between rooting percentage and cutting length, while a negative relationship was found with cutting diameter. Cutting diameter increased normally from the apical to the basal nodes while cutting length decreased in the same direction. As noted above, number of roots and not rooting percentage is normally related to cutting morphology. In *A. guachapele*, basal nodes are extremely short (3 cm-3.5 cm), woody and hollow and inappropriate for rooting. These cuttings also had a greater susceptibility to rotting, because their leaves were lying on the surface of the medium. The same negative effects of short nodes has been found in *Eucalyptus grandis* (Hoad and Leakey 1992). These authors found that a large stem dry mass or volume, with a large but not too concentrated pools of soluble and storage carbohydrates provide an adequate supply of carbohydrates without inhibiting photosynthesis; current photosynthesis may be inhibited if stems are too short. Therefore, the correlations found in this study may simply reflect the high mortality rate of these short, basal nodes. The number of roots produced by these basal cuttings was also reduced, particularly in the most basal node, highlighting once again the importance of stored reserves and assimilates produced during propagation on root development and growth.

In *C. alliodora*, when cuttings from different node positions were potted and evaluated for growth characteristics, strong positive correlations were found after three months growth between cutting diameter and total height, shoot basal diameter and height to the first whorl of branches. The first two correlations can be explained in terms of stored reserves in the cuttings, which give thicker cuttings an initial advantage. In addition, cutting diameter was also positively correlated with the number of roots produced by the cuttings, and therefore, it was expected that the greater number of roots of thicker cuttings resulted in an advantage in terms of initial growth of the plants. It was not clear why the height to the first whorl of branches was also affected by cutting diameter. It is possible that each individual internode maintains the same role previously defined within the whole plant, perhaps due to endogenous levels of growth regulators along a gradient within the stem. In this way, thinner cuttings from apical positions would produce the first tier of branches at a lower height than thicker cuttings from lower sections of the stem. The correlations between cutting diameter and shoot basal diameter and shoot height became weaker after six months and disappeared after ten months growth, as other factors which normally influence growth, such as soil fertility, type of root system, biomass production, availability of water, etc. started to play a greater role. The correlations between cutting diameter and height to the first whorl of branches remained constant, as this characteristic does not vary with time.

Post-severance treatments

Foliar area and irradiance during propagation

The cutting's ability to photosynthesize and remain turgid during the propagation period has been identified as an important factor determining rooting success (Eliasson and Brunes 1980; Leakey *et al.* 1982a; Leakey and Coutts 1989;

Leakey *et al.* 1993; Newton *et al.* 1992a; Okoro and Grace 1976). The practice of trimming the leaf aims at minimizing water loss while allowing some photosynthesis during the rooting process (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, VPD and stomatal conductance (Loach 1988a).

In *C. alliodora*, interesting interactions were found between foliar area and irradiance during propagation. Very low irradiances during propagation resulted in a low rooting percentage of cuttings with small foliar areas (10 cm^2), but an increase in rooting occurred in cuttings with foliar areas of 20 cm^2 and 30 cm^2. Under high irradiance, rooting percentage decreased with increases in foliar area. The results suggest that low rates of current photosynthesis and/or amount of stored reserves in the small leafed cuttings were insufficient to support the cutting long enough to initiate the formation of roots. Auxin transport and/or synthesis may also be reduced under conditions of low photosynthetic activity (Heide 1968; Hillman and Galston 1961; Scott and Briggs 1963; Vardar 1968). Although this aspect was not investigated, it may be important in stimulating root primordium initiation. Indeed, when the experiment was repeated using a slightly higher irradiance, no differences in rooting percentage were found between the three leaf area treatments. The importance of an active rate of current photosynthesis on rooting, as has been suggested by Davis (1988) and Leakey and Coutts (1989), was supported by the strong positive relationships found in this study between the chlorophyll fluorescence ratio of cuttings and final rooting percentage. It was also evident that cuttings display an optimal photosynthetic rate when irradiance reaches a certain peak. P_n in cuttings increased with increases in irradiance until approximately 400 μmol m^{-2} s^{-1}, to decrease with further increases in irradiance. The photosynthetic rate also decreased with increases in leaf area, particularly under high irradiance; this probably resulted from the closure of stomata which restricted gas exchange, as larger leaves are more susceptible to water loss and the associated water deficits. This reduction in photosynthesis corresponded with decreases in rooting percentage.

The number of roots produced by the cuttings showed an opposite pattern to that of rooting percentage, in relation to leaf areas and irradiance during propagation. Increases in leaf areas and irradiances resulted in increases in root numbers. This supports the view that root initiation and root development are affected by a different set of conditions, as discussed above. In this study, it was evident that total production of assimilates had a greater influence on the number of roots produced by cuttings than on rooting percentage. Therefore, it appears that a high photosynthetic activity promotes the initiation of root primordia through its effects on hormonal factors rather than nutritional effects. These factors include increased transport and/or production of auxin and other rooting cofactors to the base of the cutting, where they promote rooting (Davis 1988; Heide 1968; Hillman and Galston 1961; Kumpula and Potter 1984; Scott and Briggs 1963). Once the initial stimulus for root initiation occurs, a higher availability of stored reserves or current assimilates will determine the number of roots than can be supported by the cutting. In this regard, cuttings with large leaf areas and high irradiances during propagation would result in the production of larger number of roots in this species, providing that low irradiances are used during the initial stages of the rooting process. This is in agreement with early suggestions by Leakey (personal communication) that for some species, it may be beneficial to increase the irradiance progressively during the rooting period.

Rooting medium

The importance of the rooting medium on propagation has been known for many years (Andersen 1986; Hartmann and Kester 1983). It is agreed that the provision of water is a prime requirement, but it is also clear that oxygen is necessary for adventitious root development in cuttings (Hartmann and Kester 1983; Loach 1986). It appears that the optimal balance between air and water differs according to the species involved, the propagation system and even the weather (Loach 1988a). This is probably the reason why, generally, claimed correlations between air or water content and rooting have not been reproducible in repeated propagations (Loach 1986). Besides, since percent air and water are inversely correlated for any one medium, it is not possible to say whether high air content or low water content promoted higher rooting (Loach 1986).

In this study it appears that, in *C. alliodora*, water content was more related to rooting ability than air content. Both rooting percentage and number of roots produced by the cuttings were significantly larger in sand and gravel than in sawdust. The air content of sawdust and gravel, however, was almost identical, and the main difference between sawdust and the other two media was the larger water content of the former. No relationships were evident between the air/water ratio of the media and the rooting ability of cuttings: the air/water ratio of the media was similar for sawdust (0.57) and sand (0.33), while a much larger ratio was found in gravel (7.00).

It was interesting to find that in spite of the lower rooting of cuttings in sawdust, the photosynthetic rates of cuttings in all media were similar, with rates between 0 μmol m^{-2} s^{-1} and 6 μmol m^{-2} s^{-1}, and showed a similar pattern following increments in light. Furthermore, cuttings in sawdust showed larger chlorophyll fluorescence ratios during the first three weeks in the propagators, which indicates the proper functioning of the photosynthetic apparatus of these cuttings (Bolhar-Nordenkampf *et al.* 1989). It is possible that the larger foliar temperatures of cuttings in sand (43.1 °C) and gravel (43.3 °C), as compared with sawdust (31.4 °C) resulted in this reduction in chlorophyll fluorescence ratios, as it is known that most leaves are damaged at approximately 42 °C (Turner and Newton 1990). The larger availability of water in sawdust probably resulted in this difference in foliar temperature, through the cooling effect of water condensing on the leaves of cuttings in this medium.

It appears, therefore, that the negative effect of sawdust on rooting was more related to the excessive water content of this medium, and the subsequent rotting of cutting bases, than on the physiological condition of the aerial part of the cuttings. This result highlighted the importance in propagation of optimizing all factors that may affect rooting, as discussed by Leakey *et al.* (1993); considering only some processes that occur in the leaf does not necessarily result in greater rooting if other processes in other parts of the cutting are operating under suboptimal conditions.

Auxins

For many years it has been known that auxins increase the percentage of cuttings which form roots, but also hasten root initiation, increase the number and quality of roots produced per cuttings and increase uniformity of rooting (Blazich 1988; Hartmann and Kester 1983). The effects of auxins on rooting have been associated with an increase in cell division and enhanced transport of carbohydrates and leaf cofactors to the base of the cutting (Haissig 1974a). Exogenous auxin may also induce DNA synthesis in suitable cells (Gaspar and Hofinger 1988). It is presently unknown whether applied auxins have an indirect effect on other physiological processes that may stimulate rooting, such as water and photosynthetic relations.

In this study, rooting percentage of *C. alliodora* was considerably enhanced by increasing concentrations of indol-3- butyric acid (IBA) from 0 % to 1.6 %. *A. guachapele* generally responded equally to a range of concentrations between 0.05 % and 0.4 % IBA, showing a decrease in rooting with higher concentrations. In both species, rooting was generally very low when no IBA was applied. In *C. alliodora*, the number of roots per rooted cutting significantly increased with increasing concentrations of IBA; a similar trend was found in *A. guachapele*, although a slight reduction in root numbers was sometimes observed with the highest concentration used (0.8 %).

It was not possible, however, to establish a clear relationship between IBA concentration and water relations or production of assimilates in the cuttings. The relative water content of *C. alliodora* cuttings was unaffected by variations in IBA concentration, as the observed changes followed a similar pattern in all cuttings, regardless the IBA concentration used. Changes in relative water content appeared to be related to environmental changes during the propagation period. Similarly, no relationships were found between changes in foliar or total dry mass and final rooting. The increase in total dry mass with time indicated that photosynthesis did occur in cuttings during propagation, but the increment was similar in all treatments. It must be taken into account that dry mass is only a rough indicator of photosynthetic activity (Davis 1988) and there is also the problem, which is impossible to avoid experimentally, of calculating gains or losses based on different samples. Although the corrections used in this study attempted to reduce this problem, it is likely that only slight differences in the estimations may obscure the relatively minor differences in dry mass that may occur in the cuttings.

Increasing concentrations of IBA clearly inhibited bud growth, and a strong negative relationship was found between the percentage of cuttings with actively growing shoots at week 4 and final rooting percentage. This suggests that IBA enhanced competition for assimilates and probably other factors by the cutting base, with sink strength successively enhanced by each increase in IBA concentration. It is known that growing shoots may act as a sink for assimilates in competition with the cutting base, which may limit rooting (Davis 1988). The same result was found in *Populus tremula*, in which root growth was significantly reduced by shoot growth (Eliasson 1971). This is in agreement with the work of Phillips (1969; 1975), who found that metabolites and other growth factors are translocated to auxin-treated regions of the stem.

In *A. guachapele* it was interesting to find that, although the general response to IBA followed a similar pattern in all experiments, the optimal concentration varied. In addition, control cuttings (without IBA) showed different rooting percentages in different experiments, from less than 20 % to approximately 55 %. These differences may be due to the fact that these were preliminary experiments, using seedling material, and that no attempt was made to standardize many other factors that may influence rooting. It is also possible that seasonal changes in endogenous auxin occur in *A. guachapele*, as has been found in other species (Gaspar and Hofinger 1988), and this influenced the cuttings' response to exogenous auxin applications.

The increase in number of roots obtained with increasing concentrations of IBA found in this study is in agreement with results obtained with many other species (Mesén *et al.* 1992). In these studies, as with *A. guachapele*, the optimal concentration for root production was always higher than that for rooting percentage. This is probably related to the suggestion that each of the successive phases that occur during the rooting process has a specific associated physiology and the need for auxin in each phase may be different (Gaspar and Hofinger 1988). Little is known about the different auxin requirements in each phase, but at least two different phases of sensitivity to IAA are known (Imaseki 1985). The results of this study suggest that the IBA requirements during the initial stages of the rooting process are lower than those at later stages and that concentrations that inhibit the initiation of root primordia may become optimal for root development. It is impractical to increase the IBA concentration at some point of the rooting process and besides, it is unknown when such a treatment should be applied. However, the number of roots produced by the cuttings would be increased by using the highest possible IBA concentration within the optimal range for rooting percentage.

Clonal variation

Clonal differences in rooting ability have been noted for at least 50 years, although in many cases, clonal variation has been confounded with other nongenetic factors that are transmitted from the parent ortet to their ramets (*M-effects*) or with characteristics of the individual propagules (*m-effects*) (Haissig and Riemenschnider 1988). In this study no attempt was made to investigate clonal variation in any detail. However, *M-effects* were probably eliminated in these studies by using equal age stockplants. Similarly, *m-effects*, or characteristics of the individual propagules such as diameter, were indirectly included as design variables through allocation of cuttings in order by their position within the stem. Sorting cutting diameter by replication has been used to separate *C-effects* -nongenetic factors in general- from genetic effects (Wilcox and Farmer 1968). Therefore, differences between clones found in this study are probably genetic differences.

Little is known about genetic aspects of rooting, although substantial evidence exists that rooting by cuttings is genetically controlled (Haissig 1986). It is known that clones vary in their concentrations of endogenous auxin, rooting cofactors or inhibitors, anatomy, leaf retention and many other interacting factors that may affect rooting

(Leakey *et al.* 1982), all of which are likely to produce differences in rooting ability, as observed in the present study. Current theory suggests that lack of essential genes or their inadequate expression may preclude organ regeneration (Haissig and Riemenschnider 1988). However, little is known about the possible genetic modes of action or genetic variation in physiological and biochemical mechanisms that mediate gene expression (Haissig and Riemenschnider 1988).

Practical implications

In *C. alliodora* stockplants, low irradiance resulted in higher number of roots in subsequent cuttings but the plants grew slower and produced shorter cuttings (Chapter 5). It may be worth trying an intermediate irradiance, which could perhaps improve the quality of the cuttings produced, as compared to low light, without negatively affecting their rooting ability. Planting a shade tree among the stockplants may be a practical way of achieving this, as has been done with *Triplochiton scleroxylon* stockplants grown under *Leucaena leucocephala* (Leakey and Storeton-West 1989). Shade should also be used for growing *A. guachapele* stockplants, to stimulate the production of longer internode cuttings, which show a higher rooting ability.

In *C. alliodora* cuttings with diameters of around 5 mm-6 mm should be used to stimulate the production of higher number of roots per rooted cutting. Cutting length is not critical, but cuttings longer than 30 mm should be used (Chapter 4). Thicker cuttings also result in plants with faster initial growth and the formation of branches higher up in the stem (Chapter 6). In *A. guachapele*, on the other hand, the use of thin (around 4 mm) and long (around 40 mm) cuttings result in higher rooting percentage and higher number of roots per cutting. Short, hollow cuttings from basal sections of the stem should be avoided (Chapter 8).

Indole-3-butyric acid (IBA) should be applied to the cutting base to increase the rooting percentage and the number of roots produced by the cuttings of both species. In *C. alliodora*, the higher concentration tested (1.6 % IBA) produced the best results, both in terms of rooting percentage and number of roots per rooted cutting (Chapter 3). In *A. guachapele*, rooting percentage was equally increased by IBA concentrations of between 0.05 % and 0.2 %, while the number of roots tended to increase with increasing IBA concentrations from 0 % to 0.8 %. However, as cuttings with a concentration of 0.2 % IBA produced sufficient roots (an average of nine roots in different studies), it seems reasonable to recommend a concentration of 0.2 % IBA, to avoid the negative effect of higher IBA concentrations on rooting percentage (Chapter

8). In all these studies, IBA dissolved in methanol was used, applying 10 μl droplets of solution to the clean cut base of the cuttings.

Cuttings of *C. alliodora* rooted equally well in sand and gravel, while both the rooting percentage and the number of roots per cutting were reduced when sawdust was used. It seems that rooting in this species is negatively affected when a rooting medium with a high water content is used. Sand should be preferred for practical reasons, since the practices of making the holes, inserting the cuttings in the rooting medium and removing the cuttings for evaluation are simplified in this medium as compared to gravel (Chapter 7). In *A. guachapele* it seems that media with a low air content, such as sand, are inappropriate for rooting. Gravel, or the mixtures (50:50 p/v) of sawdust with either sand or gravel should be used, as these media increased rooting ability of this species (Chapter 8).

In *C. alliodora*, propagation without shade resulted in extremely high temperatures inside the propagator, which damaged the leaves and reduced rooting, particularly when cuttings with high foliar areas (20 cm^2-30 cm^2) were used. Excess shade (two layers of black plastic netting), on the other hand, resulted in low photosynthetic activity of the cuttings and reduced rooting, particularly in cuttings with small foliar areas (10 cm^2). Foliar areas of 30 cm^2 and irradiances of between 300 $\mu mol\ m^{-2}\ s^{-1}$ and 400 $\mu mol\ m^{-2}\ s^{-1}$ were found appropriate, to maximize photosynthetic rates and rooting ability of cuttings. Although cuttings with smaller leaf areas showed similar rooting percentages than larger leaved cuttings at the same irradiances, which represents an advantage in terms of space in the propagator, the number of roots per cutting increased with increases in leaf area (Chapter 4). Increasing the irradiance progressively during the propagation period was suggested as a way to increase the number of roots produced by the cuttings, but this aspect needs further investigation.

In both species there is a strong clonal variation in rooting ability, and a preliminary selection should be carried out based on this variable. Clones with higher rooting percentage are not necessarily those with higher number of roots. However, most clones usually produced more than three well distributed roots per cutting, which is considered appropriate and therefore, rooting percentage could be used as a criteria for initial clonal selection (Chapters 3-6).

It is evident that adventitious root formation in cuttings is a complex process, affected by a large number of interrelated factors, operating both pre- and post-severance. While some factors operate in the leaf, others are generally of greatest importance in the cuttings base. Optimizing some of these factors does not necessarily result in higher rooting, if other factors that occur in a different part of the cutting are limiting. In addition, evidence was presented that suggests that treatments which may be optimal during the stage of root initiation may not be so at later stages of root development. It is important to be aware of all the processes involved that may have an influence on rooting and the way they are affected by different treatments, in order to obtain successful rooting. The results obtained in this study shed more light on the process of adventitious root formation in cuttings, but it is clear that much research is still needed to finally understand this complex process completely.

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APPENDIX A

Table A1 Analysis of variance of percentage rooting of leafy stem cuttings of *Cordia alliodora* from three clones, treated with five different concentrations of IBA, after nine weeks in non-mist propagators. Data was collected from 600 cuttings, 40 from each clone/IBA combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	7	16.246	2.321	4.88	0.0001
Clone	2	4.090	2.045	4.30	0.0141
IBA	4	63.620	15.905	33.43	0.0001
Residual	525	249.760	0.476		
Total	574	354.559			

*Only significant ($p < 0.05$) results are presented

Table A2 Analysis of variance of the number of roots per rooted cutting in leafy stem cuttings of *Cordia alliodora* from three clones, treated with five different concentrations of IBA, after nine weeks in non-mist propagators. Data was collected from 600 cuttings, 40 from each clone/IBA combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	7	129.608	18.515	6.53	0.0001
Clone	2	69.634	34.827	12.29	0.0001
IBA	4	345.131	86.283	30.44	0.0001
Residual	219	620.739	2.834		
Total	281	1442.571			

*Only significant ($p < 0.05$) results are presented

Table A3 Analysis of deviance by stepwise regression to determine the relative influence of IBA concentration, clone, the presence of leaf in the cutting (leaf), the presence of shoot in the cuttings at week 4 (shoot), the cuttings diameter and the node position in the rooting ability of single-node, leafy stem cuttings of *Cordia alliodora* from three clones, treated with five different concentrations of IBA, after nine weeks in non-mist propagators.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Block	7	26.915	3.845	3.69	< 0.01
IBA	4	120.919	30.230	29.05	< 0.01
Leaf	1	30.570	30.570	29.37	< 0.01
Diameter	1	12.238	12.238	11.76	< 0.01
Shoot	1	14.608	14.608	14.04	< 0.01
Clone	2	11.040	5.520	5.30	< 0.01
Node position	4	4.047	1.012	0.97	ns
Residual	554	576.573	1.041		
Total	574	796.909			

Table A4 Analysis of variance of percentage rooting at week seven of leafy stem cuttings of *Cordia alliodora* from three clones, with three different leaf areas, set to root with or without shade in non-mist propagators (PE). Data was collected from 864 cuttings, 48 from each irradiance/foliar area/clone combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	7	7.239	1.034	2.73	0.0083
Clone	2	74.713	37.357	98.63	0.0001
Node position	5	4.572	0.914	2.41	0.0347
PE*clone	2	3.753	1.876	4.95	0.0073
PE*foliar area	2	10.092	5.046	13.32	0.0001
Clone*area	4	8.168	2.042	5.39	0.0003
Residual	813	307.925	0.379		
Total	863	428.777			

***Only significant ($p < 0.05$) results are presented**

Table A5 Analysis of variance of mean number of roots of leafy stem cuttings of *Cordia alliodora* from three clones, with three different leaf areas, after seven weeks in non-mist propagators (PE) with or without shade. Data was collected from 864 cuttings, 48 from each irradiance/foliar area/clone combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	7	174.235	24.891	7.48	0.0001
PE	1	13.214	13.214	3.97	0.0467
Clone	2	638.170	319.085	95.93	0.0001
Leaf area	2	20.688	10.344	3.11	0.0454
Node position	5	226.955	45.391	13.65	0.0001
PE*clone	2	148.836	74.418	22.37	0.0001
PE*foliar area	2	21.281	10.641	3.20	0.0415
Residual	572	1902.661	3.326		
Total	622	3266.915			

*Only significant ($p < 0.05$) results are presented

Table A6 Analysis of deviance for stepwise regression to determine the influence of propagation environment (PE), node position (node) and morphological characteristics of single-node, leafy stem cuttings of *Cordia alliodora* on their rooting ability, after seven weeks in non-mist propagators with or without shade.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Block	7	15.277	2.183	2.23	< 0.05
Clone	2	157.549	78.774	80.49	< 0.01
Node	5	11.282	2.256	2.31	< 0.05
Cutting diameter	1	4.281	4.281	4.37	< 0.05
Leaf area	2	8.198	4.099	4.19	< 0.05
Cutting length	1	0.276	0.276	0.28	ns
PE	1	0.022	0.022	0.02	ns
Residual	844	825.995	0.979		
Total	863	1022.881			

Table A7 Analysis of variance of percentage rooting at week six of leafy stem cuttings of *Cordia alliodora* from three clones, with three different leaf areas, set to root in non-mist propagators with or without shade (PE). Data was collected from 648 cuttings, 36 from each irradiance/foliar area/clone combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	5	27.709	5.542	13.55	0.0001
PE	1	5.213	5.213	12.74	0.0004
Clone	2	21.346	10.673	26.09	0.0001
Node position	5	5.776	1.155	2.82	0.0157
PE*clone	2	3.617	1.809	4.42	0.0124
Clone*leaf area	4	10.456	2.614	6.39	0.0001
Clone*node position	10	8.491	0.849	2.08	0.0245
Residual	599	245.023	0.409		
Total	647	334.375			

*Only significant ($p < 0.05$) results are presented

Table A8 Analysis of variance of mean number of roots at week seven of leafy stem cuttings of *Cordia alliodora* from three clones, with three different leaf areas, set to root in non-mist propagators with or without shade (PE). Data was collected from 648 cuttings, 36 from each irradiance/foliar area/clone combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	5	189.342	37.868	4.81	0.0003
PE	1	283.331	283.331	35.99	0.0001
Clone	2	545.581	272.791	34.65	0.0001
Node position	5	150.515	30.103	3.82	0.0021
PE*clone	2	120.435	60.217	7.65	0.0005
Residual	406	3196.463	7.873		
Total	454	4759.749			

*Only significant ($p < 0.05$) results are presented

Table A9 Analysis of deviance for stepwise regression to determine the influence of propagation environment (PE), clone, leaf area, node position (node) and cutting diameter of single-node, leafy stem cuttings of *Cordia alliodora* on their rooting ability, after seven weeks in non-mist propagators with or without shade

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Block	5	33.052	6.610	6.61	< 0.01
Clone	2	59.911	29.955	29.95	< 0.01
PE	1	9.405	9.405	9.40	< 0.01
Leaf area	2	7.079	3.540	3.54	< 0.05
Node	5	7.572	1.514	1.51	ns
Cutting diameter	1	0.343	0.343	0.34	ns
Residual	603	603.078	1.000		
Total	619	720.440	1.164		

