VEGETATIVE PROPAGATION OF *SHOREA LEPROSULA* MIQ. BY STEM CUTTINGS

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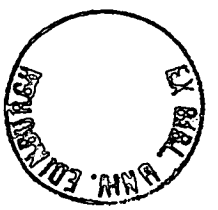

DECLARATION

I declare that this thesis is my own composition. The work presented in it is entirely my own, and has not been presented in any other thesis.

AMINAH Hamzah
(February 1995)

ABSTRACT

The thesis reports new studies on the factors affecting the rooting of single node leafy stem cuttings of *Shorea leprosula* Miq., a Dipterocarp timber tree native to South East Asia. Several aspects of vegetative propagation were investigated including treatments of the stock plants from which the cuttings were taken, propagation systems and post-severance treatments to cuttings.

Stock plants raised in 1 litre pots of forest top soil and sand (3:1), and fertilised every two weeks with 0.5 g per plant of NPK fertiliser (12%N:12%P_2O_5:17%K_2O:2MgO + Trace elements) were suitable for production of cuttings. Cuttings from stock plants raised under low irradiance of diurnal range 0 to 325 µmol photons m^{-2} s^{-1} (nominally 10% full sunlight) produced higher rooting and more roots than those from a high irradiance of 0 to 722 µmol photons m^{-2} s^{-1} (nominally 30% full sunlight).

S. leprosula stem cuttings rooted equally well in mist and non- mist propagation systems as long as a consistently low vapour pressure deficit (VPD) was maintained. A temporary increase in the VPD of more than 0.5 kPa at peak irradiance could be tolerated by *S. leprosula* cuttings. Cuttings also rooted equally well in media with either low or high water retaining capacity such as river sand, coconut fibre or a mixture of these two media. A diurnal irradiance of 0 to 360 µmol photons m^{-2} s^{-1} was adequate for rooting but 0 to 98 µmol photons m^{-2} s^{-1} resulted in low rates of net photosynthesis (P_n) and a much reduced rooting success. In the enclosed mist propagation system, misting every 1 hour with a 1 minute duration of spray, throughout the day and night, provided sufficient moisture to cuttings and maintained mean relative humidity of more than 90%. Cuttings planted in the same system with a 3 hour misting frequency tended to develop water deficit as indicated by low relative water content and stomatal conductance.

Application of 20 µg IBA to the base of each cutting accelerated the rate of rooting,

with 50% rooting at week 5 with IBA treatments compared to week 7 with the untreated cuttings. The number of roots was also increased with IBA treatments.

The effect of reducing leaf area by trimming was tested. A leaf area of 15 to 30 cm^2 retained on each cutting yielded good rooting in *S. leprosula* cuttings. A 60 cm^2 leaf area was more liable to water deficit. In general, a negative relationship between rooting and volume of cuttings was obtained, which may indicate that larger volume/diameter was not suitable for rooting, presumably due to lignification. This aspect is also discussed in relation to stored carbohydrate reserves. Thin cuttings between 0.2 to 0.4 cm rooted best. Cutting lengths of 3 cm or more are recommended for easier handling. Cuttings were found to photosynthesise prior to rooting with mean P_n values of 0.34 to 2.3 µmol CO_2 m^{-2} s^{-1} depending on the treatments used. On the whole, P_n of rooted cuttings was higher than that of cuttings which remained unrooted.

In conclusion, successful rooting of this important timber tree from stem cuttings may be obtained by using suitable cutting materials (through good stock plant management), followed by optimising the post-severance rooting conditions.

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CHAPTER 1

GENERAL INTRODUCTION

Forestry in Malaysia

Malaysia is a tropical country located north of the Equator within latitudes 1° to 7° North and longitudes 100° to 119° East (Figure 1). The total land area of Malaysia is 32.86 million hectares. As at 1990, 18.67 million ha (56.8% of the total land area) were forested. Of these, 5.51 million ha are in Peninsular Malaysia, 4.44 million ha in Sabah and 8.72 million ha in Sarawak. These forest zones comprise Permanent Forest Estate (12.55 million ha), national parks, wildlife sanctuaries and reserves (1.08 million ha) and forest reserves for agriculture conversion (5.04 million ha). The Permanent Forest Estate is further divided into protection and production forests (Thang 1991). Only the production forest (9.81 million ha) will be managed for sustained timber harvesting while the protection forest is preserved in its natural state to protect the climate, physical condition of the country, water supplies and genetic diversity.

The Malaysian tropical rainforest mainly consists of species-rich Dipterocarp forests which form 86% of the total forested land and are of vital ecological and economic importance to the country. The predominant species of the genera *Shorea, Dipterocarpus, Hopea, Dryobalanops* and *Anisoptera* produce timber which has long been known for its good quality in the world market. The other forest types are the mangrove, peat swamp, montane oak and montane ericaceous forests. The mangrove forests situated along the coastal areas are economically important in producing poles and firewood for the manufacture of charcoal. They also play an important role in the protection and conservation of the coastal ecosystem for fishery and aquaculture activities. The peat swamp forest dominates the inland swampy region and yields some species of high quality timber.

Figure 1 : Map of South-East Asia. *Shorea leprosula* is distributed from the Southern part of Thailand, throughout Peninsular Malaysia, Sumatra and North Borneo (Symington 1974).

In 1990, the forestry sector of Malaysia contributed 10% of the total export earning and ranked fourth after petroleum, oil palm and rubber (Anonymous 1990).

The issue of tropical rainforest including those in Malaysia has been the focus of world attention especially in the developed countries. Of major concern is the alarming rate of tropical forest depletion worldwide which was reported to be nearly 17 million ha annually for the period of 1986 to 1990 (Rao 1990). Like any other tropical countries in the world, Malaysia has also faced the problem of forest depletion. Intensive logging and the practice of shifting cultivation will on the other hand deplete the wood base resource in the forest zones. Statistical data show that the log supply from natural forest is not able to meet the future domestic wood based industry of the country and it is expected to be more acute for the year 2000 and beyond. Projection shows that log production will decrease from 38 million m^3 in 1990 to 20 million m^3 in 2000. Peninsular Malaysia with the present demand of 12 million m^3 of logs will be the most severely affected (Anonymous 1992).

To supplement the shortage of logs from the natural forest, the use of rubber wood from the large areas of plantation (2.0 million hectares) is an important development. The products from rubber wood are currently marketed both locally and overseas.

To slow down the rate of depletion of the wood base resource from the natural forest, the Government of Malaysia has taken measures to reduce the logging activities. Compared to the period 1981 to 1985, logging activities were expected to decrease by 32 % in the period 1986 to 1990 (Anonymous 1988). Besides that, emphasis has been put on regeneration of logged over forest. In areas where adequate stocking of economic or potentially economic species are available, the forest is left to regenerate spontaneously. Silvicultural treatment is carried out whenever necessary to aid the rehabilitation process. In this operation, undesirable moribund and defective trees incapable of producing a clear bole of at least 5 meters in length are poisoned by girdling. This treatment is favoured because there is very

little disturbance to the standing crop as few trees are girdled. At the end of 1990, the Forest Department has successfully carried out silvicultural operations of logged over forest over an area of 996,206 ha in Peninsular Malaysia, 298,600 ha in Sabah and 255,084 ha in Sarawak (Thang 1991).

In the poorly stocked forest, artificial regeneration by enrichment or line planting with relatively fast growing indigenous species such as *Shorea leprosula*, *S. parvifolia*, *S. platyclados*, *Anisoptera species*, *Dryobalanops aromatica*, *Scaphium* species and *Dyera costulata* has been carried out as an alternative measure to reforest the area. At the end of 1990, a total of 181,120 ha and 1,645 ha of poor forest have been enriched or line planted in Peninsular Malaysia and Sabah respectively (Thang 1991). Similar steps will be taken by the state of Sarawak. To counter the problem of shifting cultivation practises which are particularly common in the states of Sabah and Sarawak, migratory forest dwellers are encouraged to settle in village schemes set up by the government. In addition to the above measures, the Government of Malaysia has also set up forest plantations. Commercial establishment of forest plantation has occurred since 1957 with the planting of 779 ha of *Tectona grandis* in the northern states of Peninsular Malaysia. This was followed in late 1960's and 1970's with the planting of the fast growing tropical pines such as *Pinus caribaea*, *P. merkusii*, *Araucaria* species and about 5,558 ha have been established (Thang 1991). Then, in 1982, large scale planting known as the Compensatory Plantation project was launched. This Compensatory Plantation project is anticipated to reforest 188,200 ha of the logged over forest within fifteen years (Johari and Chin 1986). The species chosen are the fast growing utility timber with a 15 year rotation period such as *Acacia mangium*, *Gmelina arborea* and *Paraserianthes falcataria*. At the end of 1992, 50,249 ha were established in Peninsular Malaysia (Ismail 1993). In the state of Sabah, plantation forestry has started in 1973. By the end of 1990, a total of 50,262 million ha have been established with *Eucalyptus deglupta*, *P. falcataria*, *G. arborea*, *P. caribaea* and *A. mangium*. The state of Sarawak has no large scale forest plantation programme. However, Sarawak has carried out rehabilitation work on degraded

areas due to shifting cultivation and by the end of 1990, 5,933 ha were planted mainly with the species *A. mangium*, *G. arborea*, *Shorea macrophylla*, *Swietenia macrophylla* and *Araucaria cunninghamii* (Thang 1991). Among the plantation species, *A. mangium* forms more than 80% of the trees planted. It is favoured over the other species because of its superior growth performance and adaptability even on the degraded and infertile sites. However, incidence of heart rot, a disease that causes decay of the central core of the tree stem or heartwood has been reported on this species (Lee *et al.* 1988; Hashim *et al.* 1990). The concept of planting exotic timber species may have to be renewed soon.

A parallel effort which is currently been considered, for increasing the harvestable fraction of the forest, is to establish plantations of valuable and relatively fast growing indigenous trees including both Dipterocarp and non-dipterocarp species. The use of indigenous species for the plantation programmes has been hindered in the past due to unavailability of viable seeds. For example, with most Dipterocarps, the supply of seeds is irregular. In general, Dipterocarps may flower heavily every 2 to 3 years and with the occasional interval of 5 or 6 years (Burgess 1972; Cockburn 1975; Ng 1976; Whitmore 1976). But even then, this heavy flowering may not always be followed by good fruiting (Sheikh Ibrahim 1976; Ng 1976 and 1977). Heavy rain and high wind may damage the flowers resulting in few fruits being set.

The viability period of the Dipterocarp seeds is short and the seeds are incapable of withstanding desiccation, so that prolonged storage by any conventional method is problematic (Tang 1971; Tang and Tamari 1973; Tamari 1976; Sasaki 1980; Yap 1981; Tompsett 1987). In addition to these problems, the seeds are easily attacked by insects and the intensity of insect attack can be as high as 80% (Singh 1976). With the present deforestation, seed collection of Dipterocarps is becoming more problematic since mother trees are often situated in remote areas which are sometimes inaccessible. To set up a seed orchard from seedlings to solve the seed supply of Dipterocarps is rather impractical since the trees raised from seeds

normally require 20 to 30 years to reach reproductive maturity (Ng 1966) and in some species first flowering may not occur until 45 years (Ng 1976).

Vegetative propagation

In view of the above problems, it is necessary to find an alternative method of propagation to provide a regular supply of planting stock so that plantation of the indigenous species may be established. Vegetative propagation by cuttings can be one of the methods used. It is preferred to other methods of vegetative propagation because it is convenient and easy. It requires neither skill nor expensive technological facilities, whereas grafting, budding and layering require skill, are expensive, labour intensive and unsuitable for mass propagation. Micropropagation in its various forms can generate higher multiplication rates of stock plants but a high capital and skilled personnel are needed. Besides providing a regular supply of planting stock, vegetative propagation offers other advantages such as retaining the selected characteristics in the new generation, and large numbers of selected propagules from the same genotype can usually be obtained (Hartmann and Kester 1983). This is especially useful in forest tree improvement since the genotype of tree species in wild populations is highly variable and breeding cycles in all trees are long. Vegetative propagation or cloning provides an opportunity for quite rapid tree improvement if the best genotype can be identified and fixed. This is in contrast to normal breeding where the successive generations required for seedling population are impractically long. Plants produced by vegetative propagation, besides having uniform genotypes, may also be phenotypically identical if grown in the same environment. It is therefore easier for foresters to select plants that are superior in growth, form, wood quality and volume, tolerance to pests and diseases, early flowering and fruit ripening time. In terms of yield, three fold gain in wood volume has been noted for *Eucalyptus* clonally propagated by cuttings in the Congo compared to unselected seed and nearly double to those produced by selected provenance (Leakey 1987). Some progress in inducing early flowering in superior clones has been achieved in *Triplochiton scleroxylon* (Longman *et al.* 1990).

Apart from the advantages mentioned, some disadvantages may be found with vegetatively propagated plants of rooted cuttings, however these problems could be overcome as discussed below:-

1. High establishment cost. This is true at the initial stage, but in the long term it could be cheaper than seedlings. Leakey (1987) drew attention to the case of *Eucalyptus* species in Congo, where vegetative propagation by cuttings was actually cheaper.

2. The root system of cuttings is inferior to that of seedlings due to the absence of "vertical sinkers". This problem can be overcome by ensuring the formation of a radially-arranged vigorous root system on the cuttings and this could be met by good management of stock plants, the use of appropriate auxin concentration, optimum leaf area and by alleviating the physiological stresses during rooting (Leakey 1985). Experience from many clonal programmes showed that the root system of cuttings is not intrinsically inferior to those of seedlings (Leakey *et al.* 1982a). There is, so far, no evidence that adventitious roots lack the ability to form sinkers, and rooted cuttings of *Eucalyptus* species have been successfully planted in Brazil since 1980 (Spears 1985). Hence the hypothesis that root system produced by cuttings is inferior to the natural root system of seedlings cannot be supported.

3. The risks of uniformity, bringing a greater liability to pests and diseases, or other hazards in clonal plantations is widely recognised (Burdon 1989). These problem can be avoided by using a minimum number of unrelated clones for planting, for example 20 (Burdon 1989) or more clones (Zobel 1992). Alternatively, a mixture of compatible species and clones in small blocks as recommended by Libby (1982) may be the way forward. In addition, continuous renewal of operational clonal populations by discarding some clones and introducing new clones is recommended (Libby and Rauter 1984).

4. The plagiotropic plants produced in some species of the rooted cuttings can be overcome by efficient stock plant management and the use of only main stem shoot (Leakey 1985).

In conclusion, it appears that vegetative propagation by cuttings could be an effective method of producing adequate, regular planting stock especially in species where seeds are the main hindrance for reforestation, conservation and tree improvement. By selective cloning, better planting stock can be produced and it is easier and quicker to obtain large genetic gain compared to seedlings of an unselected population. The approach to clonal forestry is now gaining popular support and has been developed in tree species such as *Eucalyptus* species in Congo and Brazil (Leakey 1987), *T. scleroxylon* in West Africa (Leakey 1986) and *Gmelina arborea* in Sabah, Malaysia (Wong and Jones 1986).

Description of *Shorea leprosula* Miq.

S. leprosula belongs to the family Dipterocarpaceae and the preferred vernacular name is meranti tembaga. This species is distributed from the southern part of Thailand, throughout the Peninsular Malaysia to Sumatra and North Borneo (Symington 1974; Figure 1). It is frequently found on well drained soil in the lowland and hill Dipterocarp forests up to 600 m above sea level (Symington 1974). The species requires shade for the initial establishment but its later growth responds greatly to light (reviewed by Whitmore 1976). The tree can reach a height of 60 m and 3 m in girth. The bole is straight and cylindrical with small prominent buttress. The bark is grey brown and is regularly fissured. The chief diagnostic characters are yellowish brown leaves of the crown, elliptical leaves with a yellow tomentose lower surface. The size of the leaf is about 12.5 cm long and 5 cm wide (Symington 1974).

The tree flowers once every 2 or 3 years and so the seed supply is somewhat irregular. Viability is lost very quickly and long term storage by conventional

method is not possible. The growth rate of this species is relatively fast and it is envisaged that the tree can attain a harvestable size of 50 to 60 cm diameter at breast height in 40 years. This is based on the measurement taken on the 17 year old trees line planted in Tapah Hill forest reserves in the state of Perak, Peninsular Malaysia where the annual increment of diameter is in excess of 1.0 cm (Azman *et al.* 1991). This rate is superior to the mean diameter increment (0.8 cm per year) of 39 year old trees planted in the Forest Research Institute Malaysia (Borhan and Rahman 1987).

The timber of *S. leprosula* is classified as light hardwood. It is one of the main sources of light red meranti timber which has already established a market both locally and overseas. The wood is pinkish to light brown in colour. As a general utility timber, it is commonly used for joinery, panelling, furniture, plywood manufacture and light construction work (Choo and Lim 1983).

Objectives of the Study

1. To develop appropriate vegetative propagation facilities and protocols for the mass propagation and domestication of *S. leprosula.*

2. To study the characteristics of rooting and to determine the optimum rooting conditions for *Shorea leprosula* stem cuttings.

3. To progress towards the development of a tree improvement programme. The techniques developed can be used for future tree improvement in propagating superior clones of important indigenous hardwood tree species to enhance their productivity.

Overview of the Study

This study is a part of ODA/FRIM project. Under this project, a cutting shed with a mist propagation system was constructed in 1991 for carrying out research in vegetative propagation of forest tree species by cuttings. After returning from three months training in ITE in January 1992, I constructed six non-mist propagation systems for comparison with the present mist system used in the FRIM nursery.

To achieve the above objectives, eight experiments have been conducted with the hypothesis that different treatments given in the experiments carried out affect the rooting ability of *S. leprosula* stem cuttings by influencing their photosynthetic capacity, water relations and rooting environments.

1. Comparison between the environmental and physiological aspects of rooting in the non-mist and mist propagation systems was reported in chapter 4.

2. Since adequate mist propagation facilities had already been established in the FRIM nursery, it was decided that the rest of experiments be carried out in the mist propagation systems. The rooting environments, water relations and physiological aspects of cuttings were investigated in experiments 1 and 2 of chapter 5.

3. The physiology of rooting and post-severance treatments of leaf trimming was studied and reported in experiment 1 of chapter 6. Another post-severance treatment was the application of a range of IBA doses on cuttings and this was reported in experiment 2 of chapter 6.

4. The growth environments and physiology of stock plants and their effect on subsequent collected cuttings were studied in experiments 1 and 2 of chapter 7.

CHAPTER 2

LITERATURE REVIEW

Several studies aimed at developing protocols for the vegetative propagation of Dipterocarp tree species have been carried out (Momose 1976; 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Lo 1985; Kamis and Ng 1989; Siagan *et al.* 1989; Noraini and Ling 1993; Smits *et al.* 1994; Moura-Costa and Lundoh 1994), often with encouraging results. However, attempt to understand the stock plant management in order to maintain them in a good physiological condition has not been made. Also no detailed studies have been carried out to examine factors affecting rooting such as rooting environment, water relations and physiological aspects of cuttings. Such information is important for the management of any propagation system and for successful rooting of cuttings. It is one of the objectives of this study to examine several priority factors affecting the rooting of stem cuttings on one Dipterocarp species. The results obtained can also be used as guidelines for propagating other indigenous tree species.

Role of auxins

The purpose of treating cuttings with auxin may be to improve the rooting percentage, hasten root initiation and/or increase the number of roots produced (Hartmann *et al.* 1990). The response of root formation to auxin application was reported to vary between species (Nanda *et al.* 1970; Leakey *et al.* 1982b; Negi and Tiwari 1984; Loach 1988c; Spethmann and Hamzah 1988; Aminah 1989; Leakey *et al.* 1990; Blakesley *et al.* 1991; Mesen 1993; Wilson 1994). The benefit of auxin application has been observed in many tree species both in the mist and non-mist propagation systems. For example in an intermittent mist propagation system, Pain and Roy (1981) found that rooting of cuttings of *Dalbergia sissoo* was highest (100%) with Indole-butyric acid (IBA), 80% rooting occurred with Naphthalene-

acetic acid (NAA) and poor rooting was obtained with untreated cuttings (20% rooting). Similar response to auxin application in a mist system was obtained with stem cuttings of *Acacia mangium* where IBA at 500 and 1000 ppm produced 76% and 68% rooting respectively compared to 36% with untreated cuttings (Darus 1988).

Experiments with auxin in cuttings of *Cordia alliodora, Albizia guachapele* and *Vochysia hondurensis* planted in a non-mist propagation system, showed that optimum concentrations were 0.4%, 0.1% and 0.2% IBA respectively for the three species (Leakey *et al.* 1990). The requirement for external auxin in this experiment was especially observed in *C. alliodora* where cuttings failed to root without auxin application. However, 40% rooting occurred with untreated cuttings of *V. hondurensis*. On the other hand, cuttings of *A. guachapele* rooted equally well in all auxin concentrations tested (Leakey *et al.* 1990).

Variation in rooting response with auxin application has also been reported in Dipterocarp species (Darus and Aminah 1993; Dick and Aminah 1994). IBA application to stem cuttings of *Anisoptera scaphula, Shorea leprosula, S. bracteolata* and *Dipterocarpus charteus* did not conclusively result in improvement of their rooting when compared to untreated cuttings, in spite of juvenile cutting materials being used (Srivastava and Manggil 1981). Lo (1985) also found that juvenile cuttings of *S. macrophylla* rooted well with or without auxins of IBA, NAA and their combination at 1200 ppm and 3600 ppm concentration. Similarly, Kamis and Ng (1989) obtained no significant benefit in rooting of *S. leprosula* stem cuttings treated with several IBA concentrations. In contrast, Noraini and Ling (1993) obtained a significant improvement in rooting of *S. acuminata* and *S. parvifolia* with application of 100 and 150 µg IBA respectively. Smits (1983) achieved 100% rooting of *S. obtusa* juvenile stem cuttings soaked in 100 ppm IBA, but no comparison was made with untreated cuttings and high rooting obtained could be attributed to the use of juvenile cuttings rather than IBA treatment.

Besides influencing rooting, many workers reported a favourable effect of auxin in increasing the number of roots produced on cuttings of several tree species (Leakey *et al.* 1982b; Lo 1985; Spethmann and Hamzah 1988; Siagan *et al.* 1989; Kamis and Ng 1989; Mesen 1993). The stronger root system produced will be an advantage when these rooted cuttings are planted in the field.

Differences in the response of rooting to auxin application in the species mentioned above might be due to the presence or absence of naturally occurring auxin and other substances such as cofactors, that are necessary for rooting. It could be that in species which lack endogenous auxin, rooting is greatly increased when auxin is applied and little or no response to auxin application is obtained when naturally occurring auxin is present (Hartmann *et al.* 1990). The lack of response to the applied auxin in cuttings of some species could also be due to the environmental and morphological factors which are not optimum for rooting as noted by Loach (1988c). For example, Jen (1984) found that when the relative humidity in the propagation system was low, 16% rooting of *S. macrophylla* occurred, but when a higher relative humidity was maintained in the propagation system, 42% rooting was obtained with similar auxin concentration. Similarly, when cuttings from older seedlings of 3 to 4 years old were used, Halle and Kamil (1981) found that IBA and Indole-acetic acid (IAA) and their combinations at concentrations between 1000 and 4000 ppm did not promote the rooting of cuttings of *S. palembanica, S. leprosula, S. seminis* and *Hopea mangarawan.* In addition, a single assessment made at the end of experiment may not have revealed the true effect of a treatment, for instance Lo (1985) noted a positive effect of auxin application on *S. macrophylla* 21 days after striking, but by day 60, similar rooting levels were obtained between untreated and treated cuttings. Muckadell and Malim (1978) observed that most of the cuttings of *S. acuminatissima, S. macroptera, S. faguetiana* and *Parashorea tomentella* which had successfully rooted six week after striking died when assessed four weeks later. Therefore, frequent assessment such as every 7 to 14 days is recommended.

The method of auxin application may also influence the variability of results. Any "quick dip" method is rather imprecise as the stem diameter and water status of cuttings both influence the rate of auxin uptake. With powdered hormone, amount received by cuttings depends on the cutting diameter. A known amount of auxin applied to each cutting may be a more appropriate method (Leakey *et al.* 1982b; Noraini and Ling 1993). This is to avoid undue variations in the results obtained.

Propagation systems

The most popular system used in rooting of cuttings involves mist, either with or without enclosures. The mist system operates by providing a film of fine water droplets over the cuttings and the medium. Water loss from the cuttings is reduced by lowering both the temperature of the leaf and the surrounding air via evaporative cooling, and by humidifying the air. In this way, the difference between the water vapour pressure between the leaf and the air surrounding the cuttings is minimised (Grange and Loach 1983b; Loach 1988a,b). If the open mist system is used, there is greater fluctuation in ambient humidity and less uniform coverage of mist, due to frequent disturbance by air currents (Hartmann *et al.* 1990). The misting may often need to be more frequent to avoid water stress developing in the cuttings. For example, continuous or intermittent misting day and night is necessary for optimum rooting of *S. macrophylla* cuttings (Lo 1985). Even though water stress is less likely to be severe at night, 75% of these cuttings were dead when no misting was given at night (Lo 1985). Such intensive misting may of course lead to leaching of nutrients from the cuttings.

The above situation could be improved by covering the mist system with a polythene tent (Grange and Loach 1983a). In this system the humidity trapped in the polythene tent was reported to be more effective in reducing the leaf to air water vapour pressure difference than in the open mist system (Grange and Loach 1983a). The frequency of misting in this system is less and may reduce nutrient leaching from cuttings. This system has been shown to be effective in rooting difficult-to-

root temperate broadleaf evergreen species (Loach 1977). Superiority of closed as opposed to open mist systems in maintaining low evaporation rate is evident at all light levels (Loach 1983).

The misting frequency is important for successful rooting of each species. As mentioned earlier, cuttings of *S. macrophylla* are susceptible to water stress, and regulating the frequency of misting to alleviate water stress has greatly improved rooting performance (Lo 1985).

Although mist propagation systems have been successfully used to propagate Dipterocarp species, the system could not be used in areas where electricity or piped water are unavailable. A solar powered mist system has been constructed (Wojtusik *et al.* 1994), however, a supply of piped water is still needed to run the system.

The Forestry Research Project, Tropenbos, Kalimantan Indonesia has developed a "bubble bath" system for rooting of cuttings (Smits *et al.* 1994). In this system, the cuttings are held between two brushes and the basal end of cuttings suspended in an aqueous aerated solution of auxin. The water was aerated using porous aquarium stones which act as a bubble fractionator. This system is claimed to be successful in rooting of many Dipterocarp species (Smits 1985; Yasman and Smits 1988; Smits *et al.* 1994). Unfortunately, insufficient experimental data have been made available to support the claim. Compared to mist, this "bubble bath" system offers no advantage since the system requires piped water and electricity. Although modification has been made to generate power by a water wheel to replace the use of electricity, this is only possible if the system is built near the river (Smits *et al.* 1994). Recent report by Tolkamp and Aldrianto (1994) stated that the "bubble bath" system is more suitable for research purposes than for the mass propagation of cuttings.

An alternative system which has been tested and found effective with many tree species is the non-mist system (Tchoundjeu 1989; Leakey *et al.* 1990; Dick *et al.*

1991a; Dick and East 1992; Mesen 1993; Newton *et al.* 1993). Compared to the mist and "bubble bath" system, the non-mist system offers several advantages; it is simple and cheap to construct, does not require piped water or electricity, which is practical in areas where such facilities are not available. The non-mist is an enclosed system (a polythene frame) with a volume of water at the base of the propagator below the rooting medium. The water provides constant moisture to cuttings through capillary action. The microclimates inside this propagator was comparable to that of mist system (Newton and Jones 1993a,b; Newton *et al.* 1993; Mesen 1993). In fact, lower leaf to air vapour pressure deficit in non-mist than mist system has been recorded at the same irradiance (Newton and Jones 1993a). Certain species like *Prosopis juliflora* have been found to root better in non-mist than in the mist system, and high mortality of cuttings planted under mist can occur due to rotting (Dick *et al.* 1991a). This system has now been developed and used in propagating cuttings of several tree species in Cameroon, Kenya and Costa Rica (Leakey *et al.* 1990; Newton *et al.* 1993). A modification of the system has been used to propagate cuttings of several Dipterocarp species (Kantarli 1993; Pollisco 1994). In using the system, shading should be well regulated as heat from direct sunlight will quickly kill the cuttings (Klass *et al.* 1985; Hartmann *et al.* 1990).

Irradiance

Sufficient irradiance is needed during propagation so that photosynthetic production continues. This is often essential for the formation and development of roots in cuttings (Grange and Loach 1985; Loach 1988b). A low irradiance received by cuttings on the rooting beds can reduce or inhibit rooting, as indicated by the following examples. Rooting of *Populus tremula x tremuloides* was optimum at very low light level 8 W m^{-2} (ca. 1% full sunlight) and no rooting occurred below 2 W m^{-2} (ca. 0.3% sunlight) (Eliasson and Brunes 1980). Similar results were obtained by Klass *et al.* (1985) where 69% rooting of *Prosopis alba* was obtained when an irradiance of 520 µmol photons m^{-2} s^{-1} (ca. 26% full sunlight) was given to cuttings on the rooting bed compared to only 9% rooting when irradiance was less

than 150 μmol photons m^{-2} s^{-1} (ca. 8% full sunlight). Very low irradiance in this case was probably inadequate for photosynthesis to result in ample assimilate production for rooting. On the other hand, high irradiance is also unfavourable for rooting of cuttings (Loach and Whalley 1978; Loach and Gay 1979; Christensen *et al.* 1980; Strömquist and Hansen 1980; Grange and Loach 1985). High irradiance may exert its influence partly through raising the air and leaf temperature. When the temperature of the leaf is increased, transpiration rates will increase leading to water stress being developed in cuttings and subsequently reducing the photosynthesis (Hartmann *et al.* 1990). Increase in leaf temperature will also increase the rate of respiration which in turn enhances the depletion of stored carbohydrates available (Grange and Loach 1983b).

To avoid the above problem, shading is recommended so that the temperatures of leaf and air are lowered, a certain level of irradiance suitable for photosynthesis and carbohydrate production is maintained and also an atmosphere minimising transpirational water losses is created. Grange and Loach (1983a) stated that shading of propagation beds to give a maximum irradiance of 100 W m^{-2} (ca.13% full sunlight) will keep transpiration below 5 mg m^{-2} s^{-1} and this irradiance is still adequate for photosynthesis to take place in cuttings. In fact very little light was needed to saturate photosynthesis of unrooted cuttings (Davis and Potter 1987). The degree of shading chosen depends on the propagation systems. In the mist propagation system, less shading is necessary and cuttings could be rooted at higher irradiance than in the non-mist propagation system (Hess and Snyder 1955; Loach 1977). This is because in the mist system, leaves are always covered by a water film, part of the energy received by illuminated leaves is used to evaporate surface water, and so the increase in leaf temperature is less than when leaves are dry as in the non mist propagation system (Loach 1977). The water film covering the leaves not only cools the leaves but also restricts the water loss from within leaf tissues of cuttings.

In the non-mist propagation system, the enclosure traps more heat under high irradiance. The trapped heat rapidly increases the leaf temperature which then increases the leaf to air water vapour pressure difference resulting in more water loss from the leaves. Water losses in this case come from within the leaf tissues since an externally deposited water film is often absent and if this situation is not controlled by shading, desiccation of cuttings will result (Klass *et al.* 1985; Hartmann *et al.* 1990).

Water relations

Several researchers have indicated that for optimum rooting to occur, cuttings should be rooted in a high humidity environment. This is to minimise water vapour pressure between air surrounding the cuttings and within the leaf (Hsiao 1973; Loach 1988a,b; Hartmann *et al.* 1990). Consistent high leaf to air vapour pressure deficit in propagators caused the cuttings to be under water stress and undergo loss of turgor leading to reducing rooting (Evans 1952; Kemp 1952; Hess and Snyder 1955; Hartmann and Kester 1983). Water stress in cuttings will also affect photosynthesis by closure of stomata, thus reducing the supply of assimilates for root formation and development (Hartmann *et al.* 1990). The closure of the stomata of cuttings under water stress will lead to an increase in leaf temperature since the cooling effect through stomatal transpiration is reduced. Consequently, respiration rate will increase and this will rapidly deplete stored carbohydrates available (Hsiao 1973). Stomatal conductance has often been measured as an indication of cutting water status since it measures the degree of stomata opening. When the cuttings are not water stressed the stomata opened considerably while on propagating beds. For example Loach (1988a) found that stomata opening of *Hebe elliptica* depended on environment around the cuttings. Gilserod *et al.* (1987); Gilserod and Nelson (1989) found that an increase in relative humidity considerably widened stomatal apertures of several herbaceous plants which in turn facilitated CO_2 absorption and utilisation. Stomatal conductance of *Terminalia spinosa* ranged from 60 to 230 mmol H_2O m^{-2} s^{-1} prior to root formation under relative humidity of 90% (Newton *et al.* 1992).

Stomatal conductance of *Albizia guachepele* and *C. alliodora* in the mist and non-mist systems tended to increase with time after insertion indicating that cuttings may recover from water deficit incurred after severance (Newton and Jones 1993b). It was also noted that cuttings of arid zone species had higher stomatal conductance values compared to humid area species under similar irradiance levels which may reflect different tolerance to water deficit (Newton and Jones 1993b).

Leaf water potential is an important variable for measuring the water status of cuttings and frequently relates to rooting (Hsiao 1973). This has been demonstrated in cuttings of *Rhododendron* species where rooting was adversely affected when the leaf water potential was less than -1 MPa (Loach 1977). Water potential of about -1.0 MPa results in stomata closure in many species, and cell growth as well as wall synthesis is severely restricted below -0.5 MPa (Hsiao 1973). Darbyshire (1971) found that endogenous auxin was reduced as a result of increasing water stress from -0.2 to -1.45 MPa and consequently reduced rooting. In contrast, Grange and Loach (1984); Newton and Jones (1993b) obtained no relation between rooting and leaf water potential in cuttings of several plant species. Measurement of leaf water potential may not really reflect the water status at the base of cuttings where rooting takes place. It was suggested that measurement of water status at the cutting base may be more useful to find the actual cutting water status (cf. Grange and Loach 1985). Kemp (1952) described an extreme example where cuttings of *Pilea grandis* L. were allowed to dry slowly in the propagator until the internodal tissue shrunk, and this did not affect tissue at the base of cuttings which remained fresh and produced roots.

Alternatively, relative water content has also been used as an indication of water status in cuttings since it was found to be more correlated with photosynthesis, protein synthesis; nitrate reduction and leaf senescence in plants than water potential (Sinclair and Ludlow 1985). However, in experiments with propagation systems, Newton and Jones (1993b) found that no clear relationship was obtained in relative water content of species planted in propagation systems but differences were noted

between species. Relative water content of 80% was obtained with most species except *A. guachepele* with 56% relative water content during the first week. The authors attributed this difference in species relative water content to leaf morphology, where *T. spinosa* possesses a relatively sclerophyllous leaf compared to softer leaf of *A. guachepele* which was more susceptible to wilting. In another experiment, Newton *et al.* (1992) found that relative water content in cuttings of *T. spinosa* increased with time in the propagator which may indicate that cuttings recovered from water stress after insertion. The ability of leafy cuttings of several tropical species to recover from water deficit and eventually root was noted by Newton *et al.* (1992); Newton and Jones (1993b); Mesen (1993).

Rooting medium

Rooting media such as soil, sand, coconut fibre, sawdust, peat moss, vermiculite, perlite and their combinations have been widely used. Stem cuttings of species such as *Liquidambar styraciflua* (Bilan 1974); *Betula nigra* (Bhella 1977); *Agathis dammara* (Smits 1983) and *Acacia mangium* (Darus 1988) propagated under an intermittent mist system produced better rooting in a medium of sand/peat mixture than in pure sand. Addition of peat improves the water holding capacity of the medium which may be favourable for those species, whilst cuttings of *Dalbergia sissoo* (Pain and Roy 1981) and *Acacia albida* (Harsh and Muthana 1985) rooted in the same propagation system preferred a porous medium of coarse sand to the medium with high water holding capacity. On the other hand, Wojtusik *et al.* (1994) found that rooting of *Prosopis juliflora* was equally good in media of perlite/vermiculite (1:1) and coarse volcanic gravel (3 mm in diameter) compared to composted crop residue. The authors claimed that better rooting in the two former media in mist system was due to low bulk density which allowed rapid drainage, good aeration and easy root penetration. Compost was too dense and did not provide adequate drainage and aeration and remained water logged between misting.

The variation in rooting due to the medium was also observed in the non-mist propagation system. Addition of sawdust to increase the water holding capacity of the rooting media did not affect the rooting capacity of *C. alliodora* which rooted best in fine sand with or without sawdust (Leakey *et al.* 1990). However sawdust enhanced the rooting of *E. deglupta* cuttings. In contrast, addition of sawdust to gravel or fine sand was detrimental to cuttings of *V. hondurensis* (Leakey *et al.* 1990). Similar observations were made by Tchoundjeu (1989) in a non-mist propagation system where rooting of the stem cuttings of *Lovoa trichilioides* was significantly greater in coarse gravel than in a medium with high water holding capacity such as mixtures of coarse gravel and top soil. Cutting mortality was greatest in the latter medium which was probably due to water logging. Mesen (1993) found that rooting and number of roots of *C. alliodora* cuttings were significantly better in sand and gravel, and poor in sawdust due to the excessive water content of this medium. Cuttings of *A. guachepele* were however not affected by the different medium used, but the number of roots was significantly higher with addition of sawdust to the medium (Mesen 1993).

The normal medium which has been used to propagate cuttings of Dipterocarp species in Malaysia is river sand. Like those mentioned above, rooting also varies between species. For example Srivastava and Manggil (1981) found that coarse river sand was satisfactory for rooting of *S. bracteolata* (100%), *Anisoptera scaphula* (80%) and *Dipterocarpus charteus* (60-80%), whereas poor rooting of 40% was obtained in cuttings of *S. leprosula*. However, results in their experiment should be interpreted with caution because the cutting materials used were of different age groups. Lo (1985) obtained 80% rooting with juvenile cuttings of *S. macrophylla* in medium grade sand. Fine sand of less than 1 mm diameter was found to be lethal to cuttings of many Dipterocarp species, perhaps due to water logging (Muckadell and Malim 1978). Smits (1983) stated that *S. obtusa* stem cuttings rooted equally well in pure sand and a mixture of peat/sand, but his experiment was based on very few cutting materials and no statistical evidence was presented to support the results. Noraini and Ling (1993) obtained high rooting percentage of stem cuttings of *S.*

parvifolia and *S. acuminata* in coconut fibre and paddy husk. Many of *Shorea* species have also been successfully rooted in aerated water medium (Smits *et al.* 1994). It was claimed that the root system of these cuttings was superior as roots were observed to develop from lenticels rather than being restricted to the base of stem as in solid media (Smits *et al.* 1994). A recent report by Tolkamp and Aldrianto (1994) stated that solid media are more promising than water media for large scale propagation of Dipterocarp cuttings. The nurseries in Kalimantan Indonesia were reported to use vermiculite, sand and mixture of peat:rice dust:sand (6:3:1) for large scale propagation of several Dipterocarp cuttings (cf. Tolkamp and Aldrianto 1994).

Investigation needs to be carried out to determine suitable media for each species. This is because the suitability of the rooting medium depends greatly on the water requirement of the individual species and their tolerance to the different water holding capacities of the medium. Generalisations, that water uptake by cuttings is directly proportional to volumetric water content of the medium, where a wetter medium improves rooting, are unwise (Hartmann *et al.* 1990). What is found is that the relationship between water content of the medium to rooting of cuttings may be positive, negative or absent. Claims that the porous medium is likely to be suitable for a wet propagation system and a dry propagation system such as the non-mist needs a medium with high water holding capacity can also be misleading (Loach 1985).

Leaves

The importance of leaves in root formation and development in cuttings has been recognised especially in difficult-to-root species. For example, Bristow (1985) managed to get only 12% rooting of *Leucaena leucocephala* leafless cuttings in contrast to 82% rooting with leafy cuttings. Experiments on several Dipterocarp species such as *Anisoptera scaphula, Shorea leprosula, S. ovalis, S. bracteolata, Dryobalanops aromatica* and *D. oblongifolia* also indicated that rooting occurred

only on cuttings with leaves (Wan Kadri 1979; Yahaya 1979). In *Hopea odorata*, Aminah (1991b) obtained only one rooted cuttings out of sixty leafless cuttings. Momose (1978) recommended that at least one leaf was necessary for rooting cuttings of tropical timber species. The importance of leaves in rooting of cuttings may be due to the production of auxin and/or nutritional effect (Hartmann *et al.* 1990). The effect of nutrition in rooting has been demonstrated by Nanda *et al.* (1971) where leafless cuttings of *Populus nigra* failed to root in water but did root in glucose solution. Starch and sugar generally favoured root initiation and these could be synthesized in the leaves (van Overbeek *et al.*1946; Mahlstede and Harber 1966). The occurrence of photosynthesis in cuttings while they were on the rooting beds had been observed by Leakey *et al.* (1982b) in *T. scleroxylon,* and these authors stated that carbohydrate content was almost double in leafy cuttings than in leafless cuttings over the same time period on the rooting bed. The importance of carbohydrates for root formation was supported by results of the simulation of the carbohydrate model which showed that death occurs when cuttings of *T. scleroxylon* runs into carbohydrate deficit (Dick and Dewar 1992).

Besides the effect on nutrition and auxin production, the presence of leaves affects cuttings through their water status. It is a normal practice to trim the leaf area of the cuttings to minimise transpirational water losses. Reducing leaf area also allows more cuttings to be planted on the rooting beds. These advantages of reducing the leaf area should not affect the efficiency of photosynthesis in cuttings. There has been increasing evidence that leaf area influences the rooting of cuttings through the production of current assimilates, for example retaining 50 cm^2 leaf area in *T. scleroxylon* stem cuttings could balance both the gain in assimilate production and transpirational water loses resulting in maximum rooting success compared to cuttings with 100 cm^2 or 10 cm^2 leaf area (Leakey *et al.* 1982b; Leakey and Coutts 1989). Rooting of *A. mangium* stem cuttings with a half-phyllode yielded highest rooting (70%) followed by one phyllode (66%) and two phyllodes (46%) (Darus 1988), however the effect of assimilate gain and transpiration were not examined to support the results obtained.

Photosynthesis

The role of photosynthesis in rooting is still debatable because it is difficult to set up experiments that can reveal the influence of photosynthesis on rooting, since factors are often correlated and interactive. For instance, photosynthesis may depend upon the initial amount of carbohydrates, length of the rooting period, the amount of shoot growth during rooting and also water status of cuttings (Davis 1988). Loach (1977) observed that initial stomatal conductance of *Cornus* and *Rhododendron* cuttings was very low until root formed and the author suggested that root formation depended on carbohydrate reserves rather than current assimilates. Some species were able to root in the dark (van Overbeek *et al.* 1946; Davis and Potter 1981). However, rooting period of the species used in their studies was short and the requirement for current assimilates was less apparent. It was also reported that rooting of leafless hardwood cuttings depended on stored carbohydrates (Davis 1988). However, with soft leafy cuttings, carbohydrate reserves may not be sufficient especially in cuttings where rooting periods were sometimes 12 to 14 weeks, thus current assimilates may be important.

Elliasson and Brunes (1980) obtained no rooting of *Populus tremula x tremuloides* when grown in 2 W m^{-2} (ca. 0.3% full sunlight). Similarly Klass *et al.* (1985) obtained only 9% rooting when irradiance was less than 150 µmol photons m^{-2} s^{-1} (ca. 8% full sunlight). Full sunlight is about 800 W m^{-2} or 2000 µmol photons m^{-2} s^{-1}. Both authors felt that poor rooting may indicate that certain irradiance was needed for photosynthesis to occur although no measurement of photosynthesis was made at these irradiance levels. With the facilities for measuring photosynthesis, increasing evidence has been reported showing that photosynthesis did occur on rooting beds before rooting took place and in most cases was found to influence rooting (Cameron and Rook 1973; Elliasson and Brunes 1980; Leakey and Storeton-West 1992; Newton *et al.* 1992; Hoad and Leakey 1993; Mesen 1993). Rooting of *Pisum sativum* 'Alaska' was found to decrease by 50% when net photosynthesis was adjusted to compensation point by shading and blocking CO_2 exchange with an

antitranspirant (Davis and Potter 1988). In *T. scleroxylon* Leakey and Storeton-West (1992) found that photosynthesis occurred in cuttings and it was correlated with rooting. Similar results were obtained by Hoad and Leakey (1993) with cuttings of *E. grandis*. Cuttings of *T. spinosa* were also observed to actively photosynthesise before rooting with rates between 2 to 6 μmol CO_2 m^{-2} s^{-1} (Newton *et al.* 1992). The fact that leafless cuttings of *T. spinosa* did not root further supported the argument that current assimilates was vital for root formation although other factors from leaves such as rooting cofactors may be influencing rooting (Newton *et al.* 1992). In leaf area experiments, Mesen (1993) noted that rooting of *C. alliodora* was reduced at a larger leaf area and this corresponded to a reduction in photosynthetic rates, higher rooting in smaller leaf areas being due to high photosynthesis as well as less water deficits experienced by cuttings. From the above discussion, it is thus sensible to optimise photosynthesis of cuttings during rooting (Davis 1988).