Table A10 Analysis of variance of net photosynthetic rate (*Pn*) of leafy stem cuttings of *Cordia alliodora* from three clones, with three different leaf areas, set to root in non-mist propagators with or without shade (PE). Measurements were taken at weeks 3, 4 and 5, in a sample of six cuttings per treatment.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Week	3	18.040	6.013	9.17	0.0001
PE	1	63.303	63.303	96.55	0.0001
Leaf area	2	26.058	13.029	19.87	0.0001
Week*PE	3	6.865	2.288	3.49	0.0178
Week*leaf area	6	12.227	2.038	3.11	0.0072
PE*leaf area	2	6.759	3.379	5.15	0.0071
Residual	123	80.649	0.656		
Total	147	222.618			

*Only significant ($p < 0.05$) results are presented

Table A11 Analysis of deviance for stepwise regression to determine the influence of propagation environment (PE) and leaf area on the net photosynthetic rate of single-node, leafy stem cuttings of *Cordia alliodora* set to root in non-mist propagators with or without shade.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Week	3	18.040	6.013	6.19	< 0.01
PE	1	28.042	28.042	28.86	< 0.01
Leaf area	2	39.546	19.773	20.35	< 0.01
Residual	141	136.991	0.972		
Total	147	222.618			

Table A12 Analysis of variance of chlorophyll fluorescence ratio (*Fv/Fm*) of single-node, leafy stem cuttings of *Cordia alliodora* from three clones, with three different leaf areas, set to root in non-mist propagators with or without shade (PE). Measurements were taken at weeks 1, 2 and 3 in a sample of six cuttings per treatment.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
PE	1	0.903	0.903	261.70	0.0001
Leaf area	2	0.128	0.064	18.56	0.0010
Node position	5	0.097	0.019	5.63	0.0161
PE*leaf area	2	0.102	0.051	14.81	0.0020
PE*node position	5	0.094	0.019	5.44	0.0179
Residual	8	0.028	0.003		
Total	31	1.376			

*Only significant ($p < 0.05$) results are presented

Table A13 Analysis of variance of shoot growth of five clones of *Cordia alliodora* after sixteen weeks under low or high irradiance, with or without NPK. Data represents means of five plants from each clone/irradiance/nutrient combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Irradiance	1	970.898	970.898	11.01	0.0014
Clones	4	1957.861	489.465	5.55	0.0006
Irradiance*clone	4	890.836	222.709	2.53	0.0477
Residual	75	6612.067	88.161		
Total	90	10960.681			

*Only significant ($p < 0.05$) results are presented

Table A14 Analysis of variance of specific leaf area of *Cordia alliodora* stockplants grown under low or high irradiance, with or without NPK. Data represents the mean of five samples from each treatment.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Irradiance	1	6.481	6.481	23.27	0.0001
Irradiance*NPK	1	5.733	5.733	20.59	0.0001
NPK*clone	4	5.535	1.384	4.97	0.0012
Residual	84	23.390	0.278		
Total	99	45.169			

*Only significant ($p < 0.05$) results are presented

Table A15 Analysis of variance of net photosynthetic rate of five *Cordia alliodora* clones growing under high or low irradiance, with or without NPK. Data represents the mean of two measurements on five plants per irradiance-nutrient combination carried out on consecutive days after sixteen weeks growth.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Day	1	4.753	4.753	24.59	0.0001
Irradiance	1	12.612	12.612	65.26	0.0001
Day*irradiance	1	2.387	2.387	12.35	0.0008
Residual	65	12.562	0.193		
Total	88	36.121			

*Only significant ($p < 0.05$) results are presented

Table A16 Analysis of variance of stomatal conductance of five *Cordia alliodora* clones growing under low or high irradiance, with or without NPK. Data represents the mean of two measurements on five plants per irradiance-nutrient combination carried out on consecutive days after sixteen weeks growth.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Day	1	267949.227	267949.337	38.51	0.0001
Irradiance	1	384710.606	384710.606	55.29	0.0001
NPK	1	28475.455	28475.455	4.09	0.0472
Day*NPK	1	29042.581	29042.581	4.17	0.0451
Irradiance*NPK	1	58434.028	58434.028	8.40	0.051
Residual	65	452306.959	6958.569		
Total	88	1282150.630			

*Only significant ($p < 0.05$) results are presented

Table A17 Analysis of variance for stepwise regression to determine the influence of day of measurement, stomatal conductance (g_s), irradiance and nutrient treatment on the net photosynthetic rate per unit leaf area of *Cordia alliodora* stockplants growing under low or high irradiance, with or without NPK. Data represents the mean of two measurements on five plants per irradiance-nutrient combination carried out on consecutive days after 16 weeks growth.

Source	Degrees of freedom	Sum of squares	Mean square	F value	P
Irradiance	1	19.374	19.374	104.35	< 0.01
g_s	1	0.959	0.959	5.16	< 0.05
NPK	1	0.008	0.008	0.04	ns
Residual	85	15.781	0.186		
Total	88	36.121			

Table A18 Analysis of variance for stepwise regression to determine the influence of day of measurement, irradiance and nutrient treatment on the stomatal conductance of *Cordia alliodora* stockplants growing under low or high irradiance, with or without NPK. Data represents the mean of two measurements on five plants per irradiance-nutrient combination carried out on consecutive days after sixteen weeks growth.

Source	Degrees of freedom	Sum of squares	Mean square	F value	P
Irradiance	1	247458	247458	22.15	< 0.01
NPK	1	73710	73710	6.60	< 0.05
Residual	86	960983	11174		
Total	88	1282151			

Table A19 Analysis of variance of rooting percentage of single-node, leafy stem cuttings of *Cordia alliodora* from five clones, grown under low or high irradiance, with or without NPK, after six weeks in non-mist propagators. Data was collected from 600 cuttings, 30 from each irradiance/nutrient/clone combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	4	18.983	4.746	9.60	0.0001
Clone	4	28.605	7.151	14.46	0.0001
NPK	1	3.224	3.224	6.52	0.0109
Irradiance*clone	4	26.467	6.617	13.38	0.0001
NPK*clone	4	6.892	1.723	3.48	0.0080
Residual	545	269.539	0.495		
Total	599	369.518			

*Only significant ($p < 0.05$) results are presented

Table A20 Analysis of variance of mean number of roots per rooted cutting of leafy stem cuttings of *Cordia alliodora* from five clones, grown under low or high irradiance, with or without NPK, after six weeks in non-mist propagators. Data was collected from 600 cuttings, 30 from each irradiance/nutrient/clone combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	4	299.024	74.756	4.38	0.0019
Irradiance	1	280.953	280.953	16.45	0.0001
Clone	4	394.742	98.685	5.78	0.0002
Irradiance*NPK	1	84.328	84.328	4.94	0.0272
NPK*clone	4	292.845	73.211	4.29	0.0022
Residual	257	4390.011	17.082		
Total	311	6260.074			

*Only significant ($p < 0.05$) results are presented

Table A21 Analysis of deviance for stepwise regression to determine the influence of treatments to the stockplants, node position within the stem and clone on the rooting ability of single-node, leafy stem cuttings of *Cordia alliodora* after six weeks in non-mist propagators. Six hundred cuttings from six node positions within the stem were taken from stockplants of five clones growing under low or high irradiance, with or without NPK.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Block	4	31.211	7.803	6.19	< 0.01
Clone	4	50.321	12.580	9.98	< 0.01
NPK	1	6.027	6.027	4.78	< 0.05
Node position	5	5.966	1.193	0.95	ns
Irradiance	1	1.128	1.128	0.89	ns
Residual	584	736.163	1.261		
Total	599	830.816			

Table A22 Analysis of variance of rooting percentage of leafy stem cuttings of *Cordia alliodora* from six different positions in the stem (apical to basal), after eight weeks in non-mist propagators. Data was collected from 480 cuttings, 80 from each node position within the stem.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Block	7	5.341	0.763	2.12	0.0404
Clone	9	72.403	8.045	22.36	0.0001
Node	5	7.119	1.424	3.96	0.0016
Clone*node	45	25.522	0.567	1.58	0.0127
Residual	413	148.563	0.360		
Total	479	258.949			

*Only significant ($p < 0.05$) results are presented

Table A23 Analysis of deviance for stepwise regression to determine the influence of clone, node position and morphological characteristics of *Cordia alliodora* single-node cuttings on their rooting ability after eight weeks in non-mist propagators.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Block	7	10.312	1.473	1.64	ns
Clone	9	143.457	15.940	17.76	<0.001
Cutting length	1	20.875	20.875	23.25	<0.001
Node position	5	16.104	3.221	3.59	<0.001
Cutting diameter	1	3.773	3.773	4.20	<0.05
Residual	456	409.366	0.898		
Total	479	603.887			

Table A24 Analysis of variance for stepwise regression to determine the influence of week, stomatal conductance (g_s), irradiance (PAR), clone and node position on the photosynthetic rate per unit leaf area of single-node cuttings of *Cordia alliodora*.

Source	Degrees of freedom	Sum of squares	Mean square	Variance ratio	P
Week	3	36.505	12.169	34.55	<0.001
PAR	1	251.389	251.389	713.84	<0.001
g_s	1	59.975	59.975	170.31	<0.001
Clone	4	12.344	3.086	8.76	<0.001
Node position	5	7.951	1.590	4.52	<0.001
Residual	186	65.503	0.352		
Total	200	433.667			

Table A25 Analysis of variance for stepwise regression to determine the influence of week, irradiance (PAR), clone and node position on the stomatal conductance per unit leaf area of single-node cuttings of *Cordia alliodora*.

Source	Degrees of freedom	Sum of squares	Mean square	Variance ratio	P
Week	3	1201711	400570	20.77	<0.001
PAR	1	727408	727408	37.72	<0.001
Clone	4	290618	72654	3.77	<0.01
Node position	5	31956	6391	0.33	ns
Residual	188	3675751	19286		
Total	201	5877443			

Table A26 Analysis of variance of shoot height of *Cordia alliodora* plants from three clones and six node positions in the stem (apical to basal), after three months growth in the nursery. Data was collected from 270 plants, 15 from each clone/node position combination.

Source*	Degrees of freedom	Sum of squares	Mean square	Variance ratio	P
Block	4	866.600	216.650	3.85	0.0048
Clone	2	6468.901	3234.450	57.48	0.0001
Clone*cno	10	2107.592	210.759	3.75	0.0001
Residual	221	12435.597	56.269		
Total	242	22436.398			

*Only significant ($p < 0.05$) results are presented

Table A27 Analysis of variance of basal diameter of *Cordia alliodora* plants from three clones and six node positions in the stem (apical to basal), after three months growth in the field. Data was collected from 270 plants, 15 from each clone/node position combination.

Source*	Degrees of freedom	Sum of squares	Mean square	Variance ratio	P
Clone	2	28.328	14.164	13.30	0.0001
Node position	5	22.919	4.584	4.30	0.0009
Clone*node position	10	23.708	2.371	2.23	0.0174
Residual	221	235.417	1.065		
Total	242	318.330			

*Only significant ($p < 0.05$) results are presented

Table A28 Analysis of variance of height to the first whorl of branches of *Cordia alliodora* plants from three clones and six node positions in the stem (apical to basal), after three months growth in the field. Data was collected from 270 plants, 15 from each clone/node position combination.

Source*	Degrees of freedom	Sum of squares	Mean square	Variance ratio	P
Block	4	706.527	176.632	4.41	0.0020
Clone	2	3950.362	1975.181	49.34	0.0001
Node position	5	509.297	101.859	2.54	0.0295
Residual	194	7765.744	40.030		
Total	215	13633.951			

*Only significant ($p < 0.05$) results are presented

Table A29 Analysis of variance of rooting percentage of single-node, leafy stem cuttings of *Cordia alliodora* from three clones, set to root in three different rooting media, after six weeks in non-mist propagators. Data was collected from 360 cuttings, 40 from each clone/rooting medium combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Rooting media	2	3.125	1.563	5.64	0.0039
Clone	2	18.423	9.212	33.27	0.0001
Residual	339	93.864	0.277		
Total	359	118.374			

*Only significant ($p < 0.05$) results are presented

Table A30 Analysis of variance of mean number of roots per rooted cutting of leafy stem cuttings of *Cordia alliodora* from three clones, set to root in three different rooting media, after six weeks in non-mist propagators. Data was collected from 360 cuttings, 40 from each clone/rooting medium combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Rooting media	2	965.098	482.549	45.06	0.0001
Clone	2	350.849	175.435	16.38	0.0001
Residual	282	3020.089	10.710		
Total	302	4477.650			

*Only significant ($p < 0.05$) results are presented

Table A31 Analysis of deviance for stepwise regression to determine the influence of rooting media, clone, cutting diameter and node position on the rooting percentage of single-node, leafy stem cuttings of *Cordia alliodora* after six weeks in non-mist propagators.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Clone	2	56.388	28.194	40.28	< 0.01
Rooting media	2	10.940	5.470	7.81	< 0.01
Node position	9	7.231	0.803	1.15	ns
Block	3	0.505	0.168	0.24	ns
Cutting diameter	1	0.126	0.126	0.18	ns
Residual	342	239.375	0.700		
Total	359	314.565			

Table A32 Analysis of variance of shoot growth of six clones of *Albizia guachapele* growing under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25 % NPK), after eight weeks in controlled-environment cabinets. Treatment means for each irradiance-nutrient combination are from five plants (clones 5003 and 5005), four plants (clone 5007) or two plants (clones 5013, 5014 and 5015).

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Irradiance	1	3259.458	3259.458	27.05	0.0001
NPK	1	2367.588	2367.588	19.65	0.0001
Clones	5	2938.145	587.629	4.88	0.0017
Residual	36	4338.275	120.508		
Total	76	17409.675			

*Only significant ($p < 0.05$) results are presented

Table A33 Analysis of variance of specific leaf area of two clones (5003 and 5005) of *Albizia guachapele* growing under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25% NPK), after eight weeks in controlled-environment cabinets. Data represents the mean of 10 samples from each clone-irradiance-nutrient combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Irradiance	1	57.054	57.054	1647.83	0.0001
Nutrients	1	5.639	5.639	162.87	0.0001
Clone	1	7.913	7.913	228.54	0.0001
Irradiance*nutrients	1	3.698	3.698	106.80	0.0001
Irradiance*clone	1	0.506	0.506	14.60	0.0003
Residual	72	2.493	0.035		
Total	79	77.343			

*Only significant ($p < 0.05$) results are presented

Table A34 Analysis of variance for stepwise regression to determine the influence of day of measurement, stomatal conductance (g_s), irradiance and nutrient treatment on the net photosynthetic rate per unit leaf area of *Albizia guachapele* stockplants growing under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25% NPK) in controlled-environment cabinets. Data represents the mean of four measurements on ten plants per irradiance-nutrient combination carried out on consecutive days during the eighth week.

Source	Degrees of freedom	Sum of squares	Mean square	F value	P
Day	3	0.084	0.028	0.06	ns
g_s	1	115.006	115.006	240.13	< 0.01
Irradiance	1	28.455	28.455	59.41	< 0.01
Nutrients	1	0.340	0.340	0.71	ns
Residual	85	40.710	0.479		
Total	91	184.595	2.029		

Table A35 Analysis of variance for stepwise regression to determine the influence of day of measurement, irradiance and nutrient treatment on the stomatal conductance of *Albizia guachapele* stockplants growing under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25% NPK) in controlled-environment cabinets. Data represents the mean of four measurements on ten plants per irradiance-nutrient combination carried out on consecutive days during the eighth week.

Source	Degrees of freedom	Sum of squares	Mean square	F value	P
Day	3	3036	1012	0.64	ns
Irradiance	1	750	750	0.47	ns
Nutrients	1	195	195	0.12	ns
Residual	88	139969	1591		
Total	93	143949	1548		

Table A36 Analysis of variance of rooting percentage of single-node, leafy stem cuttings of *Albizia guachapele* from six clones, grown under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25% NPK), after three weeks in non-mist propagators. Data was collected from 432 cuttings, 18 from each node position-irradiance-nutrient combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Clone	5	13.386	2.677	5.69	0.0001
Irradiance	1	5.715	5.715	12.14	0.0006
Nutrients	1	14.245	14.245	30.26	0.0001
Node position	5	7.749	1.550	3.29	0.0065
Irradiance*nutrients	1	2.695	2.695	5.72	0.0173
Residual	331	155.842	0.471		
Total	392	225.117			

*Only significant ($p < 0.05$) results are presented

Table A37 Analysis of variance of mean number of roots per rooted cutting of single-node, leafy stem cuttings of *Albizia guachapele* from six clones, grown under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25% NPK), after three weeks in non-mist propagators. Data was collected from 432 cuttings, 18 from each node position-irradiance-nutrient combination.

Source*	Degrees of freedom	Sum of squares	Mean square	F value	P
Clone	5	452.541	90.508	3.43	0.0072
Nutrients	1	359.536	359.536	13.63	0.0004
Residual	83	2189.968	26.385		
Total	143	4534.493			

*Only significant ($p < 0.05$) results are presented

Table A38 Analysis of deviance for stepwise regression to determine the influence of treatments applied to the stockplants, clone, node position and morphological characteristics of single-node, leafy stem cuttings of *Albizia guachapele* on their rooting ability after three weeks in non-mist propagators. Cuttings from six node positions were taken from 80 stockplants of six clones growing under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25% NPK) in controlled-environment cabinets.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Block	3	3.127	1.042	0.94	ns
Nutrients	1	25.715	25.715	23.07	< 0.01
Clone	5	29.997	5.999	5.38	< 0.01
Cutting diameter	1	27.122	27.122	24.33	< 0.01
Cutting length	1	5.989	5.989	5.37	< 0.01
Irradiance	1	1.065	1.065	0.96	ns
Node position	5	5.440	1.088	0.98	ns
Residual	375	417.962	1.115		
Total	392	516.417	1.317		

Table A39 Analysis of deviance for stepwise regression to determine the influence of irradiance and nutrient treatments applied to the stockplants, clone, node position and cutting volume of single-node, leafy stem cuttings of *Albizia guachapele* on their rooting ability after three weeks in non-mist propagators. Cuttings from six node positions were taken from 80 stockplants of six clones growing under two irradiances (200 and 500 μmol m^{-2} s^{-1}) and two nutrient treatments (0.25 and 1.25% NPK) in controlled-environment cabinets.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	P
Block	3	3.127	1.042	0.92	ns
Nutrients	1	25.715	25.715	22.82	< 0.01
Clone	5	29.997	5.999	5.32	< 0.01
Irradiance	1	16.469	16.469	14.61	< 0.01
Node position	5	15.929	3.186	2.83	< 0.05
Cutting volume	1	1.421	1.421	1.26	ns
Residual	376	423.760	1.127		
Total	392	516.417	1.317		

Annex: Publications produced during period of postgraduate training

Contents

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Genetic improvement of trees in Central America, with particular reference to Costa Rica

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ABSTRACT

Central America has little or no forestry tradition other than widespread clearance and exploitation of its natural forests. Planting of trees to provide for a shortfall of timber and other products is a recent event and has only reached a significant scale in the past decade. Tree improvement activities in Central America, starting in 1977 at CATIE in Costa Rica and ESNACIFOR, Honduras, therefore developed initially in a vacuum, without the usual demand for improved seed. The Honduran project was curtailed in 1982 owing to the lack of finance. Work started again in 1987 with the CONSEFORH (COHDEFOR/ODA) project, working with a greater number of species, a wider remit, and greater emphasis on the conservation of native tree genetic resources. In Costa Rica, the Tree Improvement Project (TIP) has worked continually since 1977. Central to the approach of the TIP has been the establishment of trials on farmers' land to ensure the performance of selected provenances/genotypes on the type of land to be reforested. More recently, a DANIDA-funded bilateral project started in Nicaragua, focusing on the improvement and conservation of various native species. No formal tree improvement programmes exist in the other Central American countries, although seed stands have been established for a number of species.

Given population densities and the land tenure situation in Central America, the majority of reforestation is and will continue to be by farmers with small and medium-sized holdings, using both timber and multipurpose trees. The great diversity of climatic and edaphic conditions encountered in Central America, and the varied demands of farmers for trees, results in the need for work on a greater number of species than is normal in tree improvement programmes. These include both native species, such as *Albizia guachapele, Alnus acuminata, Bombacopsis quinata, Cedrela odorata, Cordia alliodora, Gliricidia sepium, Pinus* spp., *Swietenia macrophylla* and *Vochysia guatemalensis*, and exotics, mainly *Acacia mangium, Gmelina arborea* and various eucalypt species. The paper, with particular reference to activities in Costa Rica, reviews past, present and planned tree improvement activities in the region and the effect of some of the peculiarities of the region in developing strategies for tree improvement and domestication.

INTRODUCTION

Few areas of the tropics of a similar size to Central America show such wide variations of topography, climate, soil and vegetation. According to Holdridge's life zone classification, there are 17 major zones in Central America (Holdridge 1967). Vegetation ranges from very dry forest to rainforest, depending on the amount of rainfall, and with altitude from basal to paramo. Vavilov (1935, 1951) and Zohary (1970) identified Central America as one of the principal areas of the world for genetic diversity of plant life. This botanical wealth is shown as much in tree species as in shrubs and herbs. With a total number of more than 4 000 tree species, the region's tree genetic resources are enormous.

Various tree species now planted on a large scale in many tropical countries of the world are indigenous to Central America, e.g. *Calliandra calothyrsus*, *Cupressus lusitanica*, *Gliricidia sepium*, *Leucaena* spp., *Pinus caribaea*, *P. oocarpa* and *P. tecunumanii*. Others have been planted on a smaller scale but have great potential for use and development; e.g. *Alnus acuminata*, *Bombacopsis quinata*, *Cedrela odorata*, *Cordia alliodora*, *Juglans olanchana*, *Parkinsonia aculeata*. Furthermore, there are numerous species in the region which may have potential for use in the future. The increasing levels of deforestation endanger the genetic resources of many of the region's species. This is particularly so in the dry Pacific zone where deforestation has been extensive, species have suffered serious genetic erosion and in some cases are in danger of localized and even regional extinction (Janzen 1986).

Central America has little or no forestry tradition other than widespread clearance and exploitation of its natural forests. The high population growth rate in the region has resulted in a continuing demand for land for agriculture, ranching and forest products, leading in turn to an indiscriminate destruction of the forest. Hartshorn (1982) estimated that 60-70 kha of forest are cut each year in Costa Rica; for every hectare of plantation established, 80 ha of mature forests are cut. There is little indication of a change in this pattern and the majority of the natural forests outside protected areas will be exhausted by the end of the century.

The necessity for reforestation on a large scale as part of the solution to this critical situation has been evident for some time. Flores (1985) estimated that for the period 1985-1995, 900 kha needed to be planted in the Central American region to supply the internal demand for wood and wood-based products. The demand for seed has therefore grown in recent years and will continue to increase, even if only a part of the area needed is planted. As the number of reforestation projects in the region increases, experience shows that inappropriate source of seed, or propagative material, is a major technical cause of observed failures or low yields. Much of the seed collection in the region is carried out by farmers or other unqualified people without adequate knowledge of the best collection techniques, or of the characteristics that the trees or stands should possess. As a result, the collections are often made from badly formed trees and stands. For this reason, established plantations often show poor growth and a lack of uniformity that makes management difficult and gives reforestation a poor image. These problems have also been accentuated by the lack of knowledge about the importance of using appropriate sources of seed and a lack of information of the best sources among the region's scientific personnel and farmers.

In the past ten years reforestation has increased dramatically in Costa Rica. This was due initially to the motivation of a number of small-scale farmers and extension agents and their perceived need to meet the shortfall of timber to local communities, within an affordable price. Within this context it was important to achieve visible results early on and species were chosen which were easy to establish, such as *Bombacopsis quinata*, *Gmelina arborea* and *Tectona grandis*. Capitalizing on this initial success, planting has increased under new fiscal incentives and soft loans, directed more towards small-scale farmers (typically 2-3 ha) organized into groups. The success has snowballed, with much larger numbers now being involved, and the resultant plantations have been of a much higher quality than previously. As a result, with respect to forestry, the situation in the region as a whole is now very different from what it was 10-15 years ago. Even though the species worked on have been traditionally regarded as "industrial species", they are planted by farmers on a small scale for their own use, in a non-industrial context. Seed stands of *Gmelina arborea* were later set up using small plots, and sale of seed has become an important source of extra income for farmers.