Node position of cuttings

The distance of cuttings from the growing point of the shoot is critical in determining the rooting potential of many species. The rooting ability of cuttings of many tree species decreased with increasing distance from the main leading shoot apex. For example, Lo (1985) reported that the rooting of *S. macrophylla* stem cuttings decreased sequentially with the distance from the apex. This has also been observed by Bilan (1974) where rooting of cuttings of *Liquidambar styraciflua* was highest from the tip and decreased gradually to nil in cuttings at the bases of the branches. Similar result was obtained by Leakey (1983) with *T. scleroxylon* stem cuttings where 70% rooting was achieved for cuttings taken from apical nodes and about 10% rooting from the basal nodes. This relationship was also observed in *E. deglupta* (Davidson 1974); *Olea* species (Avidan and Lavee 1978); *A. guachepele* (Mesen 1993). The decrease in rooting towards the basal nodes might be associated to the difference in the degree of lignification as observed by Avidan and Lavee (1978) in their work with stem cuttings of *Olea* species. Cuttings from the basal nodes of the stem are usually lignified and woody and have probably undergone their

secondary growth and thickening and they either take longer time to root or may not root at all (Hartmann *et al.* 1990). Another possible reason is that the old leaves of cuttings from the basal nodes abscinded while on rooting beds before rooting could occur, as found in *T. scleroxylon* (Leakey 1983). These now-leafless cuttings will usually die when the carbohydrate reserves have been depleted. Hartmann and Kester (1983) on the other hand associated the poor rooting of cuttings towards the basal nodes with shorter cutting length which may have less carbohydrate reserves for root formation compared to longer cuttings of the apical nodes. This relationship of cutting length and rooting was further demonstrated by Leakey and Mohammed (1985) in their experiment with juvenile stem cuttings of *T. scleroxylon*. They found that rooting was positively correlated to the cutting length either sequentially increased acropetally or basipetally along the stem. But when the cuttings were cut to the same length, cuttings of the basal nodes rooted better than the apical ones. Similarly, with juvenile cuttings of *Khaya ivorensis*, cuttings of the basal nodes rooted better than those of the apical nodes (Tchoundjeu 1989). This could be because cuttings at the apical nodes of juvenile materials are still in their active growth stage where rooting would be competing with the rapidly developing shoot for carbohydrates, mineral nutrients and hormones. However, Aminah (1991a) obtained no significant difference in rooting potential between node 2 to 8 below apex of *S. bracteolata* taken from six months old seedlings. This could be due to the juvenile character of these cuttings, where differences between the node positions may be less apparent.

It is therefore important to determine which part of the stem is suitable for rooting: whether the apical, middle or basal nodes depending on the physiological condition and age of the cutting materials.

Cutting size

Good rooting is often associated with long cuttings with more than one node as observed in species such as *Tectona grandis* (Bhatnagar 1974), *E. camaldulensis*

(Geary and Hardings 1984) and *Acacia tortilis* (Dick *et al.* 1991a). Similarly in single node cuttings, good rooting was related to longer internodes as in *T. scleroxylon* (Leakey 1983; Leakey and Mohammed 1985; Leakey and Storeton-West 1992). Leakey and Mohammed (1985) have also indicated that there is a correlation between diameter of cuttings and rooting. This was demonstrated in juvenile cuttings of *T. scleroxylon*, when cuttings from different node positions were cut to the same length, basal cuttings with larger diameter rooted better than the apical cuttings with smaller diameter. Similarly, Mesen (1993) working with constant length of cuttings, found that an increase in cutting diameter (within tested range of 3 to 6 mm) generally resulted in significant number of roots. This may imply that longer or larger volumes of cuttings have more carbohydrate reserves which could be beneficial for rooting and root development. The positive relationship between rooting and volume of carbohydrate reserves obtained may indicate that the supply from current photosynthate was insufficient to support rooting (Viereskov 1988; Leakey and Coutts 1989). For example, rooting of cuttings of *T. scleroxylon* with a smaller leaf area (10 cm^2) depended to a greater extent on carbohydrate reserves than rooting in large leaved cuttings (Leakey and Coutts 1989). In contrast, Tchoundjeu (1989) in his experiment with *Lovoa trichilioides* showed that long cuttings with smaller diameter root significantly better than long cuttings with larger diameter. No significant difference was obtained between rooting of long and short cuttings with a thinner diameter (Tchoundjeu 1989). Cuttings with larger diameter could be associated with increasing secondary growth and thickening, making them less suitable for rooting (Liew 1992; Hartmann *et al.* 1990). This lignification may be more pronounced in older cutting materials.

Data available on all these aspect are still lacking and need further investigation. The information is vital to show which factor plays greater role in determining rooting either carbohydrate reserve in cuttings, which correlates with cutting size; or current assimilates from photosynthesis, so that conditions could be made available to optimise either of these two factors to result in optimum rooting

of cuttings. For good rooting both cutting length and diameter should be considered since both have been shown to influence rooting.

Stock plant nutrients

The influence of nutrients on stock plants and subsequent rooting of cuttings varies with species. Fertiliser application was necessary to maintain the growth of stock plants and production of cutting materials in *Prosopis alba*. Without fertiliser, the stock plants were found to grow slowly and few cutting materials could be obtained (De Souza and Felker 1986). In *T. scleroxylon*, application of NPK to pruned stock plants enhanced their growth; and only increased rooting ability of the lower lateral shoot but did not affect rooting of the apical lateral shoot. In another trial Leakey and Storeton-West (1992) found that there was an interaction between irradiance and fertiliser where addition of fertiliser improved rooting of cuttings from stock plants grown at high irradiance, (650 µmol photons m^{-2} s^{-1}) but poor rooting was obtained when fertiliser was applied to the stock plants raised under low irradiance (250 µmol photon m^{-2} s^{-1}). Mesen (1993) found that 0.25% of liquid NPK fertiliser applied twice wekly (20%N:20%P:20%K) coupled with low irradiance (200 µmol photons m^{-2} s^{-1}) applied to stock plants of *A. guachepele* produced suitable cutting materials for rooting. A 1.25% of similar fertiliser and irradiance of 500 µmol m^{-2} s^{-1} was detrimental to rooting of subsequent cuttings of *A. guachepele*. These cuttings shed their leaves 10 days after insertion in rooting medium, a similar result obtained with *A. falcataria* when cuttings were taken from vigorously growing stock plants (Leakey 1990). In *C. alliodora* the effect of fertiliser was more pronounced than irradiance. A 7.5 g dose per plant of NPK fertiliser (10%N:30%P:10%K) decreased rooting of cuttings relative to the unfertilised stock plants. Stock plants of *Khaya ivorensis* fertilised with high nitrogen (500 ppm N) at the rate of 200 ml per plant twice weekly had resulted in high mortality of subsequent cuttings. On the other hand, application of NPK fertiliser (800 ppm N: 500 ppm P : 300 ppm K + other elements) to stock plants at 1 litre per plant three times a week, did not enhance rooting of *Prosopis alba* cuttings compared to those of unfertilised plants (De Souza and Felker 1986).

In most cases, it seems that unrestricted fertiliser application to stock plants was unfavourable for the production of cutting materials, perhaps due to undesirable morphological and physiological characteristics of stock plants treated with supraoptimal rates of fertiliser. For example, high nutrients given to stock plants could produce leaves with low specific area (thick leaves); which may increase mutual shading of chloroplast and thus reduced the efficiency of gas exchange in cuttings (Hoad and Leakey 1993). The reduction in rooting as a result of low photosynthesis was reported by Mesen (1993) in cuttings of *A. guachepele* taken from stock plants treated with high nutrients and high irradiance. Generally, Moe and Andersen (1988) stated that suboptimal fertiliser application (for growth) resulted in cuttings that root best.

Stock plant irradiance

A certain level of irradiance is necessary for the growth of stock plants to yield suitable cutting materials for rooting (Howard *et al.* 1985; Andersen 1986). High irradiance to the stock plants in general inhibits rooting (Hansen and Eriksen 1974). The detrimental effect of high irradiance was also observed by Mesen (1993) where stock plants of *C. alliodora* grown at irradiance varying naturally from 0 to 2274 μmol photons m^{-2} s^{-1} produced fewer roots than cuttings taken from plants raised under low irradiance level (0 to 825 μmol photons m^{-2} s^{-1}). This high irradiance resulted in high levels of sugar and starch in cuttings which was unfavourable for rooting (Lovell *et al.* 1972; Hansen *et al.* 1978; Loach and Whalley 1978). The high initial carbohydrate has been shown to inhibit post severance photosynthesis in cuttings of *T. sceleroxylon* and *E. grandis* (Leakey and Storeton-West 1992; Hoad and Leakey 1993 respectively). Also high irradiance to stock plants may cause photodestruction of auxin, changes in water relations and production of rooting inhibitor/promoters (Moe and Andersen 1988).

In general, low irradiance has enhanced the rooting of subsequent cuttings in several type of plants (Hansen *et al.* 1978; Elliasson and Brunes 1980; Poulsen and

Andersen 1980; Moe and Andersen 1988). However, too low an irradiance may also inhibit growth of stock plants, for example Klass *et al.* (1985) found that 60 to 110 µmol photons m^{-2} s^{-1} was insufficient to yield cutting materials for *P. alba*. The rooting of *S. leprosula* may be affected by irradiance levels since the species in nature is shade tolerant.

Stock plant light quality

Growing stock plants at low red/far red light quality has been shown to greatly influence the subsequent rooting of cuttings of *Chrysanthemum* species (Heins and Wilkins 1979; Heins *et al.* 1980; Moe and Andersen 1988) and in *Euphorbia pulcherrima* (Hagen and Moe 1981). Recent work by Leakey and Storeton-West (1992) found that *T. scleroxylon* raised under red/far red ratio of 1.6 yielded better rooting than high red/far red of 6.3. Similarly, Hoad and Leakey (1993) found that stock plants of *E. grandis* grown under low red/far red ratio (0.4 and 0.7) produced higher rooting than those grown under high red/far red of 3.5 to 6.5. The high rooting obtained under low red/far red was associated with longer internode which may have high carbohydrate reserves. However, the results obtained above did not compare with cuttings grown under the natural red/far red daylight of 1.2. This was because Dick and East (1992) found that rooting of *Acacia tortilis* grown under low red/far red of 0.6 were not significant when compared to those grown under red/far red of 1.09 which was close to the natural red/far red of daylight, although growth of plants was enhanced under low red/far red. The photoreceptor phytochrome is known to influence extension of growth obtained under low red/far red as indicated by Morgan and Smith (1978) in their studies with herbaceous plants. In contrast, an increase in stem height and diameter of shade tolerant species of *Dacrydium cupressinum* was less apparent by reduction of red/far ratio from 1.39 to 0.34 (Warrington *et al.* 1988). Tchoundjeu (1989) found that a shade tolerant species *Lovoa trichiliodes* did not show any significant improvement in rooting of cuttings taken from stock plants raised under low or high red/far red ratio.

CHAPTER 3

GENERAL MATERIALS AND METHODS

Cutting shed

The size of the cutting shed in the nursery of the Forest Research Institute of Malaysia (FRIM) is 26 m x 10 m x 5 m. It was constructed as a steel framework with a dome shaped roof. The roof was made of 1 mm thick translucent plastic flat sheet material called Solargro (Sarlon Industries Pty. Ltd., Australia). On the inner side, a layer of black plastic netting was suspended 2 meters from the top of the roof to shade the cutting shed from overhead sunlight. The side walls of the shed are surrounded by wire netting. There are eight concrete rooting beds inside the shed and they are equipped with automatic mist sprinkler systems (Appendix A).

Mist propagation system

Preparation of the rooting bed

The rooting bed measures 1 m x 10 m x 0.2 m and it is raised to about 0.9 m above the ground to facilitate working. Drainage holes are made around the rooting bed to drain the excess water (Figure 2a). This rooting bed was washed clean with water before the rooting medium was put on it. Gravel (2 to 3 cm diameter) was placed in the bed as a layer to facilitate drainage, followed by the rooting medium (Figure 2b).

Rooting medium

River sand (60% with $\leq$ 2 mm diameter of sand particles and 40% between 2 mm and 5 mm) was used for all experiments unless otherwise stated. The sand was washed with water to remove plant materials, big stones, debris and mud before

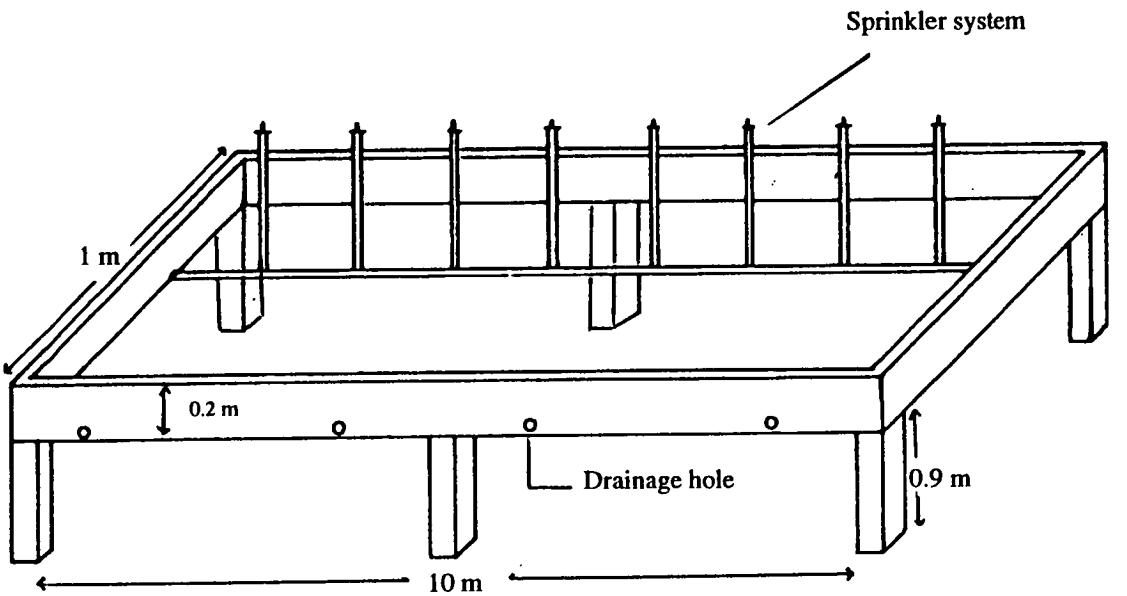


Figure 2a : Rooting bed with sprinkler system

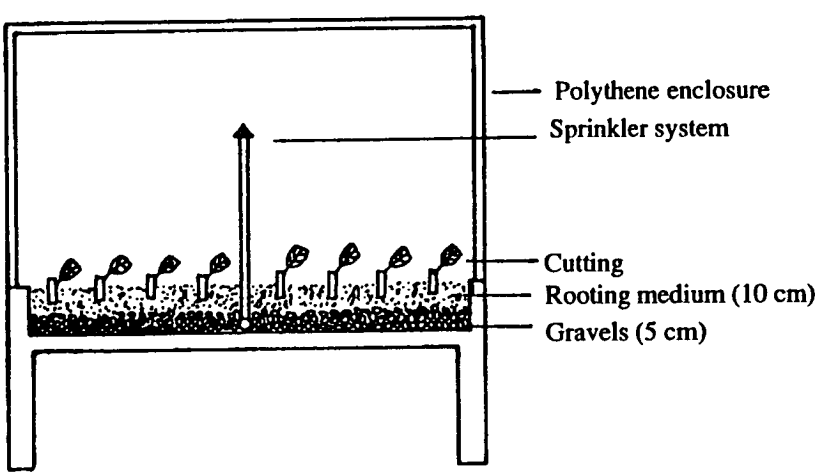


Figure 2b : Cross section of the rooting bed

being placed into the rooting beds. This rooting medium was replaced for each new experiment.

Watering system for the mist propagators

The rooting beds were kept moist by an automatic mist sprinkler system. Atomiser jets (Macpennys No.1 Type and Size No.2 from Wright Rain Ltd. UK) fed by PVC (Polyvinyl chloride) pipes were installed 118 cm apart to provide fine misting to the planted cuttings (Figure 2a). The water was drawn from the polyethylene water tank (Hercules TH64, Malaysia) by a 1.5 kilowatt water pump (Pedrello CP 190, Italy) and was passed through a water filter (RIS, James Hardie Irrigation, Ltd. Australia) before going to the sprinkler system. The automatic timers (Omron H3BA, Japan) were installed to regulate the frequency and the duration of misting for each bed. The misting frequency used for all rooting experiment except otherwise stated was every 60 minutes and the duration of misting was 1 minute. To maintain high air humidity, each misting unit was enclosed by a clear polythene sheet supported by aluminium frame (1 m x 1 m x 0.8 m) (Figure 2b and Plate 1). The enclosures were then shaded with a layer of black plastic netting.

Non-mist propagation system

Six non-mist propagators (Leakey *et al.* 1990) were placed at the north end in the cutting shed. The non-mist propagators were made of a fibre glass tank (130 cm x 75 cm x 60 cm) held by a metal frame (Figure 3). The inside of the tank was lined with clear polythene sheet raised 20 cm above the tank and firmly attached to the metal frame. The lid of propagator was made of clear polythene sheet supported by wooden frames. Rubber lining was stuck around the edge of propagator to ensure that the lid was air tight. The basal 40 cm of the propagator was filled with sand (3 cm), stones (15 cm) and gravel (15 cm) followed by appropriate rooting medium (7 cm). The propagator was filled with water to 33 cm. In order to check the water level, a clear plastic hose was attached to the propagator (Figure 3). These propagators were shaded with a layer of black plastic netting.

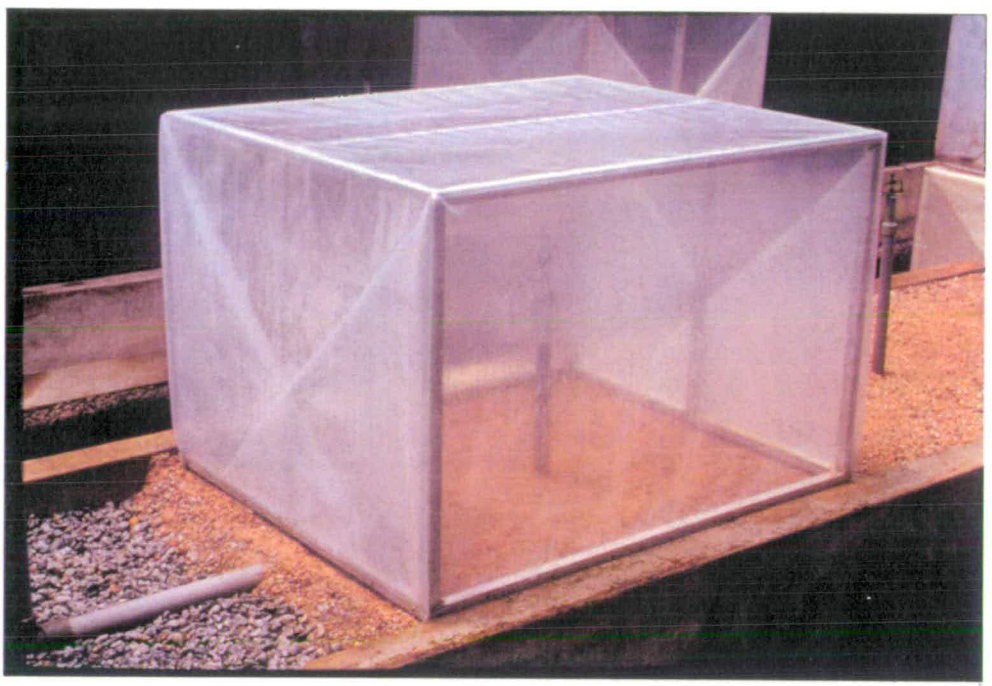


Plate 1 : Mist propagator with polythene enclosure

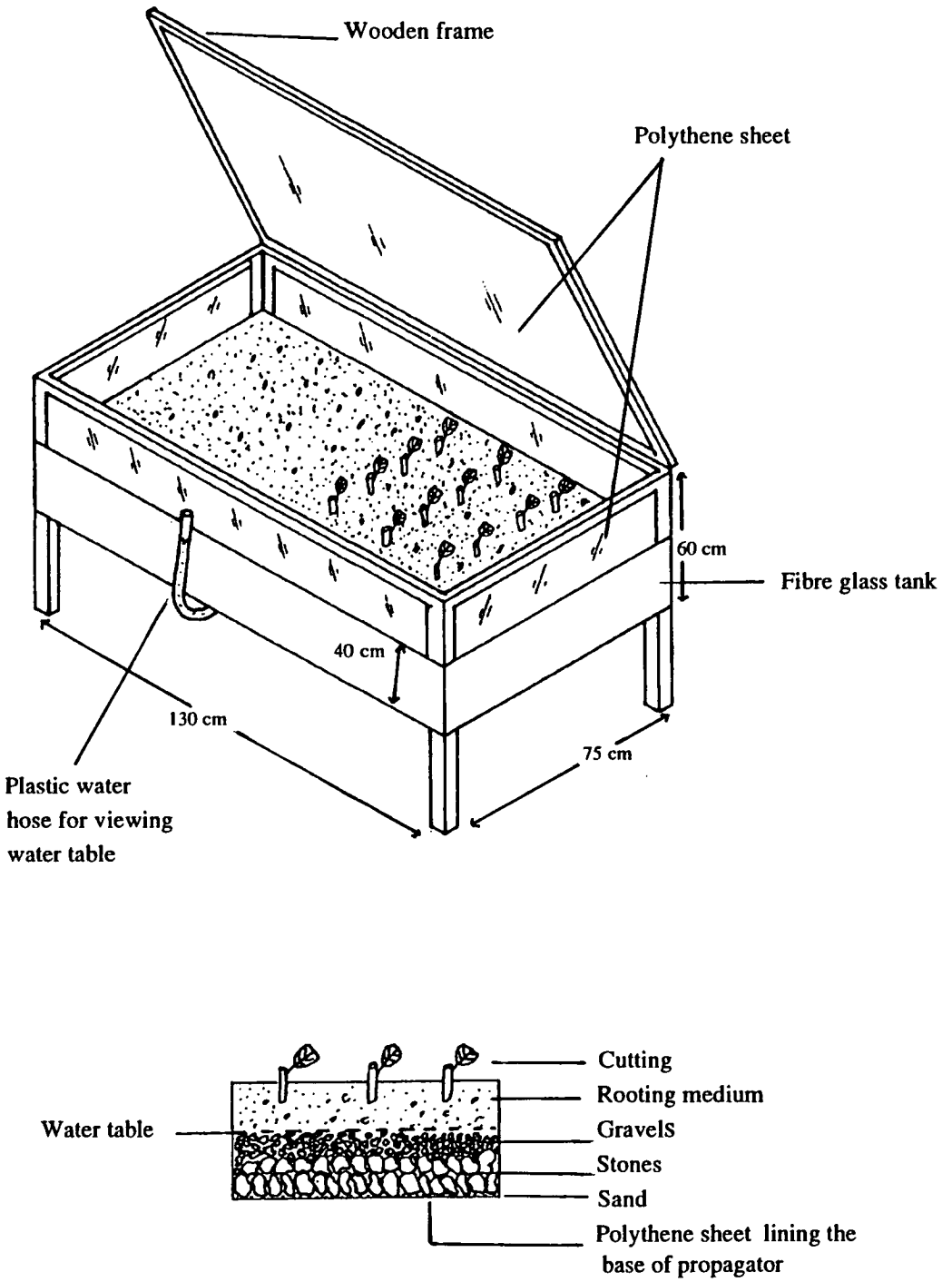


Figure 3 : Non-mist propagation system

Stock plants

The first batch of stock plants were raised from open pollinated seeds collected from three mother trees in the Forest Reserve Ulu Teranum in the state of Pahang, Malaysia. Thus the seeds produced were not expected to have a wide genetic base. The seeds were sown in the seed beds of the FRIM nursery. When the seedlings were 7 cm tall, they were transplanted into black perforated polythene bags (9 cm diameter x 17 cm height). The potting medium used to raise stock plants was forest top soil and sand in the ratio of 3:1 by volume unless otherwise stated. This potting medium is the standard medium used in the FRIM nursery and has been found suitable for raising seedlings of *Shorea leprosula* (Aminah, unpublished). The potted seedlings were kept on the transplanting beds shaded with black plastic netting (33% of full sunlight). A commercial granular compound fertiliser called NPK Blue (12%N: 12%P_2O_5: 17%K_2O: 2%MgO + Trace element, manufactured by ICI Fertilisers, Malaysia) was given to the plants at the rate of 0.5 g per plant every 2 weeks unless otherwise stated. After one year, the plants were potted into bigger polythene bags (18 cm diameter x 18 cm height). These plants were cut back to maintain supplies of coppice shoots for building up clonal populations. In addition, stock plants were also raised from coppice shoots obtained from the five year old seedlings planted in the FRIM nursery compound. Similar procedure as described above was used to pot and raise all the rooted cuttings obtained.

These potted plants were watered manually to field capacity twice a day in the morning and late afternoon except on rainy days. Weeding, insecticide and fungicide applications were carried out whenever necessary. The insecticide used was Tamaron Special (50% Methamidophos as active ingredient) manufactured by Bayer Company, Leverkusen, Germany. The fungicide applied was Benlate (50% Benomyl as active ingredient) manufactured by E.I. Du-pont, Denemours and Co. Inc. USA. Both biocides are systemic in nature.

Preparation of the cutting materials

When the plants reached a height of 40 to 50 cm (Plate 2), single node cuttings were taken from the second node down the stem unless otherwise stated. The apical undeveloped shoots were discarded as they were not suitable for cuttings. The length of the cuttings was 5 cm and the leaf area retained on each cutting was 30 cm^2 except otherwise stated. Leaf area was cut using a 30 cm^2 paper template which had been measured with leaf area meter (Delta-T series, Taiwan). The base of the cuttings was made as a right angle cut to the stem.

Preparation of plant growth regulator

Indole-3-butyric acid (IBA) was obtained in powder form from Sigma Chemical Company, USA. To prepare 10 ml of 0.2% IBA, 0.02 g IBA was weighed using an analytical balance (AA-160, Denver Instrument, USA) and was dissolved in absolute ethyl alcohol in a 10 ml volumetric flask. This preparation was used for all rooting experiments except in experiments testing the different auxin doses.

Planting of cuttings

Before the cuttings were planted, their bases were treated with ten micro-litres containing 20 µg of IBA using a micropipette (F10, Gilson Medical Electronic France). The alcohol was immediately evaporated in a stream of cold air from a fan. These cuttings were then planted in the rooting medium at a depth of 1.5 cm. The distance within and between rows was 5 cm and 10 cm respectively. This distance was used to facilitate the gas exchange measurement.

Microclimate of propagators

Air and leaf temperatures were measured using thermocouples (Type K chromel-alumel, T.C., Ltd., Uxbridge, UK); relative humidity using commercial humidity

Plate 2 : Six month old stock plant of *Shorea leprosula* raised from rooted stem cuttings

sensor (MP 100 Rotronic probes, Campbell Scientific Ltd., Loughborough, UK) and irradiance using quantum sensors (Skye Instruments Ltd., Llandrindod Wells, UK, supplied by Campbell Scientific Ltd., Loughborough, UK). A solid state data logger (21X Micrologger, Campbell Scientific Ltd., Loughborough, UK) was used to record the data from the appropriate sensors. The logger was programmed to scan each sensor every 60 seconds and to calculate and store mean readings every 5 minutes.

A polyvinyl chloride (PVC) tunnel was used to shade the humidity sensor from direct contact with water while in the propagators. For the leaf temperature, the thermocouples were supported on an aluminium label inserted in the rooting medium below the leaf with the sensor touching the under surface of the leaf. These sensors were checked daily to ensure that they are well placed in their appropriate position. Each time the lid of the non-mist propagator or the enclosures of mist propagator was opened, the cuttings were sprayed with a hand sprayer to avoid any sudden change in relative humidity.

The humidity sensor and thermocouples were calibrated using the humidity and temperature calibration chamber (HCB - 5P, C-Sun Industrial Company Ltd., Taiwan) at the Malaysian Weather Department. Irradiance sensors were checked against SKP 215/200 light sensor (Skye Instruments, UK).

Leaf-to-air vapour pressure deficit (VPD)

VPD was calculated based on the formula given in Jones (1992).

$$VPD = (e_{s(Tl)} - e)/1000$$

$$e_{s(Tl)} = a \exp (bT_l /c+T_l)*10000$$

$$e = e_{s(Ta)} * h$$

$$e_{s(Ta)} = a \exp (bT_a /c+T_a)*10000$$

VPD is leaf to air vapour pressure deficit in kilopascals (kPa)

$e_{s(Tl)}$ is saturation vapour pressure of leaf in Pascals

$e_{s(Ta)}$ is saturation vapour pressure of air in Pascals

T_l and T_a are the leaf and air temperatures in °C respectively

a,b,c are empirical coefficients where a=0.061375; b=17.502 and c=240.97

e is the air vapour pressure in Pascals

h is the relative humidity expressed as a fraction

Relative water content (RWC)

The RWC of the leaf was determined using the method described by Beadle *et al.* (1987). Leaf discs (18 mm) were taken from the cuttings using a cork borer. The fresh (FW), turgid (TW) and dry (DW) weight were measured. Turgid weight was determined after floating the discs in distilled water in covered vials for 24 hours, and these discs were dried with absorbent paper towel before weighing. The dry weight was obtained after discs were oven dried at 80 °C for 48 hours at which constant weight was obtained.

RWC was then calculated as: (FW - DW) x 100 / (TW - DW)

Photosynthetic rate (P_n) and stomatal conductance (g_s)

P_n and g_s were measured using a portable infra-red gas analyser (IRGA, LCA-3, Analytical Development Company Ltd., Hoddesdon, Herts., UK) attached to a Parkinson leaf chamber (Analytical Development Company Ltd., Hoddesdon, UK). Measurements of P_n and g_s of cuttings were made in situ. Before each measurement, the leaf surface was dried using an absorbent paper towel to avoid inaccurate g_s readings caused by free water on leaf surfaces. The thermistor was used for leaf temperature measurement and it was attached to the undersurface of the leaf with paper tape each time the measurement was made. Each measurement was recorded when the CO_2 differential readings were stable (30 to 40 seconds).

The IRGA was checked and calibrated as necessary before each session of measurement. The CO_2 was calibrated using cylinders of 350 and 500 ppm CO_2 obtained from Malaysian Oxygen, Ltd. The humidity sensor was checked using a whirling wet and dry bulb psychrometer (G.H. Zeal Ltd. London UK). The thermistor and temperature sensors were checked against a mercury bulb thermometer. Irradiance sensors were checked against SKP 215/200 light sensor (Skye Instruments, UK).

Measurements were taken after a steady differential of zero was obtained with CO_2 and relative humidity in the empty chamber which normally took 15 to 20 minutes.

Starch determination

The starch content of leaf and stem of cuttings were determined using the method of Humphreys and Kelly (1961). The stem and leaves of cuttings were separated and dried at 40 °C for 48 hours in an oven (ULM 500 Memmert, Germany). The leaves and stems were then ground into powdered form using a mill (Fritsch pulverisette 14, Germany). For the extraction of starch, ca. 400 mg of sample was weighed in a 50 ml volumetric flask, and 4.7 ml 7.2 M perchloric acid was added to the sample. It was allowed to extract for 10 minutes with periodic shaking. This sample was then diluted to 50 ml volume with distilled water and centrifuged at 1300 rpm for 20 minutes (Hettisch Universal II, Germany). After the extraction, a 10 ml aliquot was taken from the solution and placed in a 50 ml volumetric flask and a drop of phenolphthalein was added. The solution was made alkaline with 2N NaOH and then the colour was discharged with 2N acetic acid. A further 2.5 ml acetic acid was added, followed by 0.5 ml 10% potassium iodide and 5 ml 0.01N potassium iodate. The colour was allowed to develop for 15 minutes after which the volume was brought up to 50 ml with distilled water. The absorbance was measured at 650 nm against a blank of the reagent on Ultra Violet Visible Spectrophotometer (UV-160A, Shimadzu Corporation, Japan). A standard reference curve was set

up using potato starch. Percentage of starch in each sample was calculated based on oven dry weight.

Sugar determination

For the extraction of sugar, powdered oven dry leaf or stem sample was weighed ca. 50 mg in a test tube, and 5 ml of deionised water at 30 °C was added to the sample. The mixture was placed in a 30 °C water bath for 15 minutes with periodic shaking after which it was centrifuged at 4500 rpm for 15 minutes. Then, 3 ml of supernatant was taken and kept in a test tube leaving behind the pellet plus 2 ml supernatant. The extraction process was repeated twice and 5 ml supernatant was taken out after each extraction giving a total of 13 ml. This supernatant was then filtered through a 0.2 μm filter and the resulting filtrate was analysed for sugars by High Performance Liquid Chromatography (HPLC, Dionex Ltd. UK).

Nitrogen (N), phosphorus (P) and potassium (K) determinations

The method described for NPK determinations is as used in the soil laboratory in the Darwin Building, University Edinburgh, adopted from Allen (1989). A wet digestion procedure was used for digesting samples of leaf or stem. Powdered, oven dry leaf or stem was weighed ca. 100 mg in test tube. To this sample, 2 ml concentrated sulphuric acid was added followed by 0.75 ml hydrogen peroxide and when the reaction subsided another 0.75 ml hydrogen peroxide was added. The mixture was heated at 340 °C for six hours until colourless solution was obtained. It was then allowed to cool and transferred with washings to 100 ml volumetric flask and the volume was made up to the mark with distilled water. Nitrogen was determined by a gas diffusion method using flow injection analyser (Tecator 5020, Sweden). Phosphorus was also determined using flow injection analyser by a colorimetric method (Ammonium molybdate, Stannous chloride method). Potassium was determined by atomic absorption spectroscopy using a Unicam 919AA Spectrophotometer, UK).

Assessment of cuttings

Weekly assessment was carried out on cuttings starting one week after planting unless otherwise stated. At each assessment, the variables measured were: number of rooted, unrooted and dead cuttings, leaf shedding and number of roots on each cutting (Plate 3). A cutting was scored as rooted when it produced a root of 1 cm length or more and the cutting was considered dead when the whole stem turned brown. After the observation, the cuttings were replanted into the rooting medium and reassessed until the sixteenth week by which time no new roots were formed. To ensure minimal stress to cuttings during assessment, cuttings were sprayed with water before digging and after replanting. Repeated assessments on the same cuttings were made because of the limited cutting materials available, this is also a standard technique for this type of experiment (Leakey *et al.* 1982b; Leakey and Mohammed 1985). The mean accumulated number of roots per rooted cutting was calculated by dividing the total number of roots produced by the total number of rooted cuttings.

Experimental design and statistical analyses

In general, a randomised complete block design was used for all the experiments. For the experiments, a mixture of several clones were used. In all cases, clones were not well replicated since the number of plants from each clone was small. However, the number of cuttings per treatment was maintained at sixty unless otherwise stated. Full details of experimental design and layout will be described in each experiment.

To determine the significant association between treatments and morphological characteristics of cuttings, stepwise regression with analysis of deviance (Collett 1991) was carried out for binomial data of rooting using Genstat 5 (Payne *et al.* 1987). Analysis of variance for a stepwise regression was used for the number of roots per rooted cutting. For these analyses, only the statistically significant results were presented.

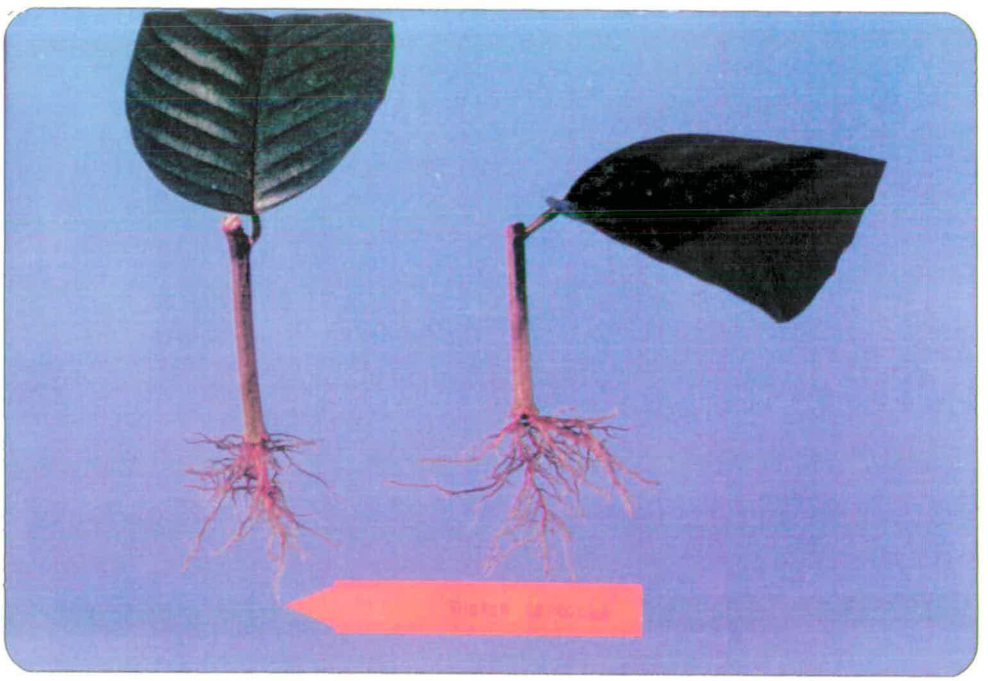


Plate 3: Rooted stem cuttings of *Shorea leprosula* six weeks after planting in the enclosed mist propagation system

For continuous data such as P_n, g_s, RWC and environmental data, analysis of variance was employed using the statistical package, SAS (1985) version 6.04 (Statistical Analysis System from SAS Institute Incorporation, Cary, North Carolina USA). The results in all analyses were considered significant when the probability level was equal or less then 5% ($P \leq 0.05$).

The graphs were drawn using Sigma Plot (Jandell Scientific, 1989).

CHAPTER 4

THE INFLUENCE OF PROPAGATION SYSTEMS AND ROOTING MEDIA ON THE PHYSIOLOGY OF ROOTING OF *SHOREA LEPROSULA* LEAFY STEM CUTTINGS

Introduction

Experimental evidence has indicated that the influence of the type of rooting medium on rooting of cuttings may be substantial but suitable rooting medium differs between species (Loach 1985; Leakey *et al.* 1990; Hartmann *et al.* 1990). In general, rooting medium should consist of inert, well aerated and moisture retaining material (Hartmann *et al.* 1990). The normal medium used for rooting cuttings of Dipterocarp species has been medium or coarse river sand (Momose 1978; Muckadell and Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Lo 1985; Kamis and Ng 1989; Aminah 1991c; Smits 1992). Other media found suitable for rooting Dipterocarp cuttings were vermiculite (Smits 1992), a mixture of sand and peat moss (Smits 1983) coconut fibre or paddy husk (Noraini and Ling 1993), and aerated water (Smits *et al.* 1994; Tolkamp and Aldrianto 1994). Among these reports, none has examined the performance of rooting in relation to the physical properties of the rooting media. Similarly, several types of propagation systems have been used for rooting Dipterocarp cuttings. Mist propagation system has been the popular choice when rooting cuttings of Dipterocarps (Momose 1978; Muckadell and Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Lo 1985; Aminah 1991c; Noraini and Ling 1993; Moura-Costa and Lundoh 1994). Other systems reported include non-mist (Muckadell and Malim 1978; Kantarli 1993; Pollisco 1994) and "bubble bath" systems (Smits *et al.* 1994; Tolkamp and Aldrianto 1994). Kantarli (1993) rooted *Hopea odorata* in a non-mist system and obtained 59 to 81% rooting depending on the height of stumps from which the coppice shoots were taken. Comparisons between propagation system have been reported, for

example Smits *et al.* (1994) found that both mist and the "bubble bath" systems were suitable for propagation of several Dipterocarps. They stated that the advantage of "bubble bath" system is that roots also arise from lenticels along the stem and this is thought to result in a stronger root system when planted out in the field (Smits *et al.* 1994). However, environmental data were not presented to enable the systems to be replicated elsewhere. Muckadell and Malim (1978) propagated Dipterocarps in non-mist and mist systems but failed to draw definite conclusions due to disturbance by animals in their experiments. Results of other experiments have indicated that the non-mist systems are just as efficient for rooting cuttings of many tropical species (Tchoundjeu 1989; Leakey *et al.* 1990; Dick *et al.* 1991a; Dick and East 1992; Mesen 1993; Newton and Jones 1993b) as long as VPD is kept below 0.5 kPa most of the time as recommended by Grange and Loach (1983a) for most broad leaved species. Experiment 1 examines how different propagation systems and physical properties of rooting medium influence microclimates, photosynthetic rates and stomatal conductance of *S. leprosula* stem cuttings. Experiment 2 further investigates rooting environments and physiological aspects of *S. leprosula* stem cuttings in the non-mist and mist propagation systems. Information on these aspects is a necessary prerequisite for an affective management of the propagation system especially that of non-mist where the environment is not highly regulated.

EXPERIMENT 1: Effect of rooting media on the rooting ability of leafy stem cuttings of *Shorea leprosula* in the mist and non-mist propagation systems.

Materials and methods

Cutting materials and experimental layout

The experiment took place in the cutting shed of the FRIM nursery in October 1993. A total of 360 single node leafy stem cuttings were taken from seven month old stock plants raised under 33% full sunlight as potted rooted cuttings. Thirty two

clones were used: 21, 24, 35, 49, 57, 58, 62, 68, 70, 78, 100, 133, 151, 157, 508, 510, 514, 515, 517, 525, 526, 528, 548, 549, 551, 552, 554, 565, 571, 576, 580, 590. Preparation of cuttings is described in chapter 3. The length and leaf area of each cutting were 5 cm and 30 cm^2 respectively. Initial diameter and node position of each cutting were recorded. Volume of the cuttings was calculated assuming a simple cylindrical shape. A 3 x 2 factorial experiment was set up with three rooting media and two propagation systems. The three media used were: 1) 100% sand; 2) 50% sand and 50% coconut fibre; 3) 100% coconut fibre. There were six enclosed polythene mist and non-mist propagators and each propagator is deemed to be a block. Each block was divided into three compartments and the media were randomly assigned to the compartments. The treatments were randomly allocated to the node positions so that there was no confounding between the treatments and the position on the stock plants from which cuttings were taken. The number of cuttings obtained per stock plant varied from 3 to 7 depending on availability of leaves on the node. Clones with less than 12 cuttings were not used for the experiment. Each treatment combination consisted of one hundred and eighty cuttings and they were randomly laid out in six blocks (30 cuttings per block). Details and illustrations of the propagation systems are described in chapter 3.

Microclimate of propagator

Temperature, relative humidity and irradiance were recorded by a data logger (21X Micrologger, Campbell Scientific, Ltd., Loughborough, UK) using respective sensors as described in chapter 3. The measurements of these data were made in two blocks which were randomly chosen from the total of six blocks of mist and non-mist propagators used in the experiment. The sensors for measurement of each variable were placed in the sand and coconut fibre compartment of each propagator chosen. There were not enough sensors to be placed in each of the media used. The data logger was programmed to scan each sensor every 60 seconds, and to calculate and store mean readings every 5 minutes. Data collection extended from day 1 to day 50 of the experiment.

Photosynthetic rate (P_n) and stomatal conductance (g_s)

P_n and g_s of cuttings prior to rooting were measured using a portable infrared gas analyser (LCA-3, ADC Ltd., Hoddesdon, UK). Four unrooted cuttings per treatment per block were randomly chosen and they were measured on days 1, 8, 14, 21 and 28 after planting in rooting media. At each time of measurement, one cutting per treatment per block was measured. Measurements of P_n and g_s were made between 09:00 to 12:00 hours.

Components of the rooting medium

To determine the components of solid, water and air in the rooting media, a 100 cm^3 sample of each medium was taken using a graduated plastic beaker on day 7 of the experiment. In every block, 4 samples of each medium in mist and non-mist propagators were taken. The volume of air in the media was determined by adding water to the sample and the amount of water required to saturate the air spaces was weighed. The total weight of all the components was recorded. The wet sample was dried at 105 °C in an oven for a period of 48 hours. The water content of media was determined by the difference between the weight of wet and dry medium. Each component of the media was expressed as percentage of the total weight.

Assessment of cuttings and statistical analyses

Assessment was made weekly until week sixteen for rooted, unrooted and dead cuttings as well as number of roots on each cutting.

Analysis of deviance for stepwise regression in Genstat 5 (Payne *et al.* 1987) was used to determine which of the recorded variables were significantly associated with rooting of cuttings. For the accumulated number of roots per rooted cutting, analysis of variance for stepwise regression was used. For stepwise regression analysis, only variables that were significantly different were shown in the tables. Analysis of

variance followed by Fisher's t test (LSD) was used to test the significant differences in the treatments for components of rooting media, P_n and g_s. The results in all the tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

In the regression analysis, the association between the variables and rooting was indicated by the regression coefficients and this association was presented graphically. The method of presentation is as follows: points on the graph were obtained from grouping of the observed rooting data with the cutting volume into appropriate class intervals, whilst the line was drawn by connecting corresponding "predicted" rooting values of individual cutting computed from the multiple regression model. In this model, other factors or variables presented in the tables were also fitted. As a result, the "predicted" line drawn through the data points may appear "wobbly or unsteady".

Results

The initial diameter and volume of cuttings were not significantly different in the treatments used. Mean diameter ranged from 0.36 to 0.37 cm while mean volume of cuttings ranged from 0.57 to 0.61 cm^3.

There was no significant difference obtained in rooting of cuttings between the media tested. Rate of rooting in different media is shown in Figure 4.1a. Significantly higher rooting was obtained in mist than non-mist system (Table B1 and Figure 4.1b). Rooting of cuttings was significantly affected by the cutting volume and the relationship was negative (Table B1 and Figure 4.1c).

Number of roots per rooted cutting was not significantly affected by either treatments or morphological characteristics of cuttings. Figures 4.2a,b show the rate of number of roots produced in the treatments used.

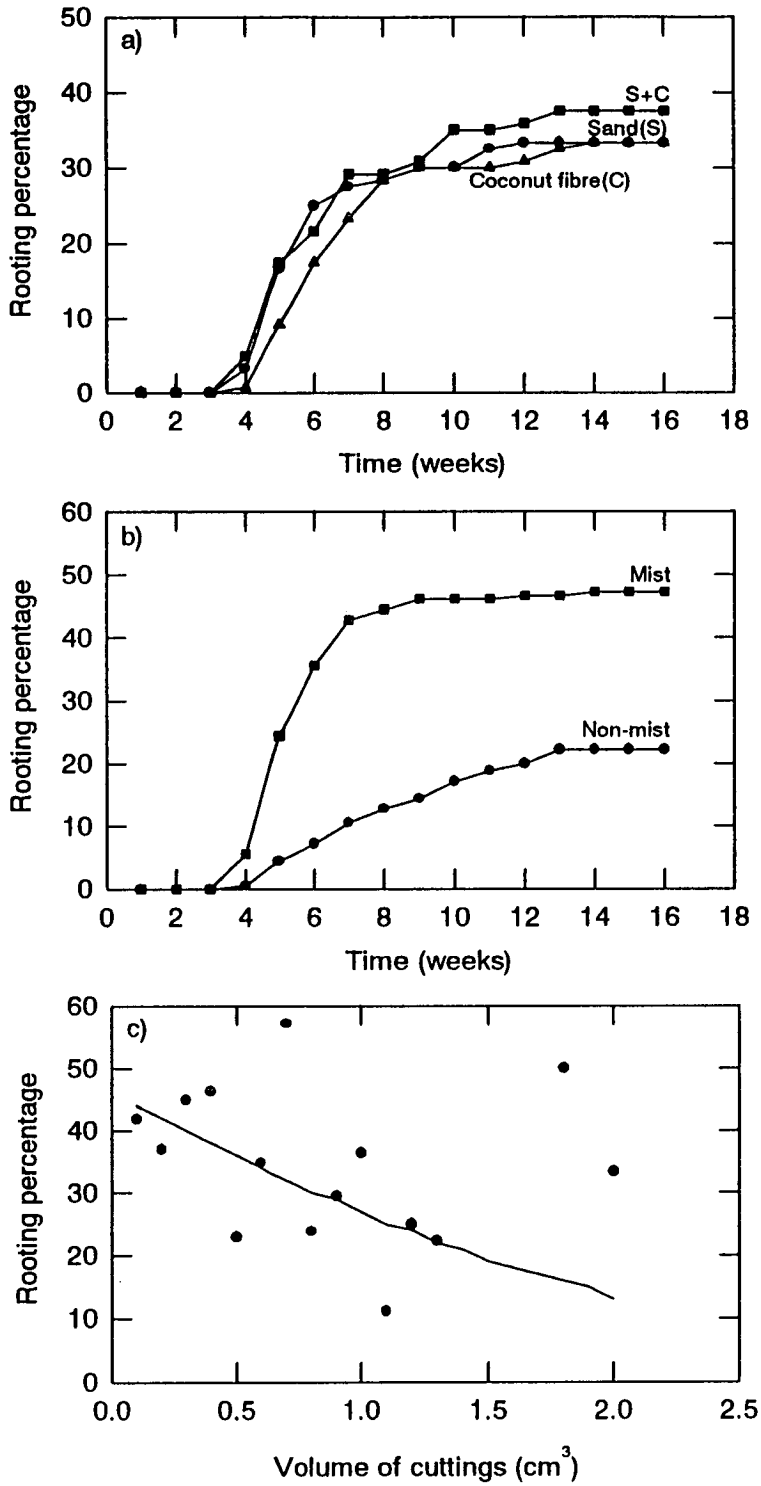
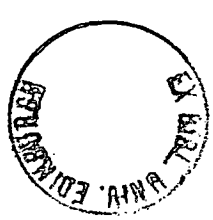


Figure 4.1 : Rooting rate of *S. leprosula* stem cuttings as affected by a) Rooting media: circle=sand(S); triangle=coconut fibre(C); square=(S+C); b) Propagation systems (n=60 per treatment combination); c) Relationship of rooting and volume of *S. leprosula* stem cuttings. Points are groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model.



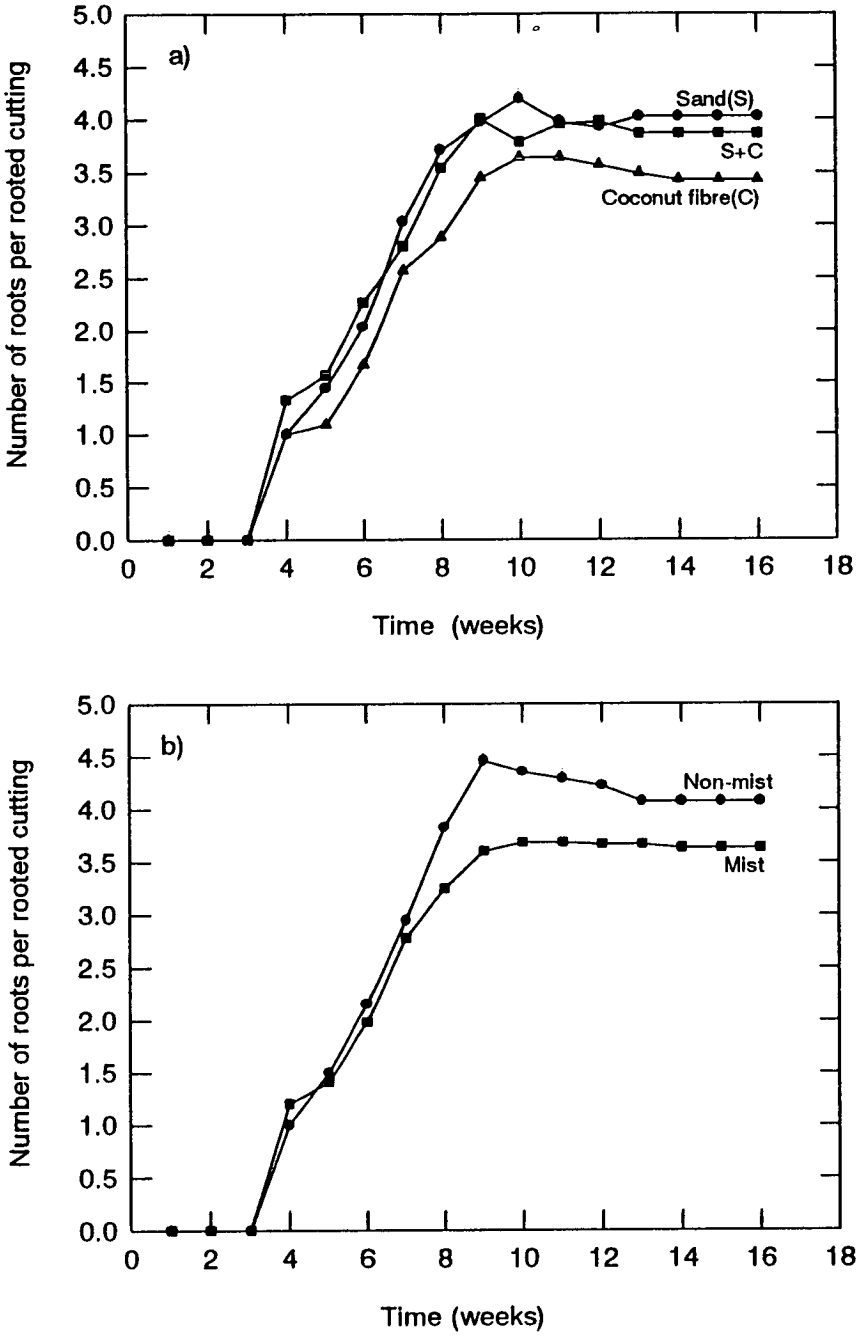


Figure 4.2 : Rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* as affected by a) Rooting media; b) Propagation systems (n=60 per treatment combination).

A significantly higher percentage of dead cuttings was found in non-mist than mist system (Table B2 and Figure 4.3a). Regression analysis indicated that mortality increased with the volume of cuttings (Figure 4.3b). Mortality in both propagation systems was related to the percentage of leaf shedded by cuttings (Figure 4.3c). The percentage of cuttings remaining unrooted was not significantly affected by any of treatments used but was significantly decreased with cutting volume (Table B3 and Figures 4.4a,b,c).

There was a significant interaction in the air, water and solid components of rooting medium and the propagation systems used (Tables B4, B5 and B6). Water and air components were highest in coconut fibre followed by the mixture of coconut fibre:sand and sand in both systems. The water content of the media was higher in non-mist than mist system, while the air content of media was higher in mist than non-mist system. The composition of each medium in both propagators is given in Figures 4.5a,b)

The environmental data collected in propagators over a period of 50 days is shown in Table 4.1. Mean VPD was very low in both propagators since this experiment was carried out in rainy season. However, daily maximum VPD was significantly higher in non-mist than mist propagators (Table B7 and Figures 4.6a,b).

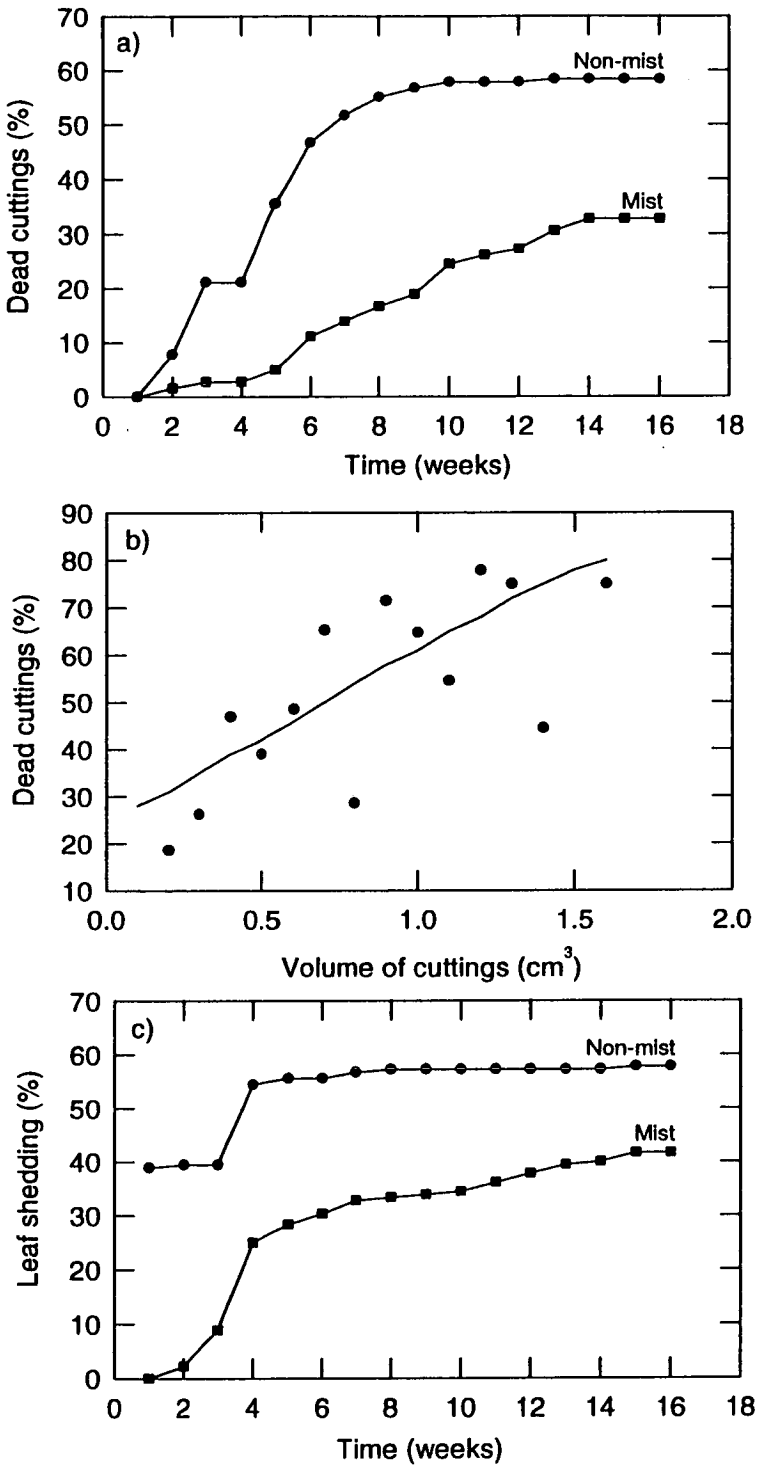


Figure 4.3 : Death rate of *S. leprosula* stem cuttings as affected by a) Propagation systems; b) Relationship of dead cuttings and volume of *S. leprosula* stem cuttings (points are groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model); c) Rate of leaf shedding of *S. leprosula* stem cuttings as affected by propagation systems (n=60 per treatment combination).

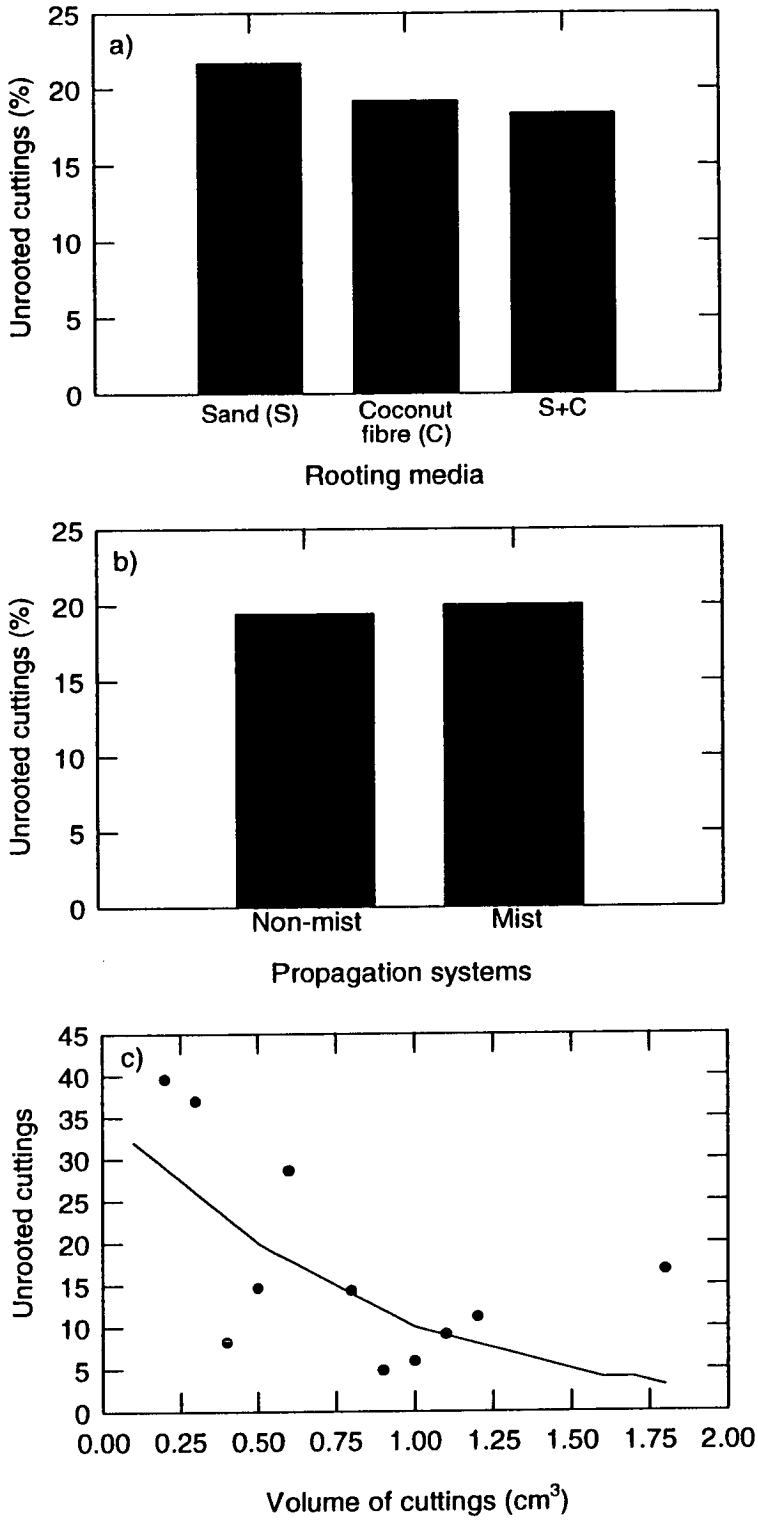


Figure 4.4 : Percentage of *S. leprosula* stem cuttings that remained unrooted at week 16 as affected by a) Rooting media; b) Propagation systems; c) Relationship of unrooted cuttings and volume of *S. leprosula* stem cuttings. Points are groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model (n=60 per treatment combination).

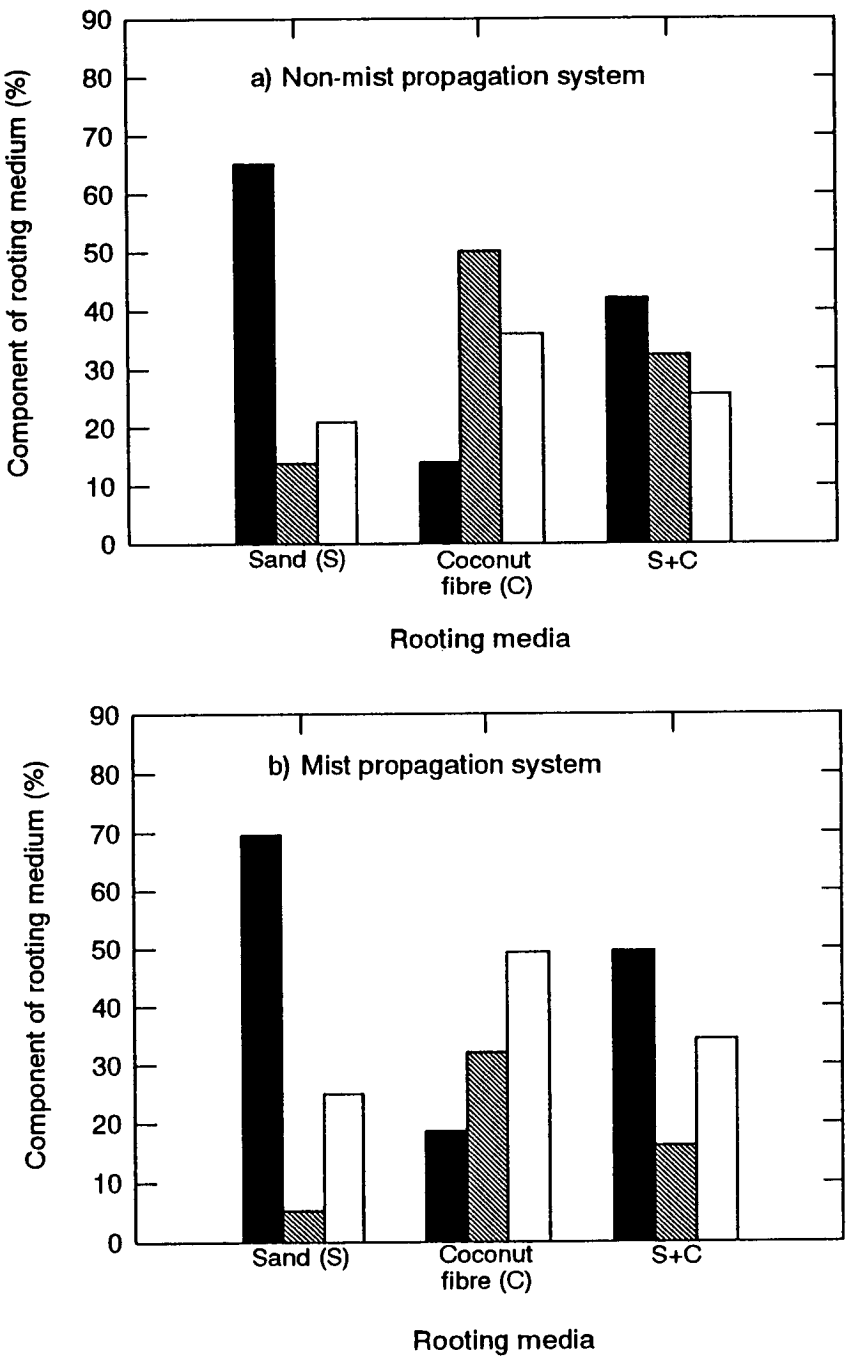


Figure 4.5 : Components of rooting media in a) Non-mist propagation system; b) Mist propagation system (solid bar=solid component; hatched bar=water component; hollow bar=air component; n=24 per treatment combination).

Table 4.1 : The environmental data in the non-mist and mist propagation systems measured from day 1 to day 50 of the experiment. Values of each variable were mean of 2 blocks calculated as 5 minutes average. Mean values for each variable were calculated over 24 hours period daily.

Propagation systems	Non-mist		Mist	
	Mean	Range	Mean	Range
Relative humidity (%)	99.48	78.85-100	99.96	85.30-100
Air temperature (° C)	27.24	23.14-37.81	26.91	23.11-37.15
Leaf temperature (° C)	27.26	23.32-37.20	27.06	23.28-36.72
VPD (kPa)	0.03	0-0.99	0.03	0-0.56
Irradiance (μmol photons m^{-2} s^{-1})	11.85	0-308.90	16.84	0-297.00

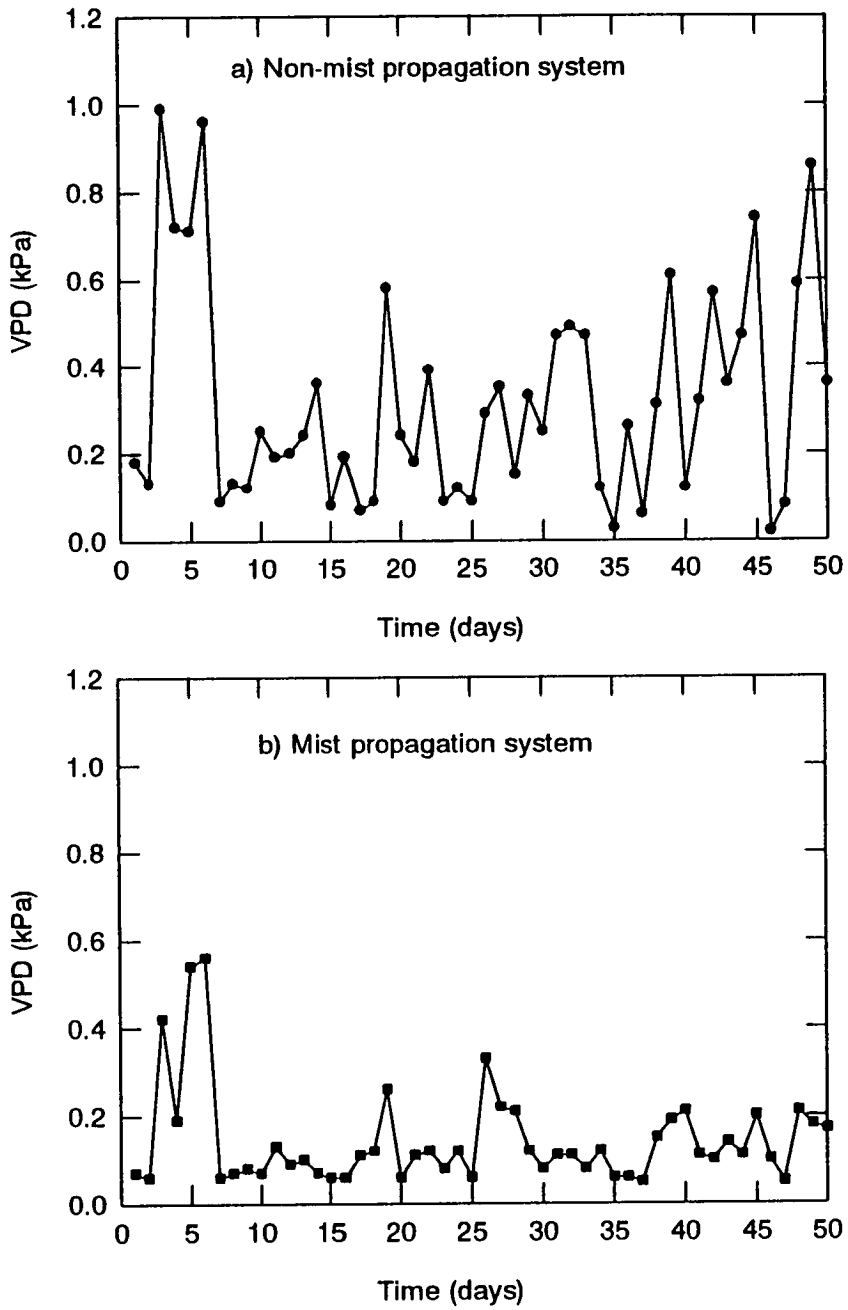


Figure 4.6 : Daily maximum VPD measured from day 1 to day 50 of the experiment in a) Non-mist propagation system; b) Mist propagation system. Maximum VPD per day was calculated as a 5 minute average.

Over the time periods of measurement, P_n of cuttings prior to rooting was not significantly influenced by the media but it was significantly higher in cuttings planted in non-mist than mist propagation systems (Table B8 and Figure 4.7a). P_n also differed significantly between days of measurement (Table B8 and Figure 4.8a). These differences were due to differences in PAR at the time of measurements (Table B9; Figures 4.7b and 4.8b). No significant difference in g_s of cuttings was obtained when measurements of P_n were made. Mean g_s ranged from 160 to 174 mmol H_2O m^{-2} s^{-1}.

Discussion and conclusions

The results of the current experiment indicate that rooting and number of roots produced by *S. leprosula* stem cuttings was not affected by any of the rooting media tested despite the differences in their physical component. Similar results have been obtained by Tolkamp and Aldrianto (1994) where no significant difference were found in stem cuttings of *S. leprosula* rooted in either aerated water or vermiculite media. Cuttings of other Dipterocarps were also reported to successfully root in several types of media having high or low water retaining capacity. For example, Smits (1983) reported that no difference in rooting of *S. obtusa* stem cuttings in sand or a mixture of sand and peat. Noraini and Ling (1993) obtained a high rooting percentage in stem cuttings of *S. parvifolia* and *S. acuminata* in coconut fibre or paddy husk. The number of roots on cutting was more in coconut fibre than paddy husk which they attributed to the physical properties of the medium although no related data had been presented (Noraini and Ling 1993). More than 80% rooting was achieved with *S. macrophylla* stem cuttings using river sand as the medium (Lo 1985). Many Dipterocarp species from the genus *Shorea* were also successfully rooted in an aerated water medium (Smits *et al.* 1994).

In other species, many workers have obtained a significant rooting response to the medium they tested. Mesen (1993) obtained significantly lower rooting percentage and fewer roots of *Cordia alliodora* stem cuttings in sawdust compared to sand

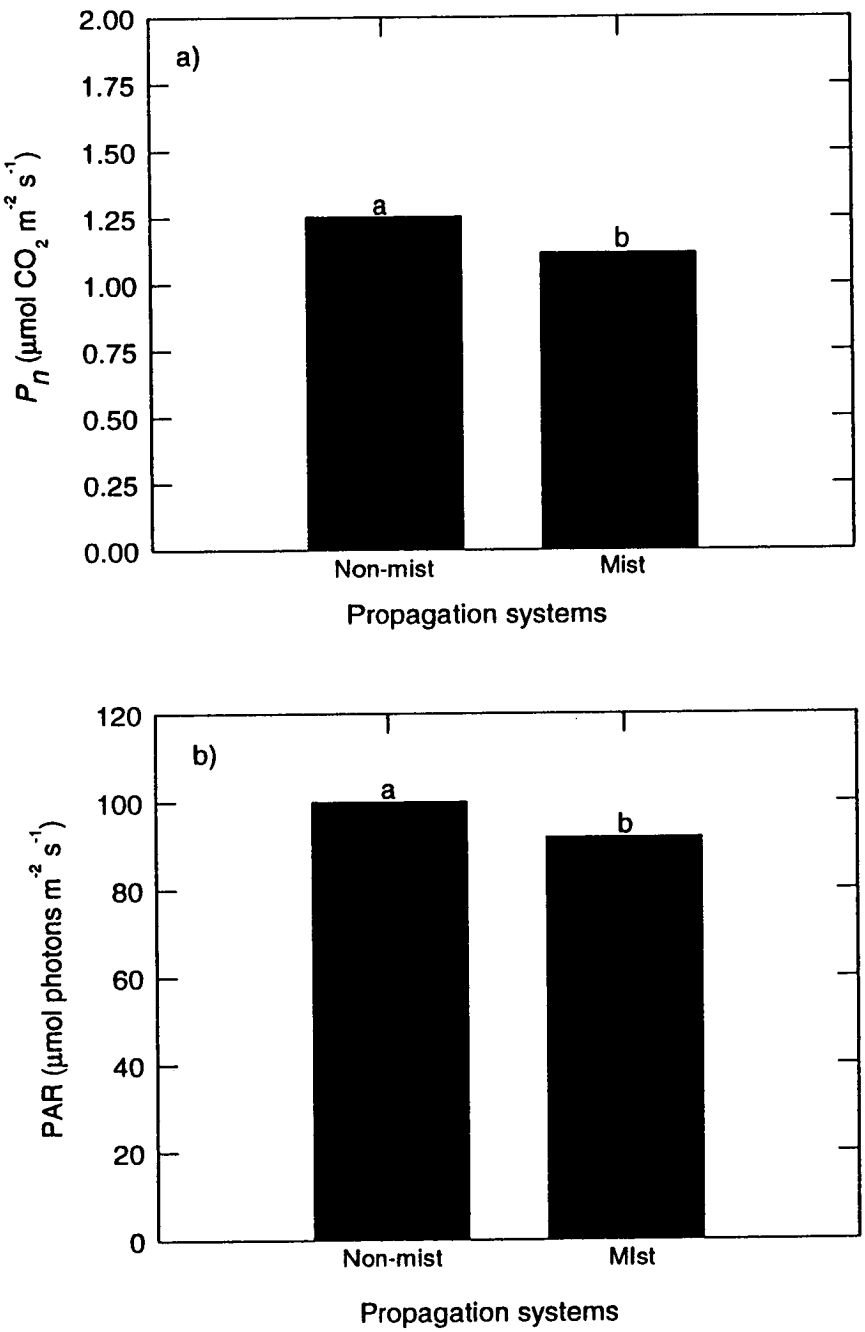


Figure 4.7 : a) Mean P_n of *S. leprosula* stem cuttings prior to rooting as affected by propagation systems; b) Mean PAR when P_n measurements were made (measurements were made on days 1, 8, 14, 21, 28; n=24 per treatment combination). Means with the same letters are not significantly different at $P \leq 0.05$.

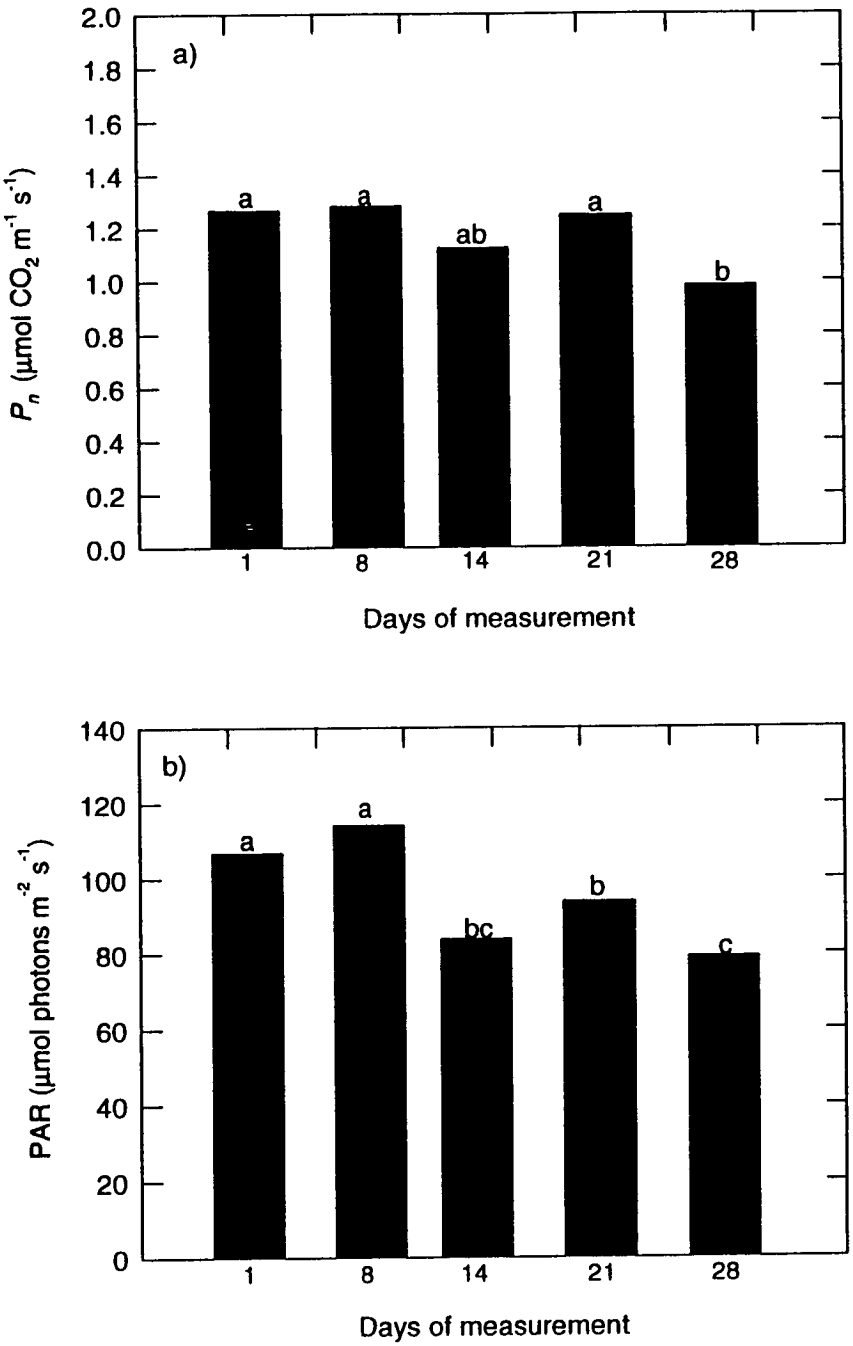


Figure 4.8 : a) Mean P_n of *S. leprosula* stem cuttings prior to rooting as affected by days of measurement; b) Mean PAR when P_n measurements were made on days 1, 8, 14, 21, 28 (n=24 per treatment combination). Means with the same letters are not significantly different at $P \leq 0.05$.

or gravel. Incorporation of sawdust into both gravel and sand was also detrimental to rooting of *Vochysia hondurensis* cuttings (Leakey *et al.* 1990). Similarly, rooting of *Lovoa trichiliodes* stem cuttings was significantly lower in a medium with higher water content (mixture of sand and soil) than the less water-retaining substrate such as sand or gravel (Tchoundjeu 1989). On the other hand, addition of sawdust to improve the water content of the rooting medium enhanced rooting of *Eucalyptus deglupta* cuttings (Leakey *et al.* 1990).

As indicated by the examples discussed above, a linear relationship between water uptake by cuttings and the water content of media as shown by Loach (1985) and Newton *et al.* (1993) was not well related to rooting. It is therefore difficult to pin point the relationship of rooting to the physical volumetric air or water content of the medium. No consistent guidelines could be made using this approach.

The effect of the design of the propagation system was more pronounced than the type of medium in the current experiment. Low rooting and high mortality of cuttings occurred in non-mist as opposed to mist propagator. Percentage of mortality corresponded to percentage of leaf shedding. The shedding of leaves was presumably due to a shock response soon after insertion. Cuttings may have experienced water stress during harvesting and the condition was worsened by the increase in VPD in the propagators which were not initially shaded as experiment was set up during rainy season. The shock response soon after insertion had also been observed in the non-mist propagators with cuttings of *A. guachepele* (Newton and Jones 1993b); *Calliandra calothyrsus* (Dick *et al.* 1994b). The sensitivity of cuttings of other *Shorea* species to water stress has been demonstrated by Lo (1985) where 75% of *S. macrophylla* cuttings were dead when misters were turned off at night. The extent of water deficit experienced by these cuttings could not be related to environmental condition since no data were presented (Lo 1985). However, the shock response soon after insertion of *S. leprosula* cuttings was less felt in the mist propagator in the current experiment since the leaves were most of the time covered with a film of water which has an advantage in lowering leaf temperature, which in turn reduced internal vapour pressure and hence decreased water loss through

transpiration. From environmental data collected, VPD in the mist was found to be consistently lower than that in the non-mist propagators, results correspond to that reported by Loach (1988a) but contradict with Newton and Jones (1993a). This could perhaps be due to the good sealing of the polythene in the enclosed mist propagators used in the current experiment compared to those of Newton and Jones (1993a). Frequent watering from the misters has an advantage of keeping higher humidity and low VPD compared to non-mist propagator which easily trapped heat during high irradiance and caused a rise in VPD.

The negative correlation between rooting and volume of cuttings showed that larger volume cuttings were less suitable for rooting and they were more prone to death. These large volume cuttings could be associated with larger diameter since cuttings were cut to the same length. Larger diameter cuttings had probably undergone secondary growth and thickening of lignin layer which may create physical barrier to root initiation (Hartmann *et al.* 1990; Liew 1992). Generally the lignified cuttings were poor rooters (Hartmann *et al.* 1990). The negative relationship between rooting and initial volume of cuttings may also reflect the fact that rooting was not influenced by the initial carbohydrate reserves; current assimilates produced by cuttings were perhaps sufficient to support rooting. Initial volume of cuttings may be associated with initial volume of carbohydrate reserves in these cuttings. Similar interpretation has been made by Leakey and Coutts (1989); Veierskov (1988). In the current experiment, cuttings were found to photosynthesise prior to rooting. Higher P_n in cuttings planted in the non-mist compared to the mist propagators was due to higher irradiance experienced by cuttings in the non-mist propagators at the time P_n measurements were made. Even though the death of cuttings that shed their leaves could be due to their inability to recover from water stress, absence of current assimilates from the leaves may partly be the reason, since these leafless cuttings eventually died when the carbohydrate reserves had been depleted. Leafless cuttings of other species have been reported to either root poorly or not root at all (Leakey *et al.* 1982b; Aminah 1991b; Newton *et al.* 1992). There has been increasing evidence showing unrooted leafy cuttings photosynthesise during propagation and

current assimilates produced were essential to rooting (Leakey and Storeton-West 1992; Newton *et al.* 1992; Hoad and Leakey 1993; Mesen 1993).

Many cuttings with a smaller volume/diameter also remained unrooted, perhaps these thin cuttings may be saturated with starch which then inhibited post-severance P_n and consequently reduced rooting as suggested by Leakey *et al.* (1994). Smaller diameter cuttings may also have less total respiratory/metabolic activity at their basal end, one of the features that had been shown to decrease rooting potential of the thin cuttings (Dick *et al.* 1994a).

From the above results, it may be concluded that *S. leprosula* stem cuttings can be rooted in any of the media tested. It is recommended that river sand medium is used because it is easily available and cheaper than coconut fibre. The current experiment also indicated that rooting of *S. leprosula* stem cuttings in non-mist was poor compared to that of mist system. However, I feel that it may not reflect the actual results since poor rooting was most probably due to the shock response to water stress soon after they were inserted in the rooting medium. Rooting of *S. leprosula* in the non-mist system merits further testing since this system offers great advantage as it is simple, cheap to construct, and does not require piped water and electricity (Leakey *et al.* 1990). The rooting ability of *S. leprosula* stem cuttings in non-mist and mist systems was tested again to confirm or refute of the results obtained; and this is reported in Experiment 2.

EXPERIMENT 2: Effect of the non-mist and mist propagation systems on the rooting ability of *Shorea leprosula* leafy stem cuttings

Materials and methods

Cutting materials and experimental layout

The experiment took place in the FRIM nursery in March 1993. A total of 204

cuttings from all the node positions with at least 30 cm^2 were taken from six month old stock plants raised under 33% full sunlight as potted rooted cuttings. Sixteen clones were used: 40, 110, 138, 153, 167, 184, 188, 191, 501, 507, 519, 537, 553, 559, 583, 589. The preparation of cuttings is as described in chapter 3. The leaf area retained on each cutting was 30 cm^2. Initial diameter, length and node position of each cutting were recorded. The prepared cuttings were planted in a medium consisting of cleaned river sand in the non-mist and mist propagation systems. The two treatments were randomly allocated to the node positions so that there was no confounding between treatments and position on the stock plants from which cuttings were taken. The number of cuttings obtained per stock plant varied from 3 to 7 depending on availability of leaves on the node. Clones with less than 4 cuttings were not used for the experiment. Each treatment consisted of 102 cuttings randomly split into two blocks with 51 cuttings per block. The non-mist propagator was constructed based on that described in Leakey *et al.* (1990) and the mist propagator was a closed polythene propagator (1 m x 1 m x 0.8 m) with a misting unit in the centre. Details and illustrations of the propagation systems used is as described in chapter 3.

Microclimate of propagator

Temperature, relative humidity and irradiance were recorded by a data logger (21X micrologger, Campbell Scientific, UK) using sensors as described in chapter 3. The sensors were placed in the centre of each block in both propagation systems. The data logger was programmed to scan each sensor every 60 seconds, and to calculate and store mean readings every 5 minutes. Data collection extended from day 1 to day 24 of the experiment .

Photosynthetic rate (P_n) and stomatal conductance (g_s)

P_n and g_s of cuttings prior to rooting were measured using a portable infrared gas analyser (LCA-3, ADC, Hoddesdon, UK). Twelve cuttings were randomly chosen

per treatment per block and they were measured on days 1, 8, 14, 21 and 28 after planting in rooting media. P_n and g_s of rooted cuttings and cuttings that remained unrooted were measured on day 63. Ten rooted and unrooted cuttings per treatment per block were randomly chosen. Measurements of P_n and g_s were carried out between 09:00 to 12:00 hours.

Assessment of cuttings and statistical analyses

Assessment was made weekly until week sixteen, for rooted, unrooted and dead cuttings as well as number of roots on each cutting.

Analysis of deviance for stepwise regression in Genstat 5 (Payne *et al.* 1987) was used to determine which of the recorded variables were significantly associated with rooting of cuttings. For this analysis, only the variables that are significantly different were shown in the tables. Analysis of variance followed by Fisher's t test (LSD) was used to test for significant differences in accumulated number of roots per rooted cutting, P_n and g_s. Results in all the tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

Results

The initial length, diameter and volume of cuttings used in the experiment did not differ significantly between treatments. Mean of these variables is given in Table 4.2.

Rooting was not significantly influenced by either the propagation systems or morphological characteristics of cuttings. Similar results were obtained by the percentage of dead cuttings or cuttings remaining unrooted. Figure 4.9a,b,c show the rooting rate, death rate and percentage of cuttings which remained unrooted at week 16 of *S. leprosula* stem cuttings in both propagation systems.

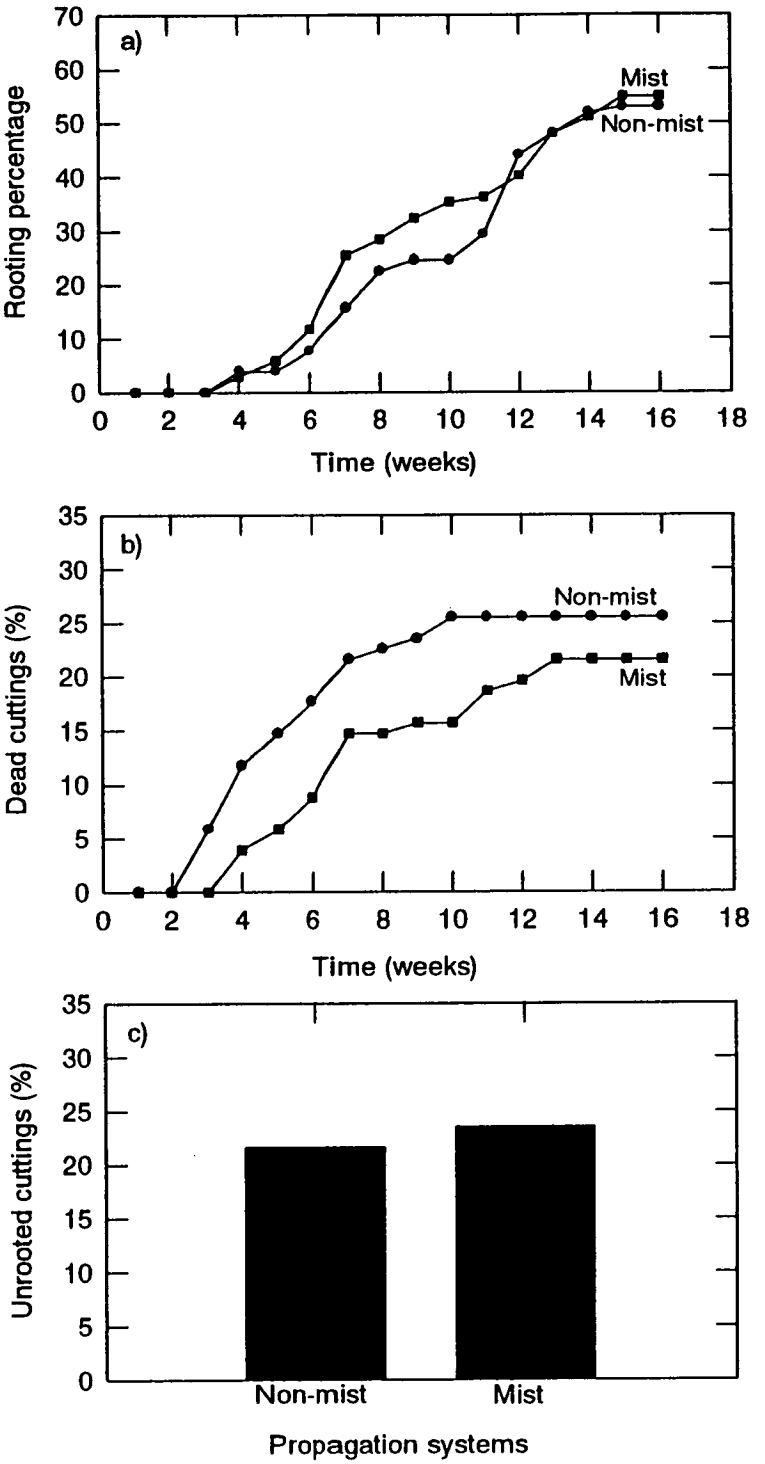


Figure 4.9 : a) Rooting rate of *S. leprosula* stem cuttings in the non-mist and mist propagation systems; b) Death rate of *S. leprosula* stem cuttings in the non-mist and mist propagation systems (circle=non-mist; square=mist system); c) Percentage of *S. leprosula* stem cuttings that remained unrooted at week 16 as affected by the propagation systems (n=102 per treatment).