The availability of propagative material of superior quality could have an important impact on tree planting in the region. Production increases in plantation forestry achieved in the past thirty years in tropical and subtropical countries, through genetic improvement techniques, have been well documented. However, the majority of these programmes, with the exception of *Leucaena leucocephala* (see Brewbaker & Sorensson, this volume), have been developed by large businesses at a purely industrial level and the potential gains from tree improvement have not been made available to medium- or small-scale farmers. Given population densities in Central America, the majority of reforestation will continue to be by small- and medium-scale landowners using both timber and multipurpose trees. It is evident that the farmers of the region plant trees for a variety of products: timber, wood for rural use (construction, carpentry, work tools, posts), protection (live fences, windbreaks), shade (for livestock, crops and the home), firewood production, recuperation of soils, and more recently to produce forage and feed livestock during the dry season, from a variety of species. For this reason and the high degree of environmental variation, tree improvement programmes are obliged to work with a greater number of species than is normal and consequently accept a slower rate of advance with any one species (see Table 1).

Past, present and future plans for tree improvement within the region have therefore developed within these constraints, taking into account the above factors. The present paper, with particular reference to activities in Costa Rica, and without any pretensions to comprehensively cover all work in the region, considers three ongoing projects with reference to (i) strategies for improvement and conservation, (ii) the state of progress, (iii) implementation and (iv) future plans. The three projects are (i) Centre for Tropical Agricultural Research and Education (CATIE)

Tree Improvement Project, (ii) CATIE Tree Crop Production and Management Project (MADELEÑA) and (iii) Conservation and Genetic Improvement of Honduras Forest Resources (CONSEFORH) Project in Honduras.

ONGOING PROJECTS FOR GENETIC IMPROVEMENT OF TREES

CATIE Tree Improvement Project

Planting of trees in Costa Rica to provide for a shortfall of timber and other products is a recent event and has only reached a significant scale in the past decade. Up until the beginning of 1983, the total area reforested in Costa Rica was estimated at 4 kha (Hartshorn 1982). Reforestation has increased during the last decade owing to a series of incentives given by the Government, to reach rates of 12-15 kha per year. However, plantation failures are high, mainly owing to poor quality of nursery stock, inadequate maintenance after planting, and poor seed quality.

The CATIE Tree Improvement Project (TIP), financed by the Overseas Development Administration (ODA) of the United Kingdom, the Department for Development Cooperation (DDC/Norway), the Swiss Office of Development Cooperation (COSUDE) and the US Agency for International Development (AID), has been active in the genetic improvement of traditional agroforestry and timber species since 1977. The TIP began, from 1977 onwards, with the establishment of provenance trials, mainly of *Pinus caribaea*, *P. oocarpa*, *Cordia alliodora*, *Gmelina arborea*, *Eucalyptus grandis*, *E. urophylla* and *Acacia mangium*. These were established as a part of international trials coordinated by organizations such as the International Union of Forestry Research Organizations (IUFRO), the Food and Agriculture Organization (FAO) of the United Nations, the Oxford Forestry Institute (OFI), the Commonwealth Scientific and Industrial Research Organizations (CSIRO) and the DANIDA tree seed centre (Denmark) . The selection of the species was based on the results from species trials and plots that were established in many parts of the country (Combe & Gewald 1979; Camacho 1981; Martínez 1981). The strategy was based on the fact that the most economical and rapid means of obtaining gains in the productivity of trees is through the use of the most appropriate species and provenances (Zobel & Talbert 1984). The trials were planted on a variety of sites throughout the country, covering the major ecological zones considered suitable for reforestation with the particular species. The project aimed also to identify any patterns of genotype x environment interaction for each species and their effect on any recommendations for the establishment of particular provenances.

At the time the project started there was little awareness of, or demand for, the information or material that the project could provide. The project therefore aimed to establish trials that would provide appropriate information, genetic material and demonstration units for the medium term when the need would become apparent.

The TIP's trials and seed orchard programme relies heavily on the active participation of smallholder farmers, both individually and in organized groups. Virtually all trials are established on small farms under written agreements with the landowners. After final thinning the trials and seed orchards are used by the individuals or groups in question for timber and/or improved seed sources. The establishment of trials on farmers' land ensures the performance of selected provenance/genotypes on the type of land to be reforested and permits the combination of the process of genetic testing, seed production and technology transfer.

An important part of the project is the strengthening of capabilities for tree improvement work throughout the region. Short courses and courses at the postgraduate level are given to improve capability within the region in the field of tree genetic improvement.

The need to increase the work of the project to ensure the uptake of its research results by the end users (i.e. tree planters of all types) has become evident. One example came from the superior growth of *Pinus tecunumanii* to both *P. caribaea* and *P. oocarpa* observed in TIP trials. The results were reported by the project as long ago as 1986 (Boshier & Mesén 1986), but the species was not officially accepted by the Costa Rican General Forestry Directorate (DGF) until 1991 as suitable for reforestation. This acceptance came only as a result of the TIP establishing a number of small demonstration plantations (1-2 ha) on farmers' land. Firm recommendations on seed sources can now be made for *Gmelina arborea*, *Acacia mangium*, *Pinus caribaea*, *P. tecunumanii* and *Eucalyptus urophylla* (Mesén 1991).

While provenance research provides a sound basis for avoiding catastrophic losses in forestry plantations, the TIP's goal is higher than simply to avoid plantation failures. Even within the best provenances, trees show a great variability in traits of economic value. Therefore, a further stage in the TIP was the selection of individual superior trees within provenances, both for the establishment of progeny tests and seed orchards in a traditional breeding programme, and for inclusion in a vegetative propagation and clonal selection programme. An additional component within the TIP's activities is the screening of *Cedrela odorata* and *Swietenia* spp. families for genetic resistance to the shoot borer (*Hypsipyla grandella*), and the eventual cloning of pest-resistant genotypes (see Newton *et al.*, this volume).

Progeny tests

The natural ranges of all the native species included in the TIP progeny test programme are not restricted to Costa Rica, but extend to many other Central and South American countries. In this regard, international cooperation for exploration, tree selection and exchange of seed becomes a critical matter. A particularly fruitful link was initiated in 1989 between the Central America and Mexico Coniferous Resource Cooperative (CAMCORE) and CATIE, through a USAID-funded project which focused on the exploration and collection of seed from superior trees of six threatened broadleaved species in Guatemala, Honduras, Colombia and Costa Rica. The species selected, *Albizia guachapele*, *Alnus acuminata*, *Bombacopsis quinata*, *Cordia alliodora*, *Sterculia apetala* and *Vochysia guatemalensis*, were chosen because all have populations in danger of extinction or genetic impoverishment and, at the same time, have exhibited commercial potential in CATIE or CAMCORE tests (Mesén & Dvorak 1992). In addition, the TIP has carried out selections within Costa Rica for a number of promising non-native species, particularly *Cupressus lusitanica, Eucalyptus deglupta* and *Gmelina arborea.* In total, 597 phenotypically superior trees have been selected and their seed established in provenance/progeny tests in a variety of suitable sites in Costa Rica.

As for the provenance trials, the provenance/progeny tests are established under cooperative agreements with farmers involved in reforestation under the Costa Rican Forestry Directorate incentive scheme. Most trials are designed in such a way that they can be converted into seedling seed orchards after the evaluation period. Thus, in addition to the basic objectives of genetic testing and provision of material for advanced selections, the trials are also used for demonstration purposes and production of improved seed, tested directly in the area where reforestation is to be carried out.

Vegetative propagation and clonal selection

It is now recognized that vegetative propagation and clonal selection offer a means to greatly enhance the yield and quality of forest products (Leakey 1987; Libby & Rauter 1984). The tremendous improvements in productivity and form of clonal eucalypts in Brazil, for example, are well known by foresters. Most advances in clonal silviculture, however, have been developed by large companies and the techniques are not available to farmers in developing countries. Since 1989, the TIP has concentrated on the development of low-cost, appropriate technology cloning techniques, through a cooperation link with the Institute of Terrestrial Ecology (ITE), Scotland. The main obstacle for the use of cloning in small-scale programmes, that of the sophisticated and expensive equipment needed, has been overcome by the use of ITE's low-technology, non-mist propagators, as described by Leakey *et al.* (1990), Leakey & Mesén (1991) and Mesén *et al.* (1992) (see also Leakey *et al.*, this volume). The TIP, through the ITE/CATIE link project, has developed considerable expertise in the use of these techniques and in understanding of the basic physiological principles, both pre- and post-severance, affecting the rooting ability of leafy cuttings. All priority species included in the programme, some of which were formerly considered to be difficult to root, are now being cloned routinely. These include *Albizia guachapele*, *Bombacopsis quinata*, *Cordia alliodora*, *Cedrela odorata*, *Gmelina arborea*, *Swietenia macrophylla* and *Vochysia guatemalensis*. The use of low-cost and easily transferred cloning methods offers excellent prospects for both the conservation and the commercial use of genetically high-quality germplasm of valuable species.

Seed stands and the Madeleña Project

The focus of the Madeleña Project is towards the development of techniques for the planting of multipurpose trees in different systems, including agroforestry, in small or relatively small farms in Central America. The main aim was to develop and strengthen the capabilities of CATIE and public and private organizations in the Central American region to allow them access, promote and disseminate on-farm, market-oriented tree crop technologies for the use and benefit of small- and medium-scale farmers and rural industries. This project developed from an initial attempt to address fuelwood shortages on a regional basis, begun in 1980, under the Fuelwood and Alternative Energy Sources Project based at CATIE. The project screened more than 100 species to determine their feasibility for fuelwood and latterly multipurpose uses. Fourteen priority species were identified and a variety of promotional, extension and training activities carried out. The exclusive focus on fuelwood was broadened under the project extension (AID/Regional Office for Central America and Panama (ROCAP) Tree Crop and Management Project) to include a mix of species and management systems which can be utilized to provide a variety of products and economic benefits.

Traditional guides to the establishment of seed stands argue that areas of 2-5 ha are recommended for seed stands (Matthews 1964). Generally in the Central American region there are few large plantations and the problems of contamination from surrounding stands of trees are reduced. Genetic constraints can, however, be avoided by the use of much smaller areas, and economies of scale may be very significant if a farmer can receive income from both the timber and the seed from his trees. This approach has worked in all the countries of the region in conjunction with each country's national institutions, and national seed banks. The project aims to establish a network of seed stands for the multipurpose species it has identified as promising and of high priority for the region.

CONSEFORH Project, Honduras

The genetic improvement programme in Honduras started in 1977 at the National School of Forest Sciences (ESNACIFOR), but was curtailed in 1982 owing to the lack of finance, leaving only a few established trials (Gibson & Romero 1988). Work recommenced in 1987 through an agreement between the Honduran Corporation for Forestry Development (COHDEFOR) and ODA with the CONSEFORH Project ("Tree Improvement and Conservation of the Forest Genetic Resources of Honduras"). CONSEFORH is working with a large number of species, both native and exotic, with particular emphasis on the conservation, evaluation and genetic improvement of native species. This involves explorations to determine the current distribution and conservation status of the species, seed collections for the establishment of genetic trials, gene conservation banks and seed orchards, establishment of silvicultural trials, and research on the properties and potential uses of the timber. Work with exotics involves the establishment of seed orchards and provenance trials.

CONSEFORH is active in the four major forest types found in Honduras, namely dry forest, cloud forest, humid forest and coniferous forest. The number of forest species in these areas is enormous, some of which (e.g. *Pinus caribaea* var. *hondurensis*) are planted on a large scale in many tropical countries of the world, or may have great potential for future use and development. Up until the present, the efforts of the project have been concentrated in the dry forest, being the most severely disturbed forest association in Honduras and where there are more than 300 tree species. Large scale ecological restoration of the type currently being implemented in the dry forest of Costa Rica is not an option in Honduras, owing to the land tenure pattern and consequent pressure from subsistence farmers (Gibson

1993). The project has thus taken a varied approach, depending on the importance and usage of the individual species. This involves collection and management of germplasm in both *in situ* and *ex situ* conservation units, while for some species the possibilities of conservation through use by local communities are also being explored (Gibson 1993).

Work initiated with priority species selected as endangered, commercially valuable and with characteristics making them acceptable to farmers and others interested in tree planting (Gibson 1993). Extensive exploration was carried out to define the distribution and status of populations and assess options for conservation of these species. Seed collections were then made, followed by the establishment of breeding seedling orchards (BSOs) as a basis for their conservation and improvement. The species in this programme included *Albizia guachepele*, *A. niopoides*, *A. saman*, *Bombacopsis quinata*, *Cordia alliodora*, *Enterolobium cyclocarpum*, *Gliricidia sepium*, *Hymanaea coubaril*, *Leucaena salvadorensis*, *Simarouba glauca* and *Swietenia humilis* (Gibson 1993). Work with pines has also involved the establishment of BSOs to ensure the conservation of endangered provenances of *Pinus caribaea.*

Most trials have been planted in one of the experimental stations that the project maintains in Honduras, which range from 100 to 1 100 m above sea level. The concentration of activities in the experimental stations allows for better maintenance and protection of trials and conservation of the local flora and fauna, and at the same time facilitates education and demonstration activities. Many valuable species grow naturally in these stations: for example *Albizia guachapele*, *Bursera simarouba*, *Enterolobium cyclocarpum*, *Guazuma ulmifolia*, *Pithecolobium dulce* and *Swietenia humilis* are common in the dry forest station. Therefore, the stations themselves offer opportunities for detailed growth and phenological studies and for the development of appropriate techniques for *in situ* conservation. At present a large proportion of the Project's resources are concentrated in this area.

CONCLUSION

In Central America, the justification for tree improvement has never been the straightforward economic one appropriate to countries and companies with large reforestation programmes; the lack of adequate levels of reforestation is precisely one of the problems which the region faces. At CATIE, at least, workers in tree improvement have long been conscious that tree improvement in Central America must lead, rather than follow, reforestation, through the provision of excellent quality material whose field performance will in itself stimulate tree planting by farmers.

This concept must be reflected in the tree improvement strategies adopted in the region. For this reason, at CATIE, the TIP is pursuing and advocating ambitious strategies of individual-tree selection based, at the highest intensity, on clonal selection and vegetative propagation. Obviously, lower-intensity activities such as provenance selection will remain crucially important. However, the best provenance contains bad as well as good genotypes, and in itself even the use of the best provenance is unlikely to achieve the sort of "quantum leap" in the quality of plantations that is necessary to completely change perceptions of and attitudes towards plantation forestry, and to stimulate reforestation on a large scale. Clonal methods have the potential to transform smallholder forestry as effectively as they have transformed industrial forestry, although different strategies of clonal deployment may need to be developed (c.f. Foster & Bertolucci, this volume), and a complementary programme of sexual improvement should be maintained.

Domestication by vegetative propagation and clonal selection is just one example of an improved production system made possible by, but not solely dependent on, genetic improvement. Workers in tree improvement in the region should be looking for other possibilities offered by genetic improvement for the development of improved production systems which meet specific problems or which offer new opportunities. An obvious example is the work on the selection for pest resistance in mahoganies mentioned above; other possibilities abound.

At a wider level, there is a need for an overall strategy of management of Central American forest genetic resources, of which the development of genetically improved forestry and agroforestry production systems would constitute only a part. The genetic base of many species is being rapidly eroded by deforestation, often before we know anything about their genetic structure or even have complete knowledge of their natural distribution. For this reason, there is now some initial work in progress in the region in genetic conservation and baseline exploration, taxonomic and population genetic studies. However, there is little coordination between these activities on a regional or even national basis. An integrated forest genetics resource management strategy might be founded upon a regional network of resource management units, each consisting of a specific type of population. These might range from national parks or reserves, with the objective of *in situ* genetic conservation, through to clonal multiplication gardens, dedicated exclusively to the production of improved germplasm for particular production systems. Between these two extremes there could be a variety of other options, such as seed stands, natural relict stands, seed orchards, etc., each with its own clearly defined role in relation to the overall genetic management of the species. Through such a network, priorities for funding and action would be made clearer, and national and regional cooperation on tree improvement and related activities could be greatly improved.

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Table 1. Tree improvement activities by species in the Central American region.

Species	Prov. trials	No. Plus trees	Prog. tests	Seed stands	Seed orch.	Cloning
Acacia mangium	10			2		x
Albizia guachapele	2	54	2		1	x
Alnus acuminata	1	52	1		1	x
Araucaria hunsteinii	2					x
Bombacopsis quinata	6	117	3	1	3	x
Calliandra calothyrsus	4					
Casuarina cunninghamiana	2					
C. equisetifolia	5					
Cedrela odorata	1	31	1			x
Cordia alliodora	8	163	5		5	x
Cupressus lusitanica	6	45	2	2	1	
Eucalyptus camaldulensis	13		4	4	2	
E. deglupta	3	55	4	1	5	x
E. grandis	3		2	2	2	
E. saligna	5		1			
E. tereticornis	2					
E. urophylla	3			1		
Gliricidia sepium	12	177	2	1		x
Gmelina arborea	9	65	8	14	8	x
Guazuma ulmifolia	2					
Inga spp.	4					x
Leucaena leucocephala	11	24	2	4		
Pinus caribaea	20	1218	6			
P. oocarpa	8		2			
P. tecunumanii	4	801	5		2	
Swietenia macrophylla	2	36	2			x
Tectona grandis	3			11		
Vochysia guatemalensis	4	61	3		3	x

Symbols: x indicates that the species has been propagated vegetatively, either by leafy cuttings or stakes. Abbreviations: prov., provenance; prog., progeny; orch., orchard.

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LOW-TECHNOLOGY TECHNIQUES FOR THE VEGETATIVE PROPAGATION OF TROPICAL TREES

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SUMMARY

Stem cuttings of five tree species from dry and semi-arid woodlands (*Acacia tortilis, Prosopis juliflora, Terminalia spinosa, Terminalia brownii* and *Albizia guachapele*) and seven species from moist tropical forests (*Cordia alliodora, Vochysia hondurensis, Nauclea diderrichii, Ricinodendron heudelotii, Lovoa trichiliodes, Gmelina arborea, Eucalyptus deglupta*) have been easily rooted in improved low-technology, high humidity polythene propagators in Kenya, Cameroon, Costa Rica and Britain. These propagators, which are cheap to construct, are very effective and have no essential requirements for either piped water or an electricity supply. Experiments have tested different rooting media, auxin applications and compared mist *versus* non-mist propagation.

Assessments of the physical and gaseous environment of the propagators has indicated ways of improving the rooting environment through an understanding of the sensitivity of the relative humidity to radiant energy and to opening the propagator for short periods (eg 2-3 minutes).

RÉSUMÉ

Des boutures de tige de cinq essences forestières de forêt claire aride et semi-aride (*Acacia tortilis, Prosopis juliflora, Terminalia spinosa, Terminalia brownii* et *Albizia guachapele*) et sept essences de forêt dense humide tropicale (*Cordia alliodora, Vochysia hondurensis, Nauclea diderrrichii, Ricinodendron heudelotii, Lovoa trichiliodes, Gmelina arborea, Eucalyptus deglupta*) ont été enracinées facilement dans des propagateurs polyéthylène à humidité élevée de technologie de base au Kenya, au Cameroun, au Costa Rica et en Grande-Bretagne. Ces propagateurs, qui ne sont pas chers à confectionner, sont très efficaces et n'ont pas d'exigences essentielles ou pour de l'eau canalisée ou pour une alimentation en électricité. Des expériences ont testé des milieux d'enracinement et des applications d'auxine différents et ont comparé la propagation sous brumisation avec la propagation sans brumisation.

Des évaluations de l'environnement physique et gazeux des propagateurs ont indiqué des moyens d'améliorer l'environnement d'enracinement par une compréhension de la sensibilité de l'humidité relative à l'énergie radiante et à l'ouverture du propagateur pour des périodes courtes (par exemple 2 – 3 minutes).

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RESUMEN

En estudios en Kenia, Cameron, Costa Rica y Gran Bretana utilizando propagadores mejorados de baja tecnología, se enraizaron fácilmente estacas de tallos de cinco especies arbóreas de zonas áridas y semi-áridas (*Acacia tortilis, Prosopis juliflora, Terminalia spinosa, Terminalia brownii* y *Albizia quachapele*) y siete especies de bosque húmedo tropical (*Cordia alliodora, Vochysia hondurensis, Nauclea diderrichii, Ricinodendron heudelotii, Lovoa trichiliodes, Gmelina arborea, Eucalyptus deglupta*). Estos propagadores son baratos de construir, muy efectivos y sin requirimientos imprescindibles de agua de caneria ni de electricidad. Los ensayos han probado diferentes medios de enraizamiento, aplicación de auxinas y la comparación de propagación con y sin nebulización.

La evaluación del ambiente físico y gaseoso de los propagadores ha indicado maneras de mejorar el ambiente de enraizamiento entendiendo que la humedad relative es sensible a la energía radiante y la apertura del propagador por períodos cortos (ej. 2 – 3 minutos).

Introduction

It is now widely realized that vegetative propagation and clonal selection offer a means to greatly enhance the yield and quality of forest products from commercial plantings in the tropics (Leakey, 1987). However, there is a need to simplify the technology so that vegetative propagation can be achieved in the absence of mains electricity and a piped water supply. In addition, in many tropical countries, the high capital and running costs of currently available mist propagation systems makes them inappropriate, except for research or large-scale commercial projects.

The environmental requirements for root initiation in leafy stem cuttings are those that minimise physiological stress in the cutting. In general terms this means using shade to lower the air temperature and, by providing a high humidity, to reduce transpiration losses. By the latter, the vapour pressure of the atmosphere surrounding the cutting is maintained close to that in the intercellular spaces of its leaf.

There are numerous propagation systems used in commercial horticulture. These are usually based either on spraying mist, fogging or enclosing the cuttings in polythene. The advantages of polythene systems have been known for many years (Loach, 1977) and they have been used to propagate tropical hardwoods with good success, particularly at the Forest Research Institute of Nigeria, Ibadan (Howland, 1975).

Recent work by the Institute of Terrestrial Ecology (ITE) and its overseas collaborators has applied and improved the design of non-mist propagators for use with a wide range of timber and multi-purpose tree species from both tropical moist forests and semi-arid areas (Leakey and Longman, 1988). Recent studies with *Triplochiton scleroxylon* cuttings under intermittent mist have indicated that rooting ability is related to the production of reflux-soluble carbohydrates, apparently derived from current photosynthesis while the cuttings are in the propagation unit (Leakey and Storeton-West, in preparation). Furthermore, it seems that the ability to produce these carbohydrates is related to the pre-severance light environment and nutrient status of the cuttings while on the stockplants. Both the total irradiance and the light quality (red:far-red ratio) are important components of the pre-severance light environment, and these factors interact with nutrient availability to influence the rates of net photosynthesis and rooting. These variables to a large extent account for the variation in the rooting ability of cuttings of *T. scleroxylon* taken from different shoots of variously-treated stockplants (Leakey, 1983).

Materials and Methods

General

Juvenile shoots of twelve tree species (Table 1) have been used as leafy stem cuttings. The studies presented here were done in either glasshouses in the UK or under nursery conditions in Costa Rica, Kenya and Cameroon. In all instances, however, the propagator temperature was between 22 – 27°C, and the cuttings were prepared as described below and set in randomized blocks. The numbers of replicate cuttings per treatment were between 24 and 117. Standard errors for percentage rooting were calculated using the procedures of Bailey (1959) for data with binomial distributions.

Table 1 Tropical tree species vegetatively propagated using simple, low-tech propagators in Costa Rica, Cameroon, Kenya and Great Britain.

Species	*Family*	*Range*	*Uses*
Gmelina arborea Roxb.	Verbenaceae	Indo-Burma region, and S.E. Asia, a Pan-tropical exotic	Timber
Eucalyptus deglupta Bl.	Myrtaceae	Tropical Australasia, a Pan-tropical exotic	Timber
Nauclea diderrichii (DeWild & Th Dur.) Merr.	Rubiceae	W. and C. Africa	Timber
Lovoa trichilioides Harms	Meliaceae	W. and C. Africa	Timber
Ricinodendron heudelotii (Baill.) Pierre ex Pax	Euphorbiaceae	W. and C. Africa	Fruit
Cordia alliodora (Ruiz & Pav.) Oken	Ehretiaceae	C. America	Timber
Vochysia hondurensis Sprague	Vochysiaceae	C. America	Timber
Albizia guachapele (Kunth) Dug.	Mimosaceae	C. America	Timber
Prosopis juliflora (Swartz) D.C.	Mimosaceae	C. Amercia	Multipurpose
Acacia tortilis (Forsk.) Hayne	Mimosaceae	W. and E. Africa	Multipurpose
Terminalia spinosa Engl.	Combretaceae	E. Africa	Multipurpose
Terminalia brownii Fresen	Combretaceae	E. Africa	Multipurpose

Preparation of cuttings

Cuttings were harvested from seedlings, managed juvenile stockplants or coppice shoots. Depending on the species, 1- to 4-node cuttings were used. These were usually about 50-60mm long and with a leaf area of about 50cm^2 (Leakey, 1985). In large leaved species, leaf areas were reduced by trimming prior to severance. The basal end of cuttings were dipped briefly in indole-3yl-butyric acid solutions (0.2-0.4% IBA in industrial methylated spirit) to a depth of about 2-5mm, and the alcohol then rapidly evaporated off in a stream of cold air from a fan (Leakey *et al.* 1982, Leakey, 1989). To minimise stress, the cuttings were inserted in the propagator as soon as they were dry. Alternatively, commercial auxin-based rooting powders "Strike" and "Seradix 2" (May & Baker Ltd) were used.