Unlike rooting, number of roots per rooted cutting was significantly enhanced in non-mist versus mist propagation system (Table B10 and Figure 4.10a). Number of roots was also significantly affected by volume of cuttings and the relationship was negative (Table B10 and Figure 4.10b).

Table 4.2 : Mean values of initial length, diameter and volume of *S. leprosula* stem cuttings rooted in the non-mist and mist propagation systems. Stock plants were grown at 33% full sunlight.

Variables	Non-mist system	Mist system	Number of samples per treatment (n)
Length (cm)	6.26a	5.82a	102
Diameter (cm)	0.46a	0.46a	102
Volume (cm^3)	1.11a	0.97a	102

Means followed by the same letters are not significantly different at $P \leq 0.05$

Environmental data on the rooting beds are shown in Figures 4.11 a,b,c,d and Table 4.3. There was significant difference in mean and maximum VPD between the two propagation systems. Maximum and mean daily VPD were significantly higher in non-mist than in mist system despite equal shading to both propagators was given (Tables B11, B12; Figures 4.11c,d). Maximum and mean irradiance were not significantly different between the two systems.

There was no significant difference between treatments in P_n and g_s of cuttings prior to rooting. Mean P_n was 2.0 and 1.9 μmol CO_2 m^{-2} s^{-1} and mean g_s was 268 and 265 mmol H_2O m^{-2} s^{-1} for cuttings in non-mist and mist propagation systems. No significant difference in PAR was obtained when the measurements were made. Mean PAR was 152 and 146 μmol photons m^{-2} s^{-1} for non-mist and mist

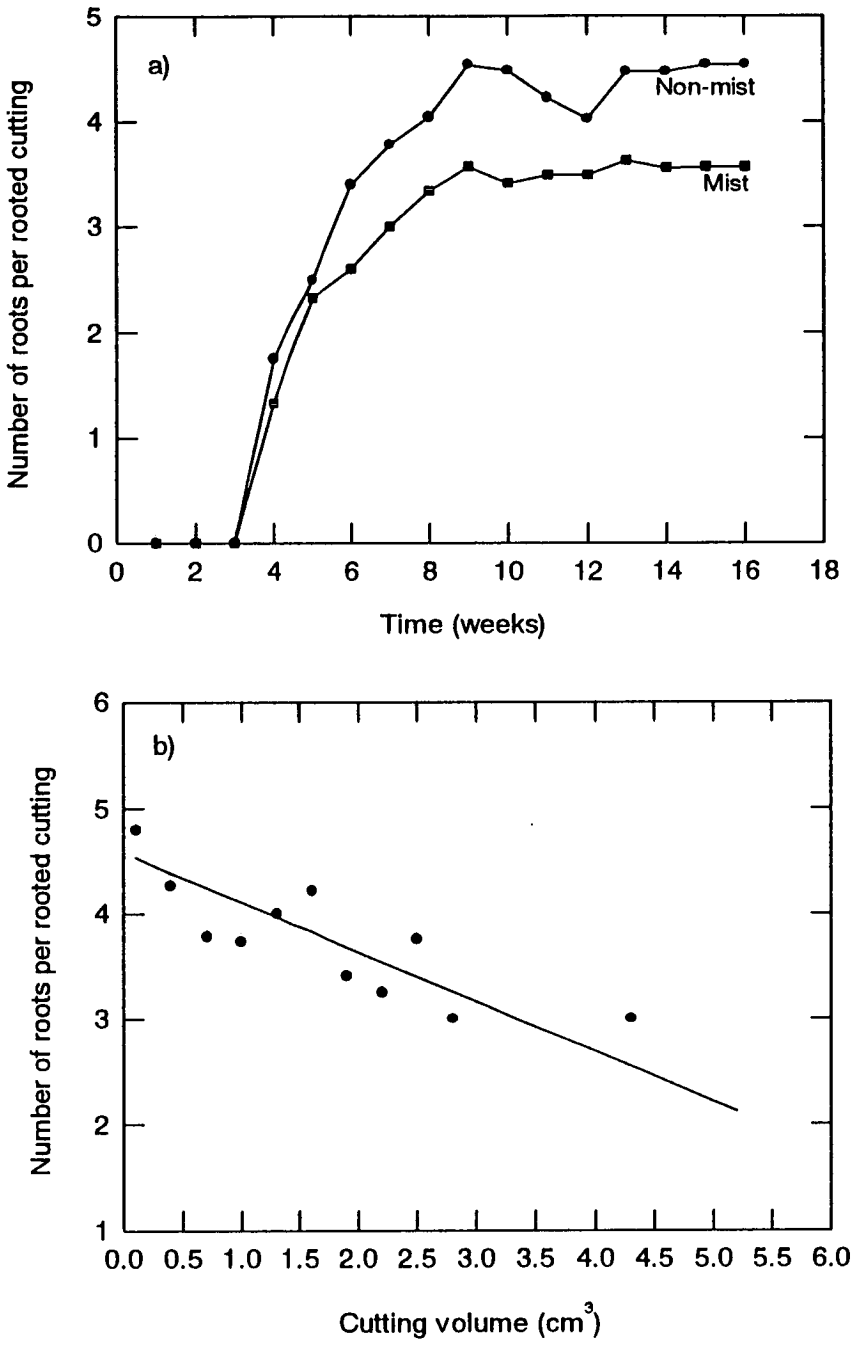


Figure 4.10 : a) Rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* (n=102 per treatment); b) Relationship of mean accumulated number of roots per rooted cutting and cutting volume of *S. leprosula.* Points represent groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model.

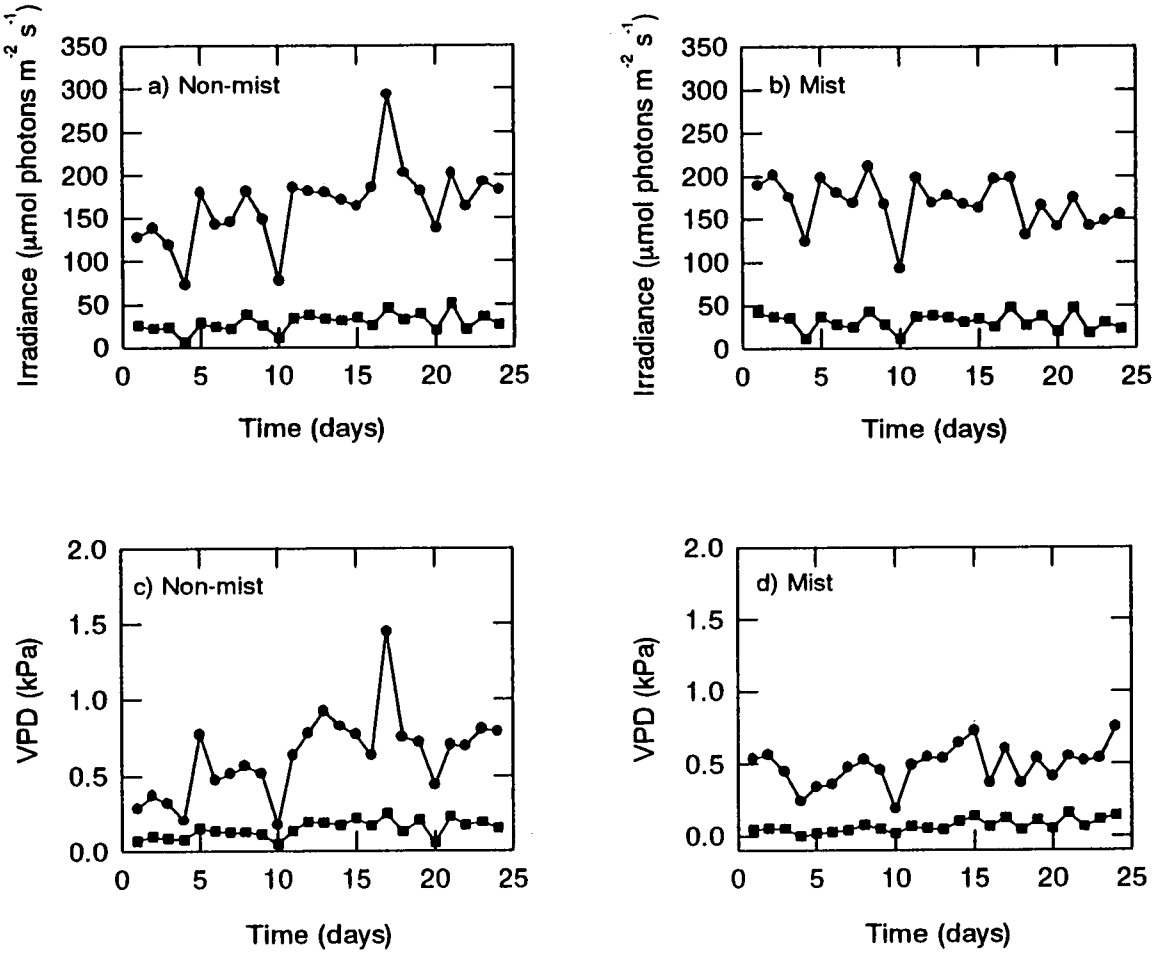


Figure 4.11 : Environmental data collected from day 1 to day 24 of the experiment. a) Daily irradiance in non-mist system; b) Daily irradiance in mist system; c) Daily VPD in non-mist system; d) Daily VPD in mist system (circle=maximum values; square=mean values). Data points of each variable represent mean values of 2 blocks per treatment calculated as 5 minutes average. Mean values of irradiance and VPD were calculated on 24 hours period daily.

propagation systems respectively. P_n was also not affected by days of measurement. Mean range of P_n between days of measurement was 1.7 to 2.2 μmol CO_2 m^{-2} s^{-1}.

P_n of rooted cuttings measured on day 63 was significantly higher than that of cuttings which remained unrooted (Table B13 and Figure 4.12a). This was associated with higher g_s (Table B14 and Figure 4.12b). No significant difference was obtained in PAR when the measurements were made. Mean PAR was 111 and 117 μmol photons m^{-2} s^{-1} for the rooted and unrooted cuttings respectively.

Table 4.3 : Environmental data in the non-mist and mist propagation systems measured from day 1 to day 24 of the experiment. Values for each variable were mean of 2 blocks calculated as 5 minutes average. Mean for each variable were values calculated over 24 hours period daily.

Propagation systems	Non-mist		Mist	
	Mean	Range	Mean	Range
Irradiance (μmol photons m^{-2} s^{-1})	28.94	0-293.00	31.35	0-211.20
Relative humidity (%)	96.49	74.85-100	99.03	87.85-100
Air temperature (° C)	28.67	23.85-38.64	28.25	23.49-38.76
Leaf temperature (° C)	28.67	23.94-37.42	28.34	23.40-39.06
VPD (kPa)	0.15	0-1.45	0.07	0-0.75

Discussion and conclusions

As stated in the introduction, Dipterocarp species have been rooted in several types of propagation systems. Results of the present experiment indicate that *S. leprosula*

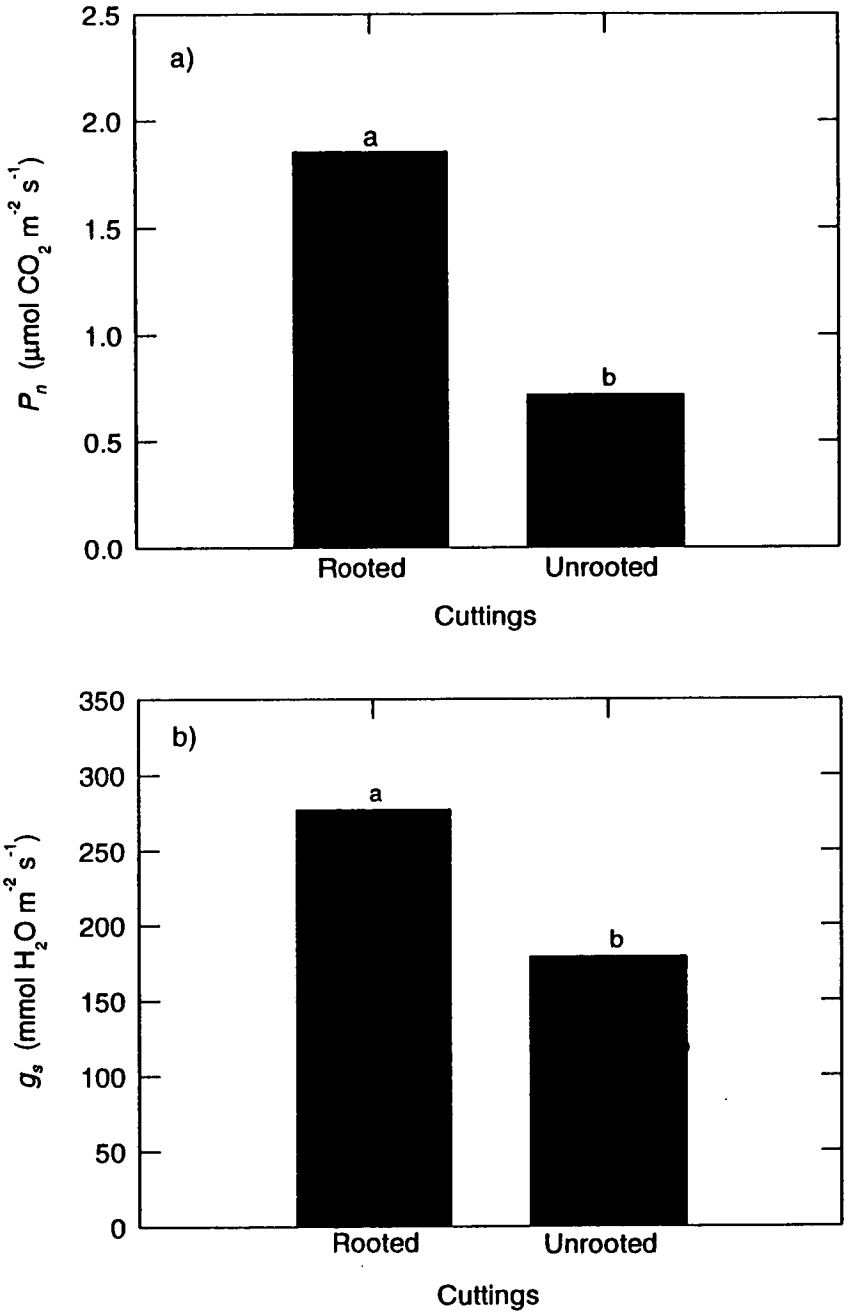


Figure 4.12 : a) Mean P_n of rooted stem cuttings and mean P_n of cuttings that remained unrooted of *S. leprosula*; b) Mean g_s of rooted stem cuttings and mean g_s of cuttings that remained unrooted of *S. leprosula* (measurements were made on day 63; n=20 per treatment for rooted and unrooted stem cuttings). Means with the same letters are not significantly different at $P \leq 0.05$.

stem cuttings root equally well in non-mist and mist propagation systems as long as the environments are conducive to rooting. Mean VPD in both propagation systems could be kept close to zero. Cuttings were kept moist during harvesting and propagators were properly shaded to avoid any shock response soon after insertion. However, periods of water deficit did occur as indicated by the daily maximum VPD which was more than the threshold level (0.5 kPa) suggested by Grange and Loach (1983a) for many broadleaved species. This temporary water deficit seemed to be tolerated by *S. leprosula* stem cuttings. The ability of cuttings of other tropical species to tolerate such temporary water deficit and eventually root has been noted in recent work by Mesen (1993); Newton and Jones (1993b).

The more roots per rooted cutting of *S. leprosula* stem cuttings from non-mist propagation system could perhaps be due to more efficient use of auxin and nutrients for root development since leaching in these cuttings is less likely to occur compared to those in mist system. This aspect has to be further investigated. Negative correlation between number of roots and initial volume of cuttings may suggest that cuttings were not influenced by initial carbohydrates reserves for root development. Such relationship may also reflect the importance of current photosynthates in root development. Now that it is possible to measure photosynthesis on rooting beds, there is increasing evidence indicating that photosynthetic rates may be appreciable even before rooting takes place, with an influence on rooting of cuttings of several tree species (Elliasson and Brunes 1980; Davis and Potter 1981; Leakey and Storeton-West 1992; Newton *et a*l. 1992; Smalley *et al.* 1991; Hoad and Leakey 1993; Mesen 1993).

P_n of rooted cuttings was higher than that of cuttings which did not root and this was associated with higher g_s. Similar results were reported by Newton *et al.* (1992); Hoad and Leakey (1993). P_n of rooted cuttings may be enhanced by the presence of roots as sink for assimilates (Wareing *et al.* 1968; Okoro and Grace 1976; Elliasson and Brunes 1980). Also the increase P_n in rooted cuttings may presumably be due to roots supplying leaves with cytokinin which may increase the

activity and/or amount of carboxylating enzymes (Okoro and Grace 1976). However, this possibility has not been critically evaluated.

Results of the present experiment confirmed that *S. leprosula* stem cuttings could be successfully propagated in the non-mist and mist propagation systems. The latter system is recommended since it offers several advantages as discussed in the earlier paragraph. This non-mist system is very practical for the nurseries established in forest areas where facilities such as electricity or piped water are unavailable.

Although the rooting of *S. leprosula* stem cuttings is equally good in non-mist and mist system, it was decided to continue the experiments in the mist propagation system since facilities have already been established and there are so far only a limited number of non-mist propagators available. As noted in the present experiment and experiment 1, it is important to understand the water deficit and physiological aspects of cuttings for effective management of any propagation system. Chapter 5 further investigates the influence of water deficit as well as the physiological and water relations of *S. leprosula* stem cuttings in mist propagation system.

CHAPTER 5

THE INFLUENCE OF ROOTING ENVIRONMENT ON THE WATER RELATIONS AND PHYSIOLOGY OF ROOTING OF *SHOREA LEPROSULA* LEAFY STEM CUTTINGS

This chapter reports the microclimates, water relations and physiological aspects of cuttings planted in the mist propagation system. The rooting environments were examined with two different approaches: i) by regulating the misting frequencies in the propagators and ii) by varying the irradiance levels in the propagators through shading.

EXPERIMENT 1: Effect of misting frequencies on the rooting ability of leafy stem cuttings of *Shorea leprosula.*

Introduction

Mist propagation systems are the most commonly used type of system in Malaysia for rooting cuttings of tree species including Dipterocarps, either with or without enclosures (Momose 1978; Muckadell and Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Lo 1985; Aminah 1991c; Noraini and Ling 1993). The variability in rooting success of Dipterocarps under the mist system, obtained in previous trials by many researchers, could not be well explained since no detailed quantification of the propagation environment or physiological processes had been made. It has been noted in many studies that newly planted cuttings prior to rooting were sensitive to water deficit which in turn affect physiological processes of cuttings and hence their rooting ability (Loach 1977; Loach 1988 a,b; Grange and Loach 1983a, 1984; Newton and Jones 1993b). The limits of tolerance to water deficits in cuttings have not been well defined, as stated in Newton and Jones (1993b). The present study was carried out to determine the extent of water deficit

affecting the physiology, water relations and rooting ability of *S. leprosula* stem cuttings by regulating misting frequencies in the respective propagators.

Materials and methods

Cutting materials and experimental layout

The experiment took place in the FRIM nursery in April 1994. A total of 396 single node leafy stem cuttings were taken from seven month old stock plants raised under 33% full sunlight as potted rooted cuttings. Twenty eight clones used were: 50, 62, 63, 68, 78, 82, 105, 106, 110, 114, 144, 172, 173, 184, 194, 507, 508, 521, 571, 578, 581, 589, 629, 634, 651, 667, 687, 692. The length and leaf area of each cutting was 5 cm and 30 cm^2 respectively. Preparation of cuttings is as described in chapter 3. The initial diameter and node position of each cutting were recorded. The prepared cuttings were planted in a medium consisting of cleaned river sand. These cuttings were then subjected to three different misting frequencies: 1 hour; 3 hours and 6 hours. The duration of each burst of misting was 1 minute. The three treatments were randomly allocated to the node positions so that there was no confounding between treatments and the position on the stock plants from which cuttings were taken. The number of cuttings obtained per stock plant varied from 3 to 7 depending on availability of leaves on the node. Clones with less than 9 cuttings were not used for the experiment. Each treatment consisted of 132 cuttings (60 and 72 cuttings per treatment were used for rooting and RWC assessments respectively); and these cuttings were randomly laid out in three blocks. Each block is a closed polythene propagator (1 m x 1 m x 0.8 m). The layout of this experiment poses some problems since no true blocking could be created. The blocks referred in this experiment was actually plots on the same rooting beds having different propagators. This was unavoidable because misting frequency could be regulated by beds and not by propagators. Therefore complete randomised block design could not be adopted. Details and illustrations of the propagation system used is as described in chapter 3.

Microclimate of propagator

Temperature, relative humidity and irradiance were recorded using a data logger (21X micrologger, Campbell Scientific, UK) with respective sensors as described in chapter 3. The sensors were placed in the centre of one block, which was randomly chosen from the total of three blocks of the respective misting frequencies. The data logger was programmed to scan each sensor every 60 seconds, to calculate and store readings every 5 minutes. Data collection extended from day 1 to day 28 of the experiment.

Relative water content (RWC)

The RWC of the leaf from different misting frequencies was determined using the method described by Beadle *et al.* (1987). Details of the method are as described in chapter 3. RWC was determined at three different times of the day (00:90, 13:00 and 17:00 hours) on days 1, 8, 14 and 21. At each time of measurement (e.g 09:00 hours) 24 discs per treatment were sampled from 6 cuttings (2 cuttings were randomly chosen per block and 4 discs were obtained per cutting). Therefore, number of cuttings harvested for RWC assessment per treatment per day was 18, giving a total of 72 cuttings per treatment (288 discs) for 4 days.

Photosynthesis rate (P_n) and stomatal conductance (g_s)

P_n and g_s of cuttings prior to rooting were measured using an infra red gas analyser (LCA-3, ADC, Hoddesdon, UK). Four cuttings per treatment per block were randomly chosen for the measurement. These same cuttings were measured on days 1, 8, 14 and 21 at three different times of the day (starting from 09:00, 13:00 and 17:00 hours; each time measurement lasted for ca. 2 hours). At each time of measurement (e.g. 09:00 hours), 12 P_n and g_s values were measured per treatment giving a total of 36 values per day (144 values per treatment for 4 days).

Assessment of cuttings and statistical analyses

Assessment on cuttings was made weekly starting one week after planting until week sixteen for rooted, unrooted and dead cuttings as well as number of roots on each cutting.

Analysis of deviance for a stepwise regression (Payne *et al.* 1987) was used to determine which of the recorded variables were significantly associated with rooting. Method for the graphical presentation of association between rooting and the corresponded variable is described in experiment 1 of chapter 4. Analysis of variance followed by Fisher's t test (LSD) was used to test significant difference between treatments for number of roots, VPD, RWC, P_n and g_s. Results in all the tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

Results

Initial diameter and volume of cuttings between treatments was not significantly different. Mean cutting diameter was 0.36, 0.39, and 0.38 cm whilst mean cutting volume was 0.56, 0.66 and 0.60 cm^3 for 1, 3 and 6 hours of misting frequencies respectively.

There was no significant difference obtained in rooting of cuttings between misting frequencies tested (Figure 5.1a). Rooting of cutting was significantly affected by initial cutting volume and the relationship was negative (Table B15 and Figure 5.1b).

The number of roots was significantly affected by the treatments (Table B16) and significantly fewer roots were obtained in 6 hours of misting interval than with the other two misting frequencies (Figure 5.2).

Mortality at week 16 was not significantly affected by treatments (Table B17 and Figure 5.3a). Regression analysis indicated that mortality of cuttings increased with

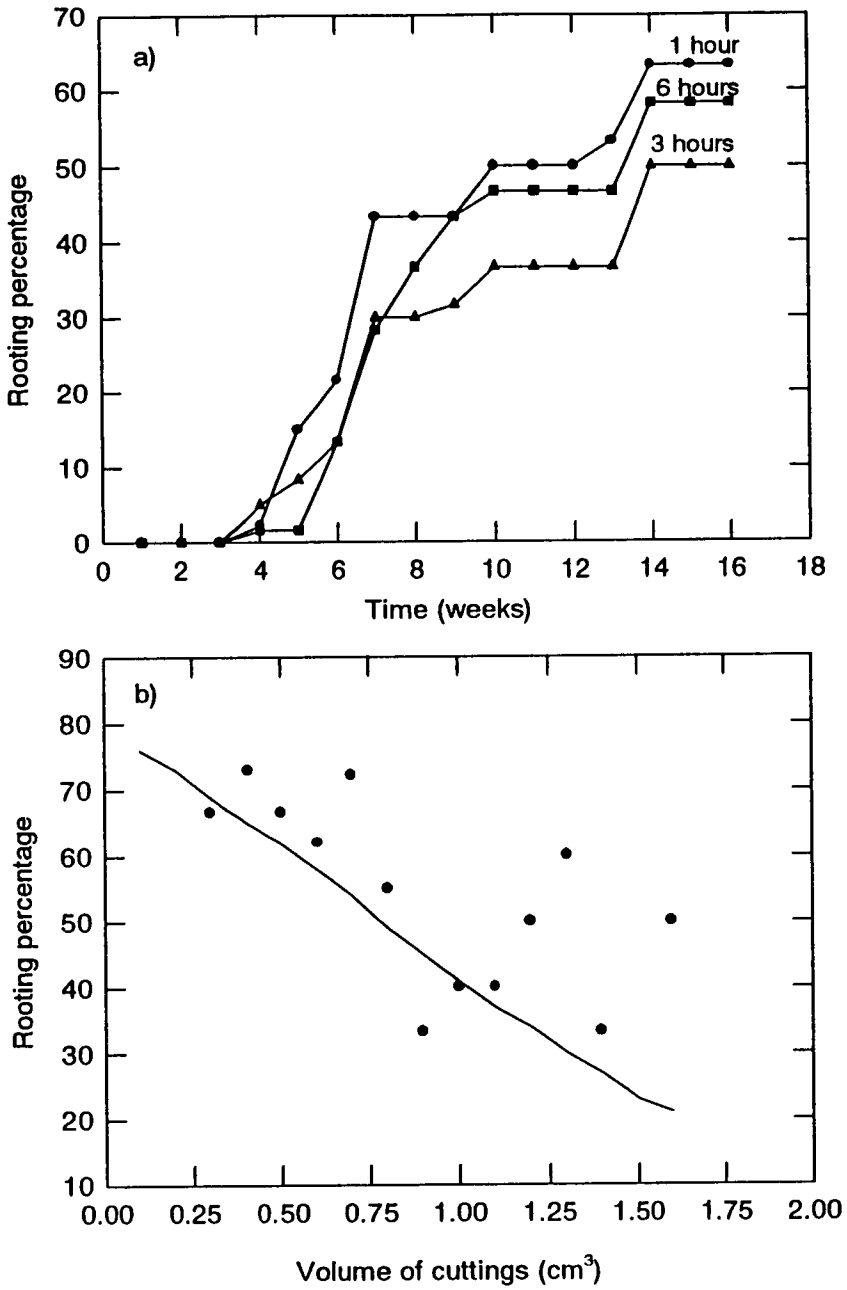


Figure 5.1 : Effect of misting frequencies on a) Rooting rate of *S. leprosula* stem cuttings (n=60 per treatment); b) Relationship of rooting and cutting volume of *S. leprosula*. Points are groups of observed data whilst line was drawn by connecting predicted values computed from the multiple regression model.

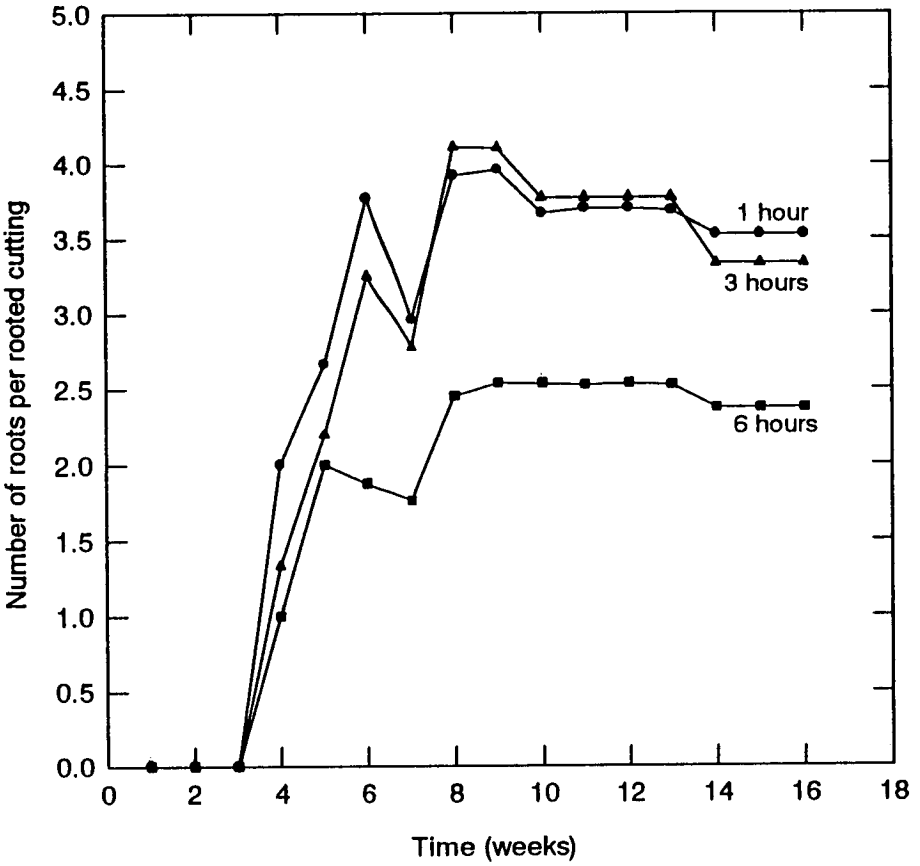


Figure 5.2 : Effect of misting frequencies on a) Rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* (n=60 per treatment).

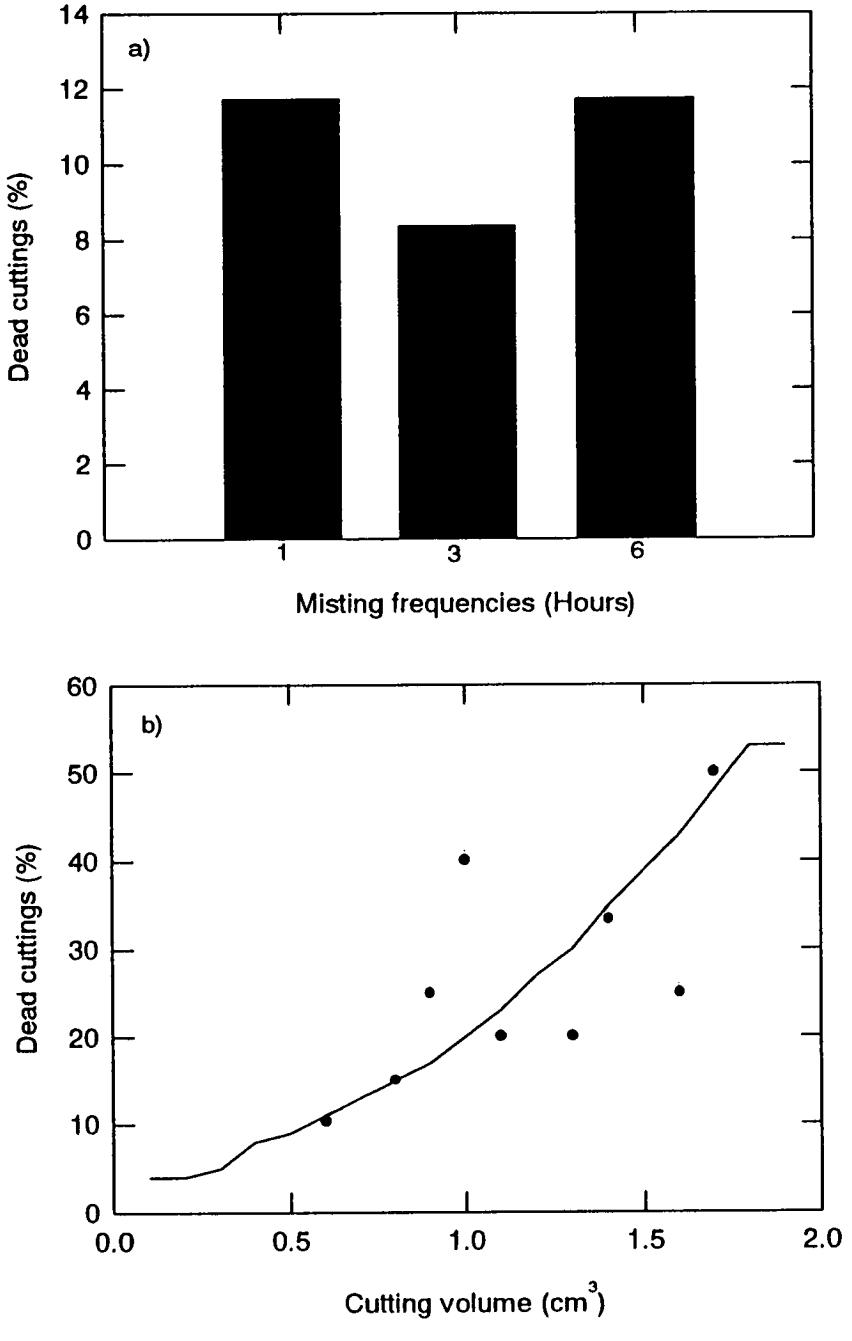


Figure 5.3 : Effect of misting frequencies on a) Mean percentage of dead cuttings at week 16 as affected by misting frequencies (n=60 per treatment); b) Relationship of dead cuttings and cutting volume of *S. leprosula*. Points are groups of observed data whilst line was drawn by connecting predicted values computed from the multiple regression model.

cutting volume (Table B17 and Figure 5.3b). Percentage of cuttings that remained unrooted at week 16 was not significantly influenced by either treatments or morphological characteristics of cuttings. Mean values of unrooted cuttings at week 16 were 25%, 42% and 30% for 1, 3 and 6 hours misting frequencies respectively.

Environmental data collected in propagators over a period of 28 days is shown in Table 5.1. There was a significant difference in daily maximum VPD and irradiance between treatments (Tables B18 and B19). Daily maximum VPD was significantly higher in 3 hours misting compared to that of 1 hour and 6 hours misting (Figures 5.4a,b,c). Despite less frequent misting, VPD in 6 hours did not differ significantly from 1 hour misting, possibly due to significantly lower irradiance received in the 6 hours treatment because of its position in the cutting shed compared with the other two treatments (Figures 5.4d,e,f).

There was a significant interaction between treatments with times of day and days of measurement on RWC of *S. leprosula* stem cuttings (Table B20). The RWC was significantly higher with 1 hour than with 3 hours misting (Figure 5.5a). The RWC recorded was highest in the morning, implying that cuttings recovered from the previous day's water deficit as indicated by lower RWC at 13:00 and 17:00 hours (Figure 5.5b). Stomatal conductance was also significantly affected by treatments, times and days of measurement. Significant interaction was also obtained on g_s between times and days of measurement (Table B21). Stomatal conductance exhibited a similar trend as RWC in response to treatments and times of measurement (Figures 5.6a,b). Significantly higher value of RWC and g_s were obtained 21 days after insertion (Figures 5.7 a,b). Mean RWC measured ranged from 79% to 92% while mean g_s varied from 80 to 216 mmol H_2O m^{-2} s^{-1} depending on treatments, times and days of measurement. P_n was significantly influenced by interactions of treatments and times; times and days of measurement (Table B22). P_n was significantly lower in 6 hours misting, presumably being associated with significantly low PAR (Table B23 and Figures 5.8 a,b). An influence of the diurnal cycle of PAR on P_n was obvious with the rate being significantly lower in the morning than afternoon and towards evening where cuttings were mainly respiring (Figures 5.9 a,b).

Table 5.1 : Environmental data in the propagators subjected to different misting frequencies measured on day 1 to day 28 of the experiment. Data for each variable was mean values of a 5 minute average. Mean value of each variable was calculated over a 24 hour period daily.

Misting Frequencies	1 Hour		3 Hours		6 Hours	
	Mean	Range	Mean	Range	Mean	Range
Relative humidity (%)	97.12	61.02-100	97.56	54.88-100	97.46	53.3-100
Air Temperature (° C)	28.34	20.20-39.04	28.73	20.16-37.95	27.92	21.07-36.20
Leaf Temperature (° C)	28.31	20.85-39.48	29.13	21.59-39.19	28.02	20.70-36.69
VPD (kPa)	0.12	0-2.28	0.18	0-3.06	0.13	0-2.05
Irradiance (µmol photons m^{-2} s^{-1})	22.46	0-204.30	25.63	0-200.00	19.22	0-184.50

Discussion and conclusions

Rooting of *S. leprosula* stem cuttings did not seem to be affected by frequencies of misting despite differences in water status of cuttings. This may suggest that differences in water status were too slight to have an effect. This was most probably so since RWC measured (>80%) was relatively high in every treatment and at different times of day. Values of RWC were comparable to those values

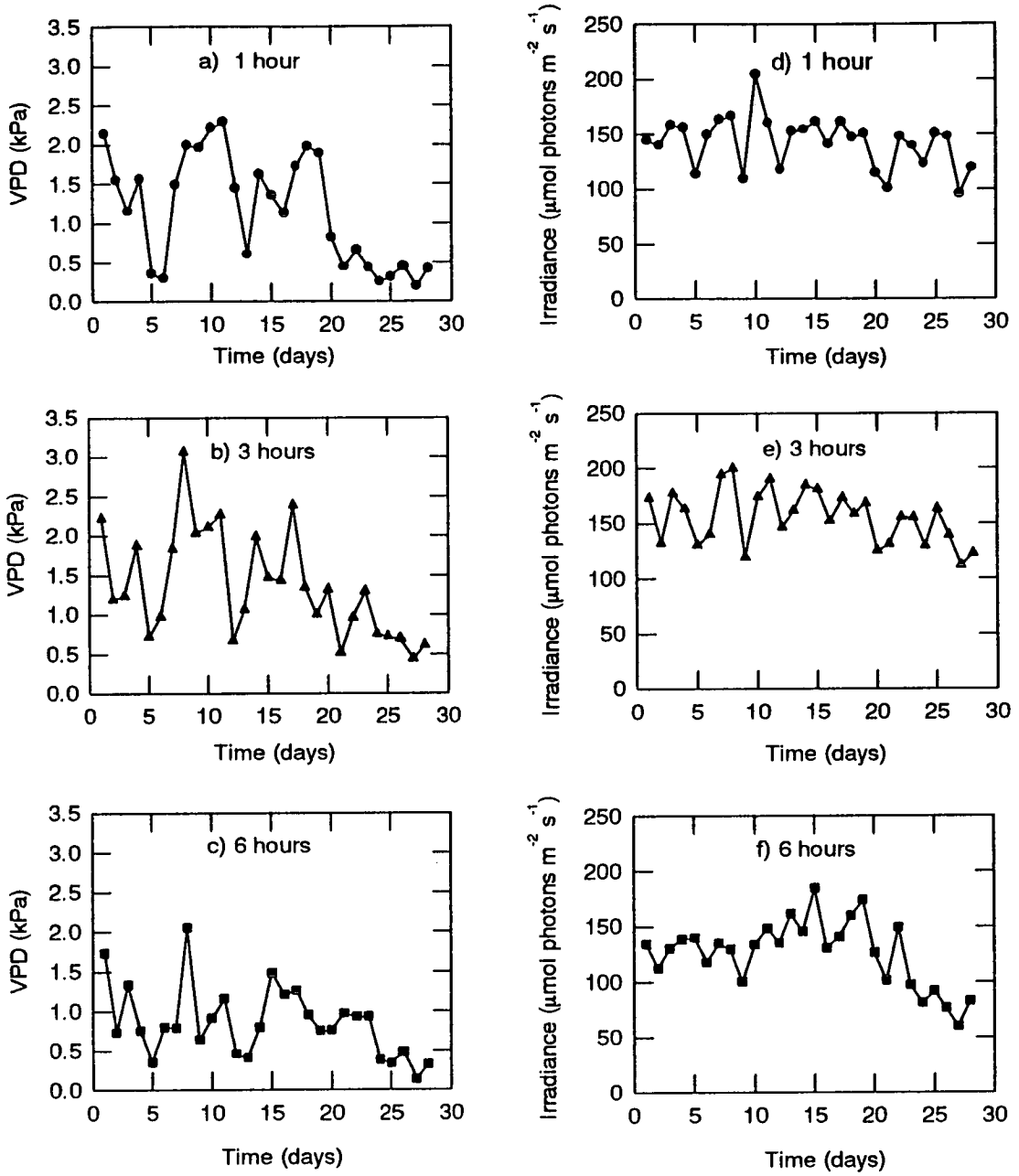


Figure 5.4a,b,c,d,e,f : Daily maximum VPD and irradiance in propagators with different misting frequencies measured from day 1 to day 28 of the experiment. Daily maximum VPD and irradiance were calculated as a 5 minute average.

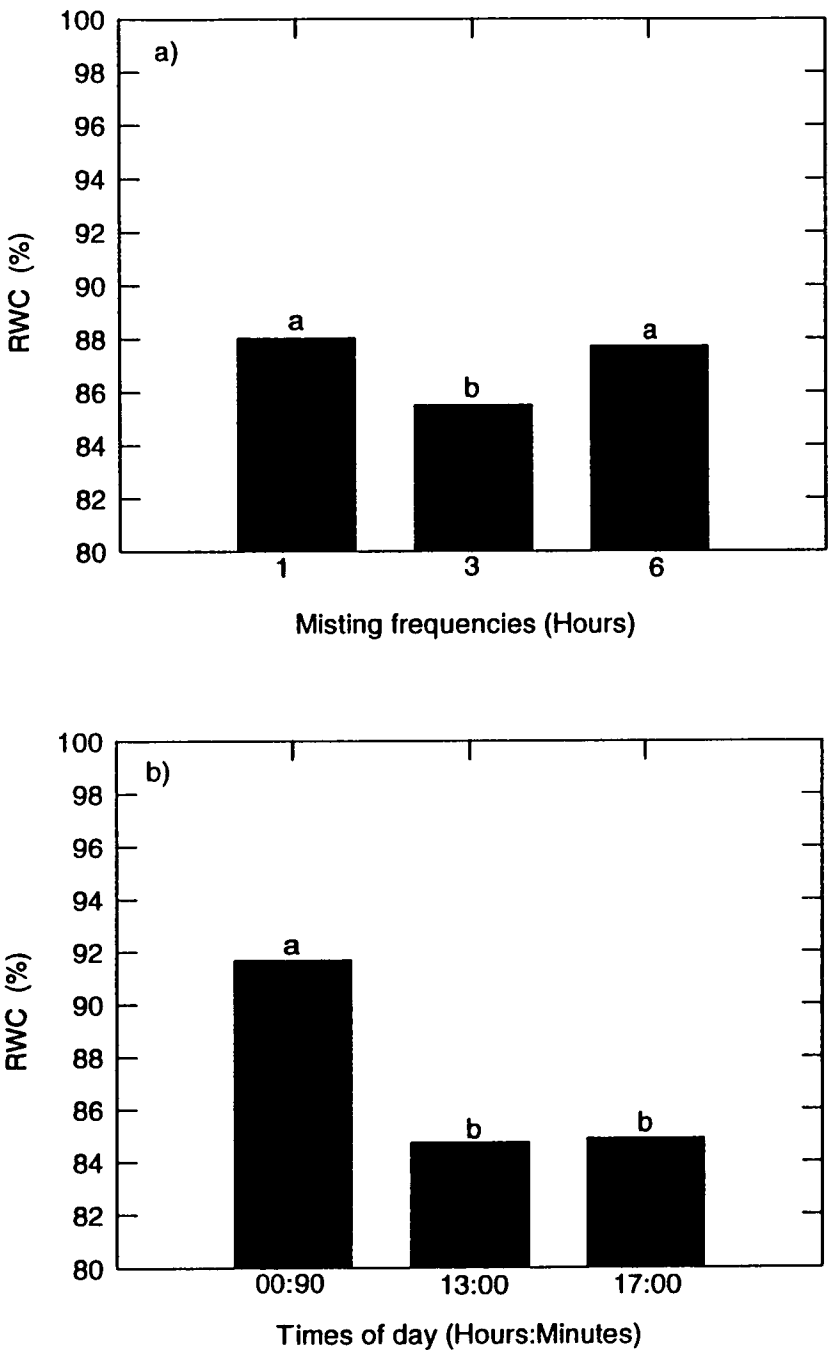


Figure 5.5 : a) Effect of misting frequencies on mean RWC of *S. leprosula* stem cuttings prior to rooting; b) Influence of times of day on mean RWC of *S. leprosula* stem cuttings prior to rooting (means with the same letters are not significantly different at $P \leq 0.05$; n=24 per treatment per time per day of measurement).