The non-mist propagator

The propagator design currently in use is based on that of Howland (1975), modified by Leakey and Longman (1988) and now further modified so that it does not require daily watering (Fig. 1). Basically, a wooden or metal frame is enclosed in clear polythene so that the base is water-tight (Leakey, 1989). The frame also provides support for the enclosed volume of water. The polythene base of the propagator is covered in a thin layer of sand to prevent the polythene from being punctured by the large stones (6 – 10 cm) which are placed on it to a depth of 10-15 cm. These stones are then covered by successive layers of small stones (3 – 6 cm) and gravel (0.5 – 1.0 cm) to a total depth of 20 cm. The gravel provides support for the rooting medium which is the uppermost layer, while the spaces between the stones are filled with water. A length of hollow bamboo provides an open cylinder inserted into the medium and stones which is used both to observe the water level and to add water if necessary. The rest of the frame is covered tightly with a single piece of clear polythene, and a closely-fitting lid is attached. Internal supports to the frame at the level of rooting medium also provide subdivisions allowing the independent use of different rooting media (Fig. 1). As a result of the studies reported here, further refinements to the design of non-mist propagators are discussed later. A similar frame to that of the non-mist propagator, with roll-up polythene sides, can be used, as in Costa Rica, as a weaning area.

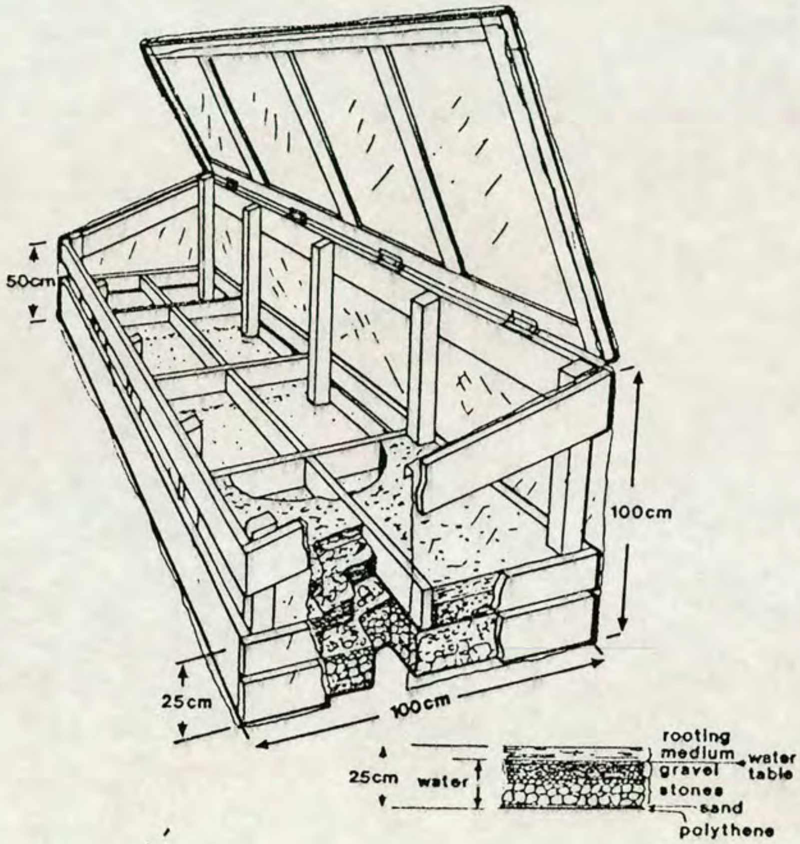


Figure 1. The design of ITE's improved non-mist propagator.

Results

The propagator environment

In tests run in ITE glasshouses, in which air temperatures were maintained at about 20°C, temperatures in non-mist propagators rose to a mid-day peak of about 34°C during bright, sunny, mid-summer weather (eg 28th July). This rise in temperature was associated with a decrease in relative humidity from about 95% to about 75% (Fig. 2).

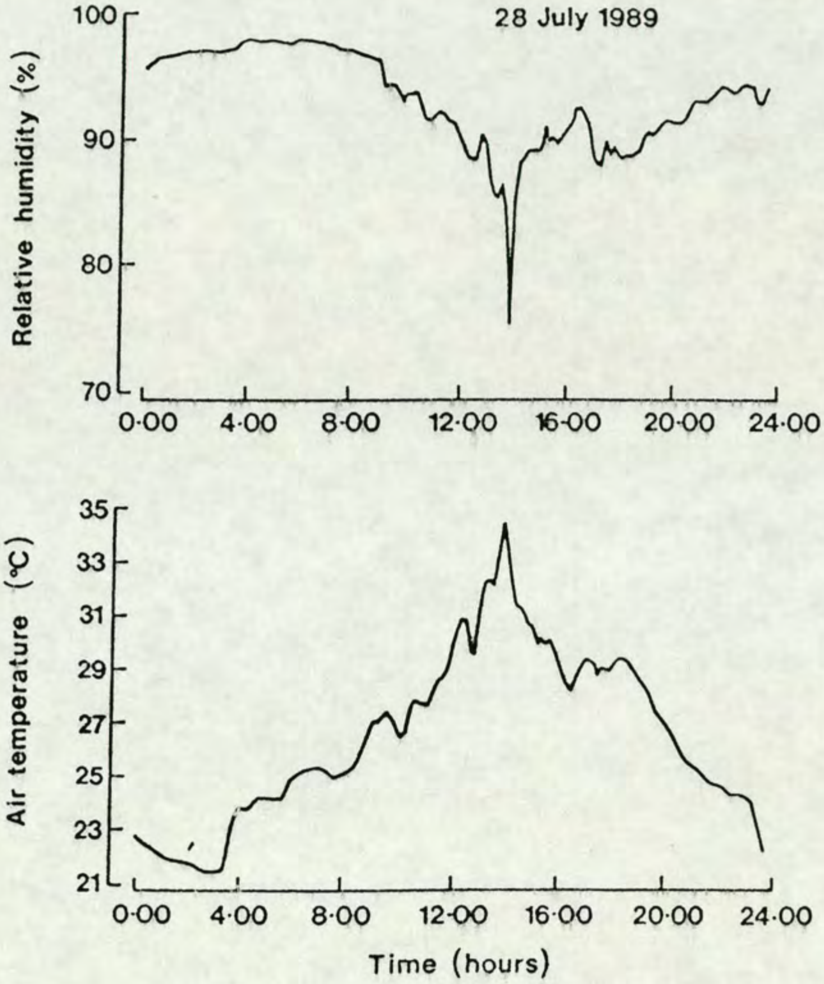


Figure 2. Effects of a rise in air temperature on the relative humidity inside a non-mist propagator.

This represents a substantial increase in the saturation vapour pressure deficit (SVPD) of the air from 0.02 kPa to 1.37 kPa. An important decrease in relative humidity also occurred when the propagator was opened for five minutes at midday (Fig. 3). In this instance, relative humidity decreased by about 40 – 50% to glasshouse ambient within two minutes,representing an increase in evaporation rate of approximately x 4.5 (SVPD = 0.45 kPa to 2.08 kPa). Relative humidity increased rapidly again following closure of the lid. Decreases in air temperature to ambient were also associated with this period of opening. Subsequent gains in the temperature resulted from closing the propagator, but the response time for temperature was considerably slower than for relative humidity.

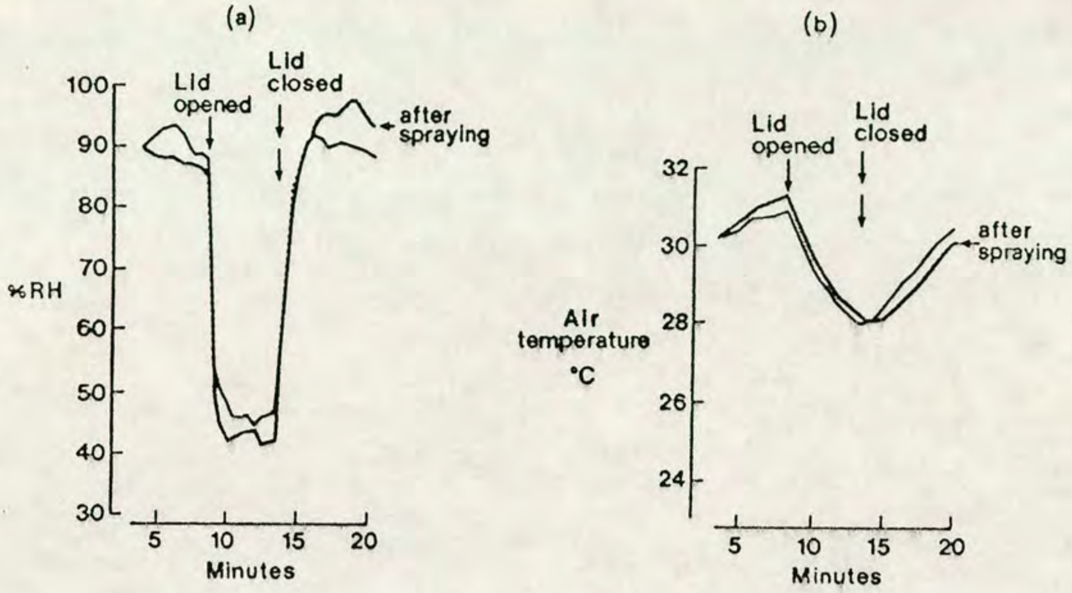


Figure 3. Effects of opening the lid of a non-mist propagator on its: a) relative humidity and b) air temperature.

When the easy-to-root species *Nauclea diderrichii* (Leakey, in press) was used for physiological studies in a non-mist propagator at Edinburgh University (Matin, 1989), it was found that cuttings had maximum rates of photosynthesis that were typical of intact plants, up to 6μ mol CO_2 m-^{2} s-^{1} at an irradiance of 1000μ mol m-^{2} s-^{1}. However, the photosynthetic capacity of these cuttings was influenced by changes in the CO_2 concentration inside the propagator. In the middle of the day, the CO_2 concentration fell to 150μ mol mol-^{1}, while by midnight it rose to 550μ mol mol-^{1}, reflecting daytime assimilation and night-time respiration, respectively.

As regards rooting media, the water-holding capacity of a fine-gravel (2 – 3mm diameter) medium was considerably increased, at the expense of the volume of the air spaces (Fig. 4), by the addition of rotted sawdust (50% by volume).

Rooting tests

In Costa Rica, studies using five tree species investigated the effects of four different rooting media (i) gravel, (ii) 50:50 gravel with sawdust, (iii) fine sand and (iv) 50:50 fine sand and sawdust. Each medium was tested with cuttings dipped in a range of IBA concentrations (0, 0.05, 0.1, 0.2, 0.4 and 0.8%). There were, however, substantial differences between species with regard to rooting success on the different media. Single-node juvenile cuttings of all five species rooted well (70 – 95%) on their best medium (Fig. 5). *Cordia alliodora* rooted best in fine sand, with or without sawdust,

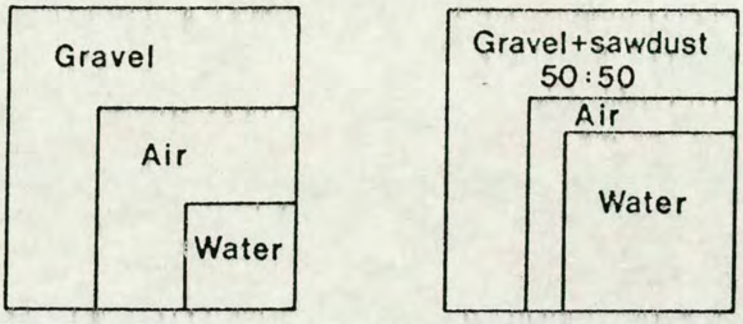
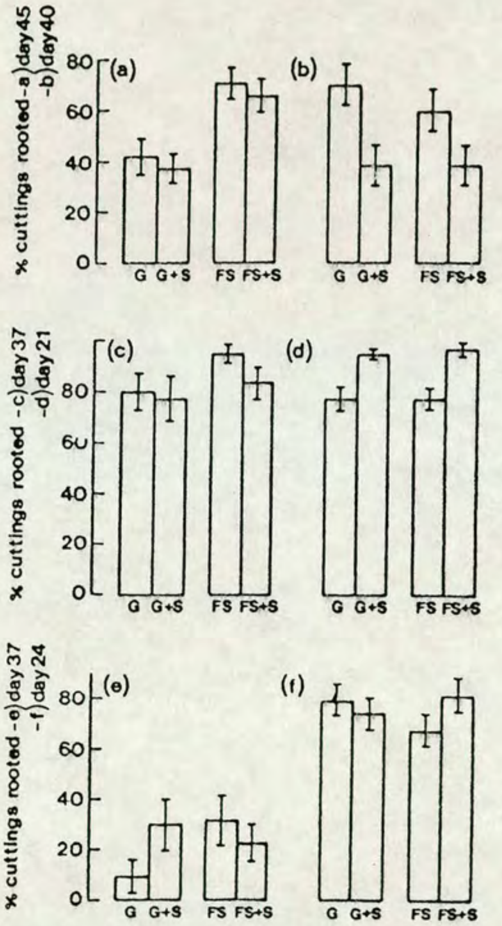


Figure 4. Relative composition by volume of a gravel rooting medium with and without sawdust.



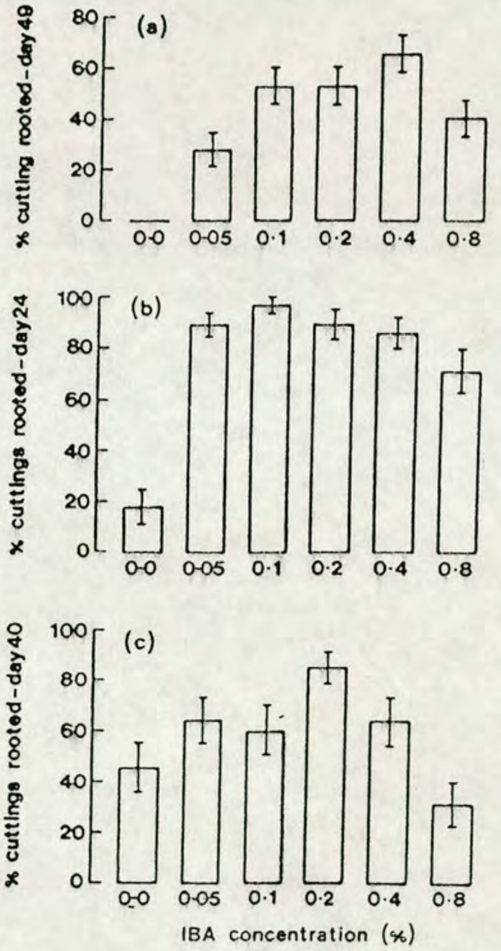


Figure 5. Effects of rooting medium (G = gravel, FS = fine sand and S = sawdust) on the rooting of leafy stem cuttings of (a) *Cordia alliodora*, (b) *Vochysia hondurensis*, (c) *Gmelina arborea* (juvenile), (d) *Eucalyptus deglupta*, (e) *Gmelina arborea* (mature), and (f) *Albizia guachapele*.

Figure 6. Effects of IBA concentrations on the rooting of leafy stem cuttings of (a) *Cordia alliodora* (b) *Albizia guachapele* and (c) *Vochysia hondurensis* in a non-mist propagator.

while rooting of *Vochysia hondurensis* cuttings was detrimentally affected by the incorporation of sawdust into both gravel and fine sand. On the other hand, sawdust enhanced the rooting of *Eucalyptus deglupta* cuttings in both gravel and sand, while *Gmelina arborea* and *Albizia guachapele* rooted well in all media. Unlike these juvenile cuttings, mature cuttings from vigorous shoots in a heavily pruned crown of *G. arborea* rooted much less well (Fig. 5e) especially in pure gravel. In juvenile *G. arborea*, a comparison between cuttings set in fine sand under an intermittent mist propagator and the non-mist propagator, showed better rooting in the non-mist propagator.

C. alliodora, *A. guachapele* and *V. hondurensis* differed in their responses to the range of IBA concentrations (0 to 0.8%). Optimal concentrations would appear to be 0.4, 0.1 and 0.2% IBA respectively for the three species (Fig. 6). In addition *C. alliodora* did not root at all without applied auxins, while rooting in untreated *V. hondurensis* cuttings exceeded 40%. Cuttings of *A. guachapele* were very responsive to all auxin treatments.

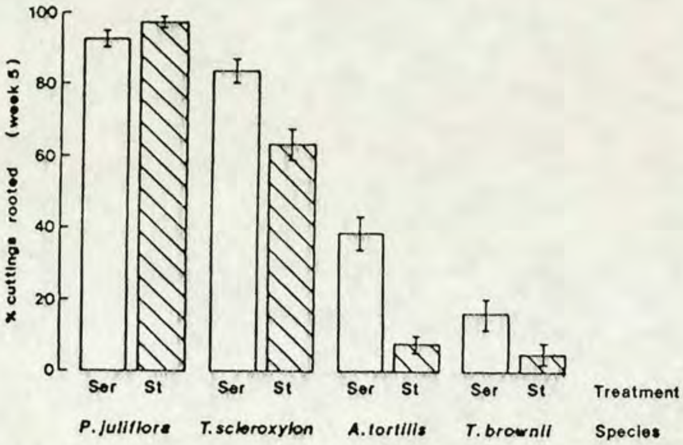


Figure 7. Percentage of cuttings of *Prosopis juliflora*, *Terminalia spinosa Acacia tortilis* and *Terminalia brownii* rooted in a non-mist propagator when treated with commercial rooting powders (Ser = "Seradix 2", St = "Strike").

Studies using non-mist propagators in Edinburgh and in Kenya, with tree species from semi-arid areas, investigated the relative merits of two commercially available rooting powders: (i) "Strike" = 0.25% NAA, and (ii) "Seradix 2" = 0.8% IBA. Seradix-treated cuttings of *Acacia tortilis*, *Terminalia spinosa* and *Terminalia brownii*, rooted better than those treated with Strike, while for *Prosopis juliflora*, rooting percentages were above 90% with both treatments (Fig. 7).

Two-node cuttings of *P. juliflora* also rooted relatively easily without applied auxins, although IBA solutions (0.4-0.8%) did hasten rooting and increase the numbers of roots formed. Long cuttings tended to root better than short ones, although there was no relationship between cutting length and its position of origin on the stockplant. Cuttings from lower on a stem generally rooted better than apical ones. An experiment comparing rooting under mist, with rooting in a non-mist propagator, clearly demonstrated the advantages of conditions without mist (Fig. 8). A third treatment in which a mist propagator was enclosed in polythene, resulted in even less rooting and higher mortalities due to rotting. When non-mist propagators have been used in Kenya to produce clonal material of *P. juliflora* for field experiments, the success rate was greater than 75%.

Like those of *P. juliflora*, cuttings of *A. tortilis* and *T. spinosa* are also much more easily rooted in the high humidity conditions of a non-mist propagator. There is, however, very considerable clonal variation in rooting ability in these species, and cuttings of *T. brownii* have so far only been rooted with a low rate of success.

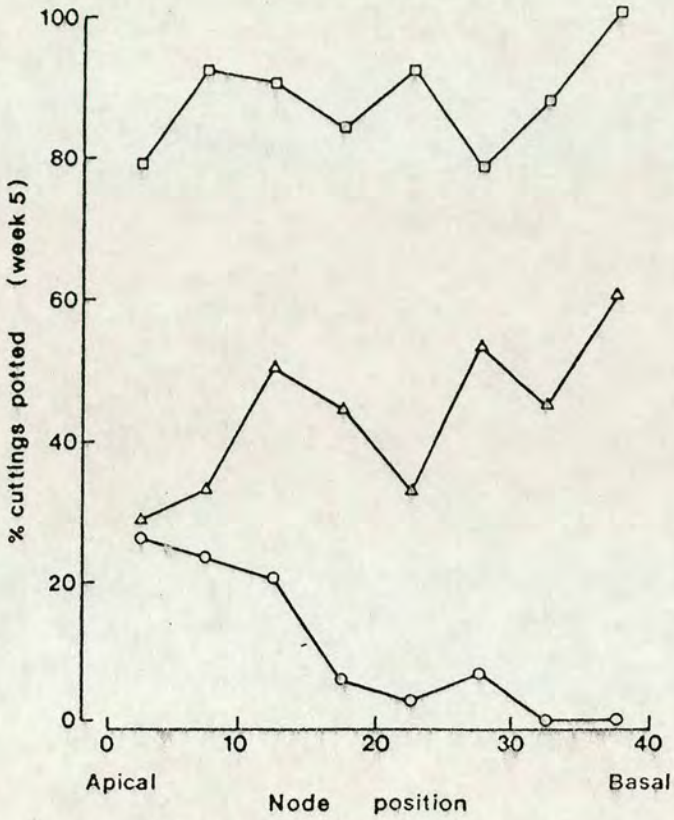


Figure 8. Effects of node position on the percentage of 2-node *Prosopis juliflora* cuttings rooted under three propagation environments (☐ = non mist, ○ = enclosed mist, ▲ = open mist).

In Cameroon, the percentage rooting of cuttings of *Lovoa trichiloides* was relatively poor (c. 40-50%), and, in addition, the results were frequently unexpected. For example, no beneficial effects on rooting were found following the application of NAA or a range of IBA concentrations (0-200 μg/cutting), either in terms of the percentage of cuttings rooted or the of roots per cutting. Furthermore, the optimum leaf area seemed to be very high at about 200 cm^2 per cutting (Tchoundjeu, 1989).

When the rooting of cuttings of *Ricinodendron heudelotii* was tested both under mist and in non-mist propagators with 'Seradix 2', rooting by day 21 was best without mist (75% *v* 50% under mist).

Discussion

While there are reports of cuttings of *Prosopis, Gmelina* and *Eucalyptus* species being rooted (Felker and Clark, 1981; Sim and Jones, 1987; Delwaulle, 1983; Delwaulle *et al*, 1983), there are apparently none on the other species tested here. *Terminalia spinosa, Terminalia brownii, Vochysia hondurensis, Albizia guachapele* and *Ricinodendron heudelotii* may not have been previously tested, while for example, a number of previous attempts to root *Cordia alliodora* have failed (Dyson, 1981). *Acacia* spp. of semi-arid/arid areas of Africa also have the reputation of being difficult-to-root (Roche *et al.* 1989). The 'low-tech', non-mist propagators described here therefore seem to provide a very practical solution to the problem of how a wide range of tropical tree species can be propagated vegetatively. The advantage of these propagators seems to be particularly great for the dry-zone species which can be very susceptible to rotting under mist. While it is clear that the environment within the non-mist propagator fluctuates considerably over the day in response to variations in ambient temperature and incident radiation, it is also clear that, by being enclosed and continuously moist, the cuttings are not subjected to the extremes of saturation and water stress that can occur when misting frequency is poorly matched to changes in the weather.

An improvement which might further stabilise relative humidity in the non-mist propagator would be to construct the lid in several sections. Then the whole propagator would not have to be opened to access the cuttings. Ideally, the propagators are opened as little as possible and especially not during the heat of the day. When propagators are opened the cuttings should be sprayed frequently with a fine spray of water from a hand-held or knapsack sprayer. During bright sunny weather, shading is essential and spraying is highly desirable to prevent low relative humidity developing at mid-day (see Fig. 2). Attention to these points of detail are likely to be particularly important when propagators are used in hot, sunny and dry areas.

The reasons why the rooting of cuttings of different species have slightly different requirements with respect to propagation media, and auxin concentration, is unknown. Studies are in progress at the Institute of Terrestrial Ecology and are aimed at the identification of fundamental principles determining rooting ability in a range of tropical tree species. In this regard it appears that stockplant light/nutrient interactions prior to severance are important (Leakey and Coutts, 1989; Leakey and Storeton-West, in preparation) affecting the subsequent capacity of unrooted cuttings to photosynthesise. This photosynthesis could also be limited to low daytime CO_2 concentrations in the propagator. Thus it may be necessary in future to enhance CO_2 diffusion into the propagators.

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LOW TECHNOLOGY VEGETATIVE PROPAGATION OF TROPICAL TREES: EXPERIENCE FROM CENTRAL AMERICA

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Abstract.

Leafy stem cuttings of a number of Central American tree species, including *Albizia guachepele, Bombacopsis quinata, Cedrela odorata, Cordia alliodora, Swietenia macrophylla* and *Vochysia hondurensis* have been successfully rooted in low-technology polythene propagators in Costa Rica. These propagators are cheap and easy to construct, and do not require piped water or an electrical supply. They are therefore particularly applicable to rural development situations. Experimental results are presented indicating the effect of different treatments applied to the cuttings, including the amount of applied IBA, the rooting medium and the propagation environment.

Introduction.

In Central America deforestation is continuing at a high rate. The urgent need for large scale reforestation efforts throughout the region has been evident for some time (Flores, 1985) but it has only been undertaken on a significant scale during the past decade (Hartshorn, 1982). Many recent attempts at re-forestation have failed, partly because of the use of genetically inappropriate sources of planting material (Mesén *et al.*, 1992b). To address this problem, the CATIE Tree Improvement Project has been active in the genetic improvement of agroforestry and traditional timber species, since its inception in 1977. The activities of the Project have primarily involved seed collection, and the establishment of progeny and provenance tests of the selected species. However, in recent years the Project has become involved in the development and application of vegetative propagation techniques aimed at the establishment of clonal plantations. The advantages of vegetative propagation and clonal selection for rapidly increasing the yield and quality of forest products are now widely appreciated (Leakey, 1987; Libby and Rauter, 1984). However, most clonal techniques are not available to farmers in developing countries because of the expensive equipment required. This obstacle has been overcome by the development of low technology propagation techniques as described by Leakey *et al.* (1990).

Low-technology propagation techniques

A wide range of tropical tree species have now been sucessfully propagated using low-technology propagation techniques, including *Albizia guachepele, Alnus acuminata, Bombacopsis quinata, Cedrela odorata, Cordia alliodora, Eucalyptus deglupta, Gmelina arborea, Swietenia macrophylla* and *Vochysia* *hondurensis* (Leakey *et al.*, 1990; Leakey *et al.*, 1992; Mesén *et al.*, 1992a). A number of these species had not been propagated before, or were previously thought to be difficult-to-root. Although this research has been based in Costa Rica, the techniques are currently being introduced to other tree improvement groups in Central America through a series of annual workshops held at CATIE.

(i) *Preparation of cuttings*
Cuttings were initially harvested from seedlings obtained from 'plus' tree seed collections from locations throughout Central America. Rooted cuttings were then established in clonal gardens and managed as coppiced stockplants, to stimulate the production of vigorous, orthotropic shoots. Shoots were usually harvested every three months, each providing 6-10 single-node, leafy stem cuttings. These were usually 5-6 cm long, with the leaf area trimmed to around 30 cm^2. Shoots were harvested during the coolest time of the day and transported to the propagation area in containers with water. Auxins such as indole-3-butyric acid (IBA) were generally applied to the base of the cuttings prior to insertion in the propagator, either in methanol solution or in powder form (e.g. commercial rooting powders such as "Strike" or "Seradix"; c.f. Leakey *et al.*, 1990). The cuttings were then inserted into the propagation medium to a depth of 15-25 mm.

(ii) *The non-mist propagator*
In all Central American countries, the high capital and running costs of currently available mist propagation systems makes them inappropriate for use in rural situations. Therefore, the project concentrated on the use of lower cost, simpler techniques, using the non-mist propagator developed by ITE (Leakey and Longman, 1988; Leakey *et al.*, 1990; Mesén *et al.*, 1992a). This design does not require an electrical supply or piped water, and is therefore particularly suitable to rural tropical areas.

The non-mist propagator is a wooden or metal frame enclosed in polythene so that the base is water tight. The polythene base is covered in a thin layer of sand, then successive layers of large stones (6 - 10 cm in diameter to a depth of 10-15 cm), small stones (3-6 cm) and gravel (0.5-1.0 cm) to a total depth of 20 cm. The rooting medium forms the uppermost layer, and may consist of sawdust, sand or fine gravel in various mixtures. Water is added to the propagator up to the base of the rooting medium, which is kept moist by capillarity. The rest of the frame is covered with a single piece of clear polythene, and a closely-fitting lid is attached. To maintain high humidities inside the propagator, the lid should only be opened when it is necessary to inspect the cuttings (Newton and Jones, 1992a).

(iii) *The propagator environment*
The effectiveness of the non-mist propagator lies in its capacity to maintain high relative humities and low vapour pressure deficits (VPD's), even at realtively high irradiances, thereby maintaining leaf turgor in the cuttings (Newton and Jones 1992a,b). In leafy stem cuttings of *C. alliodora*, for example, the relative water content of the leaves varied between 75 and 95%

during a six week period in the propagator, which was associated with rooting percentages above 80% by the end of this period (Mesén, 1991).

VPD and irradiance are positively correlated in nin-mist propagators (Newton and Jones, 1992a). At CATIE, irradiance can be in excess of 2000 μmol m^{-2} s^{-1} on a clear day, which can cause an unwanted increase in VPD inside the propagator. Therefore shading is usually necessary, and can be provided by materials such as plastic netting or palm foliage. The reduction in irradiance should not be excessive, however, as rooting is often dependent on photosynthesis during propagation (see later).

Experimental results

(i) *Rooting medium*

Substantial differences have been identified between species with regard to rooting medium (Table 1). The reasons for these differences are not well understood, but may be related to the relative composition (solids: air: water) of the media (Loach, 1988). The media utilized incorporated local and easily available substrates, following the same principles of simplicity and economy of the propagator.

Table 1. Summary of results obtained in Costa Rica on the optimum substrate requirements for the rooting of leafy stem cuttings of the project species.

Species	Rooting medium	Average rooting percentage
A. guachapele	Gravel, gravel + sawdust*	80-90%
A. acuminata	Sand	60-80%
B. quinata	Sand, sawdust	70-80%
C. alliodora	Sand, sand + sawdust*	60-90%
V. hondurensis	Sand, sand + sawdust*, gravel + sawdust*	80-90%

*50/50 by volume

(ii) *Auxin concentration*

Applied auxins hasten root emergence, and increase the percentage of cuttings which form roots, the number and quality of roots produced per cutting, and the uniformity of rooting (Hartmann and Kester, 1983). However, the exact role of auxins in stimulating adventitious root formation remains unclear. Optimum auxin concentration varies greatly between species, as the result of variation in concentrations of endogenous auxins, rooting co-factors and inhibitors etc. (Leakey, 1985).

In experiments undertaken at CATIE, IBA was applied to the clean cut base of the cuttings in 10 μl of methanol solution using a micrometer syringe. This allowed the application of a standard quantity of solution to every cutting. The alcohol was evaporated off in a stream of cold air before inserting the cuttings in the propagator (see Leakey *et al.*, 1982). Concentrations of 0, 0.05, 0.2, 0.4, 0.8 and 1.6 % IBA were generally tested (Table 2).

Contrary to all other species, *V. hondurensis* displayed the highest rooting percentages when no auxin was applied. However, the number of roots per rooted cutting increased with successive increases in auxin concentration, from three with 0% IBA to eight with 0.8% IBA. The concentration of 0.2% provided the most appropriate balance between rooting percentage and root system quality (Mesén *et al.*, 1992a; Table 2). The time of first occurrence of root emergence also varied between species, from three weeks for *A. guachapele* and *B. quinata* to around five for *V. hondurensis*. For all the species, no further rooting occurred beyond eleven weeks.

Table 2. Summary of results obtained in Costa Rica on optimum IBA concentration for the rooting of leafy stem cuttings of the project species.

Species	IBA concentration (%)	Average rooting percentage
A. guachapele	0.05-0.4	90-100%
A. acuminata	0.2	60-80%
B. quinata	0.2	60-80%
C. alliodora	0.8-1.6	60-90%
V. hondurensis	0.2	80-90%

(iii) *The physiological activity of cuttings during propagation*
Treatments such as applied IBA and rooting medium affect rooting by influencing physiological processes in the cuttings. Measurements of physiological activity are crucial for the understanding of how these processes interact, and for the determination of optimum treatments for rooting (Newton *et al.*, 1992). A detailed programme of such measurements has now been completed at CATIE, as reported elswhere (Mesén *et al.*, 1992a). Particular attention has been devoted to measurements of gas exchange in cuttings. In *C. alliodora* cuttings, rates of net photosynthesis of 4-5 μmol CO_2 m^{-2} s^{-1} were recorded at irradiances of around 300 μmol m^{-2} s^{-1} prior to root formation (Mesén *et al.*, 1992a). Results suggest that pre- and post-severance treatments should be adopted which optimise photosynthetic rates, as rooting of leafy stem cuttings is often dependent on photosynthesis during the propagation period (Newton *et al.*, 1992).

Conclusions

The CATIE Tree Improvement Project, with close collaboration of ITE, has demonstrated that clonal propagation can be adopted by small scale rural development programmes, by using low-cost, simple propagation techniques. The project produced the first reports of successful rooting for *Albizia guachapele, Alnus acuminata, Cordia alliodora* and *Vochysia hondurensis*, some of which had the reputation of being difficult-to-root. The non-mist propagators described here have been found effective for the propagation of a wide range of species from a range of different ecosystems. These techniques will enable the selection and use of genetically superior clones for use in forestry and agroforestry, both on small farms and industrial plantations.

The approach adopted by this project, combining traditional improvement techniques, such as provenance/progeny tests, with low-cost and easily transferred cloning methods, offers excellent prospects for the conservation and commercial use of high genetic quality germplasm of valuable native species. By offering improved planting alternatives to the landowner, reforestation can be stimulated, and this in turn will open new possibilities to counter further destruction of the forest genetic resources of the Central American region.

Acknowledgements

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Low technology propagation of tropical trees: rooting physiology and its practical implications

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Abstract

Leafy stem cuttings of a large number of tropical tree species (including *Albizia guachepele*, *Bombacopsis quinata*, *Cordia alliodora*, *Nauclea diderichii*, *Terminalia spinosa*, *Triplochiton scleroxylon* and *Vochysia hondurensis*) have been successfully rooted in low-technology polythene propagators in Costa Rica, Cameroon, Kenya and the UK. These propagators are cheap and easy to construct, and do not require piped water or an electrical supply.

An investigation has been made of the physiological activity of cuttings during propagation in relation to propagator microclimate. Lower vapour pressure deficits (VPDs) were maintained in the non-mist system than under mist, particularly at higher irradiances. Although cuttings were found to experience significant water deficits in both systems, resulting from the high VPD's associated with peaks in irradiance, all species were able to recover turgor prior to rooting. Cuttings of a range of species displayed high stomatal conductances (up to 400 mmol $m^{-2} s^{-1}$) when VPD's were low (less than 1.5 kPa), and average photosynthetic rates of around 2 μmol $m^{-2} s^{-1}$ were recorded in *Terminalia spinosa* cuttings prior to rooting. The microclimate inside the propagator was found to interact with routine treatments applied to cuttings. For example, the optimum leaf area for rooting uniform length cuttings of *Cordia alliodora* was found to be dependent on the irradiance regime during propagation.

Together, these results highlight the interaction between propagator microclimate, the physiological activity of the cuttings, and the post-severance treatments applied to the cuttings. Suggestions are made towards identifying the optimum post-severance treatments and propagation environment for a given species. An assessment of which experimental variables should be fully described in experimental investigations is presented.

Keywords Vegetative propagation, rooting physiology, propagator microclimate

Introduction

Vegetative propagation techniques are increasingly being applied to tropical tree species, both for genetic improvement and conservation purposes (Leakey *et al.*, 1990; see also papers in Gibson, Griffin and Matheson, 1989). A wide range of species have now been successfully propagated using the techniques developed by the Institute of Terrestrial Ecology (ITE), Edinburgh, involving the rooting of leafy cuttings in a low-technology propagation system. In order to successfully mass propagate a given species, it is necessary to understand the critical factors which influence rooting so that consistently high rooting percentages can be obtained. A number of critical factors have been identified, including (i) the propagator microclimate, (ii) the physiological status of the cuttings during propagation and (iii) the post-severance treatments applied to the cuttings. In this paper, following a brief description of low-technology propagation techniques, we describe how these environmental and physiological factors interact to influence rooting. The practical implications of these aspects are then discussed.

Low-technology propagation techniques

A wide range of tropical tree species from a range of different environments have now been sucessfully propagated using low-technology propagation techniques, including *Albizia guachepele, Bombacopsis quinata, Cordia alliodora, Nauclea diderichii, Terminalia spinosa, Triplochiton scleroxylon* and *Vochysia hondurensis* (see Leakey *et al.*, 1990; Dick *et al.*, 1992; Leakey, Newton and Dick, 1992). Many of these species have not been propagated before, or were previously thought to be difficult-to-root. The techniques are currently being applied in a number of tropical countries including Cameroon, Kenya and Costa Rica (Leakey *et al.*, 1990).

Leafy cuttings are routinely taken from seedlings, coppice shoots or managed juvenile stockplants. The cuttings consist of stem sections of 40-60 mm length, with 1- to 4-nodes, and either one or two leaves. In large leaved species, the leaves are trimmed to reduce water loss. Auxins such as indole-3-butyric acid (IBA) are generally applied to the base of the cutting prior to insertion in the propagator, either in solution (0.2-0.4% IBA in industrial methylated spirit) or in powder form (e.g. commercial rooting powders such as "Strike" or "Seradix"). The cutting is then inserted into the propagation medium to a depth of 15-25 mm.

The design of low-technology non-mist propagator currently in use is based on that of Leakey and Longman (1988), as described by Leakey *et al.* (1990). This design does not require an electrical supply or piped water, and is therefore particularly suitable to rural tropical areas. The propagator consists of a wooden or metal frame, which is completely enclosed in clear polythene so the base is water-tight. The base of the propagator is covered in a layer of sand, then successive layers of large stones (6 - 10 cm in size to a depth of 10-15 cm), small stones (3-6 cm) and gravel (0.5-1.0 cm) to a total depth of 20 cm. The rooting medium forms the uppermost layer, and may consist of sawdust, sand or fine gravel in various mixtures. The propagator is filled with water to a level a few centimetres below the surface of the rooting medium. Access is provided by a tight-fitting lid.

Successful propagation of leafy cuttings is dependent on the maintenance of suitable air and leaf temperatures, irradiances and vapour pressure deficits (VPD's) for the physiological activity of the cuttings (Loach, 1988). As water loss from the cuttings is primarily determined by VPD, quantification of variation in VPD in the propagator is particularly useful for developing management guidelines for the propagation system (Grange and Loach, 1983).

Variation in VPD in mist and non-mist propagators

When the microclimate of a low-technology non-mist system was compared with a more traditional mist unit, pronounced diurnal variation in VPD was recorded in both systems. VPD's tended to remain close to 0 kPa during nightfall, then rose rapidly to peaks of up to

VPD's under mist were consistently higher than under non-mist, reflecting the higher relative humidities and lower leaf-air temperature differences maintained in the non-mist system.

These results indicate that the non-mist system developed by ITE is at least as effective as the mist system at maintaining relatively low VPD's during periods of high irradiance. This emphasises the fact that the microclimate of non-mist propagators is comparable with other more sophisticated propagation systems, and highlights their suitability for a wide range of species.

Regression analyses between VPD and irradiance provide a basis for guidelines of propagator management. For example, in measurements taken to date in the non-mist system, irradiance in the propagator would need to be kept below approx. 100 μmol m^{-2} s^{-1} to maintain VPD's below 0.5 kPa, a threshold suggested by Grange and Loach (1983). Results also indicate that the VPD can be reduced by keeping the internal surfaces of the propagator wet, and reducing the period of opening of the propagator lid (Newton and Jones, 1992a).

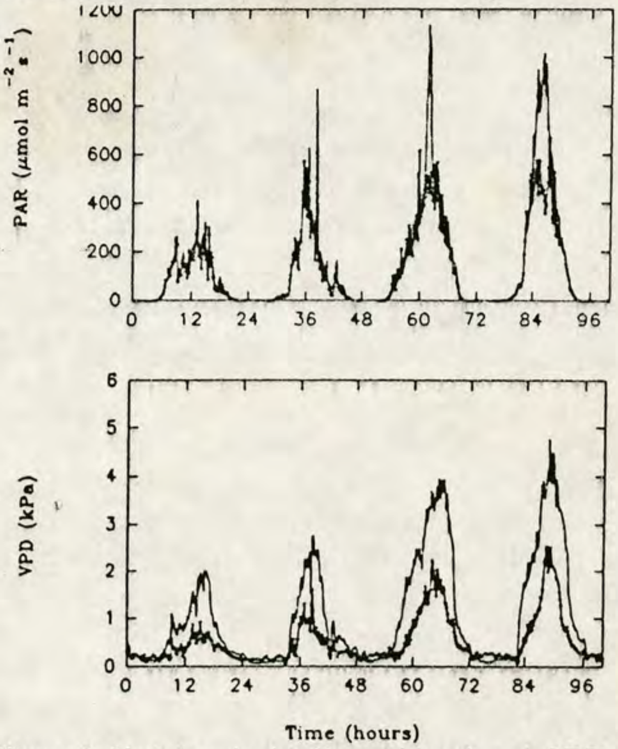

Figure 1 Variation in irradiance (above) and VPD (below) in a mist (lines) and non-mist (lines with circles) propagation system over four days, July 1991

The physiological activity of leafy cuttings during propagation

Leafy cuttings are vulnerable to desiccation during propagation, particularly prior to root development and during high VPDs (Grange and Loach, 1983). In a comparative investigation of *Albizia guachepele*, *Terminalia spinosa* (both arid-zone species), *Triplochiton scleroxylon* and *Cordia alliodora* (both humid-zone species) in polythene-covered mist and non-mist systems, water potentials measured at 14:00 h were in the range -0.4 to -2.2 MPa under mist and -0.4 to -3.1 MPa under non-mist. These values compare with the range of -0.4 to -1.2 MPa recorded for *T. scleroxylon* (Leakey and Coutts, 1989), and -0.2 MPa to -1.8 MPa obtained from three ornamental species, under conventional mist (Grange and Loach, 1985). However, in our experience, most species seem able to regain turgor following such deficits. For example, when the four species listed above were measured throughout the day, a mid-day decline in leaf water potential was frequently recorded (Fig. 2). In most cases, water potentials recovered during late afternoon or overnight. An increase in mean leaf water potential was also observed over the three weeks after insertion of cuttings of *A. guachepele* and *C. alliodora* in the propagator, from minima around -1.5 MPa to values close to -0.5 MPa (Newton and Jones, 1992b).

In this investigation, water potentials were also found to vary with the propagation system utilised and the species involved. For example, in the case of the experiment comparing *A. guachepele* and *C. alliodora*, water potentials of both species were lower under mist than under the low-technology non-mist system (Fig. 3). In addition, mean water potentials of *C. alliodora* were significantly lower than *A. guachepele,* and those of *T. scleroxylon* were lower than *T. spinosa* (Fig. 3) (Newton and Jones, 1992b). These results suggest that species from arid environments may be more tolerant of desiccation during propagation than species from humid areas.

Despite the significant water deficits incurred during these experiments, many of the cuttings subsequently rooted successfully (Newton and Jones, 1992b). Although Loach (1977) indicated that low water potentials may result in a reduction in rooting ability, the critical thresholds of water status for root development are still poorly defined. More precise information is required relating the interaction between the water status of leafy cuttings during propagation and its impact on root development. The primary effect of water deficits developed in leafy cuttings will be to restrict the photosynthetic activity of the foliage, and thereby limit the rate of carbon fixation during the propagation process.

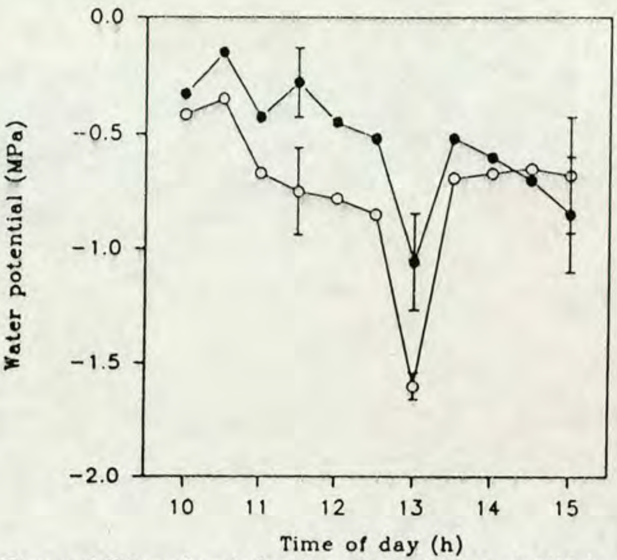

Figure 2 Diurnal variation in water potential of *Albizia guachepele* in mist (open) and non-mist (closed) propagators (n = 10 ± sem)

All of the tropical species we have so far investigated by either porometry or gravimetry have been found to actively transpire during propagation. For example, in *T. spinosa* conductances were found to be in the range 50 - 300 mmol m^{-2} s^{-1} in a non-mist propagation system; values tended to be higher after the onset of root emergence (Newton *et al.*, 1992). In a separate series of experiments comparing the responses of the four species listed above in both non-mist and polythene-covered mist systems, stomatal conductances were generally in the range 50 - 400 mmol m^{-2} s^{-1} prior to root formation (Newton and Jones, 1992b). In the case of *A. guachepele* and *C. alliodora*, conductances tended to increase with time after insertion in the propagator, highlighting the ability of the cuttings to recover from water deficits incurred following severance. When *T. spinosa* and *T. scleroxylon* were compared, conductances of the former species tended to be higher, reflecting the greater ability of this arid-zone species species to tolerate water deficits (Newton and Jones, 1992b).

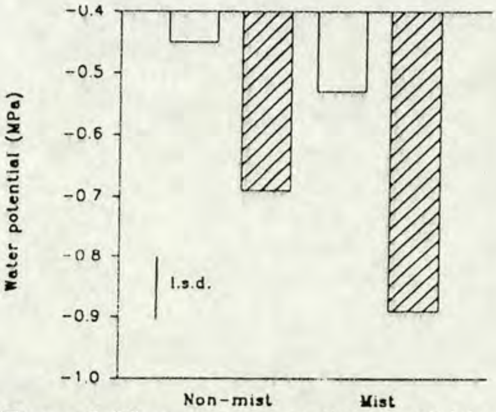

Figure 3 Mean water potential of *Albizia guachepele* (open) and *Cordia alliodora* (hatched) in non-mist and mist propagators (n > 200)

The stomatal conductances of *T. scleroxylon* and *T. spinosa* were found to be negatively related to VPD at the time of measurement (Newton and Jones, 1992b). This emphasises the effects of propagator microclimate on the physiological activity of the cuttings during propagation.

The results obtained from these investigations contrast with those of Gay and Loach (1977), who concluded from an experiment with temperate shrubs that stomatal conductances of leafy cuttings remain low (around 40 - 80 mmol m^{-2} s^{-1}) until roots have formed. The relatively high conductances recorded in our investigations are evidence for gas exchange during propagation being one of the critical factors influencing rooting.

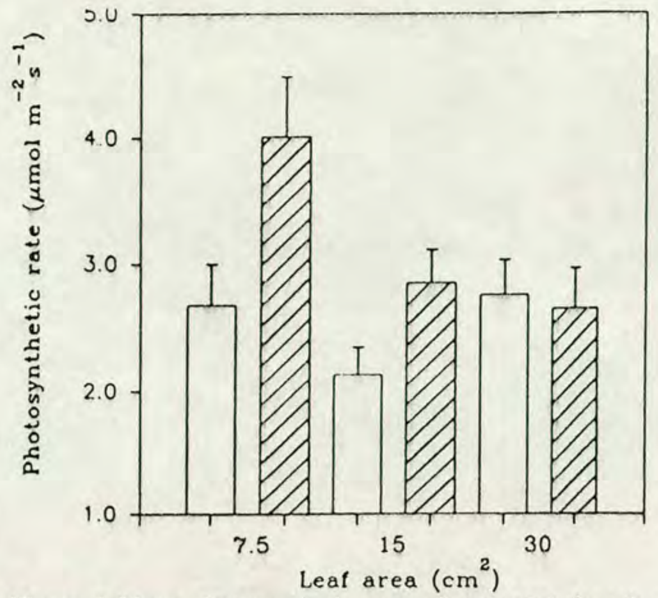

Figure 4 Mean photosynthetic rate of unrooted (open) and rooted (hatched) *T. spinosa* cuttings (n > 16 + sem)

In *Terminalia spinosa*, as in many other tropical tree species, removal of the leaf prevents rooting (Newton, Dick and Muthoka, 1992). This supports the suggestion that carbohydrates produced by the leaf are vital for root development. Although a number of authors have suggested that the production of carbohydrates during propagation is of crucial importance for rooting of leafy cuttings (see Davis, 1988), few measurements of photosynthesis have actually been made. In *T. spinosa*, all leafy cuttings measured (using an infra-red gas analyser) were found to actively photosynthesize during the period of propagation in a non-mist propagator, with a mean rate of approx. 2 μmol CO_2 m^{-2} s^{-1} and a maximum rate of over 6 μmol CO_2 m^{-2} s^{-1} (Newton *et al.*, 1992) (Fig. 4). The photosynthetic rate at any given time was found to be strongly dependent on stomatal conductance and irradiance ($P < 0.001$ in both cases). Regression analysis indicated that cuttings with larger leaf areas displayed lower photosynthetic rates during periods of high irradiance, as a result of the onset of water deficits and stomatal closure. Photosynthetic rates also tended to be higher in rooted than unrooted cuttings, as would be expected from the improved water supply to the leaf following root development (Fig. 4) (Newton *et al.*, 1992).