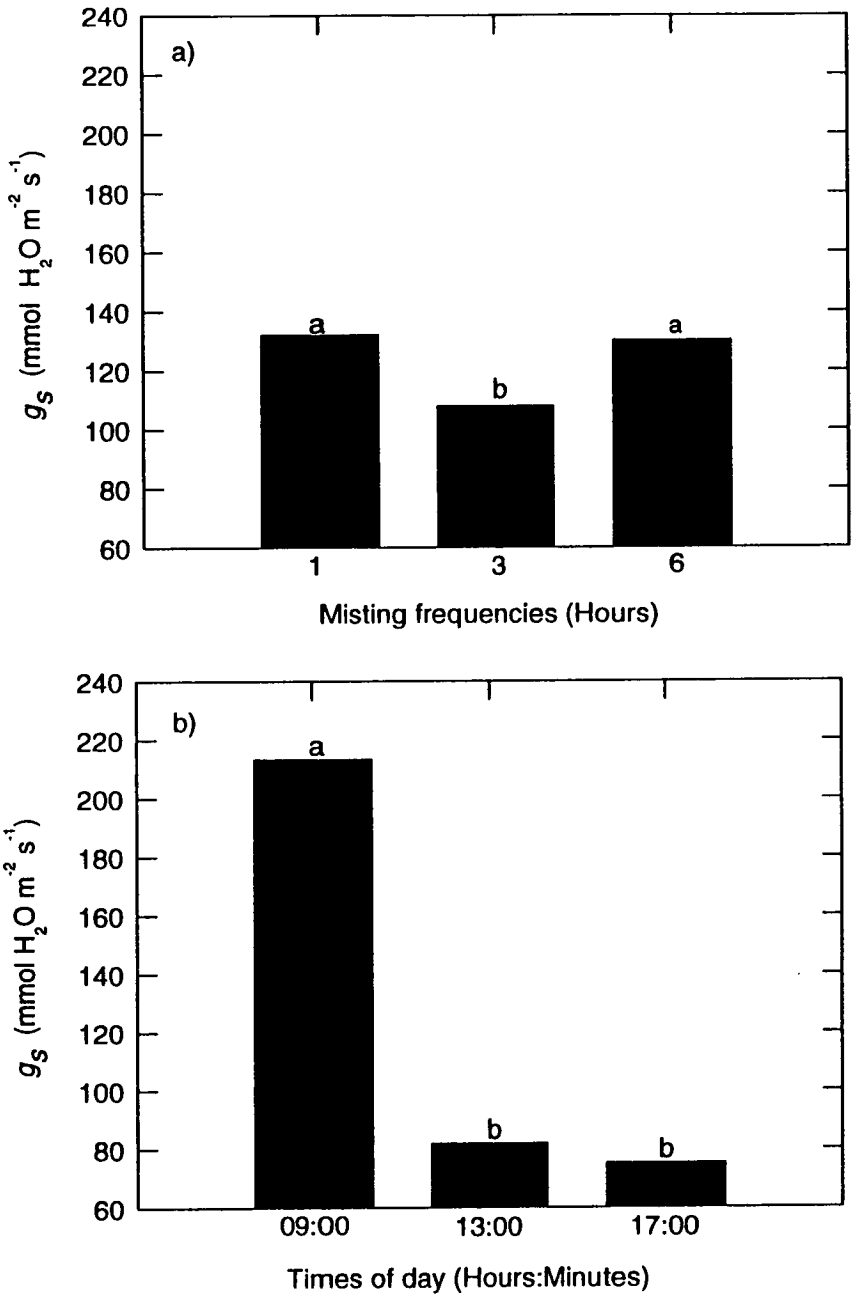


Figure 5.6 : a) Effect of misting frequencies on mean g_s of *S. leprosula* stem cuttings prior to rooting; b) Influence of times of day on mean g_s of *S. leprosula* stem cuttings prior to rooting (means with the same letters are not significantly different at $P \leq 0.05$; n=24 per treatment per time per day of measurement).

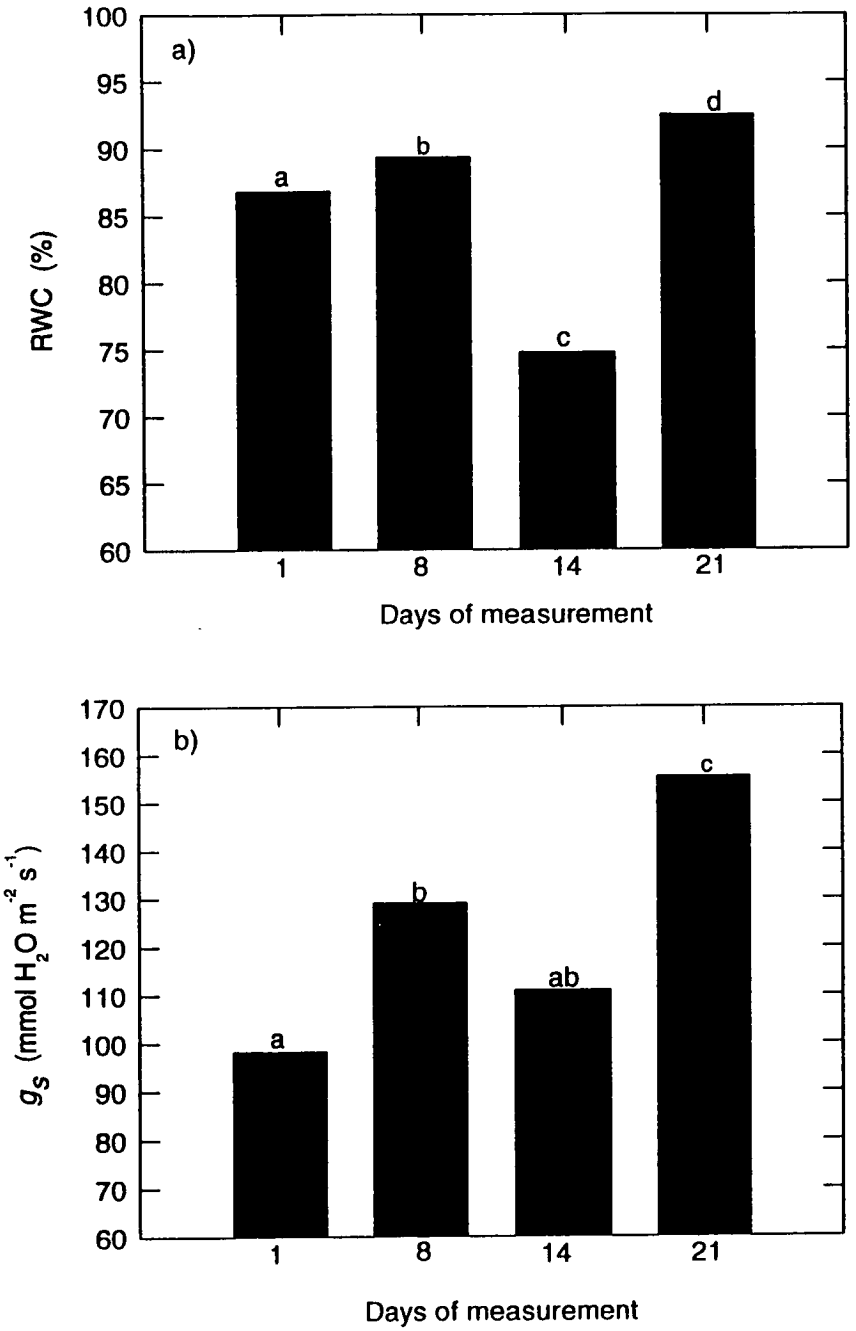


Figure 5.7 : Influence of days of measurement on a) Mean RWC; b) Mean g_s of *S. leprosula* stem cuttings prior to rooting (means with the same letters are not significantly different at $P \leq 0.05$; n=216 per day of measurement).

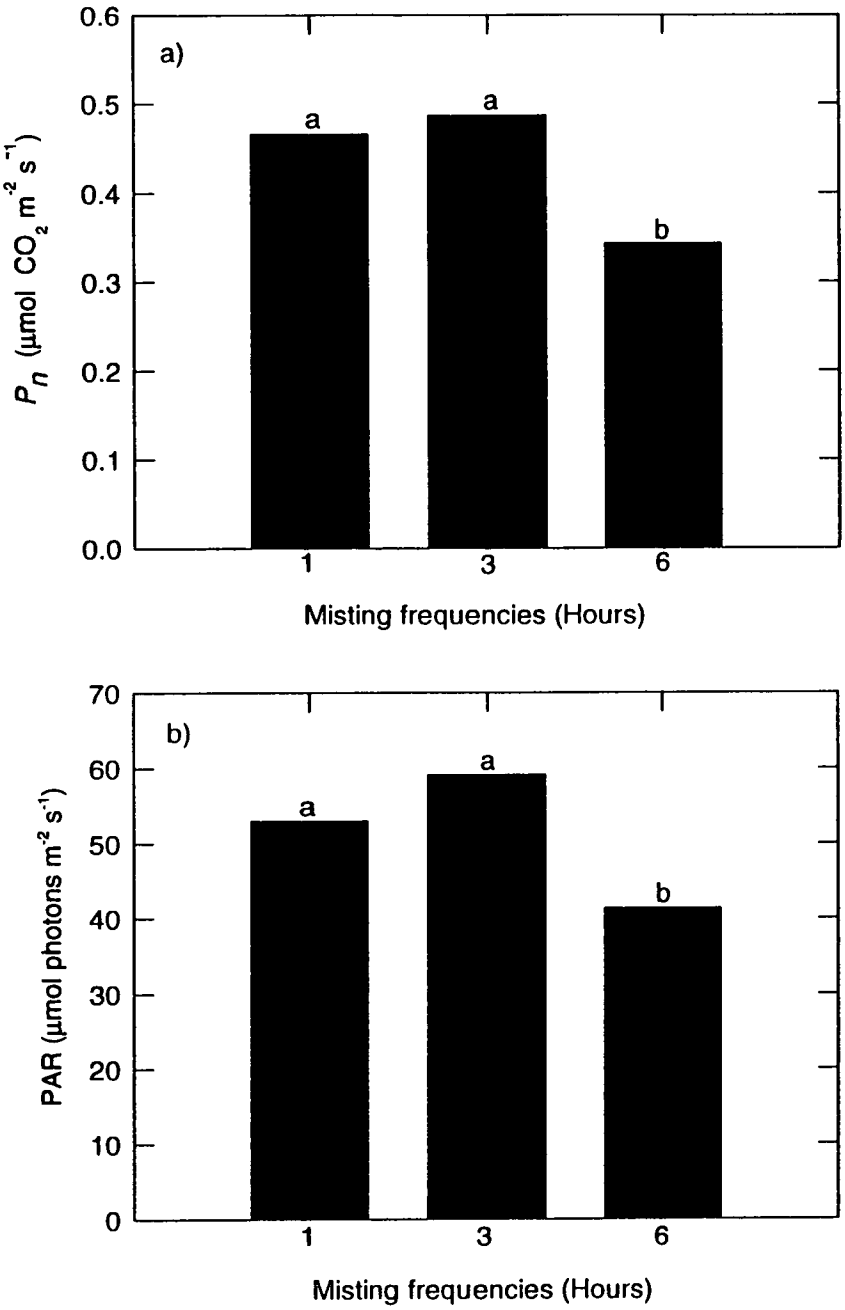


Figure 5.8 : Effect of misting frequencies on a) Mean P_n of *S. leprosula* stem cuttings prior to rooting; b) Mean PAR when the measurements of P_n were made (means with the same letters are not significantly different at $P \leq 0.05$; n=24 per treatment per time per day of measurement).

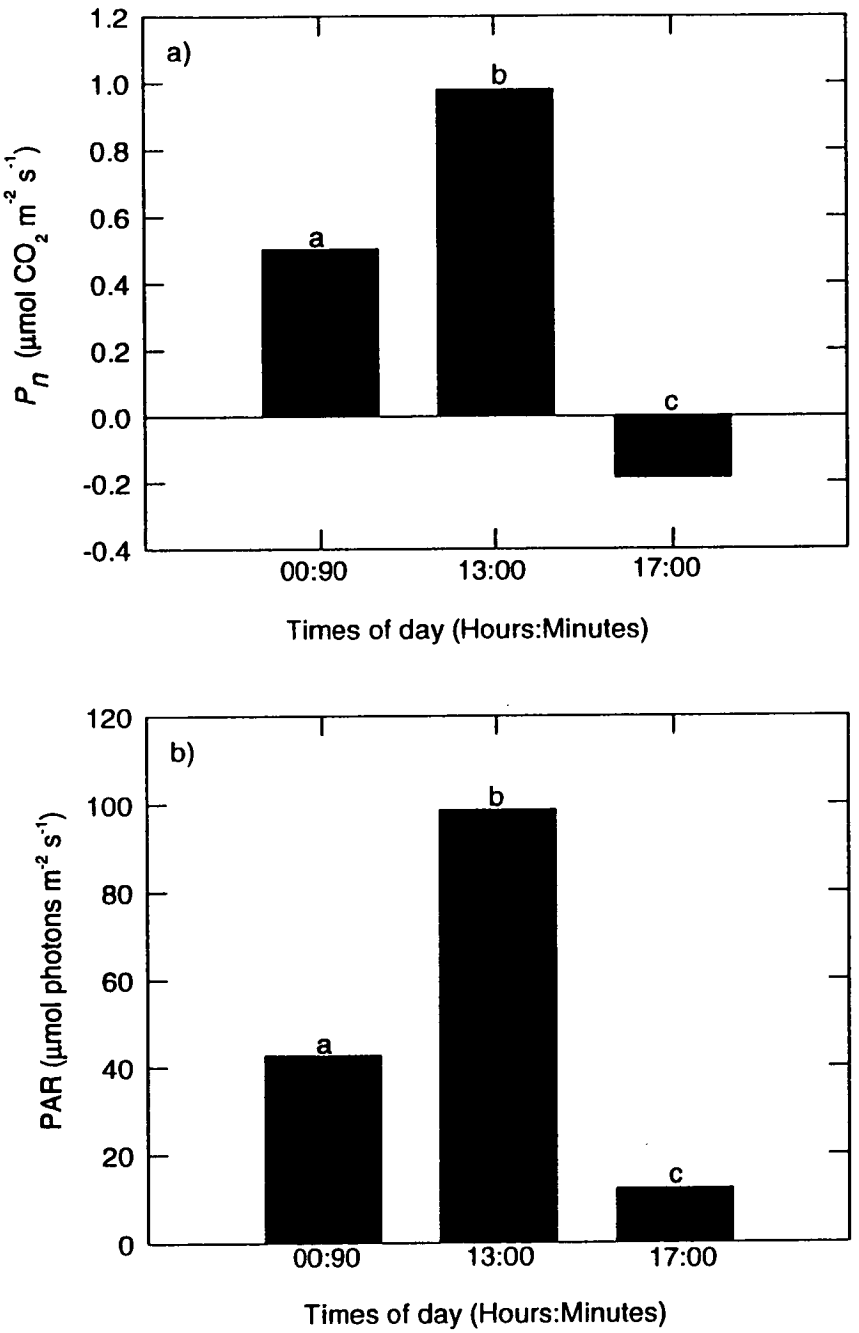


Figure 5.9 : Influence of times of day on a) Mean P_n of *S. leprosula* stem cuttings prior to rooting; b) Mean PAR when the measurements of P_n were made (means with the same letters are not significantly different at $P \leq 0.05$; n=24 per treatment per time per day of measurement).

recorded by Newton and Jones (1993b) on cuttings of several tropical species and these values may not be low enough to cause severe water deficit in cuttings. Mean g_s of unrooted cuttings was also higher (80 to 140 mmol H_2O m^{-2} s^{-1}) than those measured on *Cornus* and *Rhododendron* as reported by Gay and Loach (1977). Similar results were reported in other propagation environment studies where no appreciable effect on rooting was observed despite differences in leaf water potential (Grange and Loach 1984); and differences in RWC, g_s as well as leaf water potential (Newton and Jones 1993b). Perhaps, assessment of foliar water deficit could not reveal the actual water deficit of cuttings as cited by Grange and Loach (1985). And they suggested that water deficit in the stem base may have a greater effect on rooting.

In terms of microclimates around the cuttings, mean VPD in the different misting frequencies could be kept low, but periods of water deficit did occur as indicated by the maximum VPD in all treatments which in many cases was more than the threshold level (0.5 kPa) suggested by Grange and Loach (1983a) for many broadleaved species. This temporary water deficit however, seemed not to affect the rooting of *S. leprosula* stem cuttings. Presumably cuttings were able to tolerate this temporary water deficit and regain turgor during the nocturnal period of low VPD. This was supported by the high RWC and g_s obtained in the morning. The increase in RWC and g_s on day 21 may reflect recovery of the cuttings from water deficit experienced after insertion. The ability of cuttings of other tropical species to tolerate and recover this temporary water deficit and eventually root has been reported by Mesen (1993); Newton and Jones (1993b).

Direct comparison with Dipterocarp species could not be made since no detailed studies have been reported on microclimates around cuttings or cutting water status while on propagation beds. However, several workers have attempted to test the effect of misting on rooting. For example, Srivastava and Manggil (1981) found that intermittent mist of 5 minutes at an interval of 1.5 to 2.5 hours yielded reasonable rooting success with some Dipterocarp species while Yahaya (1979) stated that 2 minutes of misting every 2 hours was inadequate for rooting of

related species. Noraini and Ling (1993) obtained more than 65% rooting of *S. parvifolia* and *S. acuminata* in open mist with 0.5 hour interval of misting and 2 minutes duration of burst. Cuttings of these species seemed to tolerate water stress judging from the mean relative humidity in their experiment which was kept around 60% with air temperature of 27 °C (Noraini and Ling 1993). In contrast, Lo (1985) found that *S. macrophylla* required continuous misting or alternate misting during day time followed by continuous misting at night. Absence of misting at night resulted in the high mortality of 75%. The author attributed death of cuttings to water deficit experienced by cuttings at night; the results would be more informative if accompanied by environmental data.

In terms of morphological characteristics, stepwise regression analysis revealed that rooting of *S. leprosula* stem cuttings was negatively associated with initial volume of cuttings. This may indicate that cuttings with high volume were less suitable for rooting, and they were found to be more prone to death. Higher volume cuttings could be associated with larger diameter since cuttings in the present experiment was cut to the same length. Larger diameter cuttings may have increase in lignin layer and lignified cuttings were generally poor rooters as lignification may create physical barrier to rooting (Hartmann *et al.* 1990; Liew 1992). Negative relationship between volume of cuttings and rooting could also indicate that rooting was not influenced by initial carbohydrates reserves.

Cuttings planted under a misting interval of 6 hours produced the least number of roots. The low P_n obtained under this treatment may be responsible for the reduction in the number of roots. The results obtained were supported by other workers who also reported the importance of carbohydrates either from reserves or through current P_n has greater influence on root development than on root initiation which is hormonally controlled (Haaland (1976; Moe and Andersen 1988; Mesen 1993). Lower photosynthesis under 6 hours misting could possibly be due to low PAR received by cuttings. Photosynthesis was closely correlated with PAR as indicated by the P_n measured at different times of the day. The problems in experimental layout as stated in the earlier paragraph may have

contributed to the differences in PAR. Differences in PAR has also led to lower VPD in the propagator with 6 hours compared to 3 hours misting interval. Simultaneously, this difference in VPD was affecting other physiological measurements made such as RWC and g_s which were higher in cuttings planted under 6 than 3 hours misting intervals.

The findings in this experiment showed that rooting of *S. leprosula* stem cuttings was not affected by the difference in misting frequencies applied. Use of polythene enclosures enabled mean VPD to be kept below 0.5 kPa in spite of substantial differences in misting intervals applied. Temporary water deficit occurring during peak irradiance could be tolerated by *S. leprosula* stem cuttings. However, misting frequency of more than 3 hours is not recommended since there was tendency of water deficit to develop in cuttings as indicated by lower RWC and g_s values obtained compared to 1 hour misting. More frequent misting such as 0.5 hour may be worth testing. Even though improved water relations in 6 hours misting with low irradiance could be achieved, low irradiance seemed to limit photosynthetic activity of cuttings. Manipulation of shading may be beneficial in the enclosed system as direct sunlight or too much light may result in shedding of leaves or death of cuttings as noted by Grange and Loach (1985); Newton and Jones (1993b). On the other hand low light may limit photosynthetic activity of cuttings although water relations could be improved as reflected in the present experiment. Effect of irradiance regimes on rooting ability of *S. leprosula* stem cuttings will be examined in experiment 2.

EXPERIMENT 2: Effect of photon irradiance on the rooting ability of *Shorea leprosula* leafy stem cuttings.

Introduction

The primary effects of irradiance in the propagation environment are on the production of assimilates for rooting and on the water use of the cuttings (Hartmann *et al.* 1990). At high irradiance the leaf temperature is likely to

increase, causing an increase in leaf to air vapour pressure difference, and thus an increase in the transpiration rate (Grange and Loach 1983a,b; Loach 1988a,b). In cuttings which have not rooted, this increase in transpiration is likely to cause water stress, often to a lethal extent. Moreover, the warming of the air is likely to increase the saturation deficit over the leaf, exacerbating this process (Evans 1952; Kemp 1952; Hess and Snyder 1955; Loach 1988a,b; Hartmann *et al.* 1990). Low VPD of ca. 0.5 kPa is recommended for successful rooting cuttings of many broadleaved species (Grange and Loach 1983a). This may be achieved by reducing irradiance through shading of propagators (Loach 1977; Loach and Whalley 1978; Loach and Gay 1979). Although shading of propagators has also been the normal practice in rooting Dipterocarp cuttings, actual irradiance received by cuttings has not been adequately quantified due to unavailability of light sensors.(Momose 1978; Muckadell and Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Aminah 1991c; Smits *et al.* 1994). Mean irradiance of 27 W m^{-2} (ca. 3% full sunlight) and 290 μmol photons m^{-2} s^{-1} (15% full sunlight) were sufficient to yield successful rooting in some Dipterocarp cuttings (Noraini and Ling 1993; Moura-Costa and Lundoh 1994 respectively); the range of irradiance used was however not stated in both of the experiments. No prior attempt has been made to study simultaneously photosynthesis and water relations of Dipterocarp cuttings during propagation. The present experiment investigates how variation of irradiance may affect VPD, relative water content, stomatal conductance and photosynthetic rates of *S. leprosula* stem cuttings while on propagation beds.

Materials and methods

Cutting materials and experimental layout

The experiment took place in the cutting shed of the FRIM nursery in February 1994. A total of 531 single node leafy stem cuttings were taken from six month old stock plants raised under 33% full sunlight as potted rooted cuttings.

Seventeen clones were used: 40, 103, 110, 153, 514, 525, 549, 550, 551, 554, 559, 560, 580, 581, 585, 587, 590. The length and leaf area of each cutting was 5 cm and 30 cm^2 respectively. Preparation of cuttings is as described in chapter 3. Initial diameter and node position of each cutting were recorded. The prepared cuttings were planted in a medium consisting of cleaned river sand. Cuttings were subjected to three different irradiance regimes created using black plastic netting: 1) High irradiance (without net, 0 to 658 µmol photons m^{-2} s^{-1}); 2) Medium irradiance (1 layer of netting; 0 to 360 µmol photons m^{-2} s^{-1}); 3) Low irradiance (3 layers of netting; 0 to 98 µmol photons m^{-2} s^{-1}). These irradiances may vary with time of day and weather conditions. Mean red/far red ratio in these propagators was 1.1 as measured by light sensor (SKR 110 660/730, Skye Instrument, UK); the value close to that of full sunlight (1.2). The 3 irradiance treatments were randomly allocated to the node positions so that there was no confounding between the treatments and the position on the stock plants from which cuttings were taken. The number of cuttings obtained per stock plant varied from 3 to 7 depending on availability of leaves on the node. Clones with less than 9 cuttings were not used in the experiment. Each treatment consisted 177 of cuttings (60, 72, and 45 cuttings were used for rooting; RWC and dry weight assessments respectively); and they were randomly laid out in three blocks (20 cuttings per block). Each block is a closed polythene propagator (1 m x 1 m x 0.8 m) with a misting unit in the centre. Details and illustrations of the propagation system used are as described in chapter 3.

Microclimate of propagator

Temperature, relative humidity and irradiance were recorded by a data logger (21X micrologger, Campbell Scientific, UK) using sensors as described in chapter 3. The sensors were placed in the centre of one block, which was randomly chosen from the total of three blocks of the respective irradiance treatments. The data logger was programmed to scan each sensor every 60 seconds, and to calculate and store mean readings every 5 minutes. Data collection extended from day 1 to day 25 of the experiment.

Relative water content (RWC)

Method for determining RWC is as described in chapter 3. RWC was determined at three times of the day (09:00, 13:00 and 17:00 hours) on days 1, 8, 14 and 21. At each time of measurement (e.g at 09:00 hours), 24 discs were sampled per treatment from 6 cuttings (2 cuttings were randomly chosen per block; 4 discs were obtained per cutting). Number of cuttings harvested for RWC assessment per day per treatment was 18 giving a total of 72 cuttings per treatment (288 discs) for 4 days.

Photosynthetic rate (P_n) and stomatal conductance (g_s)

P_n and g_s of cuttings prior to rooting were measured using an infrared gas analyser (LCA-3, ADC, Hoddesdon, UK). A diurnal pattern of P_n was measured for a period of ten consecutive days after cuttings were planted in the rooting medium. Additional artificial light (incandescent tungsten; Tungstram 100, Romania) was used to increase the PAR regime in each treatment during measurement. Dark respiration was measured at night and early morning before the sun rose. Four cuttings were randomly chosen per treatment per block. For another set of data, the same cuttings were measured on days 8, 14 and 21 at three different times of the day (starting from 09:00, 13:00 and 17:00 hours). The measurement lasted ca. two hours for each time of measurement. At each time of measurement (e.g. 09:00 hours), 12 P_n and g_s values were obtained for each treatment giving a total of 36 values per day (108 values per treatment for 3 days).

Dry weight of leaves and stems

Fifteen cuttings were randomly harvested per treatment per block on day 1 (at 09:00 hours) giving a total of 45 cuttings per treatment. Dry weight of leaf and stem of each cutting was determined after drying in an oven (ULM 500 Memmert, Germany) at 40 °C for 48 hours.

Starch, sugar and nitrogen determinations

The method for starch, sugar and nitrogen determinations is as described in chapter 3.

Assessment of cuttings and statistical analyses

Assessment was made weekly until week sixteen, for rooted, dead and unrooted cuttings as well as number of roots on each cutting.

Analysis of deviance for stepwise regression in Genstat 5 (Payne *et al.* 1987) was used to determine which of the recorded variables were significantly associated with rooting. Analysis of variance followed by Fisher's t test (LSD) was used to test for significant differences in mean accumulated number of roots per rooted cutting, VPD, RWC, P_n, g_s, leaf and stem dry weight.

For the diurnal P_n data, a non rectangular hyperbola curve was fitted using the model described in Jarvis *et al.* (1985). The measured input variables used in the model were PAR, P_n and g_s. The parameter optimisation in Genstat 5 (Payne *et al.* 1987) was used to estimate parameter values of 1) dark respiration (R_d); 2) mesophyll conductance (g_m); 3) initial slope of P_n/PAR curve (α, apparent quantum efficiency) and 4) convexity coefficient (θ) which defines the degree of curvature between initial slope and the asymptotic value of P_n. The main assumptions in the model are:

i) P_n is related to PAR by a non-rectangular hyperbola;

$$\text{i.e.} \quad \theta(P_n+R_d)^2-(P_n+R_d)(\alpha\text{PAR}+P_{nmax}+R_d)+\alpha\text{PAR}(P_{nmax}+R_d)=0$$

ii) P_n is linearly related to C_i (internal concentration of CO_2) over the range of interest;

$$\text{i.e.} \quad P_{n\,max}=(Ci - \Gamma)g_m$$

where ($P_{n\,max}$) is the maximum P_n, Γ is the CO_2 compensation concentration and was assumed to be 50 μmol mol^{-1} (Dick *et al.* 1991b). The C_i was the mean value obtained for PAR higher than 400, 300 and 300 μmol photons m^{-2} s^{-1} for high, medium and low irradiance treatments respectively. These PAR values were chosen by testing significant difference of C_i values at different PAR levels.

iii) C_i depends on the P_n and g_s;

$$\text{i.e.} \qquad C_i = C_a - (P_n/g_s)$$

where C_a is the ambient CO_2 concentration.

The estimated parameters were then used to plot P_n /PAR curves using values for the mean g_s measured on leaves of cuttings for each irradiance treatments.

The differences between the P_n /PAR curves were determined by a combined curve analysis of variance (Ross 1981), which tests the reduction in residual variance obtained by fitting a set of individual curves and then they were compared with the residual variance from the respective common curve.

Results in all the tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

Results

The initial diameter and volume of cuttings was not significantly different between treatments. Mean cutting diameter was 0.34, 0.37 and 0.34 cm while mean volume of cuttings was 0.50, 0.57 and 0.49 cm^3 for high, medium and low irradiance treatments respectively. Both initial dry weight of leaves and stem did not differ significantly between treatments. Statistical analysis was not carried out for initial leaf and stem starch, sugar and nitrogen since inadequate samples were available. Mean values of the above mentioned variables are

presented in Table 5.2. The sugar components for each treatment is presented in Table B24.

Table 5.2 : Dry weight, starch, total sugar and nitrogen content of leaf and stem of *S. leprosula* cuttings for the respective irradiance treatments. The samples were harvested on day 1 at 09:00 hours. Cuttings were taken from stock plants growing under 33% full sunlight (leaf area=30 cm^{-2} and stem=5 cm length). These cuttings were then subjected to the these irradiance treatments: high irradiance=0-658 µmol photons m^{-2} s^{-1}; medium irradiance=0-360 µmol photons m^{-2} s^{-1}; low irradiance=0-98 µmol photons m^{-2} s^{-1}; ± standard error of mean.

	Irradiance levels (µmol photons m^{-2} s^{-1})			
	High	Medium	Low	Number of samples per treatment (n)
Leaf dry weight (g)	0.21a	0.20a	0.21a	45
Stem dry weight (g)	0.16a	0.18a	0.18a	45
Leaf starch (%)	4.30±0.14	4.45±0.74	4.56±0.37	3
Stem starch (%)	2.48±0.09	2.45±0.72	2.83±0.35	3
Leaf sugar (%)	3.10±0.78	2.44±0.43	1.65±0.43	3
Stem sugar (%)	1.35±0.26	1.57±0.38	1.11±0.08	3
Leaf nitrogen (%)	1.79±0.06	1.51±0.12	1.54±0.57	3
Stem nitrogen (%)	0.58±0.02	0.59±0.56	0.41±0.22	3

Means with the same letters are not significantly different at $P \leq 0.05$. Statistical analysis was not carried out on the starch, sugar and nitrogen values since inadequate number of samples were available.

There was a significant difference obtained in rooting percentage of cuttings between irradiance levels tested (Table B25). Significantly lower rooting was obtained in cuttings planted under low irradiance relative to the other two treatments (Figure 5.10a). Rooting of cuttings was significantly affected by initial cutting volume and the relationship was negative (Figure 5.10b).

Percentage of dead cuttings was significantly affected by treatments (Table B26). Mortality of cuttings planted under low irradiance was significantly higher than the other two treatments (Figure 5.11a). The regression analysis indicated that cuttings with high volumes were more often dead (Figure 5.11b). Death of cuttings also corresponded to percentage of leaf shedding (Figure 5.11c). Very few cuttings remained unrooted in each treatment (4 to 6 cuttings per treatment). Mean percentage of cuttings remained unrooted was 10%, 8% and 7% for high, medium and low irradiance treatments respectively.

The number of roots per rooted cutting was not significantly affected by treatments (Table B27). Figure 5.12a shows the rate of mean accumulated number of roots between treatments. Number of roots was significantly affected by initial volume of cuttings and the relationship was negative (Figure 5.12b).

Environmental data collected in propagators from day 1 to day 25 of the experiment is shown in Table 5.3. There was a significant difference in maximum VPD and PAR between treatments (Tables B28 and B29). Mean maximum VPD and PAR were significantly higher under high irradiance followed by medium and low irradiance treatments. Similar results were obtained with mean VPD and PAR (Tables B30 and B31). Figures 5.13a,b,c show the daily maximum VPD in each irradiance treatment over the 25 days period of measurement.

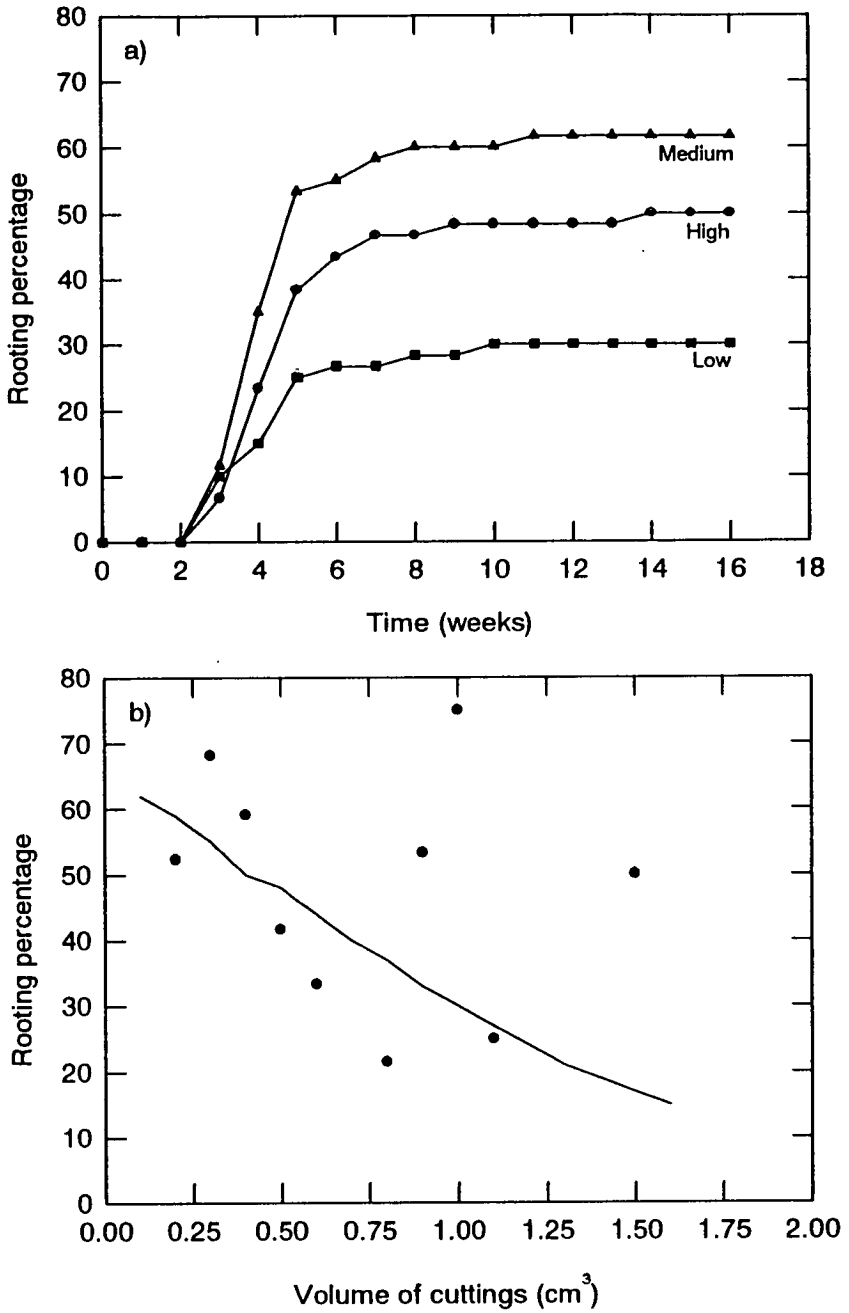


Figure 5.10 : a) Effect of irradiance on rooting rate of *S. leprosula* stem cuttings (n=60 per treatment); b) Relationship of rooting and cutting volume of *S. leprosula* stem cuttings. Points are groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model.

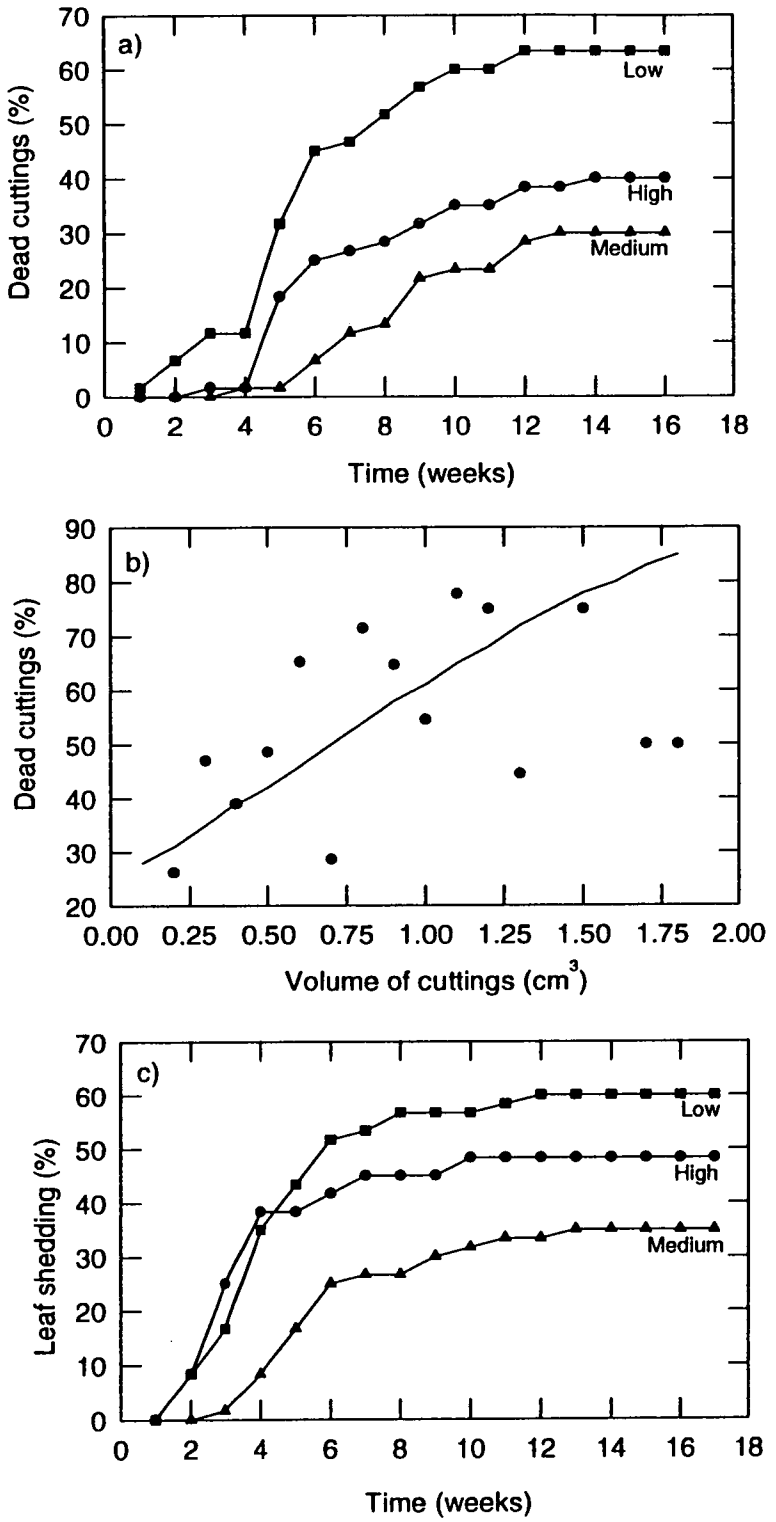


Figure 5.11 : a) Effect of irradiance on death rate of *S. leprosula* stem cuttings (n=60 per treatment); b) Relationship of dead cuttings and cutting volume of *S. leprosula*. Points are groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model; c) Effect of irradiance on the rate of leaf shedding of *S. leprosula* stem cuttings (n=60 per treatment).

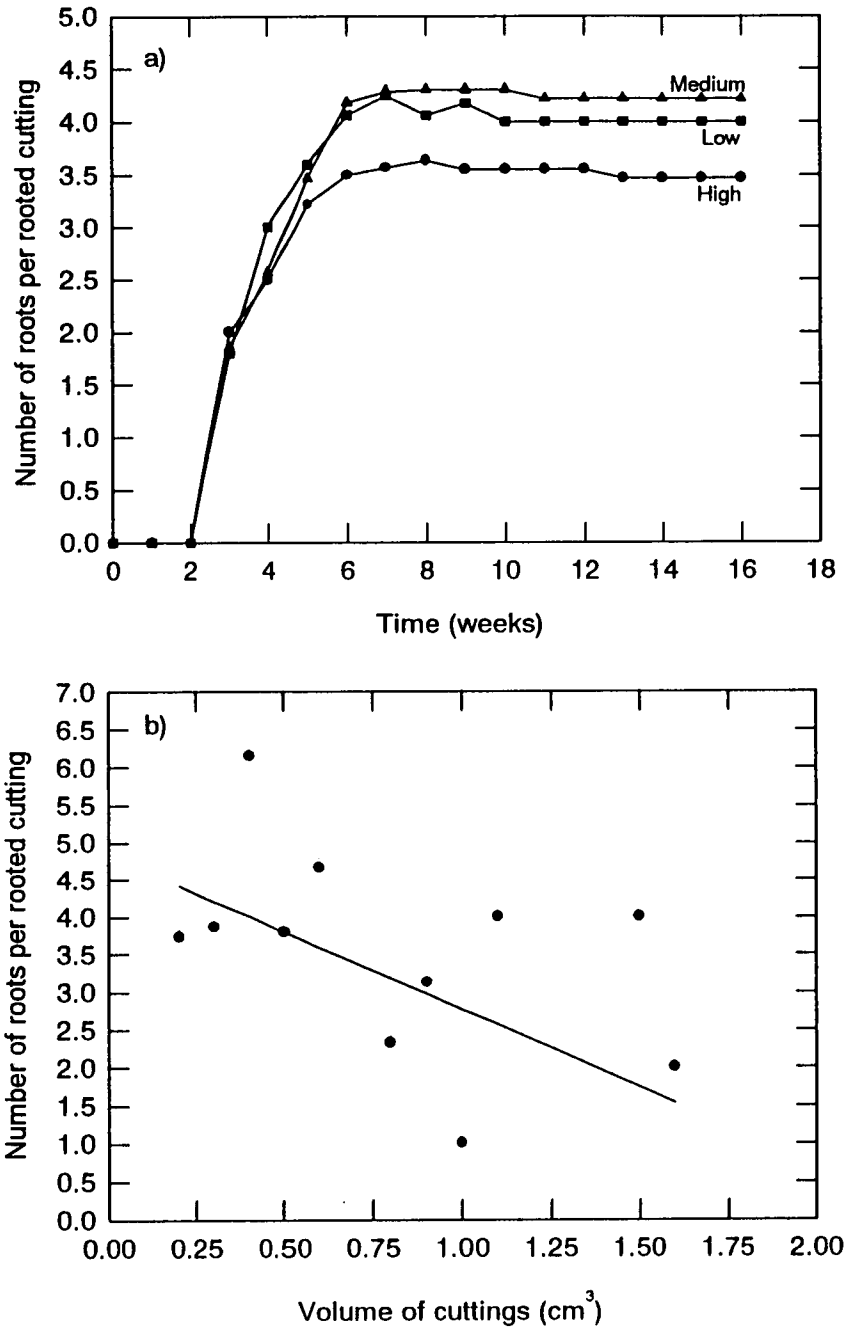


Figure 5.12 : a) Effect of irradiance on rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* (n=60 per treatment); b) Relationship of mean accumulated number of roots per rooted stem cutting and cutting volume of *S. leprosula*. Points are groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model.

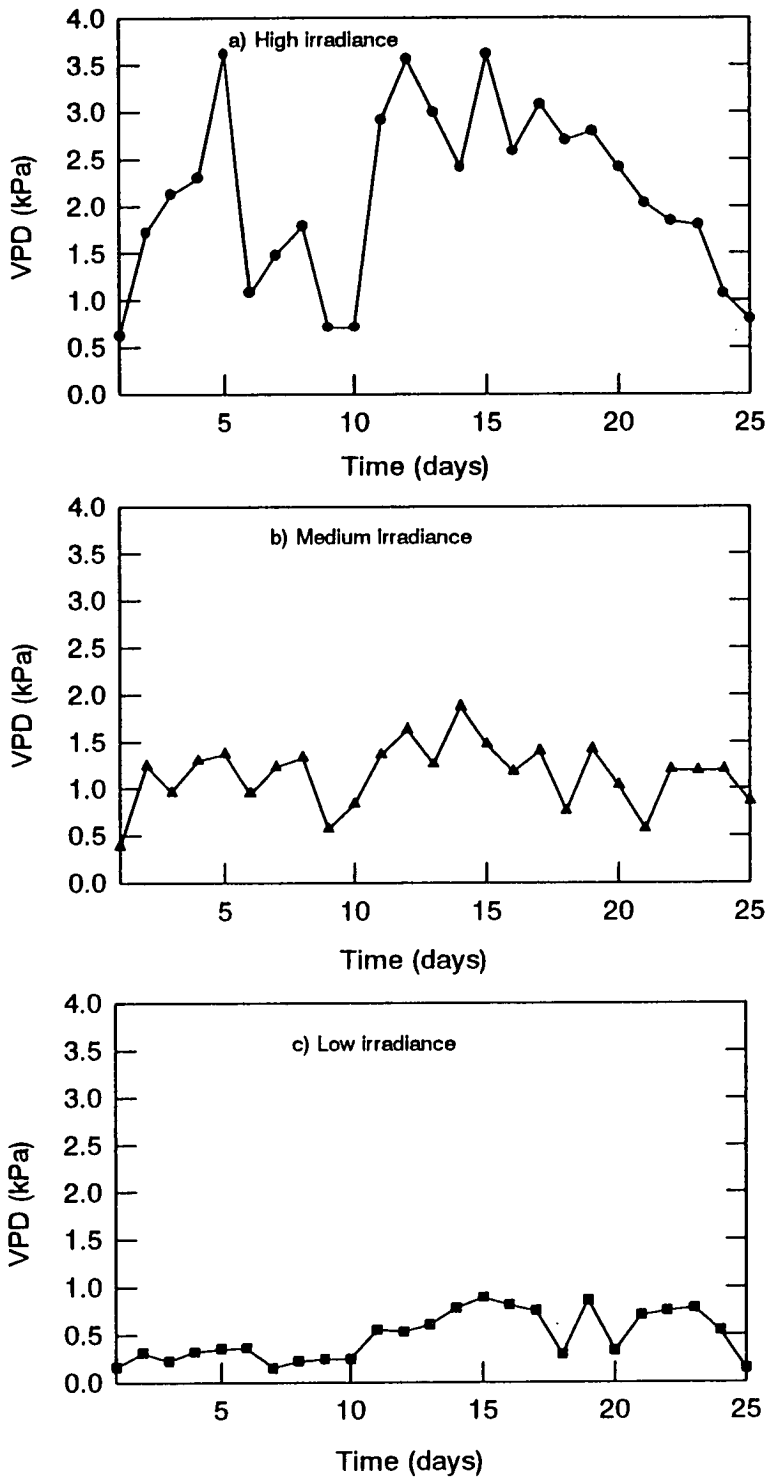


Figure 5.13 : Daily maximum VPD measured from day 1 to day 25 of the experiment. a) High irradiance level; b) Medium irradiance level; c) Low irradiance level. Maximum VPD per day was calculated as a 5 minute average.