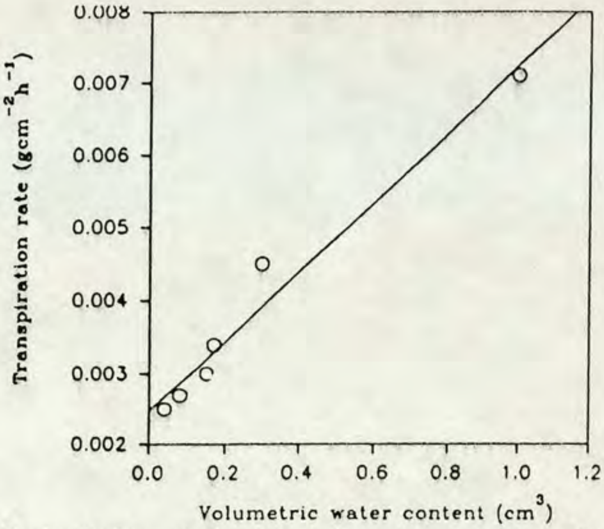

Figure 5 The relationship between transpiration rate from cuttings of C. alliodora and the volumetric water content of different media

In a separate investigation, the transpiration rate (measured gravimetrically) of *Cordia alliodora* cuttings placed in different rooting media was found to be positively related to the volumetric water content of the medium (Fig. 5; $y = 0.0048x + 0.0025$; $r^2 = 0.98$) (Newton, Mesén and Leakey, 1991). This illustrates that the water availability in the rooting medium also influences cutting water status, and may therefore also affect photosynthetic activity and rooting. The relative importance of VPD and the water content of the rooting medium in determining cutting water status has not yet been investigated, although both aspects should clearly be taken into account if rooting ability is to be optimised.

The effects of post-severance treatments applied to cuttings

Rooting of leafy cuttings can also be influenced by post-severance treatments, such as leaf area, amount of applied auxin and cutting size. For example, the rooting ability of *T. scleroxylon* was found to be positively related to cutting length (Leakey and Mohammed, 1985). Leaf areas of 50 cm^2 and 10-30 cm^2 have been found to be optimal for the rooting of *T. scleroxylon* and *Khaya ivorensis* respectively (Leakey *et al.*, 1982; Tchoundjeu, 1989). Rooting of other species, such as *T. spinosa* (Newton *et al.*, 1992) and *Nauclea diderichii* (Leakey, 1990) is apparently less sensitive to variation in leaf area. This tendency of some species to display optimum leaf areas for rooting reflects the trade-off between photosynthesis and water loss during propagation (Okoro and Grace, 1976). A number of species also display different optima for the quantity of IBA applied to the cutting base pre-severance. For example, optimum IBA concentrations in a solution of industrial methylated spirit for *Cordia alliodora*, *Albizia guachepele* and *Vochysia hondurensis* were found to be 0.4, 0.1 and 0.2% respectively (Leakey *et al.*, 1990).

The influence of the various post-severance factors on rooting can be best appreciated by considering the relative availability of carbohydrates produced pre- and post-severance. For example, the production of carbohydrate by photosynthesis during propagation is determined by the photosynthetic activity of the leaves, which depends upon leaf size and nutrient status, the water status of the cutting, and the availability of an appropriate carbohydrate sink (see Hoad and Leakey, 1992). These factors can be manipulated by the pre-severance growth environment (Leakey *et al.*, 1992).

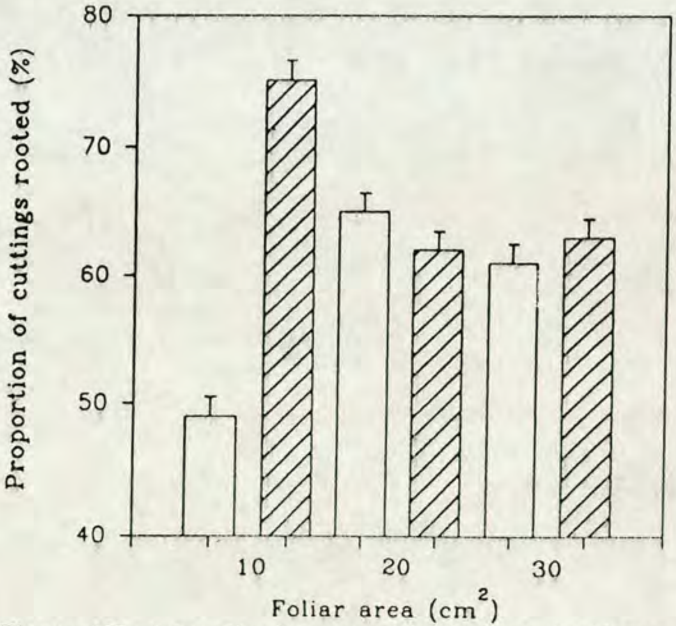

Figure 6 The interaction between cutting leaf area, irradiance regime and rooting of *C. alliodora*

Although the effects of post-severance treatments are becoming increasingly well documented, their interactive nature has largely been ignored. For example, the optimum leaf area for a given species will vary with the irradiance regime during propagation. This is illustrated by an experiment with *Cordia alliodora*, where cuttings with 20cm^2 and 30 cm^2 leaf area rooted equally well in both shaded and unshaded propagator environments, the shaded treatment receiving 7% irradiance of the unshaded propagator. However, rooting percentages of cuttings with 10 cm^2 leaf area were significantly higher under high irradiance (Fig. 6) (Newton, Mesén and Leakey, 1992). This may be explained by higher rates of photosynthesis and lower water deficits in the small-leaved, high irradiance treatment. Similarly, in *Bombacopsis quinata* and *Vochysia hondurensis*, the effect of different concentrations of applied IBA was found to depend on the rooting medium utilised (Newton *et al.*, 1991). Other more complex interactions are to be expected: the relative importance of pre- and post-severance carbohydrates will vary with the stem and leaf dimensions, and will be influenced by the propagator microclimate and rooting medium, as well as by the amount of applied IBA.

Practical implications

The fact that rooting is influenced by so many interactive factors necessitates the development of process-based models as a tool to understanding (see Dick *et al.*, 1992). In time, these modelling approaches should enable practical guidelines to be generated for individual species for any given set of conditions. In the interim, it is essential that researchers provide detailed accounts of experimental procedures, to assist in interpretation of results. For example, propagation experiments detailing the influence of a particular factor should also give information on the propagation environment, the pre-severance stockplant growth environment, the dimensions of the cuttings etc. Experimenters should seek to either remove or quantify different sources of variation, so their impact may be assessed accurately. This might include taking cuttings of standard length and leaf area, measuring cutting stem diameters, taking account of cutting origin (Leakey *et al.*, 1992) and analysing the nutritional status and carbohydrate dynamics of the cuttings during propagation (c.f. Hoad and Leakey, 1992).

As an additional layer of complexity, species and clones will differ in their responses to microclimatic factors and different post-severance propagation treatments. In order to successfully mass propagate a given species, an understanding of its physiological characteristics is desirable. These responses will determine the appropriate rooting environment and pre-severance treatments to be applied. For example, a drought-tolerant species such as *T. spinosa* is better able to tolerate a high irradiance/ high VPD propagation environment than a species from more humid areas, such as *T. scleroxylon*. Under such conditions, the drought-tolerant species might display relatively low sensitivity to variation in leaf area and variation in stem size.

Since all the various factors influencing rooting performance are interactive, the propagation and stockplant growth environment should influence which treatments are applied to the cuttings. In situations where simplified routine treatments are adopted, such as standard cutting size and leaf areas, then an attempt should be made to standardise the propagation and stockplant environments, to enhance repeatability of results. The precise treatments employed for the propagation of a particular species will depend on the physiological characteristics of the species concerned and the propagation environment utilized.

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HACIA EL DESARROLLO DE TECNICAS DE SILVICULTURA CLONAL PARA EL PEQUEÑO FINQUERO

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Roger R.B. Leakey 2/
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TRODUCCION

El interés por la silvicultura clonal (uso de opágulos vegetativos de clones seleccionados ıra el establecimiento de plantaciones) ha nido en aumento en los últimos años. Las zones de este crecimiento radican en que, con aumento del conocimiento sobre el tema, es da vez mayor el número de especies que ıeden ser propagadas vegetativamente así mo a la concientización acerca de las ortunidades que ofrece la clonación para ilizar y explotar la variabilidad genética rectamente (Leakey *et al.*, 1990). Existen rios ejemplos (Aracruz Celulose, Brasil; Centre chnique Forestier Tropical, Congo; Forest esearch Institute of Nigeria) donde se han grado aumentos extraordinarios en la calidad y oductividad de las plantaciones, al igual que na reducción del tiempo de rotación, mediante uso de clones superiores. De este modo, el so de clones permite eliminar algunos de los oblemas biológicos, principalmente en cuanto producción de semillas, que afectan la forestación con muchas de las especies opicales (Leakey, *et al*, 1982b).

Los mayores avances en la silvicultura onal se han dado en las grandes compañías forestadoras, y las técnicas desarrolladas no an estado al alcance de los pequeños grupos ırales en países en desarrollo, que general- ente no cuentan con el capital necesario para acer grandes inversiones. Es necesario mplificar y abaratar la tecnología para ponerla disposición del pequeño finquero.

Tradicionalmente se han utilizado varias formas de propagación vegetativa en mejoramiento genético; de ellas las más conocidas son los injertos y los estacones lignificados para el establecimiento de huertos semilleros clonales. Este material fisiológicamente adulto es ideal en estos casos, puesto que reduce el ciclo de producción de semillas y produce árboles bajos, de copas amplias, que facilitan la recolección de las mismas. Por el contrario, la propagación vegetativa para el establecimiento de plantaciones, requiere del uso de material fisiológicamente juvenil, el cual dará origen a árboles de crecimiento ortotrópico normal, adecuados para la producción de madera. A nivel operacional, normalmente se utilizan estacas originadas de rebrotes de tocones, rebrotes basales de árboles en pie o plántulas jóvenes, entre otros. Estas estacas se caracterizan por su tamaño pequeño (generalmente 5-8 cm de longitud y 0,5-0,8 cm de diámetro), condición suculenta y la presencia de hojas.

Numerosos factores anatómicos, fisiológicos y ambientales afectan el enraizamiento de estacas (Hartmann y Kester, 1968). Todos ellos deben ser optimizados para un enraizamiento exitoso (Leakey y Mesén, 1991a); sin embargo, la minimización del estrés hídrico en las estacas es considerado como el punto fundamental en el proceso (Loach, 1988). El efecto más inmediato del estrés hídrico es el cierre de los estomas, lo que a su vez restringe la fotosíntesis y la producción consecuente de carbohidratos, el crecimiento y la división celular y la translocación de metabolitos a los primordios radicales en desarrollo. Es probable que el estrés hídrico también reduzca el suministro de cofactores, los cuales sinergizan con las auxinas en la formación de raíces adventicias (Loach, 1988).

Existen varios sistemas de propagación que logran minimizar el estrés hídrico, usualmente basados en aspersión automática o nebulizadores. Sin embargo, es posible lograr la propagación exitosa con sistemas más simples y económicos, inclusive en condiciones de ausencia de electricidad y agua de cañería. El Proyecto Mejoramiento Genético Forestal-PMGF del CATIE, en cooperación con el Instituto de Ecología Terrestre-ITE de Escocia, ha adaptado y utilizado con éxito un propagador de sub-

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irrigación, basado en un diseño utilizado para la propagación de latifoliadas tropicales (Leakey, Chapman y Longman, 1982). El trabajo del PMGF en este campo se ha enfocado al desarrollo de las técnicas óptimas de propagación, incluyendo estudios fisiológicos antes y durante la propagación, así como al desarrollo de técnicas de selección clonal temprana. Como resultado de esto, se han identificado los requisitos básicos para el enraizamiento de varias especies y se han desarrollado sistemas simples de manejo y propagación del material, que puedan ser adoptados a nivel operacional por pequeños grupos de desarrollo rural.

Este artículo describe los avances logrados hasta la fecha en este campo, para varias especies forestales.

MATERIALES Y METODOS

Los estudios presentados en este trabajo, se llevaron a cabo tanto en invernaderos de ambiente tropical en el ITE, Escocia, como en el vivero del PMGF en el CATIE, Turrialba, Costa Rica. Se han realizado trabajos con las siguientes especies:

Albizia guachapele (guayaquil, careto real)
Bombacopsis quinata (pochote, cedro espino)
Cordia alliodora (laurel)
Eucalyptus deglupta (deglupta)
Gmelina arborea (melina)
Swietenia macrophylla (caoba)
Vochysia hondurensis (chancho blanco, San Juan)

El material vegetativo utilizado consisitió en estacas juveniles con hoja, de 5 cm de longitud, obtenidas de rebrotes de setos manejados o de plántulas.

En todos los casos la hormona utilizada fue el ácido indol-3-butírico disuelto en metanol, aplicado a la base de las estacas mediante inmersión rápida (2 segundos) en la solución o con microjeringa de 10 µl, con evaporación inmediata del metanol en una corriente de aire frío (ver Leakey *et al.*, 1982a). El segundo sistema se utilizó en los ensayos de dosis de auxina, puesto que permite un control exacto de la cantidad y la concentración aplicada a todas las estacas, independientemente de las variaciones en el diámetro de las mismas, su pubescencia, el grado de transpiración, etc.

Durante los ensayos se utilizó un microprocesador Campbell 21 X (Campbell Scientific Ltd., Leicester, Reino Unido), para el registro de las condiciones ambientales, con mediciones permanentes a intervalos de 15 minutos. Para las mediciones de tasas de fotosíntesis y conductividad estomática se utilizó un analizador de gas infrarojo tipo LCA-3 (ADC Ltd., Hoddesdon, Reino Unido), conectado a una cámara foliar Parkinson, mediante la cual se realiza la medición basada en una área foliar estándar de 6,25 cm^2. Durante las mediciones, se extrajo la estaca del medio y la base de la misma se mantuvo sumergida en agua. El uso de este equipo permite identificar los requerimientos básicos de las especies para diseñar entonces sistemas simples de fácil aplicación práctica.

Para todos los ensayos se utilizó un diseño de bloques completos al azar con arreglos factoriales, con un número variable de repeticiones para lograr un mínimo de 40 estacas por combinación. El error estándar de los porcentajes de enraizamiento se calculó mediante la fórmula de Bailey (1959) para datos con distribución binomial, o mediante la prueba de t, según se indique, ambos con una probabilidad del 5%. Para datos de número de raíces por estaca, se determinó el error estándar del promedio.

El propagador de sub-irrigación

El propagador utilizado (Fig. 1) está basado en un diseño realizado por Howland (1975), modificado por Leakey y Longman (1988), con nuevas modificaciones realizadas a la luz de las experiencias en el CATIE (Leakey *et al.*, 1990). Para su construcción se utiliza un marco de madera o metal forrado con un plástico resistente, para la retención del agua y la conservación de humedad. En el fondo del propagador se coloca una capa fina de arena de 1-2 cm (para prevenir la ruptura del plástico) y una capa de piedras gruesas de 6-10 cm (diámetro) hasta una altura de 10-15 cm. Las piedras se cubren con una capa de grava hasta una altura de 20 cm. Finalmente se coloca la última capa de 5 cm de espesor, compuesta por el sustrato a utilizar, y se adiciona agua hasta una altura de 20 cm (hasta la base del sustrato). Para observar el nivel de agua o para adicionar más agua si es necesario, se utiliza un pequeño cilindro (plástico, bambú, etc) insertado verticalmente a través de las diferentes capas. El marco se cubre con una tapa, también forrada de plástico, que ajuste lo mejor posible para evitar la pérdida de humedad. Varias divisiones internas proporcionan soporte adicional al marco, y a la vez permiten la evaluación de sustratos diferentes dentro del mismo propagador (Fig. 1).

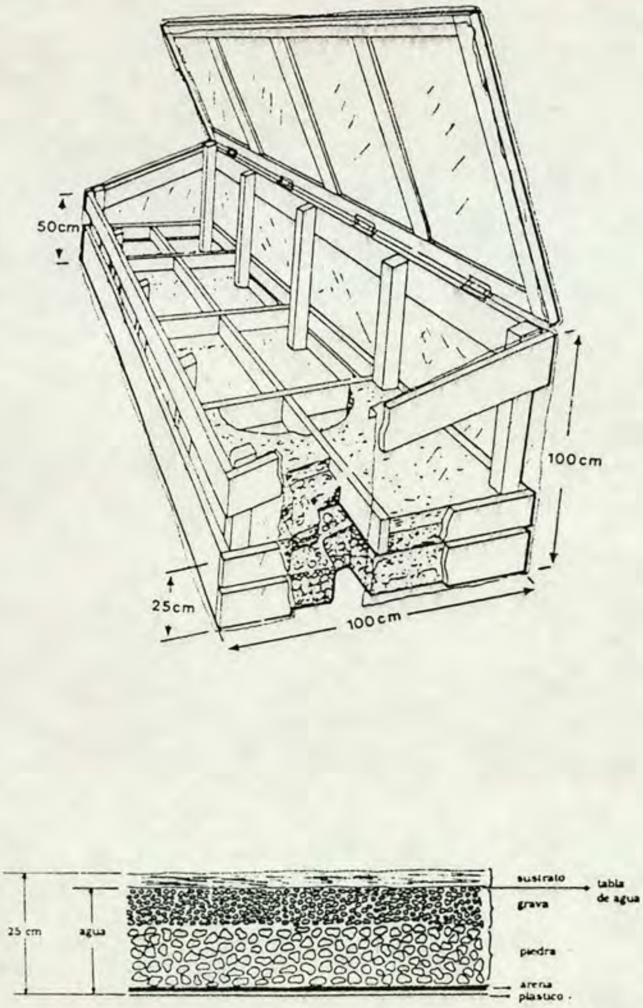

Fig. 1. El propagador de sub-irrigación

RESULTADOS

El ambiente de enraizamiento

La efectividad del propagador de sub-irrigación parece radicar en su capacidad de minimizar el estrés hídrico, protegiendo a las estacas de las fuertes variaciones ambientales externas, experimentadas bajo condiciones normales en los trópicos. La Figura 2 ilustra las variaciones típicas en humedad relativa (HR) para un período de cuatro días (25-28 de octubre, 1991) bajo las condiciones de Turrialba; mientras la humedad relativa externa fluctuó entre 75 y 100%, la humedad relativa dentro del propagador se mantuvo en el rango 85-100%.

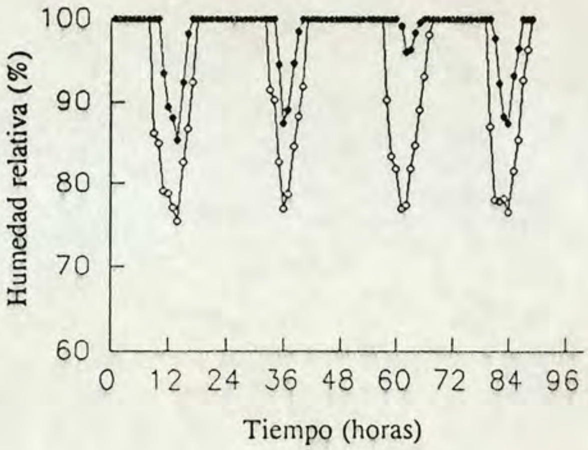

Fig. 2. Variaciones típicas en la humedad relativa externa (o) y dentro del propagador de sub-irrigación (●) para un período de cuatro días en Turrialba, Costa Rica.

Esta menor variación en la HR tiene influencia directa sobre las estacas, que logran mantener una condición de turgencia a lo largo del período de enraizamiento. El contenido relativo de agua (una medida del grado de turgencia de las hojas) (Beadle *et al.*, 1987), se evaluó, para estacas de *C. alliodora* a lo largo de seis semanas y varió típicamente entre 75 y 95% (Mesén, 1991), lo cual reafirma los efectos benéficos sobre las estacas, del ambiente de alta humedad relativa dentro del propagador. El mantenimiento de la turgencia es crítico durante las primeras semanas, cuando las estacas aún no han desarrollado raíces que puedan compensar grandes pérdidas de agua por transpiración.

Las variaciones en humedad relativa están asociadas a variaciones en irradiación (y su efecto sobre la temperatura); los aumentos en la irradiación van seguidos de disminuciones en la humedad relativa. El ámbito de irradiación que reciben las estacas puede ser controlado mediante el uso de sombra. En Turrialba, bajo condiciones de plena exposición, la irradiación puede alcanzar valores superiores a 2000 μmol m^{-2} s^{-1} en días soleados, mientras que mediante el uso de sombra (p.ej: una capa de sarán, hojas de palma, etc.) la irradiación se mantiene normalmente por debajo de los 600 μmol m^{-2} s^{-1} (Fig. 3). Por lo tanto, el uso de sombra durante la propagación es benéfico, al aumentar la humedad relativa dentro del propagador y reducir la tasa de transpiración de las estacas.

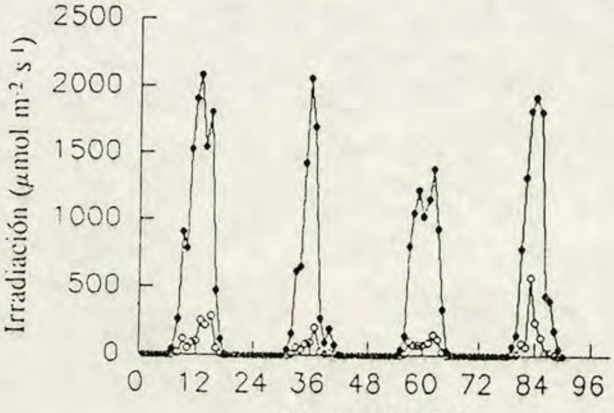

Fig. 3. Variaciones típicas en irradiación dentro de un propagador a plena exposición (●) y un propagador bajo sombra de sarán (o), en Turrialba, Costa Rica.

Sin embargo, la reducción en la irradiación no debe ser excesiva, por la reducción consecuente en la tasa fotosintética de las estacas y en la cantidad de metabolitos disponibles para la formación de raíces. El rango de irradiación óptima varía con las especies. En *C. alliodora*, por ejemplo, se han encontrado tasas altas de fotosíntesis (4-5 μmol CO_2 m^{-2} s^{-1}) dentro del propagador, a irradiaciones cercanas a los 300 μmol m^{-2} s^{-1} (Fig. 4), similares a las informadas para esta misma especie por Ramos y Grace (1990). Esto significa que el permitir irradiaciones mayores no va a aumentar la tasa fotosintética de las estacas, pero sí aumenta la temperatura dentro del propagador, se reduce la humedad relativa y en consecuencia, aumenta la pérdida de agua por transpiración.

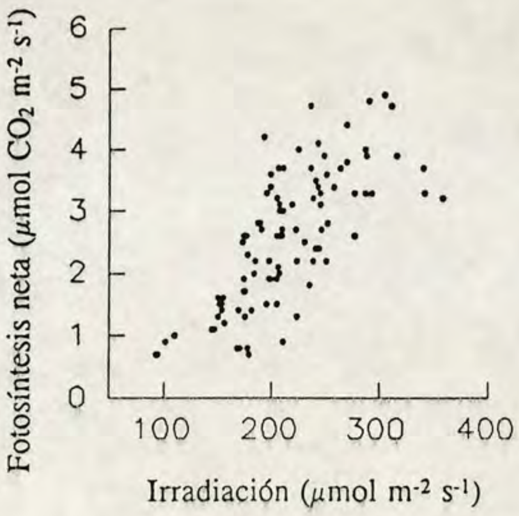

Fig. 4. Curvas fotosintéticas de respuesta a la luz para estacas no enraizadas de *C. alliodora* en propagadores de sub-irrigación, a los 21 días del establecimiento.