Table 5.3 : Environmental data in the enclosed mist propagators subjected to different irradiance levels measured from day 1 to day 25 of the experiment. Data of each variable was calculated as a 5 minutes average. Mean value for each variable was value calculated over a 24 hour period daily.

Irradiance levels (μmol photons m^{-2} s^{-1})	High		Medium		Low	
	Mean	Range	Mean	Range	Mean	Range
Relative humidity (%)	96.55	50.74-100	99.48	67.97-100	99.93	78.94-100
Air temperature (° C)	29.21	22.23-44.79	28.47	22.53-41.82	28.30	22.04-40.85
Leaf temperature (° C)	29.24	22.04-46.35	28.74	22.09-42.70	28.37	22.04-41.7
VPD (kPa)	0.26	0-3.62	0.15	0-1.87	0.03	0-0.89
Irradiance (μmol photons m^{-2} s^{-1})	76.34	0-658.30	41.81	0-359.90	10.03	0-98.00

There was a significant interaction between treatments applied with times of day and days of measurement on RWC of *S. leprosula* stem cuttings (Table B32). RWC was significantly higher in low irradiance followed by medium and high irradiance treatments (Figure 5.14a). RWC recorded was highest in the morning, implying that cuttings had recovered from the previous day's water deficit as indicated by lower RWC at 13:00 and 17:00 (Figure 5.14b). Significantly higher RWC value was obtained 14 and 21 days after insertion (Figures 5.14c). Stomatal conductance was not significantly affected by the treatments but significant interaction was obtained on g_s between times and days of measurement (Table B33). Similar to RWC, g_s was highest in the morning (Figure 5.15a). Mean RWC ranged from 78 to 87% while mean g_s varied from

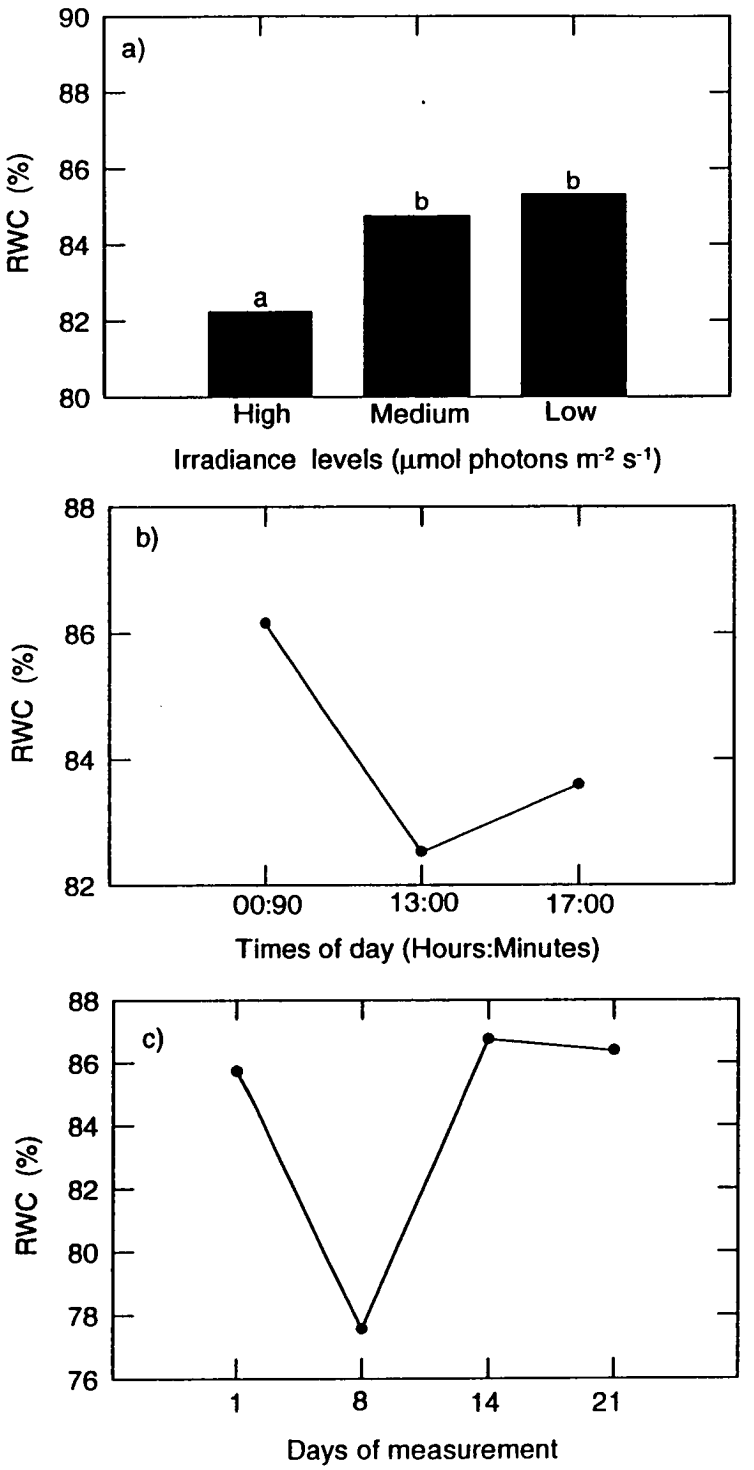


Figure 5.14 : a) Effect of irradiance levels on mean RWC of *S. leprosula* stem cuttings prior to rooting; b) Effect of times of day on mean RWC of *S. leprosula* stem cuttings prior to rooting; c) Effect of days of measurement on mean RWC of *S. leprosula* stem cuttings prior to rooting (n=24 per treatment per time per day). Means with the same letters are not significantly different at $P \leq 0.05$.

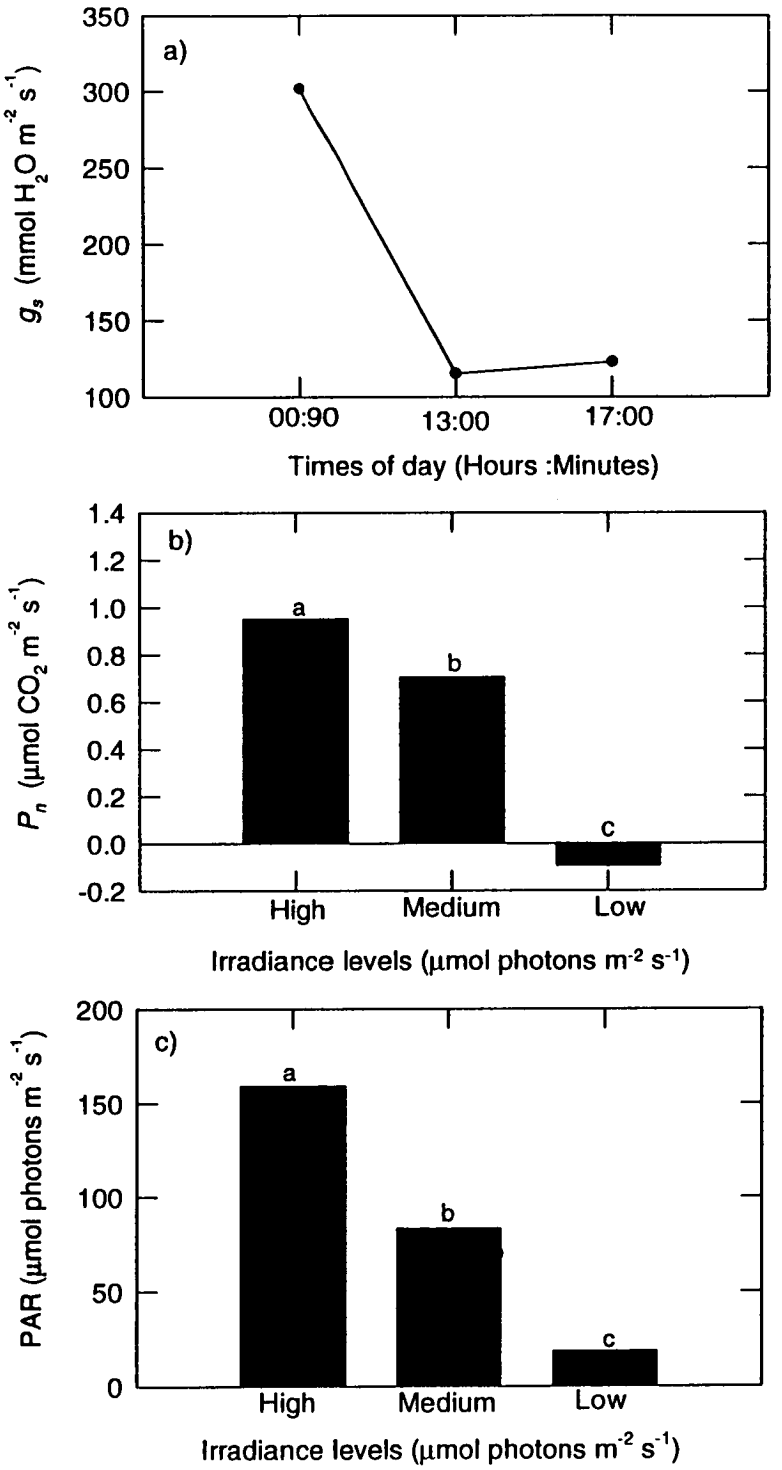


Figure 5.15 : a) Effect of times of day on mean g_s of *S. leprosula* stem cuttings prior to rooting; b) Effect of irradiance levels on mean P_n of *S. leprosula* stem cuttings prior to rooting; c) Effect of irradiance levels on mean PAR when measurements of g_s and P_n were made (n=12 per treatment per time per day for g_s, P_n and PAR). Means with the same letters are not significantly different at $P \leq 0.05$.

115 to 302 mmol H_2O m^{-2} s^{-1} depending on treatments, times and days of measurement. P_n was significantly influenced by interaction of treatments and times of day; times and days of measurement (Table B34). The rate was significantly lower in low than medium or high irradiance treatments (Figure 5.15b), corresponded with significantly low PAR (Figure 5.15c and Table B35).

The model of Jarvis *et al.* (1985) was fitted to the P_n data from the three irradiance levels. The resulting estimates of θ, α, g_m, R_d and $P_{n\,max}$ are given in Table 5.4. R_d was significantly higher in high irradiance levels followed by medium and low irradiance levels.

Combined curve analyses indicated that there was a significant difference in the overall predicted P_n curves (Table B36). The PAR level for maximum photosynthesis to occur was around 400 µmol m^{-2} s^{-1} as in indicated in high irradiance treatment after which higher PAR levels did not result in further increase in P_n of cuttings (Figures 5.16a,b,c).

Table 5.4 : Estimated parameter values from the theoretical model (Jarvis *et al.* 1985) and the maximum P_n ($P_{n\,max}$) for *S. leprosula* stem cuttings prior to rooting planted under 3 irradiance levels in the enclosed mist propagators. Measurements of P_n was made for a period of 10 days after cuttings were planted on the rooting beds. Artificial tungsten light was given to the cuttings at the time of measurements to increase the maximum irradiance levels in each treatment (n=228 and n=48 for P_n and R_d respectively; α=initial slope of P_n / PAR curve (apparent quantum efficiency); g_m=mesophyll conductance; θ=convexity coefficient).

Irradiance levels (µmol photons m^{-2} s^{-1})	α(mol CO_2 $photon^{-1}$)	g_m(mol CO_2 m^{-2} s^{-1}	R_d (µmol CO_2 m^{-2} s^{-1})	θ	$P_{n\,max}$ (µmol CO_2 m^{-2} s^{-1})
High irradiance	0.06	0.0092	0.85	0.00	2.54
Medium irradiance	0.03	0.0083	0.77	0.94	2.29
Low irradiance	0.04	0.0083	0.71	0.13	2.43

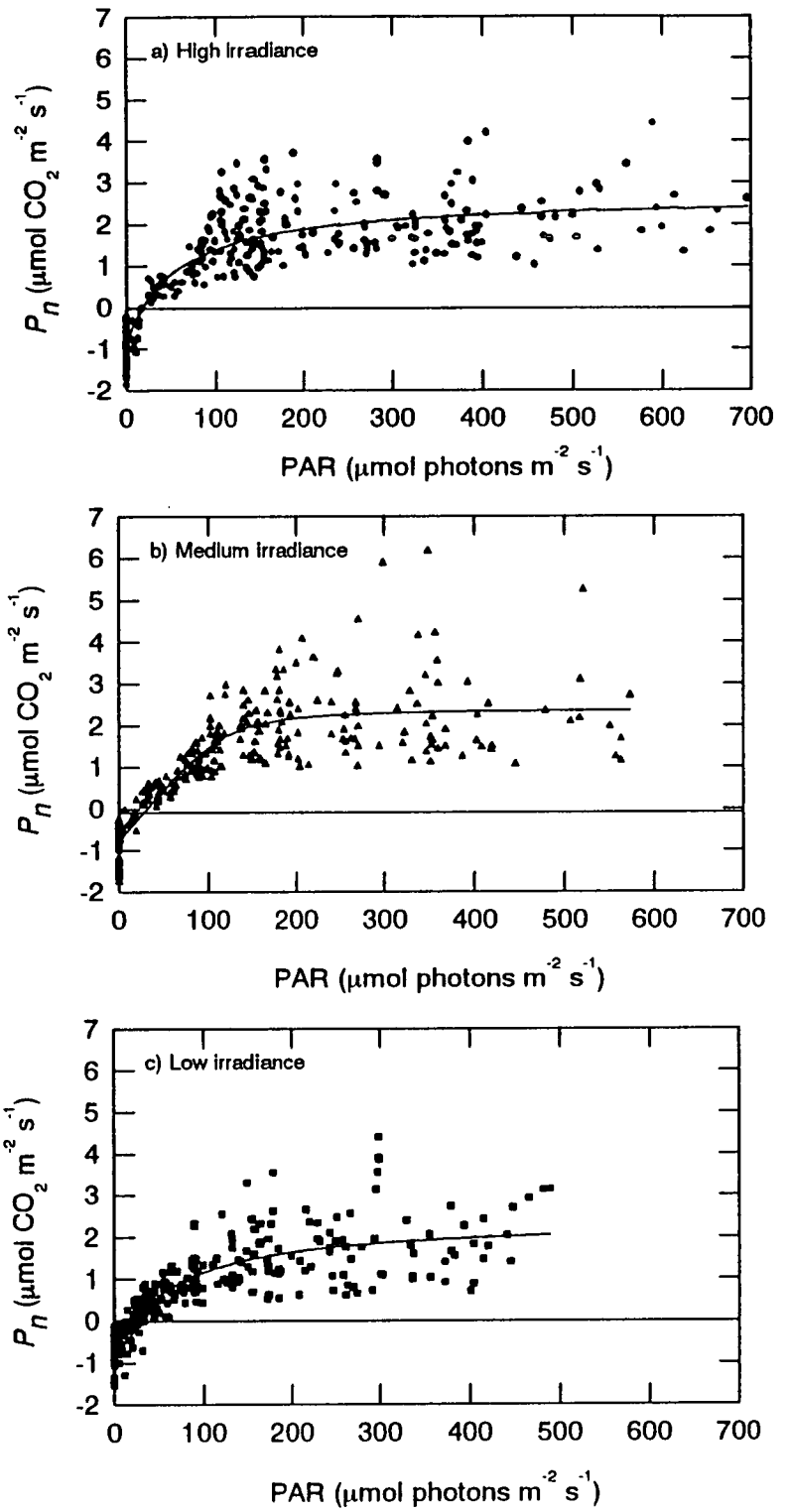


Figure 5.16 : P_n versus PAR curves of *S. leprosula* stem cuttings prior to rooting. a) High irradiance level; b) Medium irradiance level; c) Low irradiance level (n=228 and n=48 per treatment for P_n and R_d values respectively. Measurements were made for a period of 10 days; maximum irradiance for each treatment was given from artificial tungsten light).

Discussion and conclusions

Rooting of *S. leprosula* stem cuttings was greatly affected by the irradiance treatments; and a reduction in rooting percentage was obtained with low irradiance treatment (0-98 μmol m^{-2} s^{-1}; ca. 5% full sunlight). Poor rooting under low irradiance has also been obtained by other workers, for example rooting of *Populus tremula* x *tremuloides* and *Salix caprea* x *viminalis* was unsuccessful at irradiance less than 2 W m^{-2} (ca. 0.3% full sunlight) (cf. Eliasson and Brunes 1980). Only 9% rooting was obtained in cuttings of *Prosopis alba* at irradiance less than 150 μmol m^{-2} s^{-1} (ca. 8% full sunlight) (Klass *et al.* 1985). The authors supposed that a certain level of photosynthesis was essential for rooting although no measurement on photosynthesis w as made under these irradiance levels. Poor rooting under low irradiance in the present experiment may also be associated with low P_n. There is increasing evidence which indicates that photosynthesis does occur prior to rooting and influences rooting in cuttings of several tree species (Davis 1988; Leakey and Storeton-West 1992; Newton *et al.* 1992; Hoad and Leakey 1993; Mesen 1993). Besides affecting the carbon budget of cuttings, low P_n may indirectly reduce rooting by slowing down basipetal transport of auxin and other rooting cofactors which may originate from leaves and buds (Kampula and Potter 1984; Salisbury and Ross 1985).

In certain species like *Hibiscus rosa sinensis* L., its leafy cuttings rooted equally well in darkness and moderate levels of irradiance (van Overbeek *et al.* 1946). However, rooting of *Hibiscus* cuttings occurred within such a short period of a week that the need for current photosynthate may be less apparent. On the other hand, if the rooting period was quite long, cuttings with substantial carbohydrate reserves as in *Picea abies* L (Karst), may require additional photosynthate when the reserves were depleted (Strömquist and Eliasson 1979). Similar reasoning could account for the need of current photosynthate to support rooting of *S. leprosula* stem cuttings with rooting period of 12 to 14 weeks.

Mortality of cuttings was also high (63%) at the low irradiance level. This could indicate that carbohydrate reserves were insufficient to support rooting, and cuttings died when the reserves were depleted. The death of cuttings was also associated with high percentage of leaf shedding (60%). Shedding of leaves under low irradiance treatment could be due to carbohydrate depletion since leaves were below the light compensation point.

There were differences between irradiance treatments in estimated parameters obtained from the P_n model. The g_m value was slightly higher in high irradiance than the other two treatments. In general, these g_m values were comparable to other climax species of *Blighia sapida* and *Strombosia pustulata* with mean g_m of 0.008 mol CO_2 m^{-2} s^{-1} (Riddoch *et al.* 1991). Low values of θ in the high and low irradiance levels could represent the extent of mutual shading of chloroplast. High θ value obtained in medium irradiance may be due to the irradiance measured was not the one that was hitting the chloroplast. The α value in high irradiance level was slightly higher than the other two treatments suggesting that the quantum efficiency was sensitive to differences in irradiance levels. These results were contrast to those obtained by Riddoch *et al.* (1991); Ramos and Grace (1990); Kwesiga *et al.* (1986) where α values were insensitive to the growing conditions. R_d in low irradiance have been found to be lower than that of high irradiance. Lower respiration was also obtained in shaded than unshaded leaves (Bazzaz and Pickett 1980).

Low rooting in high irradiance relative to the other two irradiance levels could be due to cuttings experiencing water deficit as indicated by higher VPD and leaf shedding compared with the medium irradiance level. Also at high temperature the carbohydrates may be used for maintenance respiration rather than for root formation (Hartmann *et al.* 1990). Besides that, photodestruction of auxin, changes in concentration of rooting inhibitors and/or promoters could occur in cuttings under the high irradiance levels (Moe and Andersen 1988). Mesen (1993) working with *C. alliodora* stem cuttings, showed that under high

irradiance (0-1460 μmol photons m^{-2} s^{-1}), rooting also decreased significantly compared to that of lower irradiance level (0-339 μmol photons m^{-2} s^{-1}). The author associated low rooting under high irradiance range with water deficit experienced by cuttings as well as damage of photosynthetic apparatus as indicated by a decline in chlorophyll fluorescence in leaves of cuttings (Mesen 1993).

Besides treatments, stepwise regression analysis revealed that, rooting of *S. leprosula* stem cuttings was negatively associated with initial volume of cuttings. Cuttings with a high volume rooted poorly and were more prone to death. High volume cuttings may also be associated with a larger diameter since cuttings in the present experiment were cut to the same length. Larger diameter cuttings could have probably undergone secondary growth and thickening of lignin layer which may create physical barrier to root initiation (Hartmann *et al.* 1990; Liew 1992). Lignified cuttings were generally poor rooters and they will eventually die when the carbohydrate reserves were depleted (Hartmann *et al.* 1990). On the other hand, a negative relationship between rooting and initial volume of cuttings may imply that rooting was not influenced by the initial carbohydrates, suggesting that rooting is more influenced by carbohydrates formed after severance (Veierskov 1988). Also it is possible that the reserved starch was not converted to sugar for root initiation. The same reasoning as discussed above for rooting could cause the negative correlation between number of roots and initial volume of cuttings.

In terms of rooting environment, mean VPD in different irradiance regimes could be kept low, but periods of water deficit did occur as indicated by the maximum VPD especially in propagator with medium and high irradiance treatments which was more than the threshold level (0.5 kPa) suggested by Grange and Loach (1983a) for many broadleaved species. This temporary water deficit however, seemed to be less detrimental to rooting of *S. leprosula* stem cuttings as indicated in medium irradiance treatment. Even in high irradiance level of 0-658 μmol m^{-2}

s^{-1}, 50% rooting was obtained despite maximum VPD of 3.6 kPa. Presumably cuttings could tolerate this temporary water deficit and regain turgor with decreased VPD at night. Regain in turgor from previous the day's water deficit was also reflected in high RWC and g_s in the morning. The increase in RWC on day 14 and 21 may indicate that cuttings were recovering from a water deficit experienced after insertion. The ability of cuttings of other tropical species to tolerate and recover such temporary water deficit has also been observed by Newton and Jones (1993b); Mesen (1993).

The pronounced effect in the present experiment was a great reduction in rooting and high mortality of *S. leprosula* stem cuttings obtained under low irradiance. As discussed above, a reduction in photosynthesis may be the causal effect. Hence it is important in practical applications to regulate shading so as not to limit photosynthetic activity. The irradiance regime of 0-360 μmol m^{-2} s^{-1} (ca. 0-18% full sunlight; propagator with one layer netting) is the recommended range for rooting *S. leprosula* stem cuttings. With other Dipterocarp cuttings, >60% rooting of *S. acuminata* and *S. parvifolia* with average irradiance of 27 W m^{-2} (ca. 3% full sunlight) (Noraini and Ling 1993); and 73% to 88% of *Dryobalanops lenceolata* stem cuttings rooted under 290 μmol m^{-2} s^{-1} (ca. 15% full sunlight) (Moura-Costa and Lundoh 1994). A higher irradiance range of 0-658 μmol m^{-2} s^{-1} (ca. 0-33% full sunlight; propagator without net) in the present experiment may not be of benefit to stem cuttings of *S. leprosula* since photosynthetic activity seemed to saturate at PAR level of ca. 400 μmol photons m^{-2} s^{-1} (ca. 20% full sunlight). Irradiance higher than 400 μmol photons m^{-2} s^{-1} was also associated with a decline in P_n of *C. olliodora* stem cuttings (Mesen 1993). Perhaps varying leaf areas of cuttings within this irradiance level could further maximise photosynthesis and minimise water stress of *S. leprosula* cuttings and this will be examined in experiment 1 of chapter 6. Both irradiance and leaf area are among the factors influencing photosynthetic activity and water status of cuttings on the rooting beds as indicated by Newton and Jones (1993b); Mesen (1993).

CHAPTER 6

DETERMINATION OF ROOTING POTENTIAL OF *SHOREA LEPROSULA* STEM CUTTINGS BY APPLYING POST-SEVERANCE TREATMENTS OF LEAF TRIMMING AND INDOLE-3-BUTYRIC ACID (IBA)

The rooting potential of cuttings was examined by trimming the leaf areas to 15 cm^2, 30 cm^2 and 60 cm^2. The relationship between rooting ability with photosynthesis, transpiration rates and VPD was studied. This was to find an optimum leaf area to strike a balance between production of assimilates via photosynthesis and water loss through transpiration. The other post-severance treatment investigated was the effect of a range of IBA doses (0, 20, 40, 60 and 80 µg per cutting) on the rooting ability of *S. leprosula* stem cuttings.

EXPERIMENT 1: Effect of leaf areas on the rooting ability of *Shorea leprosula* stem cuttings

Introduction

The strong stimulatory effect of the presence of leaves on root initiation has generally been attributed to the production of carbohydrates via photosynthesis (Leakey *et al.* 1982b; Davis 1988; Leakey and Coutts 1989; Smalley *et al.* 1991; Newton *et al.* 1992). However, endogenous auxin and rooting cofactors synthesised by the leaves may also influence root initiation, although their role in promoting rooting has remained controversial (Haissig 1974; Hartmann *et al.* 1990). Too much leaf area in cuttings can however be deleterious since transpiration may lead to excessive water deficits which impair rooting or cause

mortality before root formation takes place (Gay and Loach 1977, Grange and Loach 1983a,b; Leakey 1985; Loach 1988a; 1990; Hartmann *et al.* 1990). Leaves of cuttings are usually trimmed to minimise this effect, but too much trimming may reduce photosynthesis and limit rooting. Thus, the concept of an "optimal" leaf area has been proposed and applied (Leakey *et al.* 1982b; Leakey and Coutts 1989; Tchoundjeu 1989). In Dipterocarps, these ideas have not been considered, although arbitrary leaf trimming is commonly used in practice (Lo 1985; Siagan *et al.* 1989; Smits *et al.* 1992, 1994; Noraini and Ling 1993). The current experiment investigates how variation of leaf area may affect stomatal conductance, transpiration and photosynthetic rates of *S. leprosula* stem cuttings during rooting process.

Materials and methods

Cutting materials and experimental layout

The experiment took place in the cutting shed of the FRIM nursery in February 1993. A total of 360 single node leafy stem cuttings were taken from six month old stock plants raised under 33% full sunlight as potted rooted cuttings. Sixteen clones were used: 517, 521, 533, 539, 549, 551, 554, 560, 565, 571, 572, 573, 578, 579, 580, 585. Leaves of cuttings were trimmed to 15, 30 and 60 cm^2 using paper templates which had been measured with leaf area meter (Delta-T series, Taiwan). The 3 leaf area treatments were randomly allocated to the node positions so that there was no confounding between the treatments and the position on the stock plant from which cutting was taken. Only cuttings with a leaf area of at least 60 cm^2 were used for this experiment to avoid smaller leaf area being allocated to lower node positions. The number of cuttings obtained per stock plant varied from 3 to 7 depending on availability of leaves on the node. Clones with less than 18 were not used for the experiment. The preparation of cuttings is as described in chapter 3. Initial diameter and node position of each cutting were recorded. All cuttings were cut to 5 cm length. The prepared cuttings were

planted in a medium consisting of cleaned river sand. Each treatment consisted of 120 cuttings (60 cuttings each were used for rooting and dry weight assessments). These 60 cuttings were split into 10 cuttings per block. Both node and treatments were randomly laid out in six blocks using random numbers. Each block is a closed polythene propagator (1 m x 1 m x 0.8 m) with a misting unit in the centre. Details and illustrations of the propagation system used are as described in chapter 3.

Microclimate of propagator

Temperature, relative humidity and irradiance were recorded by a data logger (21X micrologger, Campbell Scientific, UK) using sensors as described in chapter 3. The sensors were placed in the centre of two blocks which were randomly chosen from the total of six blocks. The data logger was programmed to scan each sensor every 60 seconds and to calculate and store mean readings every 5 minutes. Data collection extended from day 1 to day 13 of the experiment.

Photosynthetic rate (P_n) and stomatal conductance (g_s).

P_n and g_s of cuttings prior to rooting were measured using an infrared gas analyser (LCA-3, ADC, Hoddesdon, UK). Three cuttings were randomly chosen for each treatment per block and measured on days 14, 21 and 28 after planting in the rooting media. No measurement was made on days 1 and 7 because the gas analyser was out of order. P_n and g_s of rooted cuttings and cuttings that remained unrooted were measured on day 63 (3 rooted and unrooted cuttings per treatment per block were randomly chosen). Measurements of P_n and g_s were made between 09:00 to 12:00 hours.

Dry weight of leaves and stems

Two destructive samples of cuttings prior to rooting were made on day 1 and day 28 of the experiment. Five cuttings per treatment per block were randomly harvested at 09:00 hours on each day giving a total of 30 cuttings per treatment per day. Dry weight of leaf and stem of each cutting was determined after drying in an oven (ULM 500 Memmert, Germany) at 40 °C for 48 hours.

Starch, sugar and nitrogen determinations

Since the amounts of stem and leaf samples were small, the samples from two blocks were combined giving three samples each of stem and leaf. Method for determining starch, sugar and nitrogen of the leaf and stem is as described in chapter 3.

Assessment of cuttings and statistical analyses

Assessment was made weekly until week sixteen, for rooted, unrooted and dead cuttings as well as number of roots on each cutting.

Analysis of deviance for stepwise regression in Genstat 5 (Payne *et al.* 1987) was used to determine the association between recorded variables and rooting. Analyses of variance followed by Fisher's t test (LSD) was used to test for significant differences in the mean accumulated number of roots, leaf and stem dry weight, P_n, g_s and transpiration rates. Results in all the tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

Results

The initial volume and diameter of cuttings between treatments was not significantly different. Mean volume of cuttings was 0.71, 0.59 and 0.69 cm^3

while their mean diameter was 0.41, 0.37 and 0.40 cm for 15, 30 and 60 cm^2 respectively.

Leaf area did indeed affect rooting percentage of cuttings (Table B37). Significantly lower rooting was obtained in cuttings with 60 cm^2 leaf area than the other two treatments (Figure 6.1a). However, number of roots per rooted cutting was not significantly affected by treatments (Figure 6.1b). Neither rooting nor number of roots was influenced by the morphological characteristics of cuttings.

The proportion of cuttings failing to root was significantly affected by treatments (Table B38). The lowest percentage was obtained in cuttings with 15 cm^2 followed by 30 and 60 cm^2 leaf area (Figure 6.2a). Very few cuttings were dead with mean mortality of 3% to 10% (Figure 6.2a).

Leaf shedding was significantly affected by treatments as indicated by the Chi-square test. Highest percentage of leaf shedding was obtained in cuttings with 60 cm^2 than 30 or 15 cm^2 leaf area treatments (Figure 6.2b).

Mean VPD could be maintained close to zero (Table 6.1). However, the daily rise in VPD (as a 5 minutes average) exceeded the threshold level of 0.5 kPa suggested by Grange and Loach (1983a) for many broadleaved species (Figure 6.3).

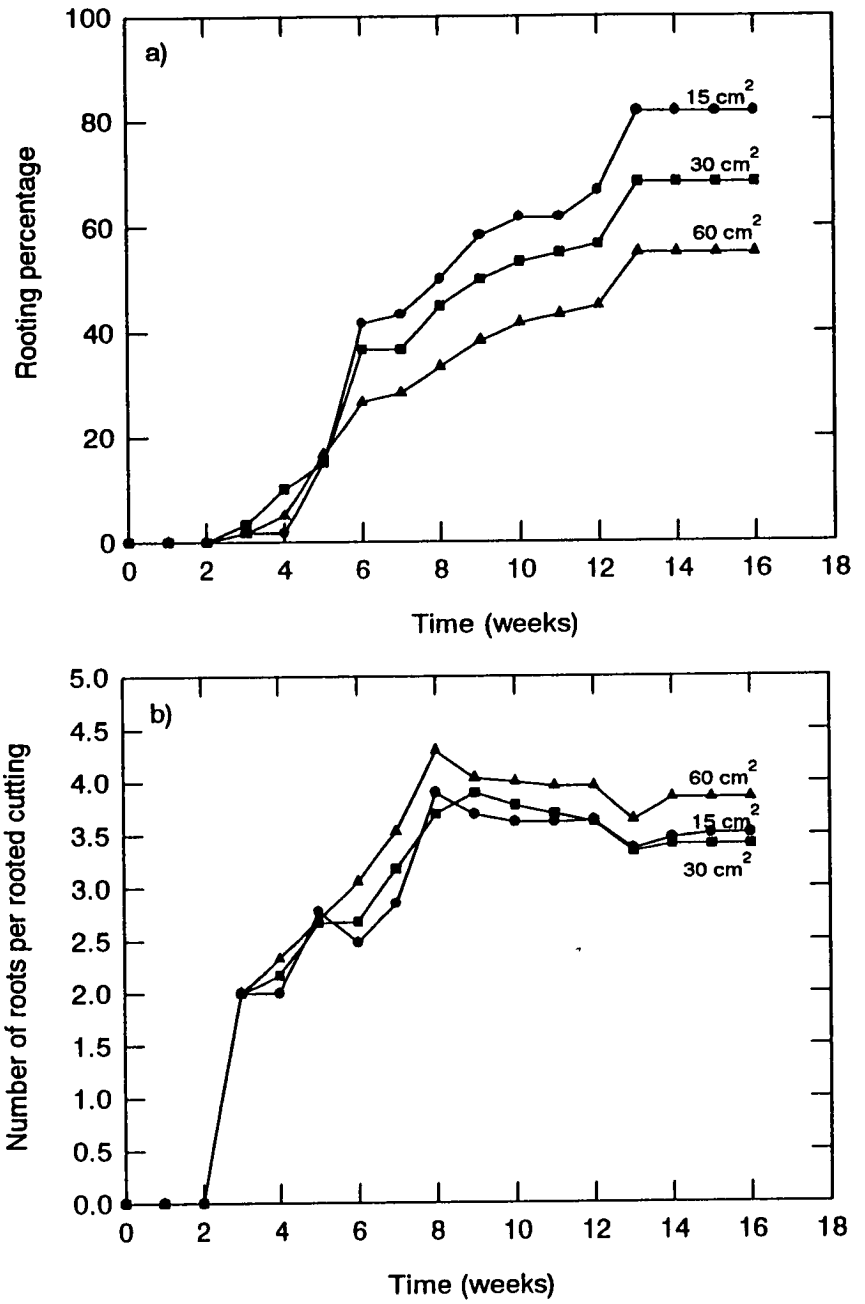


Figure 6.1 : Effect of leaf areas on a) Rate of rooting (n=60 per treatment); b) Rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* (n=60 per treatment; circle=15 cm^2; square=30 cm^2; triangle=60 cm^2).

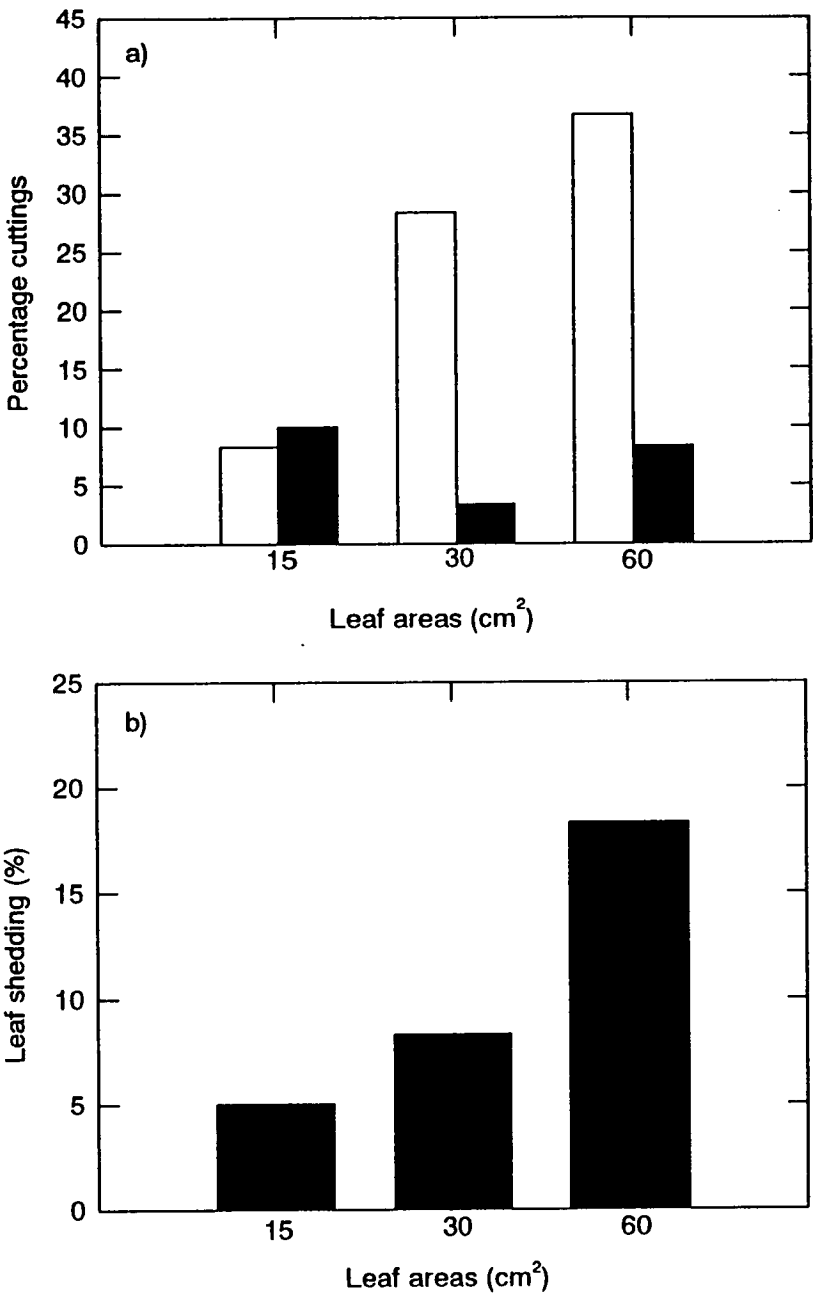


Figure 6.2 : a) Effect of leaf areas on stem cuttings that remained unrooted and dead stem cuttings of *S. leprosula* at week sixteen (hollow bar=unrooted cuttings; solid bar=dead cuttings); b) Effect of leaf areas on leaf shedding of *S. leprosula* stem cuttings (n=60 per treatment).

Table 6.1 : Environmental data on rooting beds in an enclosed mist system measured from day 1 to day 13 of the experiment. Volume of each variable were calculated as 5 minutes average. Mean values of each variable were values calculated 24 hours period daily.

	Mean	Range
Relative humidity (%)	99.04	79.65-100
Air temperature (° C)	29.14	23.86-41.80
Leaf temperature (° C)	29.05	24.00-40.67
VPD (kPa)	0.00	0-1.04
Irradiance (μmol photons m^{-2} s^{-1})	39.55	0-384.10

There was no significant difference between treatments or days of measurement in the P_n and g_s per unit leaf area of cuttings prior to rooting. Mean P_n were 1.81, 1.81 and 1.84 μmol CO_2 m^{-2} s^{-1} for cuttings with 15 cm^2, 30 cm^2 and 60 cm^2 leaf area respectively. However, a significant difference was obtained in P_n per leaf between treatments (Table B39). The highest rate was in cuttings with leaf area of 60 cm^2 followed by 30 and 15 cm^2 (Figure 6.4a). Similar results were obtained with transpiration rates of cuttings per leaf (Table B40 and Figure 6.4b).

Significantly higher values of P_n and g_s were obtained in rooted cuttings relative to those which remained unrooted (Tables B41, B42 and Figures 6.5a,b). There was no significant difference in PAR between treatments obtained when the measurements of P_n and g_s were made. Mean PAR ranged from 161 to 178 μmol photons m^{-2} s^{-1}.

Dry weight of leaf per unit leaf area and per leaf were significantly different between treatments (Tables B43 and B44). There was no significant different in

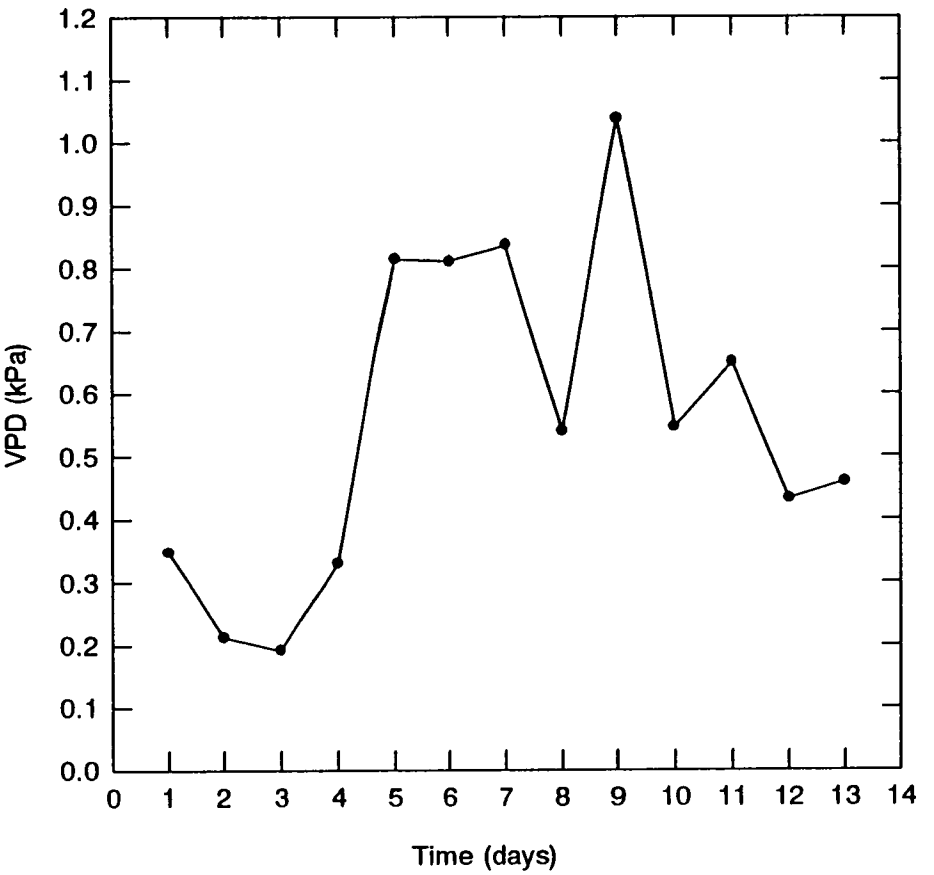


Figure 6.3 : Daily maximum VPD in the propagators measured from day 1 to day 13 of the experiment. Each data point corresponds to the maximum VPD per day calculated as a 5 minute average.

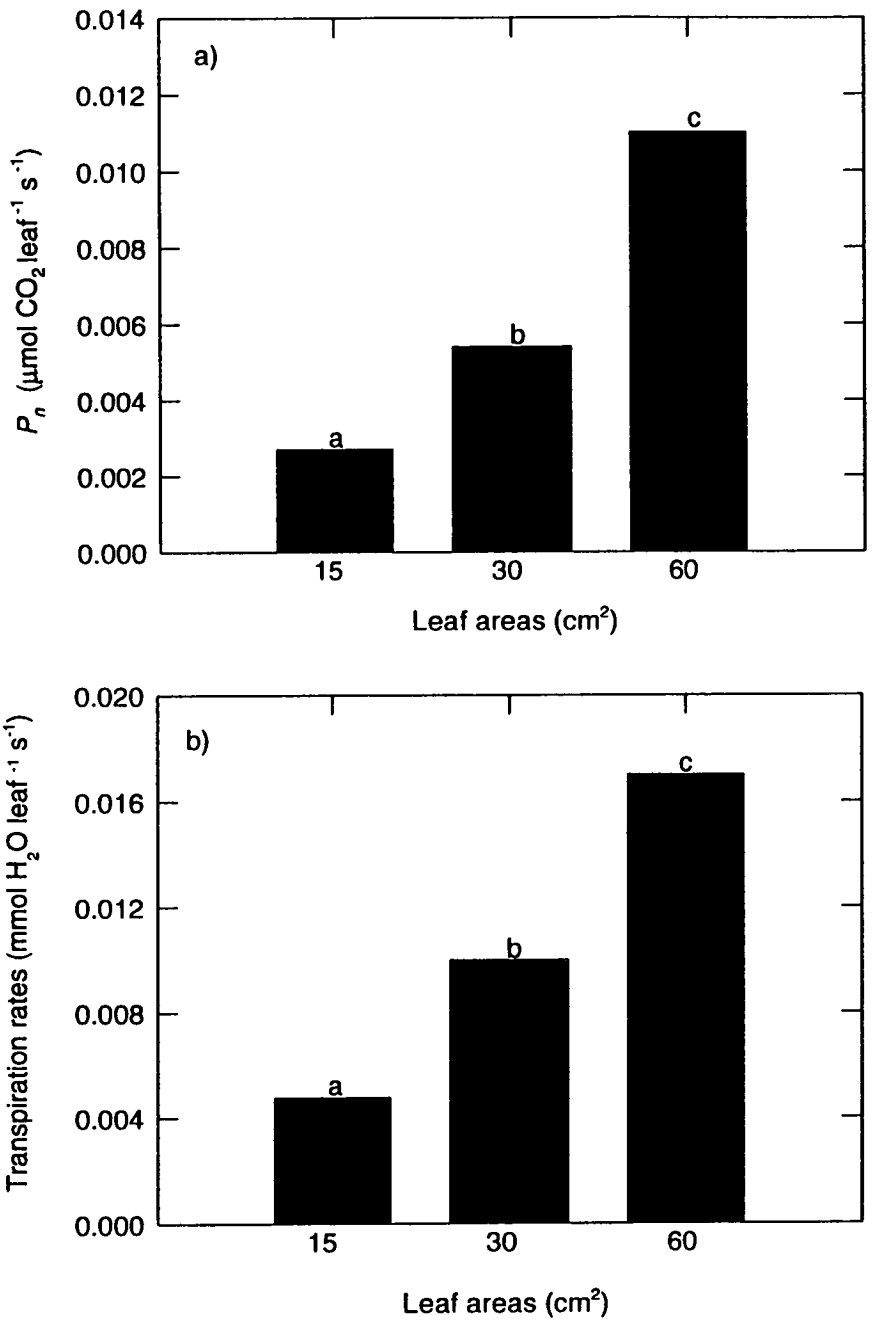


Figure 6.4 : a) Mean P_n per leaf of cuttings prior to rooting; b) Mean transpiration rates per leaf of cuttings prior to rooting as affected by leaf area treatments of *S. leprosula* stem cuttings (n=54 for 15 cm^2 and 30 cm^2 leaf areas, n=53 for 60 cm^2 leaf area since one leaf was dropped on day 28). Means with the same letters are not significantly different at $P \leq 0.05$.

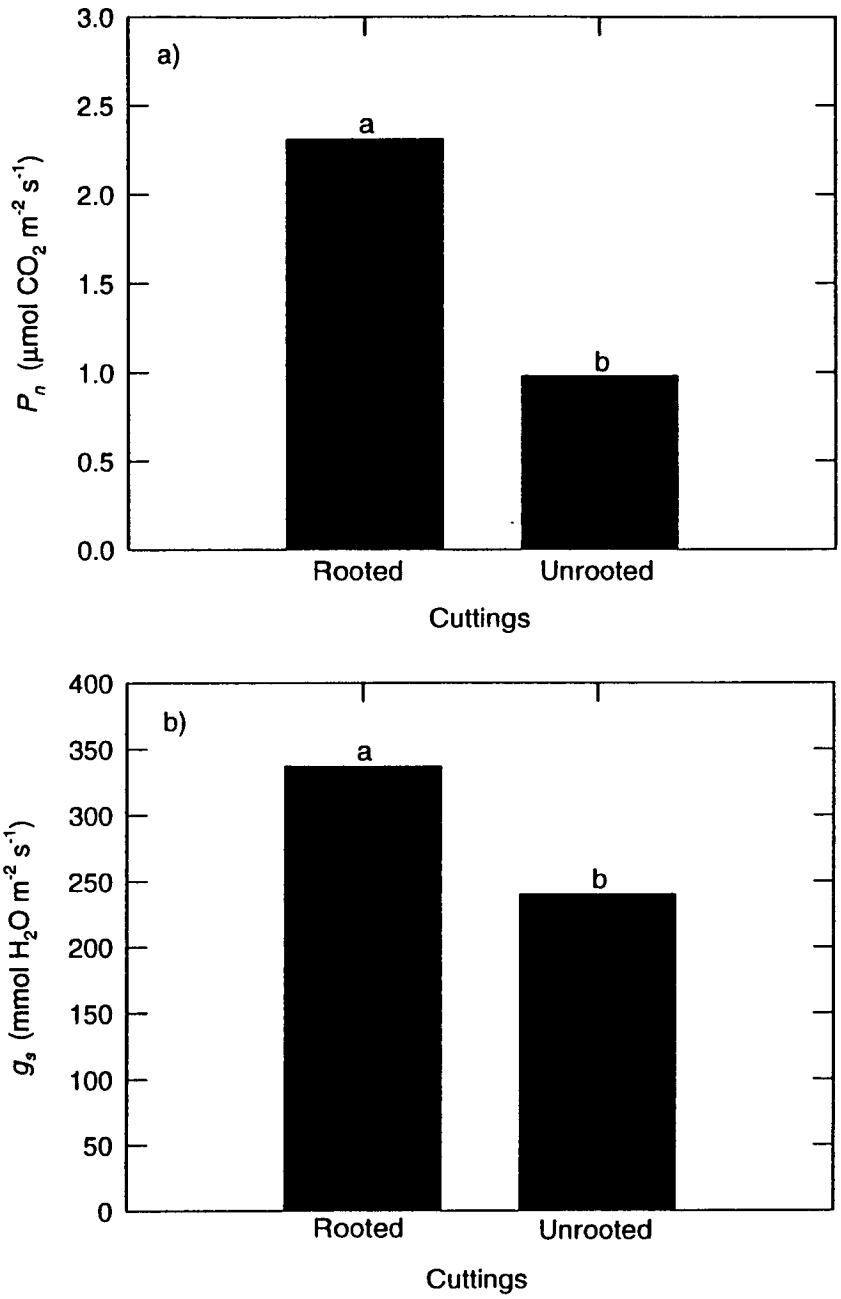


Figure 6.5 : a) Mean P_n; b) Mean g_s of rooted cuttings and cuttings that remained unrooted of *S. leprosula*. Measurements were made on day 63 (n=18 per treatment of rooted and unrooted cuttings). Means with the same letters are not significantly different at $P \leq 0.05$.

the stem dry weight of cuttings between treatments (Table 6.2). Between days of measurements, only leaf weight was significantly higher on day 28 than day 1 (Table B43).

No statistical analysis was carried out on starch, sugar and nitrogen values of leaf and stem of cuttings since inadequate samples were available. Leaf and stem nitrogen were not determined on day 28 because no sample was left. Means of these variables are presented in Table 6.2. The components of sugar for each treatment is given in Table B45.

Discussion and conclusions

Variation in leaf areas affected the final rooting percentage of *S. leprosula* stem cuttings where highest rooting was obtained with 15 cm^2 followed by 30 cm^2 and 60 cm^2. This finding is consistent with results of *Triplochiton scleroxylon* (Leakey *et al.* 1982b) and *Khaya ivorensis* (Tchoundjeu 1989) where a clear optimum leaf area was apparent, at 50 cm^2 and 10-30 cm^2 respectively. However, cuttings of *Terminalia spinosa* (Newton *et al.* 1992) and *Nauclea diderrichii* (Leakey 1990) displayed no tendency towards an optimal leaf area. On the other hand, response of rooting of *Cordia alliodora* stem cuttings to leaf areas was observed only with variation of irradiance level in propagator (Mesen 1993). He found that cuttings with 10 cm^2 leaf area rooted significantly less than those with 20 cm^2 and 30 cm^2 at PAR level of 0-88 µmol m^{-2} s^{-1}; and the opposite response was obtained at irradiance level of 0-1278 µmol photons m^{-2} s^{-1} (Mesen 1993). The primary influence of leaf areas was on leaf water status and photosynthetic activity of cuttings (Leakey *et al.* 1982b; Leakey and Coutts 1989; Newton *et al.* 1992). In the current experiment, rooting decreased with increasing leaf areas despite higher P_n obtained. The low rooting in 60 cm^2 leaf area could be due to more water being lost in these cuttings as reflected by high transpiration rates; which could be more severe during periods of water deficit. This was supported

Table 6.2 : Mean values of dry weight, starch, total sugar and nitrogen of leaf and stem of *S. leprosula* cuttings for the respective leaf area treatments on day 1 and day 28 of the experiment (length of stem= 5 cm). Samples of cuttings were taken at 09:00 hours on each day. (n=30 per treatment for stem dry weight; n=3 per treatment for stem and leaf starch, total sugar and nitrogen except for stem sugar on day 28 where n=2 for 60 cm^2 leaf area treatment); ± standard error of mean.

	Day 1			Day 28		
Leaf area (cm^2)	15	30	60	15	30	60
Leaf weight (g)	0.13a	0.21b	0.38c	0.14a	0.23b	0.41c
Stem weight (g)	0.32a	0.29a	0.35a	0.32a	0.26a	0.33a
Leaf starch (%)	5.16±1.46	3.74±0.99	5.74±1.83	5.08±1.93	8.40±0.86	8.41±2.23
Stem starch (%)	4.71±1.52	3.29±0.67	3.43±0.66	3.33±0.42	4.25±0.55	5.79±0.54
Leaf sugar (%)	3.77±0.44	4.83±0.30	3.07±0.66	2.44±0.63	4.27±0.15	2.82±0.69
Stem sugar (%)	1.77±0.54	1.07±0.36	1.08±0.30	1.73±0.19	2.51±0.11	3.34±0.15
Leaf nitrogen (%)	1.39±0.12	1.32±0.05	1.59±0.54	-	-	-
Stem nitrogen (%)	0.54±0.05	0.57±0.02	0.57±0.01	-	-	-

Means with the same letters of each variable on day 1/day 28 are not significantly different at $P \leq 0.05$. Statistical analysis was not carried out for starch, sugar and nitrogen since inadequate samples were available.

- : Samples were not available for the nitrogen determination on day 28.

by higher percentage of leaf shedding especially in cuttings with 60 cm^2 leaf area which may indicate that these cuttings suffered water deficit more than those with 30 cm^2 and 15 cm^2 leaf areas. The negative effect of water deficit on P_n in cuttings with large leaf areas was not demonstrated since the measurements ended at ca. 12:00 hours where water deficit in cuttings may have been about to occur. The results obtained seemed to indicate that it is necessary to strike a balance between photosynthesis and transpiration for optimum rooting to occur, which is in agreement with that suggested by Okoro and Grace (1976); Elliasson and Brunes (1980); Leakey *et al.* (1982b); Leakey and Coutts (1989); Newton *et al.* (1992). Poor rooting in cuttings with 60 cm^2 leaf area in the current experiment could also be due to the greater quantities of substance inhibitory to rooting being formed and accumulated in their stem during periods of high irradiance (Elliasson and Brunes 1980).

The importance of retaining a certain leaf area for successful rooting Dipterocarp cuttings has also been realised as several workers trimmed the leaves of cuttings to either half or one third of their size (Lo 1985; Siagan *et al.* 1989; Smits 1992; Smits *et al.* 1994; Noraini and Ling 1993). A 30 cm^2 leaf area seemed to be adequate for rooting *Dryobalanops lanceolata* stem cuttings although no attempt has been made to relate between carbon gain through photosynthesis and water losses via transpiration (Moura-Costa and Lundoh 1994). My earlier experiment with *Hopea odorata* also indicated that there was a tendency towards optimum leaf areas for better root development, although the results obtained were confounded to the different size of cuttings (Aminah 1991b).

Many researchers have indicated that photosynthesis does occur before rooting and influences rooting of cuttings of several tree species (Eliasson and Brunes 1980; Davis and Potter 1981; Smalley *et al.* 1991; Leakey and Storeton-West 1992; Newton *et al.* 1992; Hoad and Leakey 1993; Mesen 1993). The increment in leaf dry matter on day 28 in all treatments in the current experiment indicates that photosynthesis occurred in cuttings before rooting took place. This however

did not reflect in the stem dry weight which showed no difference between the two days of measurement. Similar results were obtained by Mesen (1993) where total dry matter of *C. alliodora* was similar to initial weight after six weeks in propagation beds despite correction made to overcome variation in the determination of dry matter weight. Dry matter increment was only a rough indication of occurrences of photosynthesis in cuttings (Davis 1988) and also there may be problems which was unavoidable experimentally in estimating gains or losses over time based on different samples and mixture of clones. However, cuttings of *T. scleroxylon* displayed a substantial increment in dry weight at several intervals of days in propagation beds (Leakey *et al.* 1982b; Leakey and Coutts 1989; Leakey and Storeton-West 1992). The dynamic of starch and sugar could not be well interpreted in the current experiment as data was not statistically analysed due to inadequate samples available. In general, there was an increase in leaf and stem starch as well as total stem sugar for the 30 and 60 cm^2 leaf area treatments from day 1 to day 28. Such increment was however not observed in the 15 cm^2 leaf area.

P_n of rooted cuttings was higher than that of cuttings which remained unrooted and this was associated with higher g_s in rooted than unrooted cuttings. Similar results were obtained by Hoad and Leakey (1993); Newton *et al.* (1992). P_n of rooted cuttings may be enhanced by the presence of roots as sink for assimilates (Wareing *et al.* 1968; Okoro and Grace 1976; Elliasson and Brunes 1980). Also the increase in P_n of rooted cuttings could be due to roots supplying leaves with cytokinin which may increase the activity and/or amount of carboxylating enzymes (Okoro and Grace 1976).

The fact that there was no influence of morphological characteristics of cuttings on rooting and number of roots in the present experiment may be due to the restriction of taking the cuttings from the upper and middle nodes. Only cuttings with at least 60 cm^2 leaf area was used as to avoid the smaller leaf area being allocated to the lower node positions where leaf area tend to be smaller than the

upper nodes.

Environmental data indicated that mean VPD in propagators could be kept low, but periods of water deficit did occur as indicated by the maximum VPD which was more than the threshold level (0.5 kPa) suggested by Grange and Loach (1983a) for many broadleaved species. This temporary water deficit seemed to affect the rooting of cuttings with larger leaf (60 cm^2) rather than smaller leaf areas of 15 and 30 cm^2 as discussed in the earlier paragraph.

The current experiment demonstrates the importance of trimming leaf areas for optimum rooting of *S. leprosula* stem cuttings. Leaf areas between 15 cm^2 and 30 cm^2 are more favourable for successful rooting of *S. leprosula* stem cuttings. Larger leaf area of 60 cm^2 is not recommended under the PAR range studied (0-384 μmol photons m^{-2} s^{-1}) since this may induce water deficit and consequently lower rooting. Mesen (1993) working with *C. alliodora* stem cuttings demonstrated that larger leaf area could give better rooting under low PAR and vice versa. For practical application, the information on leaf areas is required in rooting any cutting to avoid wastage of space in rooting bed occupied by large leaf areas. On the other hand, labour and time spent in trimming the leaves could be saved in cuttings where rooting is not responsive to leaf area treatment.

EXPERIMENT 2 : Effect of indole-3-butyric acid (IBA) on the rooting ability of leafy stem cuttings of *Shorea leprosula*

Introduction

The effect of auxin has been the subject of investigation in many studies on the rooting of cuttings (Loach 1988c; Hartmann *et al.* 1990; Blakesley *et al.* 1991). The optimum auxin concentration for the rooting of cuttings has been reported to vary between species/clones (Leakey *et al.* 1982b; Leakey 1985; Hartmann *et al.* 1990; Blakesley *et al.* 1991). Among the auxins tested, IBA has been found to

be the most effective in promoting rooting of cuttings in a large number of tropical tree species (Pain and Roy 1981; Leakey *et al.* 1982b and 1990; Darus 1988; Tchoundjeu 1989; Mesen 1993). Similarly, IBA has been shown to be most suitable for rooting of cuttings in several Dipterocarp species (Smits 1983; Lo 1985; Aminah 1989; Kamis and Ng 1989; Pollisco 1994). Many auxin trials on *S. leprosula* stem cuttings have been reported, but none has critically evaluated the influence of auxin on root initiation (Muckadell and Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Srivastava *et al.* 1986; Aminah 1989; Kamis and Ng 1989; Siagan *et al.* 1989; Liew 1992). The present study was carried out to determine the effectiveness of several IBA doses on the rooting of *S. leprosula* stem cuttings.

Materials and methods

Cutting materials and experimental layout

The stock plants were raised from seeds collected from three trees in the Forest Reserve Ulu Teranum in the state of Pahang Malaysia. When the germinated were approximately 7 cm tall, they were transplanted into black perforated polythene bags (9 cm x 17 cm height). The potting medium used was forest top soil and sand in the ratio of 3:1 by volume. To every cubic meter of the medium, 1.2 kg triple superphosphate (46% P_2O_5) and 1.6 kg ground magnesium limestone (33%) were added. The potted seedlings were kept on the transplanting beds shaded with plastic netting. The average mid-day irradiance on a sunny day under this shade was 770 µmol photons m^{-2} s^{-1} (ca. 33% full sunlight) as measured with a SKP 215/200 light sensor (Skye Instruments, UK). Granular compound commercial fertiliser "NPK Blue" (12%N: 12%P_2O_5:17%K_2O:2MgO + Trace element) was applied at the rate of 1 g per seedling per month. In January 1992 when the seedlings were ten months old, sixty seedlings were randomly selected for the experiment. The average height of the selected seedlings was 62 cm. Single node cuttings were taken from these stock plants from the second to

sixth node down the stem. The apical undeveloped shoots were discarded as they were not suitable for cuttings. Five cuttings were taken from one stock plant and each node was systematically allocated to each of the five indole-3-butyric acid (IBA) treatments enabling each node position to be equally represented in each treatment. This was to reduce morphological variation as cuttings materials were taken from many non-clonal seedlings. The length of the cuttings was 5 cm and the leaf area retained on each cutting was 30 cm^2. The leaf area was cut using a 30 cm^2 template made of graph paper which was measured with leaf area meter (Delta-T Series, Taiwan). The base of the cuttings was cut at a right angle to the stem and was treated with one of five IBA doses: 0, 20, 40, 60 and 80 µg. The IBA formulation was prepared by dissolving in absolute ethyl alcohol which was diluted to 50% with distilled water. The control, without IBA, was 50% ethyl alcohol. The IBA was applied to each cutting using a micropipette (F10, Gilson Medical Electronic, France). The alcohol was immediately evaporated in a stream of cold air from a fan. These treated cuttings were inserted into the rooting medium of cleaned river sand in a closed polythene mist propagation system. The plastic enclosures were then shaded with a layer of black plastic netting. The average mid-day photosynthetically active irradiance under this shade on a sunny day was 275 µmol photons m^{-2} s^{-1} (14% of full sunlight). Details and illustration of the propagation system is described in chapter 3. Each treatment consisted of sixty cuttings and they were laid out in six blocks. Each block consisted of five plots with ten cuttings of each treatment per plot. The treatments were randomly allocated to plots within a block. The node was held on the rooting beds in sequential order of its position on the plants in order to simplify the lay out. Unfortunately this resulted in node and cutting position on the rooting bed being confounded and therefore preventing statistical analysis to be carried out. Ideally, the node positions on rooting bed should have been randomised. The initial diameter of each cutting base was measured using digital calliper (CD-6, Mitutoyo Corporation Japan).

Assessment of cuttings and statistical analyses

Assessment was made every two weeks for rooted, unrooted and dead cuttings as well as number of roots on each cutting. After the observation, the cuttings were replanted into the rooting bed and reassessed until the tenth week by which time the rooted cuttings were potted. The remaining unrooted cuttings and the cuttings with root primordia were replanted for further observation on rooting at week twelve when the experiment was terminated.

The number of cuttings rooted at each assessment was analysed using a modification of the cumulative distribution analysis formally presented by Hunter *et al.* (1984) and developed by Brain and Butler (1988). The problems with this type of data are that there is serial correlation between values at successive recording times and that the counts are not normally distributed. However, the underlying variable (the time to rooting) can be analysed by fitting its cumulative distribution function to the empirical cumulative distribution derived from the observed data. A maximum likelihood analysis was performed using Genstat 5 (Payne *et al.* 1987). The time to rooting in all cases appeared to be normally distributed with no transformation of the time axis. If time of recording is z, the cumulative distribution (F) is:

$$F(z) = N(b(z-m))$$

where m is the mean time to rooting, b is the inverse of the standard deviation of time to rooting and N is the cumulative normal distribution function with zero mean and unit variance. The significance difference between treatments was determined using the Chi-square test on the differences of treatment deviances.

Analysis of deviance (Payne *et al.* 1987) was carried out at the final week of assessment to determine the significant influence of treatments on rooting

percentage. Analysis of variance (Payne *et al.* 1987) was carried out to determine treatment differences in initial stem diameter and number of roots. The results in all tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

Results

Analysis of variance showed that there was a significant difference between treatments in the initial diameter of cuttings used (Table B46). Mean diameter of cuttings assigned to the 0 µg IBA treatment (3.22 mm) was significantly smaller than that of cuttings used for treatments 40 µg (3.33 mm) and 60 µg (3.32 mm) IBA. However, no significant difference was found between initial diameter of cuttings used for 0 µg IBA and those used for 20 µg (3.26 mm) and 80 µg (3.28 mm) IBA. No significant difference was also found in initial diameter among the IBA treated cuttings.

Figure 6.6 presents the fitted cumulative distribution function for rooting response to occur for each of five IBA treatments. Hence predicted rooting percentage could be estimated with time. From Figure 6.6, it can be seen that stem cuttings of *S. leprosula* treated with IBA initiated rooting earlier than the untreated cuttings. The onset of rooting was staggered over several weeks until week ten, after which little new rooting was obtained. The predicted time taken for 50% rooting to occur was significantly shorter in cuttings with IBA treatments compared with untreated cuttings (Table 6.3).

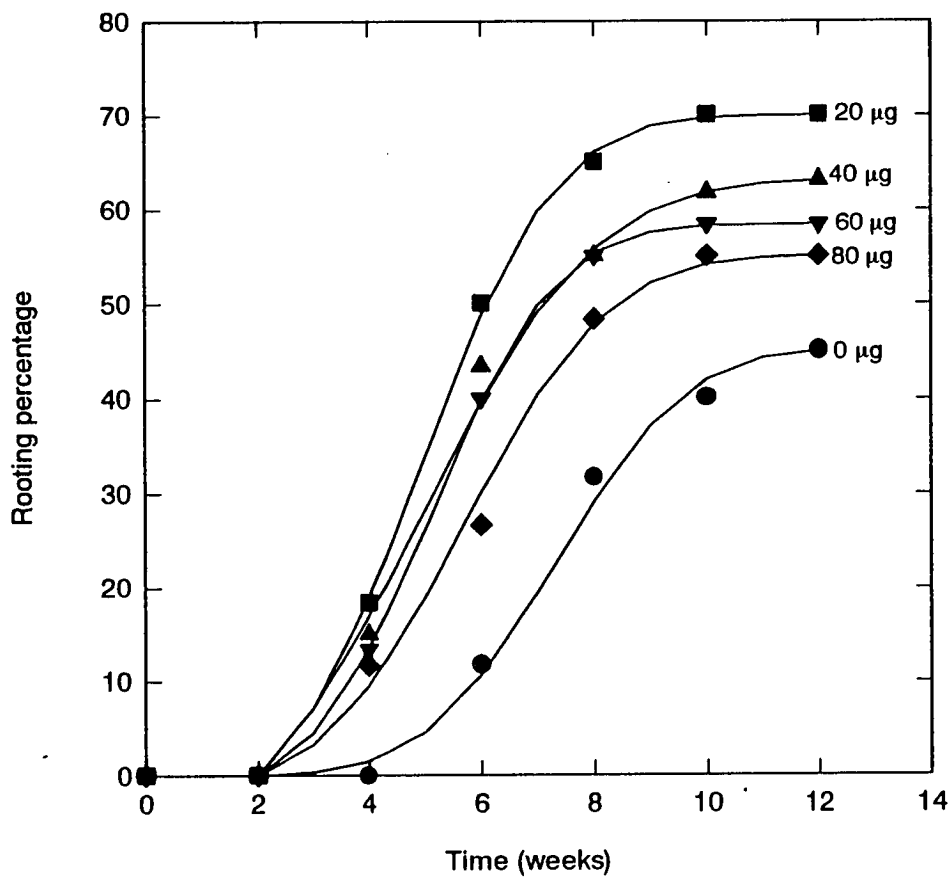


Figure 6.6 : Fitted cumulative distribution function of rooting response of *S. leprosula* stem cuttings treated with five different IBA doses (n=60 per treatment).

Table 6.3 : The time taken for fifty percent rooting response to occur in *S. leprosula* stem cuttings treated with five different IBA doses (n=60 per treatment).

Treatments	Weeks
0 µg IBA	7.31
20 µg IBA	4.92
40 µg IBA	4.93
60 µg IBA	5.13
80 µg IBA	5.69

Analysis of deviance carried out on rooting at week twelve revealed that IBA treatments did not significantly influence the final rooting percentage of *S. leprosula* stem cuttings. The accumulated rooting percentage at week twelve for cuttings with IBA treatments of 20, 40, 60 and 80 µg was 70%, 63%, 58% and 55% respectively, while the rooting percentage of the untreated cuttings was 45%. Analysis of deviance also indicated that there was no significant difference between treatments in either dead cuttings or cuttings that did not root at week twelve. It can be seen that a high percentage of cuttings remained unrooted (43%) in untreated cuttings (0 µg IBA). Among the IBA treatments, a higher percentage of cuttings remained unrooted when treated with 60 and 80 µg IBA compared with those receiving 20 and 40 µg IBA. The percentage of dead cuttings ranged from 10 to 17% (Figure 6.7).

Analysis of variance showed that IBA treated cuttings produced significantly more roots than the untreated cuttings throughout the assessment period. Table B47 shows the analysis of variance on the number of roots at week ten. The mean accumulated number of roots per rooted cutting at week ten for 20, 40, 60 and 80 µg IBA was 5.1, 5.3, 4.8 and 4.8 respectively compared to 3.1 for cuttings without IBA treatment. Figure 6.8 shows that IBA treated cuttings produced more roots than the untreated cuttings throughout the assessment period.

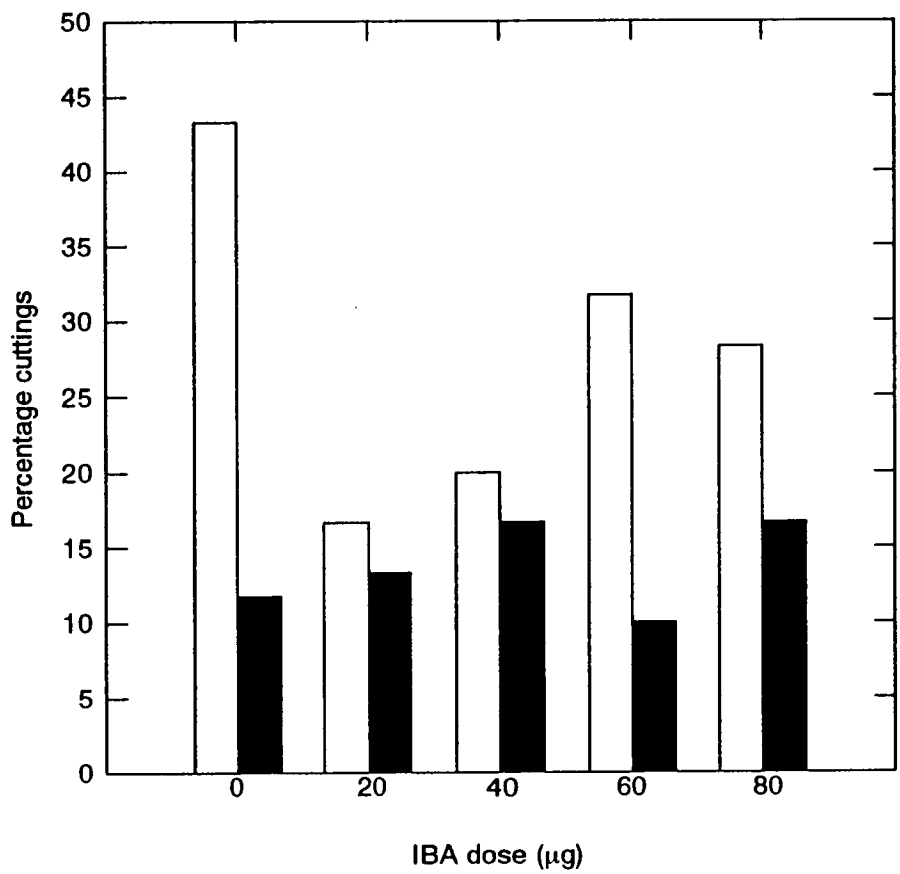


Figure 6.7 : Effect of IBA doses on the percentage of dead cuttings and on percentage of cuttings that remained unrooted of *S. leprosula* at week twelve (hollow bar=unrooted cuttings; solid bar=dead cuttings; n=60 per treatment).

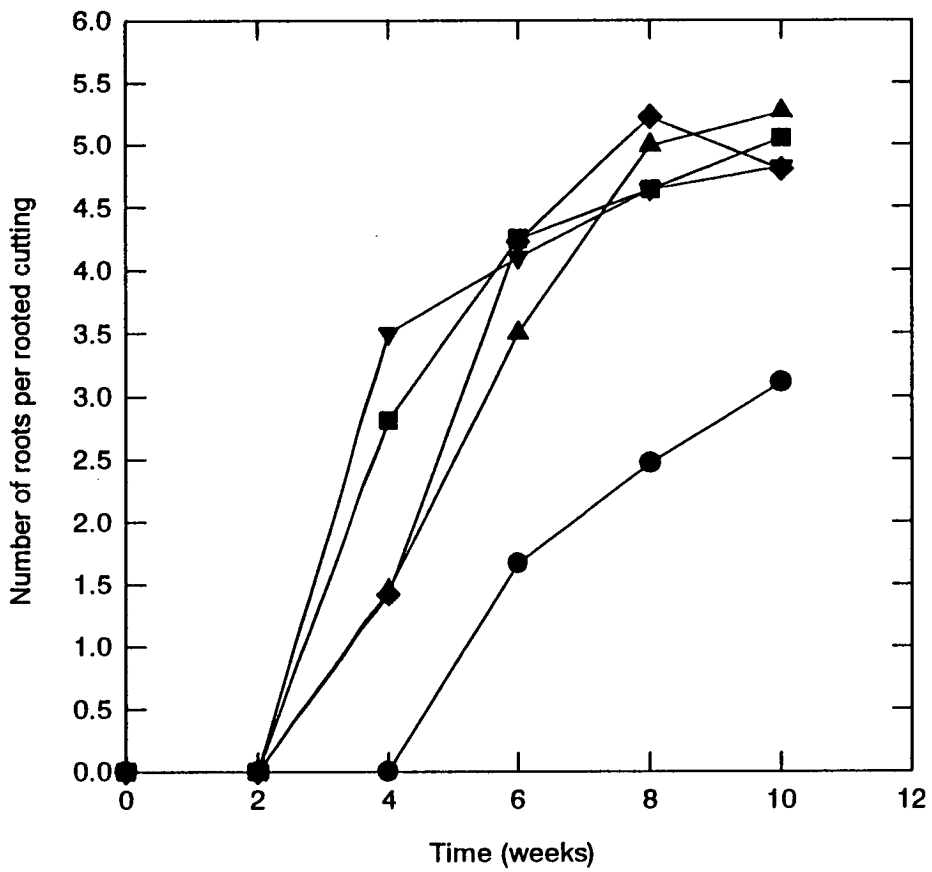


Figure 6.8: Effect of IBA doses on the rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* (circle=0 μg; square= 20 μg; triangle up=40 μg; triangle down=60 μg; diamond=80 μg IBA per cutting; n=60 per treatment).

Discussion and conclusions

External hormone application to the base of cuttings has been shown to improve rooting of many difficult-to-root species (Morsink and Smith 1974; Leakey *et al.* 1982b; Darus 1988; Kamis and Ng 1989; Siagan *et al.* 1989; Pollisco 1994). Injecting the hormone into the xylem of the stock plants of *Triplochiton scleroxylon* also improved the rooting performance of cuttings taken from these stock plants (Leakey 1993).

The results of the present experiment showed that the final rooting percentage (week 12) was not significantly affected by the treatments. These results were in agreement with those obtained in previous trials with *S. leprosula* (Srivastava and Manggil 1981; Halle and Kamil 1981) where no improvement in final rooting of cuttings treated with IBA was achieved. However, with more detail assessments, the present experiment has demonstrated the significant response in the rate of rooting of cuttings treated with IBA. Similar results were reported by Lo (1985) where auxin application also enhanced the rate of rooting of *Shorea macrophylla* although the final rooting was not affected by the auxin application. Recent report by Pollisco (1994) also noted that IBA applications hastened the rooting of cuttings of five Dipterocarp species but the results were not statistically analysed. The application of IBA may have an indirect influence by enhancing the speed of translocation and movement of sugar to the base of cuttings and consequently stimulate rooting (Haissig 1974, 1982; Patrick 1979).

Practically, speeding up the process of adventitious root formation is considered an advantage, as the earlier the cuttings were able to form roots, the greater the chances for them to survive. This aspect of rate of rooting has often been neglected in auxin experiments. A single assessment made at the end of an experiment could not reveal the effect of auxin in accelerating rooting of cuttings (Muckadell and Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Siagan *et al.* 1989; Kamis and Ng 1989; Noraini and Ling 1993). In

addition, the number of cuttings used in several auxin trials of Dipterocarp species were so few and poorly replicated that the effect of auxin dose could not be statistically detected (Muckadell and Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Smits 1983; Kamis and Ng 1989).

Application of IBA also enhanced the number of roots developed on each rooted cutting of *S. leprosula* as indicated by the greater number of roots produced compared to the untreated cuttings. This may have an advantage by enhancing good anchorage when planted in the field. Similar results have been observed in other studies with *S. leprosula* cuttings (Kamis and Ng 1989; Siagan *et al.* 1989). Besides the effect of IBA, the diameter of cuttings may have influenced the development of the number of roots on the cuttings since the initial diameter of cuttings used for 0 µg IBA was smaller compared with those used for IBA treatments. Larger diameter cuttings may have more carbohydrate reserves for root development compared to smaller diameter cuttings. It has been shown by Mesen (1993) that an increase in cutting diameter of *Cordia alliodora* resulted in significant increase in the number of roots. The importance of stored carbohydrates in cuttings for root development had also been observed by Veierskov and Andersen (1982); Veierskov *et al.* (1982). However, differences in cutting diameter had no effect on the rooting percentage of *C. alliodora* cuttings (Mesen 1993).

Higher doses of IBA in the present experiment were not detrimental to cuttings as indicated by the mortality which was 10 to 17% among all the IBA doses used. The detrimental effect of IBA was also not observed on juvenile cuttings of *S. macrophylla* where a higher concentration of 10,800 ppm IBA resulted in only an average of 11% mortality (Lo 1985). Hartmann and Kester (1983) stated that IBA could be used in a wide range of concentrations without giving toxic effect to the cuttings. But the higher levels of 60 and 80 µg IBA used in the present experiment may be less suitable for rooting the juvenile cuttings of *S. leprosula* because many cuttings remained unrooted even twelve weeks after

planting in the rooting bed. A higher auxin dose (200 µg per cutting) had been shown to inhibit rooting in cuttings of certain clones of *Triplochiton scleroxylon* (Leakey *et al.* 1982b).

From the trend of the results obtained in the present experiment and from the economic point of view, 20 µg IBA per cutting is recommended for rooting of juvenile stem cuttings of *S. leprosula.* A lower dose of 5 to 10 µg IBA could be further tested to confirm the results. In other species, higher doses of IBA such as 100 µg and 150 µg were reported to significantly improve rooting of *S. acuminata* and *S. parvifolia* stem cuttings. However, lower IBA doses were not tested in their experiments (Noraini and Ling 1993).

As seen from all the rooting experiments carried out in the present study, the morphological characteristics have substantial influence on rooting of *S. leprosula* stem cuttings. Therefore management of stock plants is vital to produce suitable cutting materials for rooting. Nutrients and irradiance had been reported to influence the rooting of subsequent collected cuttings (Moe and Andersen 1988; Leakey and Storeton-West 1992; Mesen 1993). These aspects were examined in chapter 7.

CHAPTER 7

THE INFLUENCE OF STOCK PLANT GROWING ENVIRONMENTS ON THE MORPHOLOGICAL AND PHYSIOLOGICAL CONDITIONS OF *SHOREA LEPROSULA* AND SUBSEQUENT ROOTING ABILITY OF LEAFY STEM CUTTINGS

The morphology and physiology of stock plants were influenced by their growth environments and this aspect was investigated by treating the stock plants of *S. leprosula* with different levels of i) NPK fertilisers and ii) irradiance. The rooting potential of the subsequent cuttings collected from these stock plants were tested.

EXPERIMENT 1: Effect of two fertiliser levels on stock plant growth of *Shorea leprosula* and their subsequent effect on the rooting ability of leafy stem cuttings

Introduction

Several workers have reported a species-dependent response of tropical forest tree seedlings to fertiliser application (Anthony 1971, Sundralingam 1977, 1982, 1983; Mendoza and Glori 1976; Mel Zwierink 1983; Sundralingam *et al.* 1985). Observations in the FRIM nursery also indicated that the newly potted seedlings or rooted cuttings of several Dipterocarp species were stunted and required a supplement of fertiliser (Aminah, unpublished). Similarly, the need for nutrients in raising stock plants for production of regular cutting materials has been widely recognised in a number of plant species (Enright 1963; Leakey 1983; Lo 1985; De Souza and Felker 1986; Blazich 1988; Moe and Andersen 1988; Yasman and Smits 1988; Tchoundjeu 1989; Leakey and Storeton-West 1992; Mesen 1993).

At the same time, unrestricted application of fertiliser is however not recommended; for example, high rates of nitrogen fertiliser application to *Khaya ivorensis* has yielded cuttings which suffered high mortalities (Tchoundjeu 1989). The effects of fertiliser application to stock plants on the subsequent rooting ability of cuttings were reported to be inconsistent between species (Moe and Andersen 1988). The present experiment was carried out to examine the influence of fertiliser application on morphological and physiological characteristics of *S. leprosula* stock plants, and the rooting ability of subsequent cuttings.

Materials and methods

Stock plants

The experiment took place in the FRIM nursery in August 1992. The planting materials were raised from stem cuttings taken from coppice shoots of five year old seedlings. The newly rooted cuttings were potted in black perforated polythene bags (9 cm diameter x 17 cm height). Potting mixture consisted of forest top soil and sand in the ratio of 3:1 (standard potting mixture in the FRIM nursery). Seven clones were used: 525, 549, 550, 559, 581, 587 and 590. These plants were treated every two weeks with 0.5 g or 1.5 g per plant granular compound fertiliser (NPK Blue, 12%N: 12%P_2O_5 :17%K_2O :2%MgO + Trace elements, manufactured by ICI Fertilisers, Malaysia). Each treatment consisted of 42 plants and they were randomly arranged in six blocks on transplanting beds. The beds were shaded with black plastic netting. The red/far red ratio measured was 1.16, almost similar to that of full sunlight (1.2). Clones were equally represented in each treatment and block. Plants were watered to field capacity twice daily in the morning and late afternoon except on rainy days. Weeding, insecticide and fungicide applications were made whenever necessary. The insecticide used was Tamaron special (50% Methamidopos active ingredient, Bayer Company, Leverkusen, Germany). The fungicide was Benlate (50%

benomyl active ingredient, E.I. Du-pont, Denemours and Co. Inc. USA). Both biocides are systemic in nature.

Environments of the stock plants

Temperature, relative humidity and irradiance were recorded on a data logger (21X micrologger, Campbell Scientific, UK) using sensors as described in chapter 3. The sensors were placed in the centre of three blocks which were randomly chosen from the total of six blocks. The data logger was programmed to scan each sensor every 60 seconds, to calculate and store mean readings every 5 minutes. Data collection extended from day 1 to day 6 of the experiment.

Assessments of stock plants and statistical analyses

Initial height (from base of the new shoot to the apex) and basal diameter of new shoot on each plant were measured. Successive measurements of height and diameter were made every two weeks until the experiment was terminated 22 weeks after planting. Then number of nodes, leaf area and P_n were also measured. For determination of leaf area, the length and width of leaves along the stem were measured on 12 plants (two plants randomly selected per treatment per block). Leaf area was then calculated using the equation developed for *S. leprosula* plants grown in the FRIM nursery as described in experiment 2 of this chapter (Equation: y=0.33+0.60x, where y=leaf area in cm^2; x=product of length and width in cm^2). P_n was measured on first fully developed leaf from the apex of each experimental plant using a portable gas analyser (LCA-3, ADC, Hoddesdon, UK). One leaf per treatment per block was measured at a time. Number of nodes was counted on all the experimental plants.

Analysis of variance was carried out on height, diameter, leaf area, node positions, P_n and g_s and PAR of experimental plants followed by Fisher's t test (LSD).

Rooting of stem cuttings

The treatments were arranged on the propagation bed by randomly selecting stock plants after 22 weeks treated with fertiliser. The number of stock plants varied between 4 to 7 per block per treatment depending on the number of cuttings per stock plant. Cuttings from all the node positions with at least 30 cm^2 leaf area were taken. Initial diameter, length and node position of each cutting were recorded. The preparation of cuttings is as described in chapter 3. The prepared cuttings were planted in a medium consisting of cleaned river sand. Node positions were held in sequential order on the rooting beds as they were on stock plants. The lay-out of each cutting from the stock plants is illustrated in Appendix C. Clones were not uniformly replicated in each block because some of the stock plants were dead; therefore, the effect of clones will not be analysed in the rooting experiment. Each treatment consisted of 162 cuttings (126 and 36 cuttings for rooting and dry weight assessments respectively). These cuttings were randomly split into six blocks with 27 cuttings per block. Each block is a closed polythene propagator (1 m x 1 m x 0.8 m) with a misting unit in the centre. Details and illustrations of the propagation system used are as described in chapter 3.

Microclimate of propagator

Temperature, relative humidity and irradiance were recorded by using a data logger (21X micrologger, Campbell Scientific, UK) using sensors as described in chapter 3. The sensors were placed in the centre of two blocks which were randomly chosen from the total of six blocks. The data logger was programmed to scan each sensor every 60 seconds, to calculate and store mean readings every 5 minutes. Data collection extended from day 1 to day 23 of the experiment.

Photosynthetic rate (P_n) and stomatal conductance (g_s)

P_n and g_s of cuttings prior to rooting were measured using a portable infrared gas analyser (LCA-3, ADC, Hoddesdon, UK). Four cuttings were randomly chosen per treatment per block and they were measured on days 1, 8, 14 and 28 after planting in rooting media. No measurement was made on day 21 because the gas analyser was out of order. P_n and g_s were measured on day 63 on the rooted cuttings and cuttings that remained unrooted. Only cuttings from blocks 1,3,4,5 and 6 were taken for measurement since most of the cuttings in block 2 were rooted and the number of unrooted cuttings left to be sampled was inadequate. Three rooted and unrooted cuttings per treatment per block were randomly chosen.

Dry weight of leaves and stems

Destructive samples of cuttings were harvested on day 0 17:00 hours after the experiment was laid-out on the rooting beds. Six cuttings per treatment per block were randomly harvested giving a total of 36 cuttings per treatment. Dry weight of leaf and stem of each cutting was determined after drying in an oven (ULM 500 Memmert, Germany) at 40 °C for 48 hours.

Nitrogen, phosphorus and potassium (NPK) determinations

Initial NPK of leaves and stem were determined using the method as described in chapter 3.

Assessment of cuttings and statistical analyses

Assessment was made weekly until week sixteen, for rooted, unrooted and dead cuttings as well as number of roots on each cutting.

Analysis of deviance for stepwise regression in Genstat 5 (Payne *et al.* 1987) was used to determine which of the recorded variables were significantly associated with rooting percentage. Analysis of variance followed by Fisher's t test (LSD) were used to test for significant differences in mean accumulated number of roots per rooted cutting, leaf and stem dry weight, P_n and g_s. Results in all the tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

Results

Stock plants

The environmental data of the stock plants is as shown in Figures 7.1a,b,c,d,e.

Initial height and diameter of shoot did not differ significantly between treatments. Mean height was 4.0 cm and 4.2 cm for 0.5 g and 1.5 g fertiliser respectively while mean diameter was 0.1 cm for both treatments. But there were significant differences between clones in initial height and diameter of the shoot (Tables B48 and B49). The final height and diameter (at week 22) were significantly different between treatments and clones (Tables B50 and B51). Clones 549 were tallest and had the largest diameter at the initial and final week of experiment (Figures 7.2a,b and 7.3a,b). Plants treated with 1.5 g fertiliser were taller and larger in diameter than those of 0.5 g (Figures 7.4a,b). No significant difference was obtained between treatments in the number of nodes, leaf area and P_n of stock plants measured at week 22. Mean values of the above mentioned variables are given in Table 7.1. The number of dead plants was three (7.1%) and seven (16.7%) for 0.5 g and 1.5 g fertiliser treatment respectively. The Chi-square test indicated that the dead plants were not significantly different between treatments.

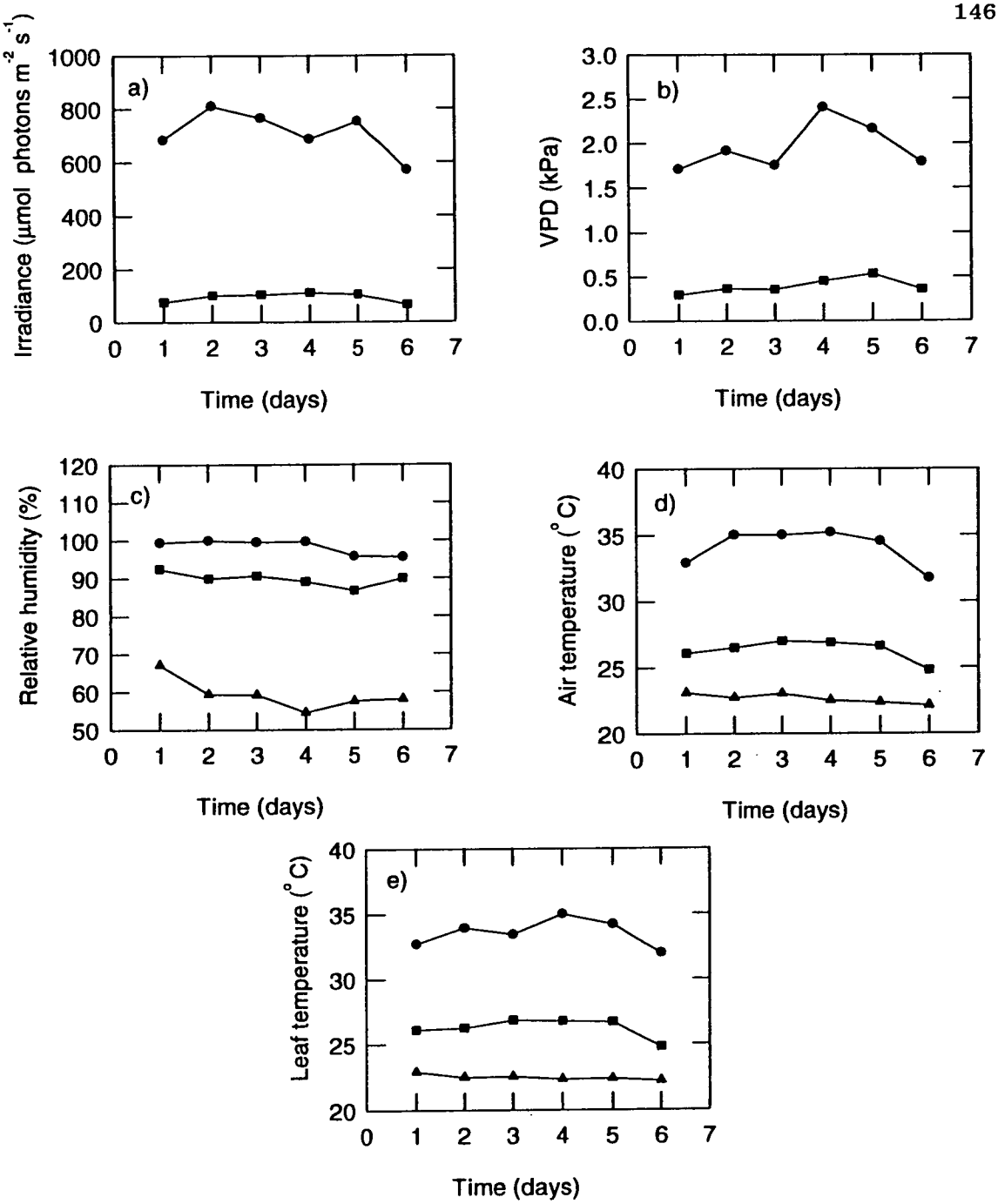


Figure 7.1 : Environmental data of *S. leprosula* stock plants measured from day 1 to day 6 of the experiment a) Irradiance; b) VPD; c) Relative humidity; d) Air temperature; e) Leaf temperature. Each data point per variable corresponds to mean values of three blocks calculated as a 5 minute average. Mean values were calculated over 24 hours period daily (circle=maximum values; square=average values; triangle=minimum values for each variable). Minimum values for irradiance and VPD are not displayed because values are zeros or close to zeros.