Bajo condiciones tropicales, el propagador de sub-irrigación también mantiene las temperaturas del aire y del sustrato dentro del rango normal para el enraizamiento de especies forestales (20-35 °C y 18-30 °C, respectivamente. Ver Fig. 5). La apertura de la tapa del propagador puede reducir la humedad relativa dentro del mismo, por lo cual es conveniente asperjar las estacas con agua cuando la tapa deba mantenerse abierta.

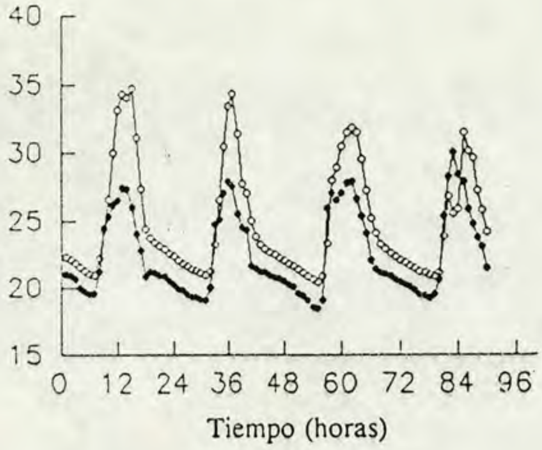

Fig. 5. Variaciones típicas en las temperaturas del aire (o) y del sustrato (●) en propagadores de sub-irrigación bajo sombra de sarán en Turrialba, Costa Rica.

Ensayos de enraizamiento

Sustratos de enraizamiento

Siguiendo la misma filosofía de simplicidad y economía en el sistema de propagación, la investigación en este aspecto se ha basado en el uso de sustratos de bajo costo y fácil adquisición, tales como arena, grava y aserrín. Si bien otros sustratos (vermiculita, perlita, turba, etc.) se mencionan normalmente en la literatura como medios efectivos para el enraizamiento de estacas (ver Hartmann y Kester, 1968), los costos de importación pueden resultar prohibitivos para la mayoría de los proyectos de desarrollo rural. La efectividad de un medio de enraizamiento, radica en su capacidad de retener agua, pero con aireación adecuada, proveer soporte adecuado a las estacas, sin impedimentos para el crecimiento radical y encontrarse libre de patógenos (o permitir una desinfección adecuada) (Hartmann y Kester, 1968), todo lo cual se logra con los sustratos utilizados en estos estudios.

En los ensayos del PMGF se han encontrado diferencias sustanciales entre especies en su capacidad de enraizamiento en diferentes sustratos. *G. arborea* ha enraizado mejor en arena que en grava o en las mezclas de estas con aserrín (Fig. 6a), mientras que arena fue el peor sustrato para *A. guachapele* (Fig. 6b). *C. alliodora* enraizó mejor en arena y en la mezcla arena-aserrín (1:1 p/v) (Fig. 6c), mientras que *V. hondurensis* enraizó bien en arena o en las mezclas con aserrín (Fig. 6d). La adición de aserrín a la arena y a la grava mejoró el enraizamiento de *E. deglupta* (Fig. 6e), y fue, el aserrín junto con la arena, el mejor sustrato para *B. quinata* (Fig. 6f). Con *S. macrophylla* se probaron varias combinaciones arena:grava y se obtuvo el mejor enraizamiento en sustratos con contenidos de arena del 50% o mayores (Fig. 7).

La razón de las "preferencias" de diferentes especies por diferentes sustratos no se conoce aún y probablemente estén relacionadas con la composición relativa (sólidos:agua:aire) de los sustratos, la cual presenta variaciones considerables (Fig. 8).

Concentración de AIB

Aunque el ácido indol-3-acético (AIA) es la auxina natural que se encuentra en las plantas, dos compuestos relacionados, el ácido indol-3-butírico (AIB) y el ácido α-naftalenacético (ANA) han sido utilizados con éxito en la promoción del enraizamiento de la mayoría de las especies forestales informadas en la literatura. De estos, el AIB es generalmente más efectivo, tiene las ventajas de ser más fotoestable que el AIA y, al ser insoluble en agua, se mantiene por más tiempo en el sitio de aplicación y mantiene su efectividad por períodos más largos (Hartmann y Kester, 1968). Además, las plantas poseen mecanismos que reducen y/o nulifican el AIA, ya sea conjugándolo con otros compuestos o destruyéndolo, no así con el AIB (Gaspar y Hofinger, 1988). Por su parte, el ANA con frecuencia presenta problemas de toxicidad.

El efecto de las auxinas en la promoción del enraizamiento ha sido reconocido por muchos años y documentado en cientos de artículos (ver por ejemplo, Hartmann y Kester, 1968; Blazich, 1988). Sin embargo, no se conoce con exactitud su papel en el proceso. Además de los efectos directos de las auxinas sobre la división celular,

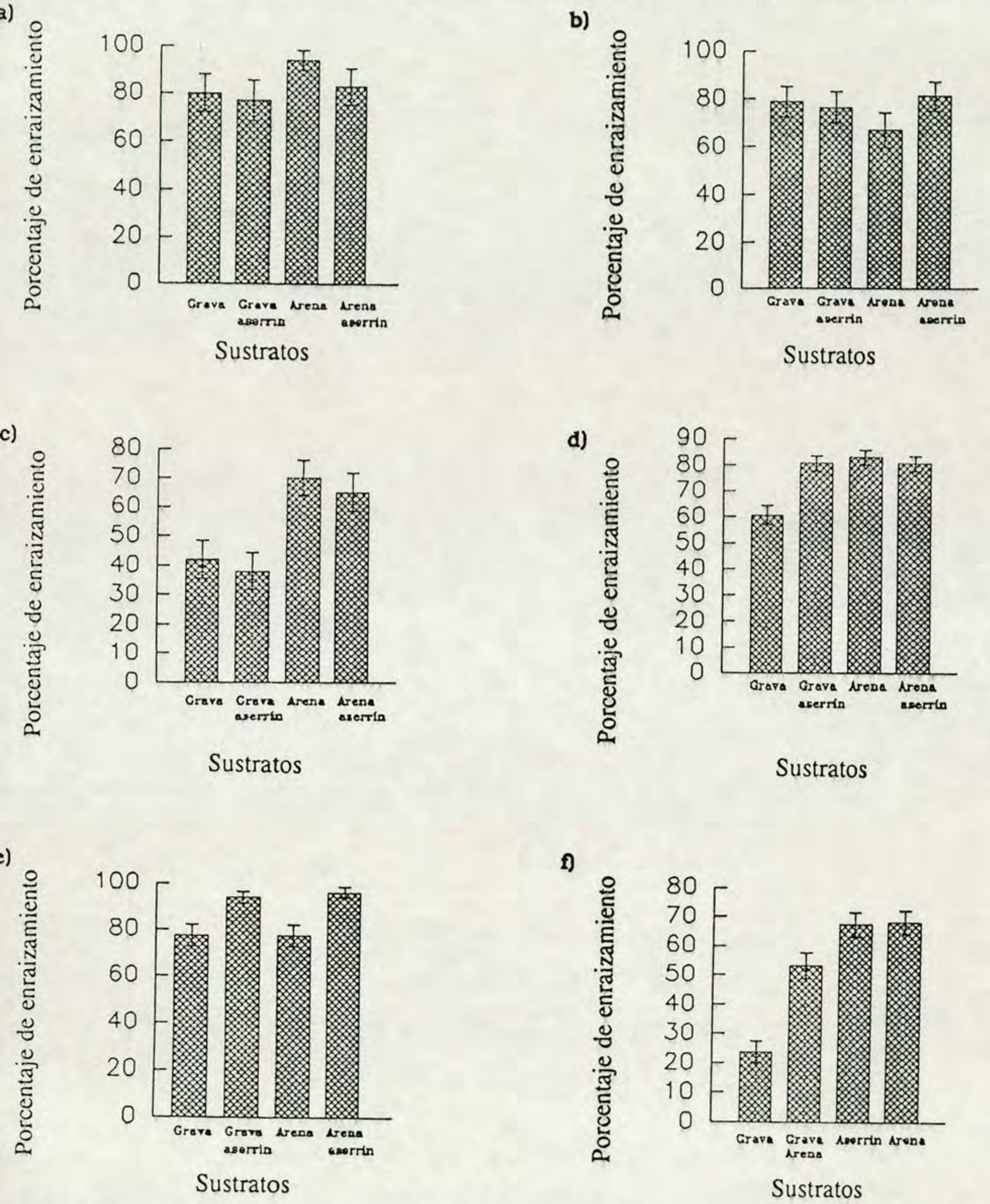

Fig. 6. Efecto del sustrato en propagadores de sub-irrigación sobre el enraizamiento de estacas de *a) G. arborea* a los 37 días; b) *A. guachapele* a los 24 días; c) *C. alliodora* a los 45 días; d) *V. hondurensis* a los 63 días; e) *E. deglupta* a los 21 días; f) *B. quinata* a los 63 días en propagadores de sub-irrigación. Barras = error estándar.

sus efectos benéficos han sido asociados con un aumento en la tasa de transporte de carbohidratos y cofactores foliares a la base de las estacas, donde promueven la iniciación y desarrollo de las raíces (Haissig, 1974); actualmente está bien establecido que los metabolitos y otros factores de crecimiento se translocan hacia regiones tratadas con auxina (Phillips, 1969; 1975). Bajo condiciones de baja actividad fotosintética, el transporte y/o la síntesis de auxinas pueden verse reducidos; por lo tanto, la fotosíntesis también puede afectar indirectamente el enraizamiento, al afectar el suministro de auxinas a la base de la estaca (Davis, 1988). Otro efecto de las auxinas, asociado con la formación de raíces, es su capacidad de estimular la síntesis de ADN en ciertas células (Gaspar y Hofinger, 1988). El efecto de las auxinas sobre la formación de raíces adventicias es probablemente el resultado de la interacción de estos y posiblemente otros procesos.

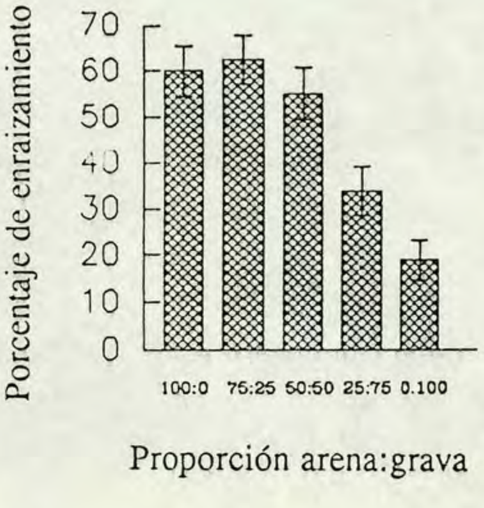

Fig. 7. Efecto de diferentes combinaciones de arena y grava en el sustrato sobre el enraizamiento de estacas de *S. macrophylla* a los 77 días en propagadores de sub-irrigación. Barras = error estándar.

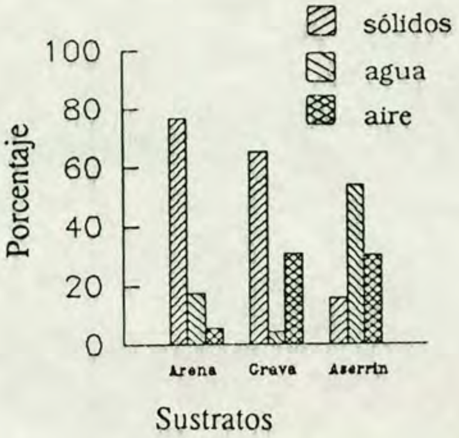

Fig. 8. Proporción relativa por volumen de sólidos, agua y aire de tres de los sustratos utilizados comúnmente en los estudios de enraizamiento.

Tomando en cuenta lo anterior, no es sorprendente encontrar grandes variaciones entre especies en cuanto a sus respuestas a diferentes concentraciones de AIB. *V. hondurensis* es un caso interesante por cuanto presentó el mayor porcentaje de enraizamiento sin aplicación de AIB (Fig. 9a); sin embargo, el sistema radical formado mejoró con concentraciones crecientes de AIB (Fig. 9b), por lo cual se sugiere utilizar la concentración de 0,2%. Por el contrario, tanto *A. guachapele* (Fig. 9c) como *C. alliodora* (Fig. 9d) mostraron un enraizamiento muy pobre en ausencia de AIB y enraizaron mejor con las concentraciones de 0,1 y 1,6% respectivamente. La concentración de 0,2% presentó los mejores resultados en *B. quinata* (Fig. 9e) y *S. macrophylla* (Fig. 9f). No se han realizado ensayos de concentración de AIB para *E. deglupta*; sin embargo, en el ensayo de sustratos (Fig. 6e) se utilizó una concentración estándar de 0,2%, lográndose un enraizamiento promedio de 90%.

Area foliar

En estacas suculentas es bien conocido el efecto benéfico de las hojas sobre el enraizamiento, el cual ha sido asociado con la actividad fotosintética y el suministro de metabolitos a los primordios radicales en

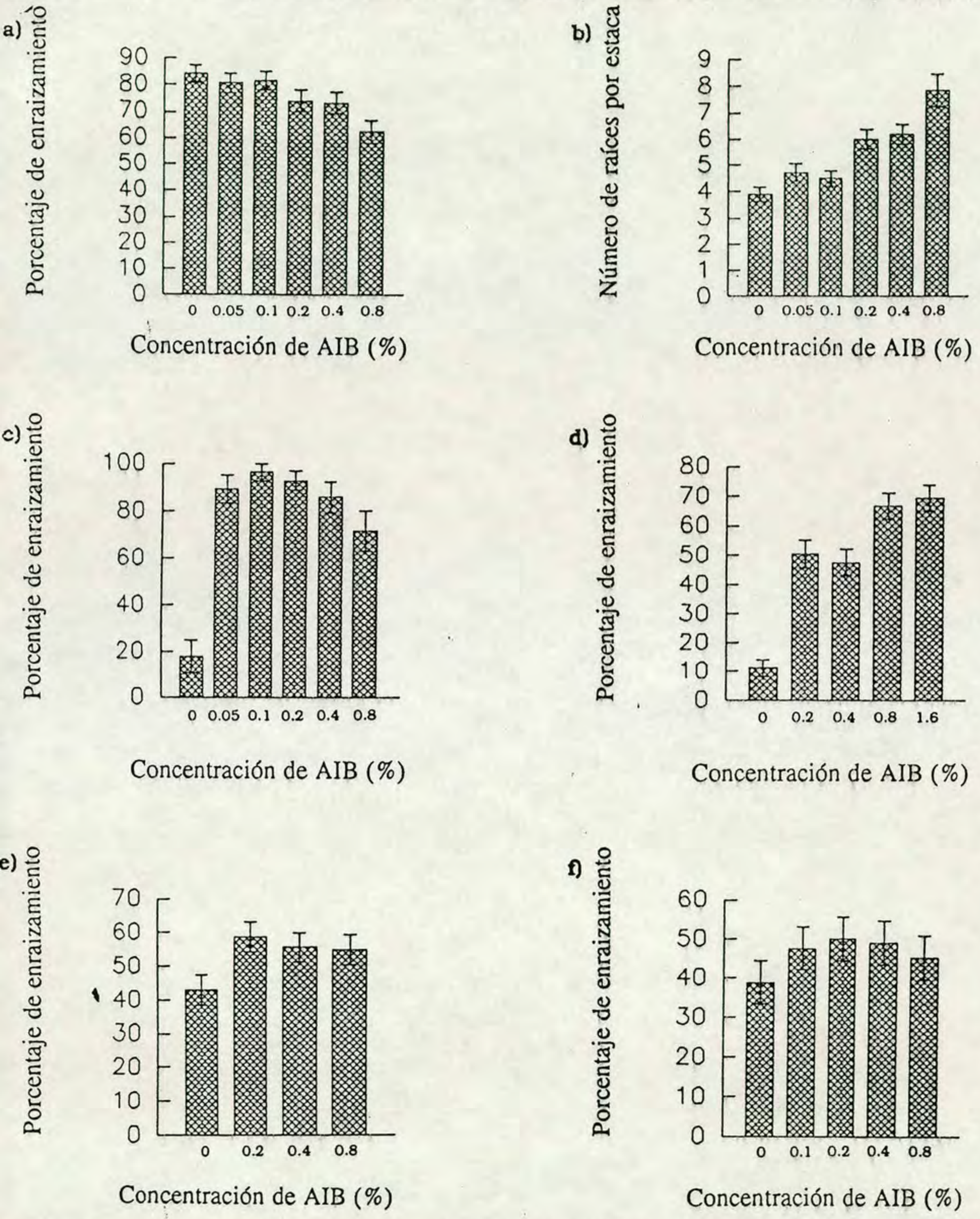

Fig. 9. Efecto de la concentración de AIB sobre a) el porcentaje de enraizamiento y b) el número promedio de raíces en estacas de *V. hondurensis* a los 63 días; el porcentaje de enraizamiento de estacas de c) *A. guachapele* a los 24 días; d) *C. alliodora* a los 40 días; e) *B. quinata* a los 63 días; f) *S. macrophylla* a los 77 días en propagadores de sub-irrigación. Barras = error estándar.

esarrollo (Hartmann y Kester, 1968). Otras ustancias producidas en las hojas, llamadas en érminos genéricos cofactores foliares, también xperimentan un transporte basípeto en las stacas y permiten o estimulan la iniciación y lesarrollo de los primordios radicales (Haissig, 974; Hartmann y Kester, 1968). El tipo y antidad de cofactores, en realidad, parece leterminar parcialmente que las estacas inicien a producción de raíces con facilidad, con lificultad o que no las produzcan del todo Haissig, 1974).

No todos los efectos de las hojas son enéficos, puesto que la pérdida de agua por ranspiración puede causar problemas de lesecación en las estacas (Leakey, 1985), lgunas veces a un grado tal que sobreviene la nuerte de las mismas (Hartmann y Kester, 1968). La pérdida de agua también causa una educción en la fotosíntesis debida al cierre de stomas (Loach, 1988). La práctica común de educir el área foliar de la estaca pretende ninimizar la pérdida de agua por transpiración, ero a la vez permitir la fotosíntesis durante el periodo de enraizamiento. En estacas atalogadas como "difíciles de enraizar", la btención de este balance parece ser crítico y en realidad puede determinar el éxito o el fracaso de a propagación (Leakey, 1985).

Para la mayoría de las especies incluidas en este trabajo se ha utilizado un área foliar de 30 cm^2 con buenos resultados. Con *V. hondurensis*, sin embargo, se obtuvo el mejor porcentaje de enraizamiento con un área foliar de 60 cm^2 (Fig. 10). *C. alliodora*, por su parte, presentó una interacción interesante entre área foliar e irradiación, cuando se probaron dos rangos de irradiación máxima (100 y 1300 µmol m^{-2} s^{-1}) y tres áreas foliares (10, 20 y 30 cm^2). En el tratamiento de mayor irradiación, el área foliar de 10 cm^2 presentó el mayor porcentaje de enraizamiento (75%), mientras que a baja irradiación, las estacas con la misma área foliar presentaron el menor enraizamiento. No hubo diferencias entre tratamientos de irradiación para las demás áreas foliares (Fig. 11). Esto parece apoyar la necesidad de lograr un balance óptimo entre fotosíntesis y transpiración en las estacas. Con irradiaciones bajas es de esperar que no haya problemas de pérdida de agua por transpiración, pero el área de 10 cm^2 tal vez fue insuficiente para una fotosíntesis adecuada.

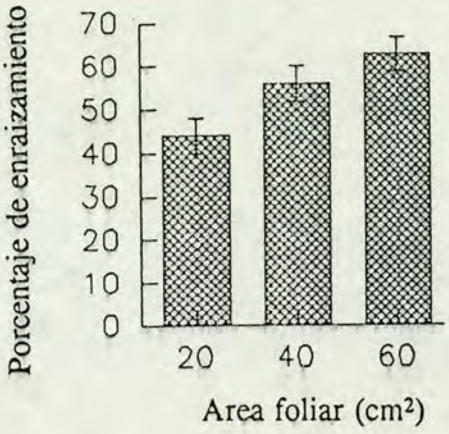

Fig. 10. Efecto de tres áreas foliares sobre el enraizamiento de estacas de *V. hondurensis* después de 81 días en propagadores de sub-irrigación. Barras = error estándar.

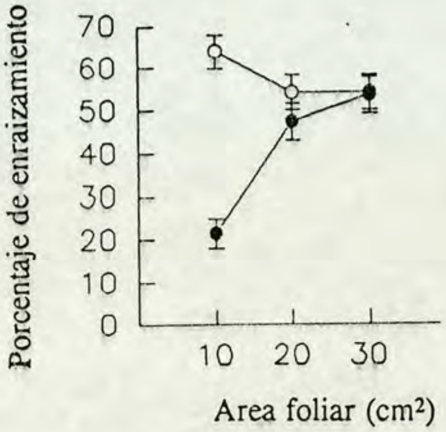

Fig. 11. Efectos del área foliar sobre el enraizamiento de estacas de *C. alliodora* en propagadores bajo irradiaciones máximas de 2100 µmol m^{-2} s^{-1} (o) y 600 µmol m^{-2} s^{-1} (●) después de 42 días en propagadores de sub-irrigación. Barras = t (DMS) $_{0.05}$.

Por otro lado, en el propagador a plena exposición, es probable que la reducción en el porcentaje de enraizamiento en las estacas con áreas foliares mayores, estuviera asociado con una mayor pérdida de agua por las hojas.

ISCUSION

La propagación vegetativa de clones eleccionados permite obtener las mayores anancias genéticas en productividad en el nenor tiempo posible (Libby y Rauter, 1984). Es sí mismo, una herramienta valiosa para la onservación, multiplicación y aprovechamiento e germoplasma en peligro de extinción y ermite eliminar la dependencia del uso de emillas, cuyo abastecimiento es una limitante eria en muchas latifoliadas tropicales. Los écnicos forestales conocen muy bien el roblema de producción de semilla de buena alidad de *B. quinata*, por ejemplo, o la nposibilidad de almacenar semilla de *Vochysia* pp., especies que pueden ser propagadas sin ificultad mediante el uso de estacas enraizadas. i bien existe una vasta literatura sobre la ropagación de especies como *E. grandis*, *riplochiton scleroxylon* y otras, los trabajos del MGF y el ITE están generando los primeros nformes sobre la propagación exitosa de la nayoría de las especies nativas de América entral incluidas en este artículo. Así mismo, stos trabajos demuestran la factibilidad de nplementar la silvicultura clonal a nivel del equeño productor. El sistema de propagación e sub-irrigación descrito aquí puede roporcionar una solución práctica para la ropagación de una gran variedad de especies restales, aún en condiciones rústicas y de falta e capital, como las que enfrentan la mayoría de s programas pequeños de desarrollo rural.

Existe una serie de riesgos que se asocian ormalmente a la silvicultura clonal, como los oblemas asociados con la producción de stemas radicales pobres y la creación de andes áreas genéticamente homogéneas isceptibles a epidemias. El primer problema se iede eliminar completamente mediante el anejo adecuado del material por enraizar y la lección cuidadosa del material por plantar, mo ha sido demostrado en los programas más anzados de silvicultura clonal mencionados al icio del documento. El segundo problema, por parte, no es exclusivo de la silvicultura clonal incluso, al existir conciencia de los riesgos, es ás fácil evitarlos mediante el control cuidadoso l "pedigrí" y el uso de sistemas silviculturales ropiados. Hay que recordar que el uso de millas *per se* no garantiza heterogeneidad genética; es común observar grandes plantaciones originadas de semilla de uno o pocos árboles madres, donde todos los individuos están genéticamente relacionados, o el uso continuo por años, de semilla originada de una pequeña área semillera. Sin embargo, nadie parece preocuparse demasiado por este hecho, debido a la falsa seguridad que brinda el uso de semilla, sin importar su origen ni su grado de consanguinidad. Es claro que un manejo irresponsable de la silvicultura clonal puede llevar a problemas serios de homogeneidad genética, en su caso más extremo, mediante el uso exclusivo en grandes áreas de un "super clon". Es por esto que es vital adoptar estrategias adecuadas que minimicen los riesgos. La discusión de este tipo de estrategias merece un artículo separado; basta indicar que el PMGF está trabajando en el desarrollo de estrategias apropiadas y ya se han producido algunos lineamientos básicos (Leakey y Mesén, 1991b).

Mediante el uso de los propagadores de sub-irrigación, se pueden esperar porcentajes de enraizamientos entre 70 y 100% para las especies descritas, utilizando el sustrato y la concentración de AIB indicada en cada caso. El preparado comercial "Seradix", basado en el AIB, es igualmente efectivo para la mayoría de las especies y puede utilizarse cuando no sea posible adquirir el AIB en forma pura. Algunos aspectos adicionales que deben ser considerados son los siguientes: i) las estacas deben estar sanas y vigorosas, provenientes de tallos ortotrópicos juveniles de plántulas, rebrotes de tocones o setos manejados; la técnica puede no ser exitosa si se utiliza material fisiológicamente adulto; ii) en el caso de los rebrotes de tocones o setos manejados, es recomendable realizar cosechas periódicas antes de que los rebrotes crezcan demasiado, para evitar la lignificación excesiva en las estacas; una altura del rebrote de 50 cm se considera apropiada para la mayoría de las especies, con lo cual se obtienen 6-10 estacas por rebrote; iii) es importante proteger los propagadores de la luz directa del sol, mediante el uso de sarán, hojas de palma, etc.; iv) el proceso de propagación, desde la colecta de las estacas hasta su inserción en los propagadores debe realizarse en el menor tiempo posible, y en todo momento las estacas deben mantenerse húmedas y frescas para evitar su desecación; v) cuando las raíces alcancen 1 cm de longitud se puede iniciar el transplante de las estacas, evitando que las raíces se encurven hacia arriba; vi) hay que recordar que las nuevas plantas vienen de un ambiente de alta humedad, y un cambio demasiado brusco puede provocar su desecación y muerte. Es necesario trasladar las plantas a un ambiente húmedo y fresco durante las primeras tres a cuatro semanas para permitir su endurecimiento. A partir de este momento, el material puede manejarse mediante los métodos tradicionales de vivero.

El método de propagación descrito aquí ha sido probado con éxito en más de 100 especies de diferentes ecosistemas y que tienen una gama amplia de usos (Leakey *et al.*, 1982a). Si bien cada especie tiene sus requerimientos particulares en cuanto a concentración de auxina, sustratos, tipo y tamaño de estaca, etc., el sistema básico descrito proporciona un buen punto de partida para enfrentar la propagación de especies nuevas.

Los estudios fisiológicos con algunas de las especies proporcionan cada vez más información sobre el proceso de enraizamiento, y sobre los factores de mayor importancia práctica. El conocimiento científico de estos factores proporciona la base para diseñar sistemas de manejo simples y de fácil aplicación. Este conocimiento es de gran importancia ante el aumento creciente en el uso de clones superiores para la reforestación operacional.

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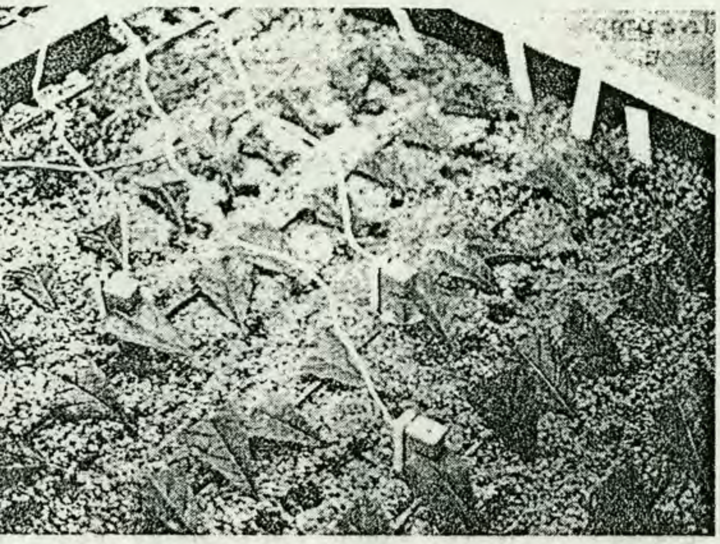
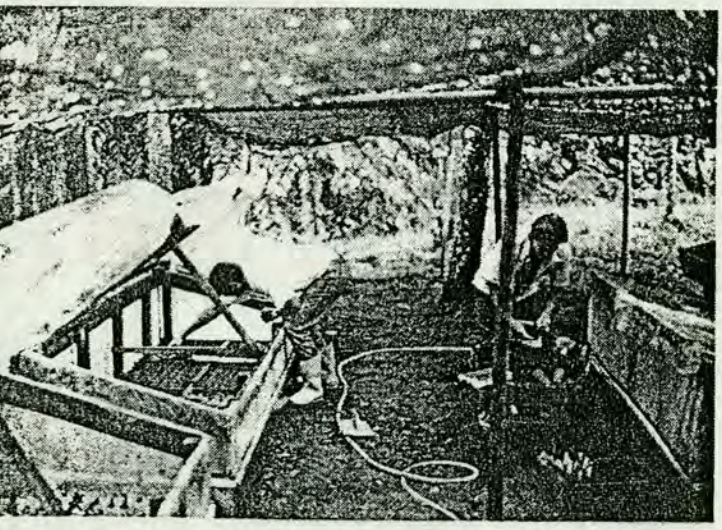


Las estacas de *C. alliodora* son evaluadas durante el proceso de propagación para determinar el potencial hídrico de las estacas y su relación con la capacidad final de enraizamiento.

Numerosas especies, como *C. odorata* son fácilmente enraizadas mediante estacas juveniles en los propagadores de sub-irrigación.



Los propagadores de sub-irrigación pueden ser establecidos en condiciones rústicas, lo que permite su transferencia inmediata a finqueros o programas pequeños de desarrollo rural.

EL CHASQUI No.28:19-24

El PROYECTO MEJORAMIENTO GENETICO FORESTAL DEL CATIE Y SU PAPEL EN LA REGION CENTROAMERICANA

Eugenio Corea [1]
Francisco Mesén [2]
Jonathan Cornelius [3]

INTRODUCCION

A pesar de los esfuerzos realizados en los últimos años, la deforestación continúa siendo un problema grave en América Central. Esta conlleva, además de los conocidos e importantes efectos negativos sociales y ambientales, una seria amenaza sobre la actividad económica del sector forestal. Ante el eventual agotamiento de los bosques productores de la región, la industria maderera entraría en un período de crisis que, entre otros efectos, generaría una fuerte presión adicional sobre las áreas naturales legalmente protegidas. Por otra parte, la alternativa de importar productos forestales aumentaría el grado de dependencia y la salida de divisas, con el consiguiente perjuicio para la economía de los países.

Ante esta situación, los gobiernos de la región y varios organismos de ayuda internacional han puesto un gran aporte técnico y económico para promocionar y desarrollar el cultivo de especies forestales. Como resultado, la tasa de reforestación ha aumentado en los últimos años y la tendencia parece mantenerse. Sin embargo, gran parte de las plantaciones no han mostrado los resultados esperados, con las consiguientes pérdidas ecológicas y económicas y el efecto colateral de desestímulo a la reforestación. La experiencia en América Central y en muchos países tropicales ha demostrado que una de las principales razones para el fracaso o la baja productividad de plantaciones forestales ha sido el uso de germoplasma inadecuado. Es indispensable incorporar definitivamente el componente genético como un elemento esencial del cultivo de árboles, si se quiere lograr un desarrollo acelerado y sostenible del sector forestal de la región.

El potencial del mejoramiento genético forestal es enorme. Esta situación ha sido comprendida y aprovechada por varios países tropicales que cuentan con programas muy exitosos. Por ejemplo, en el Congo se logró triplicar la producción del volumen de madera con clones altamente seleccionados de híbridos de varias especies de *Eucalyptus*. En Brasil y en Colombia se han obtenido resultados similares y los árboles son cosechados actualmente a los seis años de edad en varias compañías.

En América Central la actividad en mejoramiento genético forestal ha venido creciendo en los últimos años, aunque no con la velocidad y continuidad requerida. Muchas de las acciones han sido puntuales y de efecto limitado. Es necesario y urgente desarrollar e implementar una estrategia regional integral para la conservación, desarrollo y uso de los recursos genéticos forestales.

EL PROYECTO

El Proyecto Mejoramiento Genético Forestal -PMGF es ejecutado por el Centro Agronómico Tropical de Investigación y Enseñanza-CATIE y financiado por el Ministerio Noruego de Cooperación para el Desarrollo-MDC, la Administración Británica para el Desarrollo en Ultramar-ODA y la Agencia para el Desarrollo Internacional-AID de los Estados Unidos. Los principales objetivos del proyecto son:

1) La identificación y desarrollo de germoplasma genéticamente superior.
2) El desarrollo de técnicas de propagación vegetativa de bajo costo, apropiadas para programas rurales.
3) La conservación genética de especies o poblaciones amenazadas.
4) La capacitación en el área de mejoramiento genético forestal.
5) La difusión de tecnología e información.

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El PMGF ha sido un proyecto pionero en la gión desde que inició sus actividades en 1977. urante los primeros años, el trabajo estuvo rigido principalmente hacia la prueba y elección de especies y procedencias de pinos opicales. Desde entonces el PMGF ha npliado sustancialmente su ámbito de acción, atando de satisfacer las múltiples necesidades la región centroamericana. El crecimiento a ocurrido principalmente en los siguientes spectos:

Una mayor diversidad de zonas ecológicas en donde se trabaja.

Un aumento significativo en el número de especies.

Un mayor número de áreas de investigación básica y aplicada en conservación, mejoramiento y genética forestal.

Capacitación y enseñanza.

Divulgación de la información.

Zonas ecológicas

Hasta la fecha el PMGF ha establecido 90 idades experimentales de campo, que arcan una gran variedad de condiciones máticas y edáficas. Los ensayos han sido antados en suelos que van desde mpactados, muy ácidos, fuertemente iviados y erosionados hasta suelos fértiles ropiados para sistemas forestales y roforestales altamente productivos. Las nas ecológicas abarcan desde el bosque seco pical hasta el bosque pluvial tropical y el sque nublado montano.

Las especies

En los últimos años, a través de varios oyectos nacionales y regionales, se ha ntificado un gran número de especies con tencial. Sin embargo, muchas se encuentran davia en la fase de validación o no han mplido aún su primer ciclo de rotación. La periencia acumulada en la región no permite, muchos casos, definir con certeza cuál pecie es la más apropiada. Este hecho, nado a la alta diversidad ecológica, los ltiples objetivos de producción y las posibles fluctuaciones del mercado, han determinado que el PMGF trabaje con un gran número de especies, tratando de cubrir el mayor ámbito de situaciones posibles. La idea es mantener un abanico amplio de opciones abiertas, en vez de concentrar los esfuerzos en unas pocas especies. De esta forma, se beneficia, además, a un mayor número de usuarios y no solamente a algunas compañías reforestadoras. Actualmente se trabaja con 18 especies de las cuales 11 son nativas de América Central (Cuadro 1).

3. Areas de investigación

En la actualidad se trabaja en seis áreas prioritarias de investigación:

a) Exploración, selección y conservación de germoplasma.

b) Selección de procedencias.

c) Selección de descendencias.

d) Selección por resistencia contra el barrenador de las meliáceas.

e) Desarrollo de técnicas de propagación vegetativa de bajo costo.

f) Desarrollo de técnicas de selección temprana.

a) Exploración, selección y conservación de germoplasma.

Consciente de la pérdida acelerada de los recursos genéticos forestales de la región, el PMGF ha desarrollado una labor pionera de exploración, rescate y conservación *ex-situ* de poblaciones e individuos únicos y amenazados. En este sentido, el Proyecto ha trabajado en estrecha colaboración con la Cooperativa de Recursos Genéticos Forestales de Centroamérica y México-CAMCORE, el Instituto Forestal de Oxford-OFI y el Instituto de Ecología Terrestre de Escocia-ITE, en un esfuerzo regional inter-institucional por conservar, evaluar y utilizar dicho germoplasma.

Cuadro 1. Especies y áreas de investigación del Proyecto Mejoramiento Genético Forestal.

Especies	Exploración y selección	Conservación	Selección procedencias	Selección descendencias	Huertos semilleros	Propagación vegetativa
Acacia mangium		x	x			x
Albizia guachapele	x	x	x	x	x	x
Alnus acuminata	x	x	x	x	x	x
Araucaria hunsteinii		x	x			x
Bombacopsis quinata	x	x	x	x	x	x
Cedrela odorata	x	x	x	x		x
Cordia alliodora	x	x	x	x	x	x
Cupressus lusitanica	x	x		x	x	
Eucalyptus deglupta	x	x		x	x	x
Eucalyptus grandis		x	x	x	x	
Eucalyptus saligna			x			
Eucalyptus urophylla		x	x			
Gmelina arborea	x	x	x	x	x	x
Pinus caribaea		x	x	x		
Pinus oocarpa		x	x	x		
Pinus tecunumanii	x	x	x	x	x	
Swietenia macrophylla	x	x	x	x		x
Vochysia hondurensis	x	x	x	x	x	x

Hasta la fecha el PMGF ha seleccionado más de 400 árboles superiores (plus) de 32 procedencias, de nueve especies nativas en Costa Rica, Honduras y Trinidad-Tobago (Cuadro 1). Este germoplasma, junto con las selecciones y recolecciones realizadas por CAMCORE, OFI y el ITE en otras áreas de América Central y del Sur, está siendo conservado y evaluado por el Proyecto en forma de semilla, bancos de conservación, ensayos genéticos, huertos semilleros, plantaciones de validación y plantaciones semilleras. Todas estas opciones funcionan en mayor o menor grado como unidades de conservación *ex-situ*.

Las actividades de conservación *ex-situ* del PMGF abarcan también especies exóticas de mucha importancia para la región, tales como *A. mangium*, *G. arborea* y varias especies de *Eucalyptus*.

Por otra parte, durante las actividades de exploración, selección y recolección, el PMGF ha efectuado una importante labor de fomento de la conservación *in-situ* de rodales o árboles valiosos o con características únicas, a través de la concientización de comunidades y principalmente de los propietarios de dicho material. En muchos casos los propietarios se muestran muy interesados y orgullosos de contribuir a la preservación de los recursos genéticos forestales. En otros casos el poder del dinero de las industrias madereras ha sido más fuerte.

b) Selección de procedencias

Generalmente, las poblaciones de una especie presentan diferencias genéticas que afectan caracteres de importancia económica. Las diferencias pueden ser desde pequeñas hasta dramáticas. Por ejemplo, en Costa Rica el PMGF ha encontrado diferencias de hasta 400% en producción de madera entre procedencias de especies de pinos.

Hasta la fecha, el PMGF ha establecido más de 50 ensayos de procedencias de 16 especies (Cuadro 1) lo que ha permitido la selección de procedencias superiores para la reforestación y para la continuación del proceso de mejoramiento. Con las procedencias seleccionadas se han establecido plantaciones piloto con el fin de evaluar su comportamiento bajo condiciones normales de manejo, para la producción de material en futuras selecciones y su eventual conversión en rodales semilleros.

Selección de descendencias

Normalmente, una vez identificadas las mejores procedencias se seleccionan árboles plus dentro de las mismas. El valor genético de estos árboles se evalúa a través de ensayos de descendencias. Estos ensayos tienen la ventaja adicional de que pueden ser convertidos en huertos para la producción de semilla mejorada al final del período de evaluación.

Actualmente el PMGF trabaja en la evaluación de descendencias de 14 especies y ha establecido hasta hoy 30 ensayos de campo. Esta actividad aumentará fuertemente en los próximos años.

Selección por resistencia contra el barrenador de las meliáceas

Con seguridad, la razón principal para que en América Tropical no se planten intensivamente las especies de cedros y caobas nativas en lugar de otras especies menos valiosas, es el ataque del barrenador *Hypsipyla grandella*. Se han realizado muchos esfuerzos para controlar esta plaga sin resultados económicamente aplicables. Sin embargo, una vez más, el componente genético no ha sido considerado.

El PMGF ha retomado el problema y ha iniciado la búsqueda de genotipos resistentes o con una alta capacidad de recuperación al ataque, principalmente en *C. odorata* y *S. macrophylla*. Para ello se han establecido ensayos de procedencias-descendencias con estas especies y los resultados después de un año de evaluación son realmente promisorios. Si estos resultados se mantienen, el Proyecto pretende y está en capacidad de desarrollar poblaciones y principalmente clones resistentes y altamente productivos a un plazo relativamente corto. Indudablemente, el efecto que esto produciría en la actividad forestal de la región sería muy significativo.

e) Desarrollo de técnicas de propagación vegetativa de bajo costo

El desarrollo de la silvicultura clonal ha sido sin duda el logro más importante de las grandes empresas forestales durante los últimos quince años. Sin embargo, la tecnología generada por estas compañías no ha estado disponible ni es apropiada para programas de desarrollo rural.

El Proyecto cree firmemente que el pequeño y mediano agricultor no sólo merece que se le brinde el mejor material genético sino que también se le proporcionen las técnicas necesarias para su utilización óptima. Por esta razón, en cooperación con el Instituto de Ecología Terrestre de Escocia, se ha desarrollado un sistema simple y barato de propagación vegetativa que puede ser fácilmente utilizado por los agricultores. El sistema consiste en propagadores sin aspersión, de fácil construcción, hechos de madera y plástico, que no necesitan electricidad, agua de cañería o sistemas de riego, por lo que pueden ser construidos e instalados a muy bajo costo en cualquier zona rural. Hasta el momento el Proyecto ha probado este sistema con 11 especies (Cuadro 1) logrando porcentajes de enraizamiento generalmente superiores al 80%. Esta técnica es también sumamente útil para la conservación de germoplasma valioso de especies tropicales.

f) Desarrollo de técnicas de selección temprana

El período de evaluación de germoplasma forestal en experimentos de campo es generalmente largo en comparación con la mayoría de los cultivos agrícolas. Como alternativa, el PMGF está iniciando el desarrollo de técnicas de selección temprana fundamentadas en la existencia de posibles correlaciones entre las características morfológicas y fisiológicas de la planta en vivero y su posterior comportamiento en el campo. La aplicación de técnicas de selección temprana no elimina la fase de evaluación de germoplasma en experimentos de campo, pero sí reduce el número de genotipos a evaluar a un grupo selecto con una mayor probabilidad de tener un comportamiento superior en campo. Para aquellas características que muestren una alta correlación, las ganancias genéticas que se obtengan a través de la selección temprana serán muy importantes.

La selección temprana sería muy útil también en los viveros tradicionales de la región y tendría un impacto importante en las plantaciones operacionales.

4. Capacitación y enseñanza.

Indudablemente la formación y capacitación de profesionales, técnicos y reforestadores en general es fundamental para el desarrollo forestal de la región. Sin embargo, las instituciones de educación generalmente no han incorporado en sus programas el componente genético como un elemento esencial de la silvicultura y del manejo forestal. Las consecuencias de este vacío de formación han sido evidentes en muchos proyectos de reforestación.

Ante esta situación, el PMGF ha desarrollado un programa de capacitación y enseñanza dirigido especialmente a profesionales y técnicos que laboran en instituciones de educación superior, proyectos de mejoramiento y reforestación y bancos de semillas. El programa incluye un curso corto anual sobre técnicas de mejoramiento, entrenamiento en servicio y la formación de profesionales a nivel de pregrado y posgrado mediante el curso del programa de maestría del CATIE sobre "Genética y Mejoramiento Forestal", la conducción de tesis y trabajos especiales. También se ha preparado un manual sobre "Mejoramiento Genético Forestal con Referencia Especial a América Central" el cual sirve de texto para los cursos de posgrado y capacitación. Este manual será publicado próximamente como libro.

5. Divulgación de información

El PMGF tiene entre sus objetivos poner la información generada al alcance del mayor número posible de usuarios y fomentar el flujo de información entre las instituciones involucradas en el desarrollo forestal de la región. La divulgación de la información se realiza a través de publicaciones en la prensa, series técnicas, charlas, artículos en revistas y boletines, etc.

Por otra parte, también se publica y distribuye gratuitamente el noticiero "Mejoramiento Genético y Semillas Forestales para América Central" que ha funcionado como un canal efectivo para la difusión de la información generada sobre el tema en los países de la región. Este noticiero ha tenido gran aceptación, incluso más allá de América Central. Actualmente se distribuye à 1000 usuarios en más de 25 países en todo el mundo.

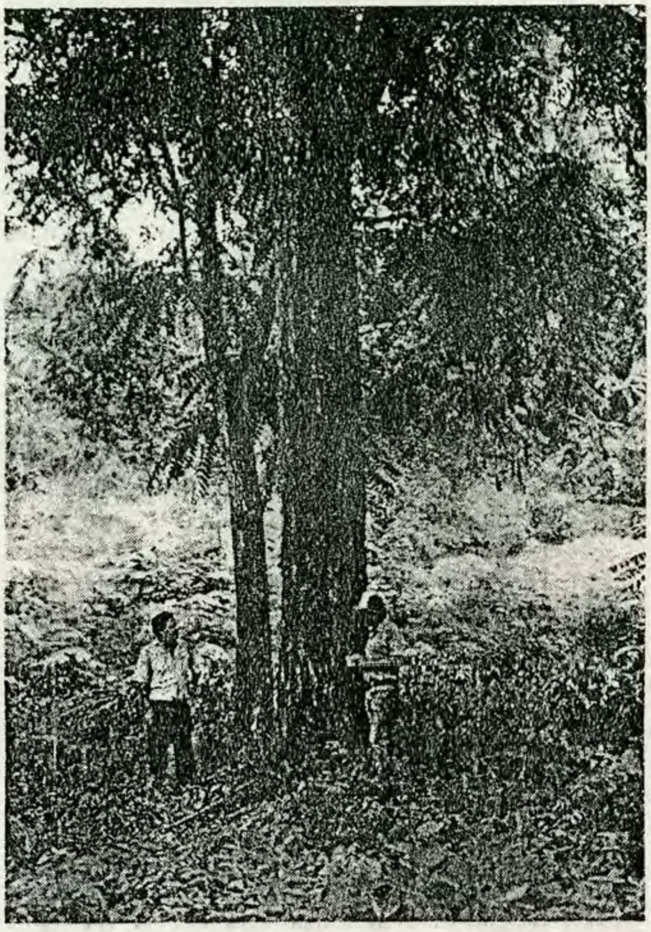
EL FUTURO

Hasta ahora, principalmente por razones de presupuesto, la mayor parte de las actividades de investigación de campo del PMGF han estado concentradas en Costa Rica. Sin embargo, el Proyecto esta diseñando una estrategia de expansión al resto de los países de la región de tal forma que se aproveche la experiencia obtenida durante los 15 años de actividad en Costa Rica.

En el área de producción de semilla jorada el PMGF contará pronto con una plia red de huertos semilleros y plantaciones nilleras de procedencias superiores para la yoria de las especies prioritarias. El PMGF isidera que en el futuro estos huertos y ntaciones deberían ser manejados por los icos nacionales de semillas forestales para iar las necesidades de los paises.

Por otra parte, la primera fase para el arrollo de una silvicultura clonal adecuada a condiciones de los agricultores de América ntral ya ha sido concluida. El Proyecto enta ahora con técnicas simples y baratas a la propagación exitosa y masiva de todas s especies prioritarias. El siguiente paso será tranferencia de dicha tecnologia y la ntificación y desarrollo de clones superiores. este sentido se está diseñando una estrategia ė contemple la participación de instituciones cionales y organizaciones rurales en el arrollo de una silvicultura clonal para el queño y mediano agricultor. Esto incluye en a primera fase la participación activa del TIE en el diseño de estrategias de joramiento, capacitación y supervisión técnica programas pilotos de aplicación de la ricultura clonal con grupos organizados de ricultores. Estos programas piloto brindarían bases para el desarrollo y aplicación de una rategia general.

El PMGF continuará ampliando y aleciendo sus actividades de capacitación y señanza y el área de difusión de la ormación. En este sentido se estimulará una yor participación de los técnicos y decisores los programas de capacitación y enseñanza como en la publicación de sus actividades e estigaciones en manejo y utilización de los ursos genéticos forestales. ■



La selección de árboles superiores, como el *Albizia guachapele* que se muestra en la foto, y la recolección de sus semillas son algunas de las actividades principales del Proyecto MGF dirigidas a la conservación, mejoramiento y uso de especies valiosas nativas de América Central.