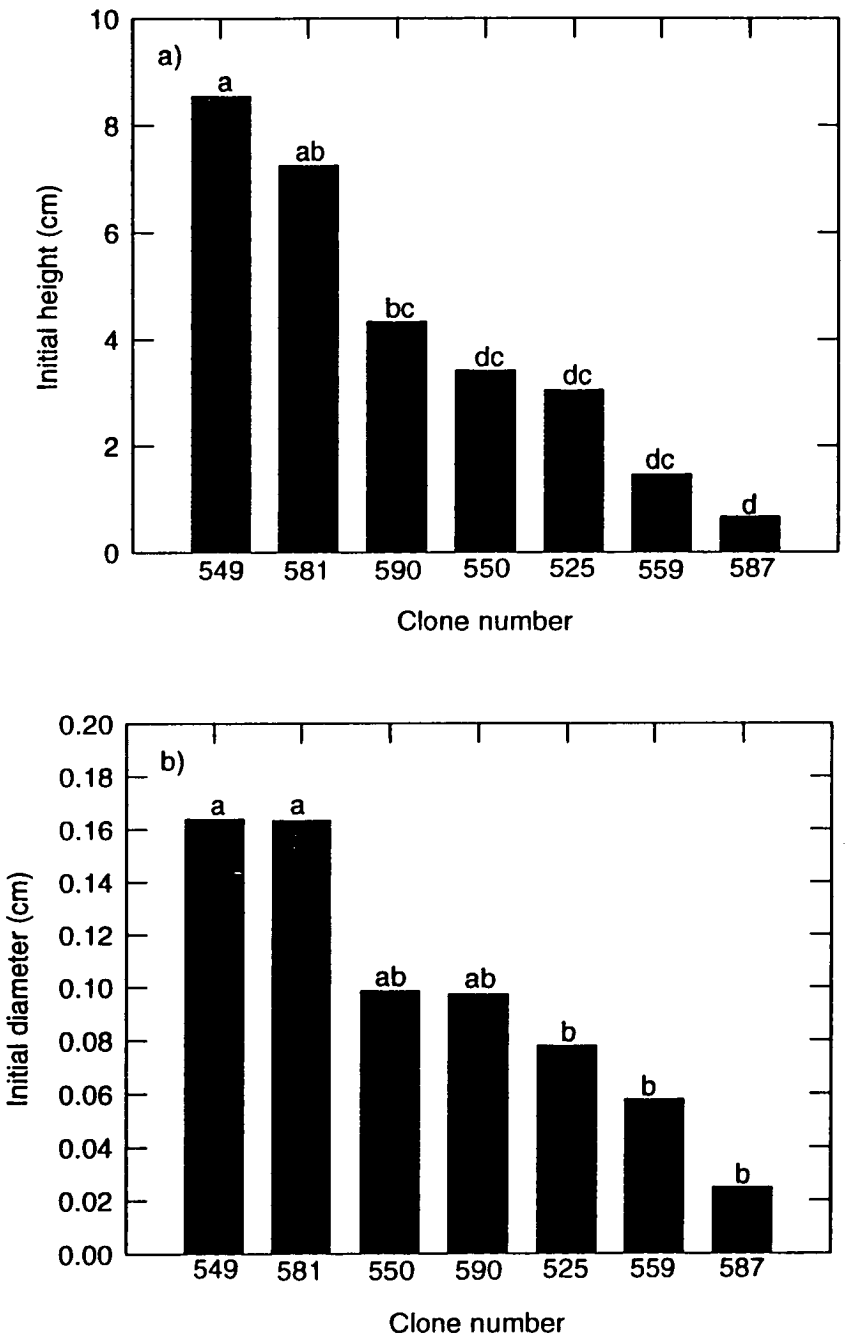


Figure 7.2: Clonal variations on mean a) Initial height; b) Initial diameter growth of *S. leprosula* potted stock plants raised from rooted cuttings measured on day 0 (n=14 per clone). Means with the same letters are not significantly different at $P \leq 0.05$.

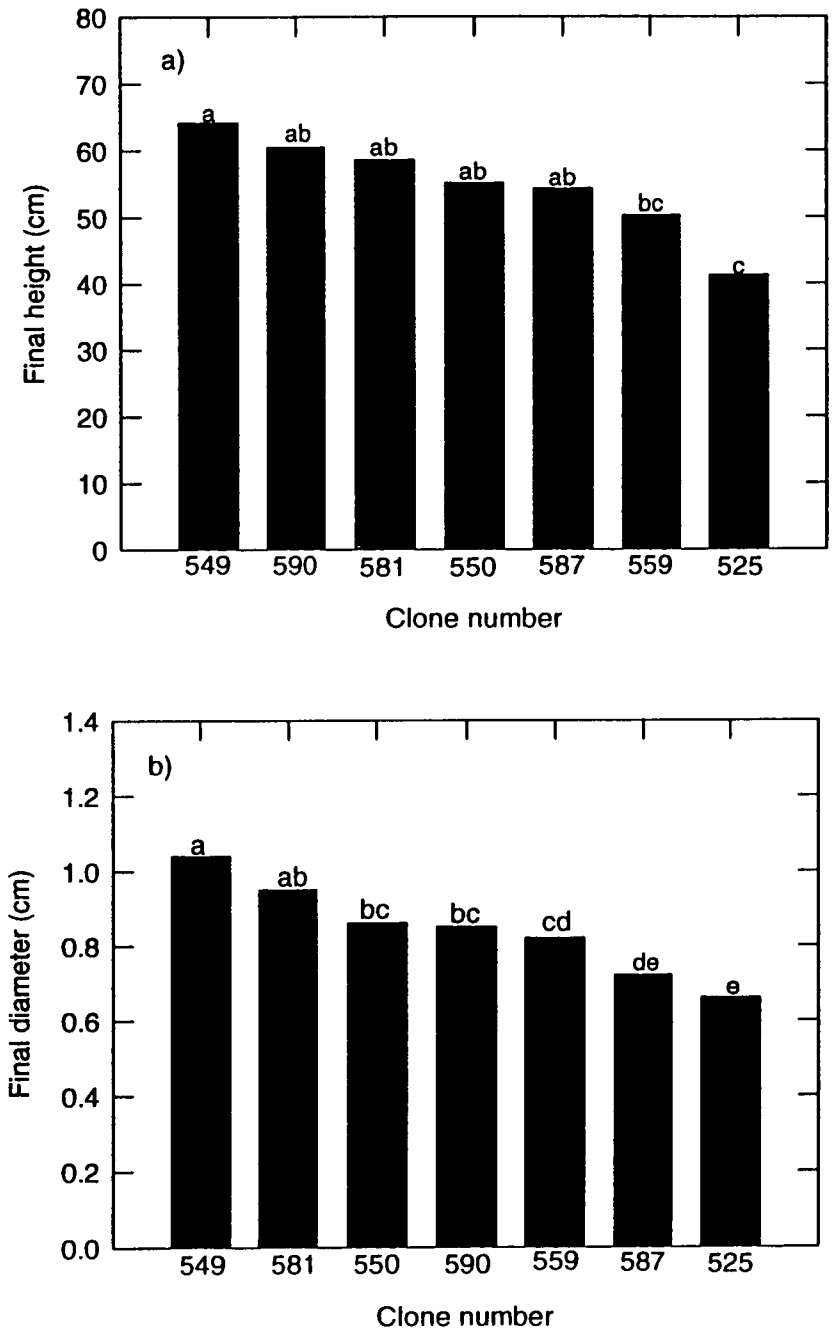


Figure 7.3 : Clonal variations on mean a) Final height; b) Final diameter growth of *S. leprosula* potted stock plants raised from rooted cuttings measured at week 22 (n=12 for clones 549,581; n=11 for clones 550,525; n=10 for clones 590,559; n=8 for clone 587; differences in number of clones were due to death of stock plants). Means with the same letters are not significantly different at $P \leq 0.05$.

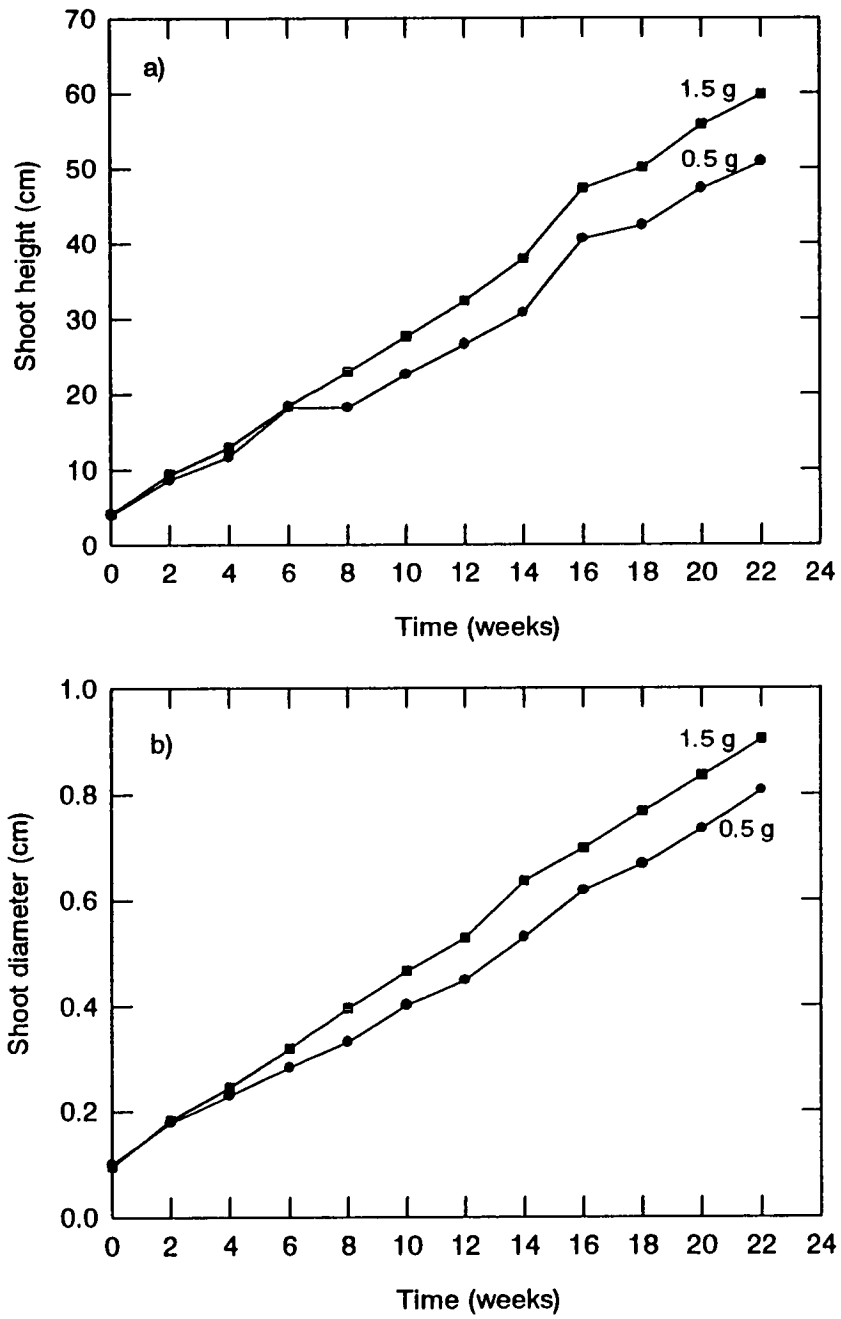


Figure 7.4 : Effect of two fertiliser levels on the a) Rate of mean height growth; b) Rate of mean diameter growth of *S. leprosula* potted stock plants raised from rooted cuttings (n=42 per treatment).

Table 7.1 : Mean number of nodes, leaf area, P_n, g_s and PAR measured at week 22 on potted stock plants of *S. leprosula* treated with 0.5 g and 1.5 g fertiliser (n=39 and 35 for stock plants treated with 0.5 g and 1.5 g fertiliser respectively, 3 and 7 plants were dead from each treatment respectively).

Fertiliser levels	0.5 g per plant	1.5 g per plant
Number of nodes	7.59a	7.86a
Leaf area (cm^2)	74.36a	65.04a
P_n (μmol CO_2 m^{-2} s^{-1})	5.19a	5.15a
g_s (mmol H_2O m^{-2} s^{-1})	235.90a	214.00a
PAR (μmol photons m^{-2} s^{-1})	226.97a	275.77a

Means with same letters for each variable are not significantly different at $P \leq 0.05$

Stem cuttings

The initial length, diameter and volume of cuttings from the 1.5 g treatment were significantly longer, larger in diameter and volume than those of 0.5 g fertiliser treatment (Tables B52, B53 and B54). Initial dry weight of leaf (30 cm^2 area per cutting) and stem was not significantly affected by treatments. Initial leaf and stem NPK were higher in cuttings from plants treated with 1.5 g than 0.5 g fertiliser. Statistical analysis was not carried out on the NPK values since inadequate samples were available. Mean values of all the above mentioned variables are given in Table 7.2.

The final rooting percentage and mortality of cuttings at week 16 were not significantly affected by either fertiliser levels or morphological characteristics of cuttings. Figure 7.5a,b show the rooting and death rates of *S. leprosula* stem cuttings as affected by the treatments respectively. The cuttings which remained

unrooted were significantly more in cuttings from 1.5 g than 0.5 g fertiliser treatment (Table B55, Figure 7.5c).

Table 7.2 : Mean initial diameter, length and volume of cuttings; initial dry weight; nitrogen, phosphorus, potassium of leaf and stem of *S. leprosula* cuttings taken from potted stock plants treated with 0.5 g and 1.5 g fertilisers. Cuttings were harvested on day 0 at 17:00 hours after the experiment was laid-out (leaf area of each cutting=30 cm^2). The stock plants were grown at 33% full sunlight; $\pm$ standard error of mean.

Fertiliser levels	0.5 g per plant	1.5 g per plant	Number of sample per treatment (n)
Diameter(cm)	0.61a	0.68b	126
Length (cm)	6.21a	7.33b	126
Volume (cm^3)	1.72a	2.47b	126
Leaf weight (g)	0.24a	0.25a	36
Stem weight (g)	0.63a	0.66a	36
Leaf nitrogen (%)	1.42±0.05	1.66±0.08	6
Stem nitrogen (%)	0.41±0.05	0.58±0.04	6
Leaf phosphorus (%)	0.16±0.01	0.30±0.02	6
Stem phosphorus (%)	0.18±0.02	0.28±0.01	6
Leaf potassium (%)	0.40±0.04	0.71±0.04	6
Stem potassium (%)	0.36±0.04	0.65±0.06	6

Means with the same letters for each variable are not significantly different at $P \leq 0.05$. Statistical analysis was not carried out on the NPK values since inadequate samples were available.

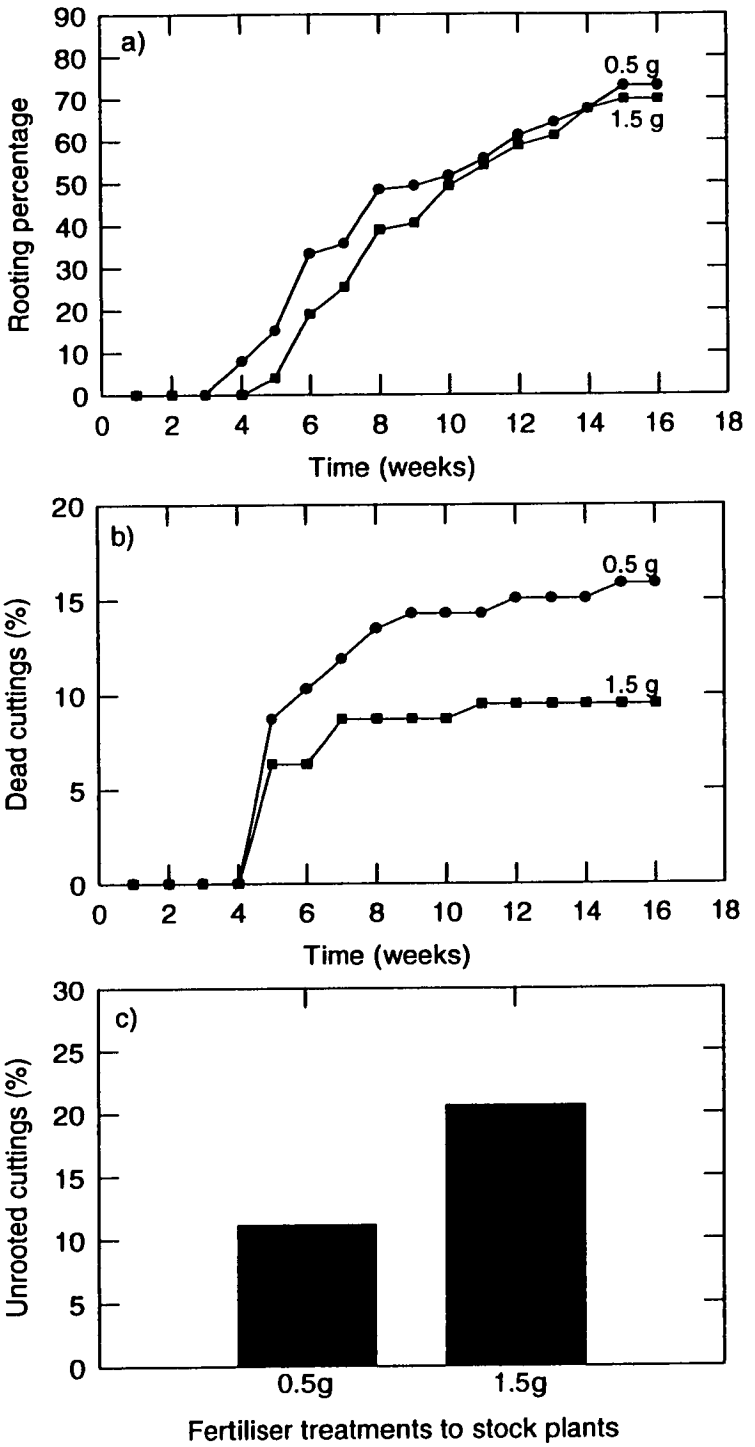


Figure 7.5 : Influence of fertiliser applications to *S. leprosula* stock plants on a) Subsequent rooting rate; b) Subsequent death rate of *S. leprosula* stem cuttings (circle =0.5 g; square=1.5 g fertiliser); c) Mean percentage of subsequent stem cuttings of *S. leprosula* that remained unrooted at week 16 (n=126 per treatment).

Number of roots per rooted cutting was not significantly affected by treatments (Figure 7.6a) but it was significantly affected by diameter and the relationship was negative (Table B56 and Figure 7.6b).

There was no significant difference between treatments in P_n and g_s of cuttings prior to rooting. Mean P_n was 2.3 and 2.2 μmol CO_2 m^{-2} s^{-1} and mean g_s was 246 and 314 mmol H_2O m^{-2} s^{-1} for cuttings from 0.5 g and 1.5 g fertiliser treatments respectively. The PAR values obtained when the measurement of P_n and g_s were made was not significantly different. Mean PAR was 109 and 94 μmol photons m^{-2} s^{-1} for cuttings from 0.5 g and 1.5 g fertiliser treatments respectively. There was a significant difference between days and P_n of cuttings prior to rooting and this was related to differences in PAR (Tables B57 and B58; Figures 7.7 a,b).

P_n of rooted cuttings measured on day 63 was significantly higher than that of cuttings which remained unrooted (Table B59; Figure 7.8). No significant difference in PAR and g_s was obtained between treatments when the measurements of P_n were made. Mean PAR ranged between 91 to 92 μmol photons m^{-2} s^{-1}; while mean g_s ranged from 295 to 318 mm H_2O m^{-2} s^{-1}.

Environmental data collected in propagators from day 1 to day 23 of the experiment is shown in Figures 7.9a,b,c,d,e. A longer period was not possible as sensors were needed for other experiments. Mean VPD could be maintained close to zero. However, the daily maximum VPD exceeded the threshold level of 0.5 kPa suggested by Grange and Loach (1983a) for many broadleaved species.

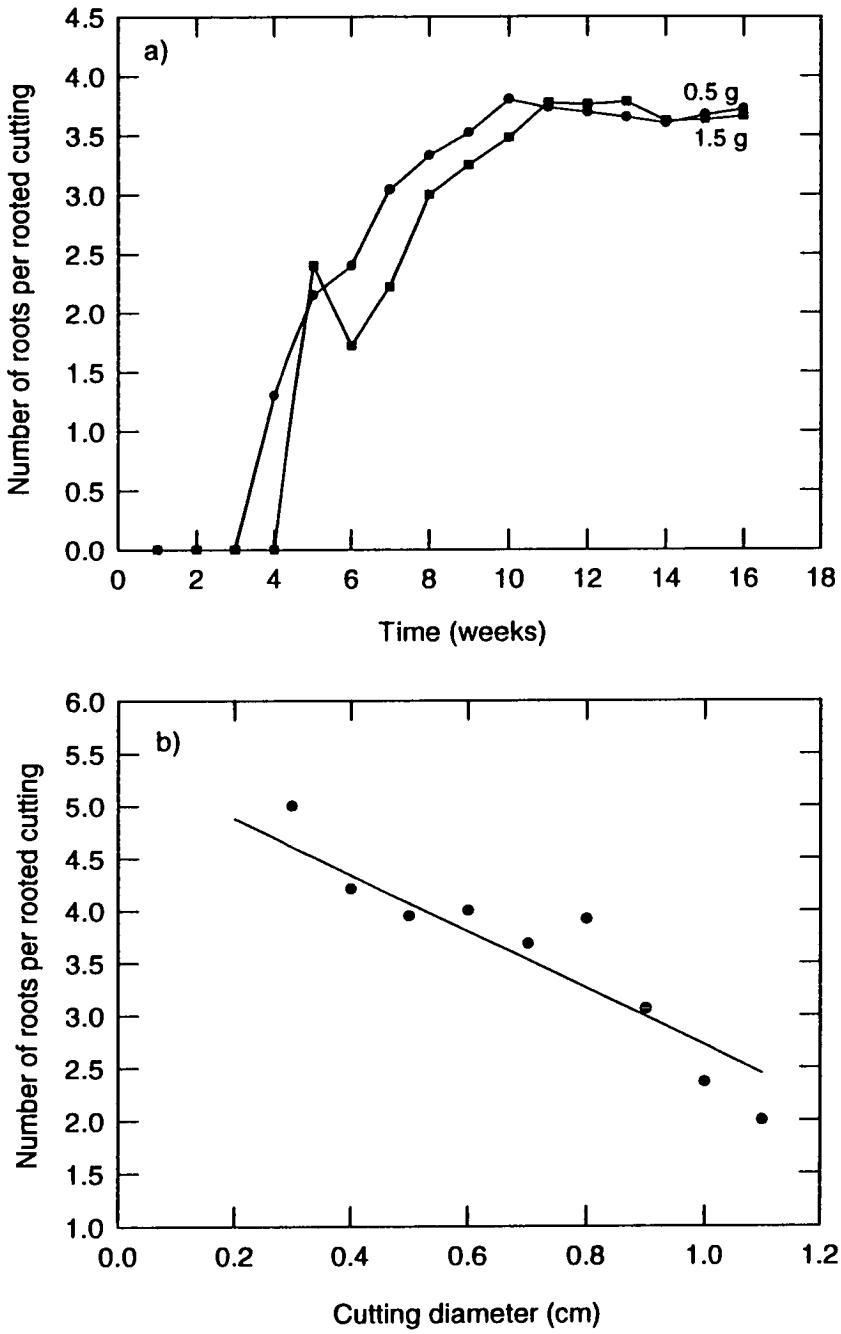


Figure 7.6 : Influence of fertiliser applications to *S. leprosula* stock plants on a) Rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* (circle=0.5 g; square=1.5 g fertiliser; n=126 per treatment); b) Relationship of mean accumulated number of roots per rooted cutting with diameter of *S. leprosula* stem cuttings. Points are groups of observed data whilst the line was drawn by connecting predicted values computed from the multiple regression model.

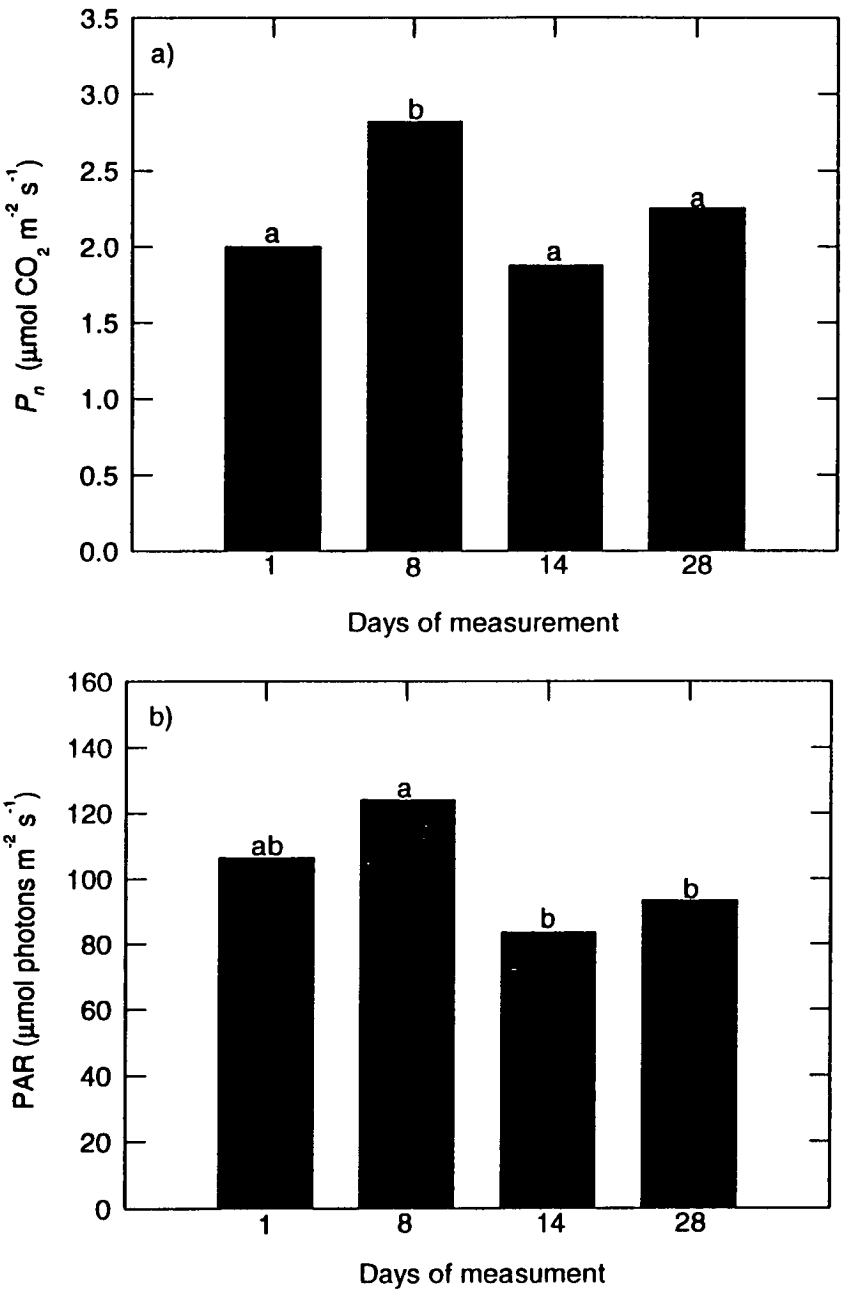


Figure 7.7 : Influence of days of measurements on a) Mean P_n of *S. leprosula* stem cuttings prior to rooting; b) Mean PAR when the P_n measurements were made (n=48 per day; means with the same letters are not significantly different at $P \leq 0.05$).

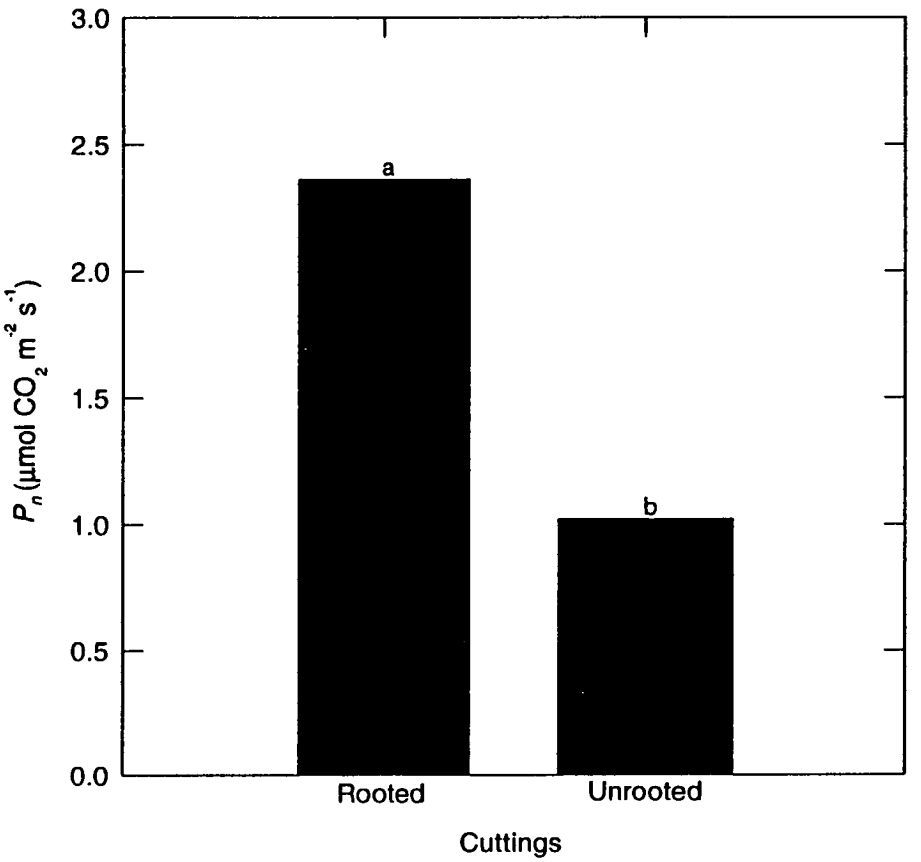


Figure 7.8 : Mean P_n of rooted cuttings and cuttings that remained unrooted of *S. leprosula*. Measurements were made on day 63 in blocks 1,3,4,5,6 since most of the cuttings in block 2 were rooted and number of unrooted cuttings left to be sampled were inadequate; (n=15 per treatment for rooted and unrooted stem cuttings). Means with the same letters are not significantly different at $P \leq 0.05$.

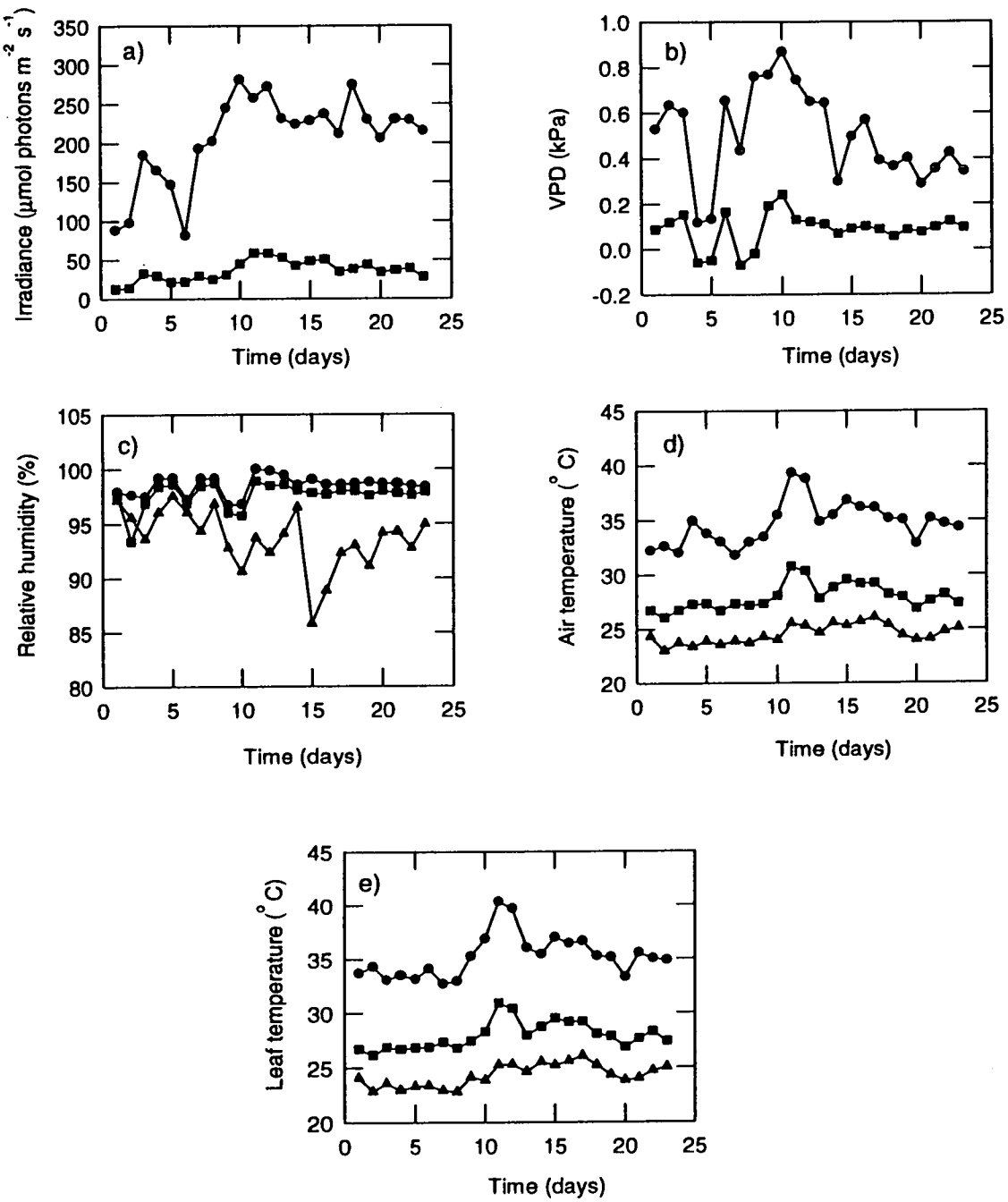


Figure 7.9 : Environmental data in enclosed mist propagators measured from day 1 to day 23 of the experiment. a) Irradiance; b) VPD; c) Relative humidity; d) Air temperature; e) Leaf temperature. Each data point per variable corresponds to mean value of two blocks calculated as a 5 minute average. Mean values were calculated on a 24 hour period daily (circle=maximum values; square=average values; triangle=minimum values of each variable). Minimum values of irradiance and VPD are not displayed because values are zeros or close to zeros.

Discussion and conclusions

In the present study height and diameter growth of *S. leprosula* stock plants were enhanced by higher fertiliser rate (1.5 g per plant per 2 weeks). However, both rooting percentages and number of roots of cuttings taken from these stock plants were not affected by either of the fertiliser treatments (0.5 g or 1.5 g per plant per 2 weeks). The importance of nutrients applied to stock plants is widely recognised, but their effects on subsequent rooting of cuttings have often been inconsistent and depended on plant species (Moe and Andersen 1988). For example, application of NPK fertiliser to pruned stock plants of *Triplochiton scleroxylon* enhanced their growth but improved the rooting of cuttings from lower lateral shoots; and had no affect on cuttings from apical lateral shoots (Leakey 1983). In another experiment, addition of fertiliser improved rooting of *T. scleroxylon* cuttings from stock plants grown at high irradiance (650 µmol photons m^{-2} s^{-1}); but poor rooting of cuttings was obtained when plants grown at low irradiance of 250 µmol photons m^{-2} s^{-1} were applied with fertiliser (Leakey and Storeton-West 1992). Cuttings of *Albizia guachepele* rooted better when stock plants were grown under low irradiance (200 µmol photons m^{-2} s^{-1}) and low dose (0.25% per plant) of fertiliser (20%N:20%P:20%K). On the other hand, rooting was reduced when stock plants were treated with high irradiance (500 µmol photons m^{-2} s^{-1}) and high dose (1.25% per plant) of similar fertiliser (Mesen 1993). In another instance, Mesen (1993) found that effect of fertiliser on rooting was more pronounced than irradiance. Application of NPK fertiliser (7.5 g per plant per 2 weeks) to stock plants of *C. alliodora* grown under shade or full sunlight was detrimental to rooting of subsequent cuttings compared to those from non fertilised plants. It seemed that high dose of fertiliser application to stock plants was not favourable for rooting of subsequent cuttings. Moe and Andersen (1988) stated that in general, stock plants which were suboptimally fertilised would yield cuttings that root best. Results of the present experiment suggest that the high rate used (1.5 g per stock plant per 2 weeks) may have not been at supraoptimal level as no negative effect in rooting of subsequent cuttings of *S.*

leprosula was observed. A supraoptimal fertiliser application has been associated with reduction in P_n as indicated by Mesen (1993) with *A. guachepele*. High nutrients also could result in leaves with low specific area (thicker leaves); which may increase mutual shading of chloroplast and reduce efficiency of gas exchange (Hoad and Leakey 1993). The low P_n may indirectly reduce rooting by slowing down the basipetal transport of auxin (Scott and Briggs 1963; Kampula and Potter 1984) and other cofactors (Salisbury and Ross 1985) which may originate from leaves to the base of cuttings for root initiation.

The work on stock plant fertilisation and subsequent rooting in Dipterocarps has not been reported. However, Yasman and Smits 1988 reported that routine fertiliser application is necessary for maintenance of production of cutting materials. Lo (1985) applied slow released fertiliser (12%N:12%P:17%K:2%Mg + Trace elements) to *S. macrophylla* stock plants and 80% rooting of subsequent cuttings has been achieved. In others no mention has been made as how the stock plants were fertilised (Momose 1978; Halle and Kamil 1981; Omon *et al.* 1989; Siagan *et al.* 1989; Kantarli 1993; Noraini and Ling 1993; Moura-Costa and Lundoh 1994).

Negative correlation between number of roots and initial diameter of cuttings may suggest that cuttings depended to a greater extent on carbohydrates formed after severance rather than the carbohydrate reserves. This result was contrasted to that of Mesen (1993) who found that number of roots of *C. alliodora* was positively related to the cutting diameter. With measurement of P_n of cuttings on the rooting bed, there is increasing evidence which indicated that photosynthesis occurred before rooting and contributes to rooting cuttings of several tree species (Eliasson and Brunes 1980; Davis and Potter 1981; Smalley *et al.* 1991; Leakey and Storeton-West 1992; Newton *et al.* 1992; Hoad and Leakey 1993; Mesen 1993).

P_n of rooted cuttings was higher than that of cuttings which remained unrooted. Similar results were obtained by Newton *et al.* (1992); Hoad and Leakey (1993). P_n of rooted cuttings may be enhanced by the presence of roots as sink for assimilates (Okoro and Grace 1976; Wareing *et al.* 1968; Elliasson and Brunes 1980). Also roots may supply leaves with cytokinin which could increase the activity and/or amount of caboxylating enzymes, hence increase in P_n (Okoro and Grace 1976).

In terms of microclimates around the cuttings, the mean VPD in propagators was successfully kept low, but periods of water deficit occurred as indicated by the maximum VPD which was more than the threshold level (0.5 kPa) suggested by Grange and Loach (1983a) for many broadleaved species. As in previous experiments, this temporary water deficit was not detrimental to rooting of *S. leprosula* stem cuttings. Similar observations were made by Mesen (1993); Newton and Jones (1993b) with other tropical species.

Although 1.5 g fertiliser applied to stock plants has enhanced their growth, no added advantage in the rooting of subsequent cuttings was obtained. Hence, from economic point of view 0.5 g per plant of NPK fertiliser (12%N: 12%P_2O_5 :17%K_2O: 2%MgO + Trace elements) applied every two weeks to potted stock plants is recommended for production of cutting materials in rooting of *S. leprosula*.

EXPERIMENT 2: Effect of two photon irradiance levels on stock plant growth of *Shorea leprosula* and their subsequent effect on the rooting ability of leafy stem cuttings.

Introduction

Many authors have demonstrated that the morphology and physiology of stock plants and the subsequent rooting of collected cuttings can be influenced by

variation in irradiance given to the stock plants (Hansen and Eriksen 1974; Hansen *et al.* 1978; Leakey 1983; Moe and Andersen 1988; Leakey and Storeton-West 1992; Mesen 1993). The effects of increasing irradiance during stock plant growth on the subsequent rooting of cuttings have been variable; irradiance may inhibit, delay or promote rooting or have no effect (Moe and Andersen 1988). Experimental evidence had indicated that stock plants require a certain level of irradiance to produce cuttings that root well but the optimum level varied between species (Moe and Andersen 1988). For example, at an irradiance of 60 to 100 μmol photons m^{-2} s^{-1}, no stock plant growth of *Prosopis alba* occurred and growth was marginal at 190 μmol photons m^{-2} s^{-1} (Klass *et al.* 1985). An irradiance of 520-560 μmol photons m^{-2} s^{-1} was needed for growth of *Prosopis alba* stock plants to provide sufficient cutting materials for a rooting experiment (Klass *et al.* 1985). Irradiance ranging between 0 to 2274 μmol photons m^{-2} s^{-1} promoted the growth of *Cordia alliodora* but did not influence the subsequent rooting compared to the lower irradiance (0 to 825 μmol photons m^{-2} s^{-1}). Similarly, growth of Dipterocarp seedlings requires certain level of irradiance for good growth, Sasaki and Mori (1981) showed that the height of *Vatica odorata*, *Hopea helferi* and *Shorea talura* grown under low irradiance of 10 to 15% full sunlight over a year growing period was 20, 18 and 10 cm respectively. Maximum height increment of *V. odorata* and *H. helferi* could be obtained at 32% and 32-52% of full sunlight respectively (Sasaki and Mori 1981). However, no experimental data has been presented on how Dipterocarp cuttings respond to stock plant irradiance treatments. The present experiment examines whether levels of irradiance applied to the stock plants may affect the morphological and physiological characteristics of *S. leprosula* stock plants and rooting ability of subsequent collected cuttings.

Materials and methods

Stock plants

The experiment took place in the FRIM nursery in June 1992. The planting materials were raised from stem cuttings taken from ten month old seedlings. The newly rooted cuttings were potted in black perforated polythene bags (9 cm diameter x 17 cm height). Potting mixture consisted of forest top soil and sand in the ratio of 3:1 (standard potting mixture in the FRIM nursery). Thirty clones each with two plants were: 9, 23, 24, 31,32,35, 37, 38, 39, 42, 45, 49, 54, 55, 57, 68, 70, 74, 75, 76, 81, 82, 84, 100, 133, 143, 151, 157, 162, 183). Five clones each with four plants were: 15, 21, 58, 78, 168. These plants were distributed equally in each of the two irradiance treatments: low irradiance (0-325 μmol photons m^{-2} s^{-1}) and high irradiance (0-722 μmol photons m^{-2} s^{-1}). Each treatment consisted of 40 plants and they were randomly arranged in two blocks. The high and low irradiance conditions were created by covering a wooden frame box (1.5 m x 1 m x 1 m) with one and two layers of black plastic netting respectively. These boxes were placed in open areas avoiding effects of shade from other objects. Mean red/far red ratio measured with a light sensor (SKR 110 660/730, Skye Instruments, UK) was 1.1, close to that of full sunlight (1.2). Granular compound fertiliser (NPK Blue, 12%N: 12%P_2O_5 :17%K_2O :2%MgO + Trace elements) was applied at the rate of 0.5 g per plant per two weeks. Maintenance of these stock plants is as described in experiment 1 of this chapter.

Environments of the stock plants

Environmental data: temperature, relative humidity and irradiance were recorded by data logger (21X micrologger, Campbell Scientific, UK) using sensors as described in chapter 3. The sensors were placed in the centre of three blocks which were randomly chosen from the total of six blocks. The data logger was programmed to scan each sensor every 60 seconds, to calculate and store mean

readings every 5 minutes. Data collection extended from day 1 to day 3 of the experiment. Another set of data collection was made for a period of ten days towards the end of experiment starting on day 180 until day 189.

Assessments of stock plants and statistical analyses

Initial height (from base of the new shoot to the apex) and basal diameter of the new shoot on each plant were measured. Successive measurements of height and diameter were made every two weeks until the experiment was terminated 30 weeks after planting. Then number of nodes was counted on all the experimental plants. Leaf area and photosynthetic rates were also measured. For determination of leaf area, the length and width of leaves along the stem were measured on 20 plants (five plants randomly selected per treatment per block from mixed clones). Leaf area was then calculated using the equation developed for *S. leprosula* plants grown in the FRIM nursery. (Equation: $y=0.33+0.60x$, where y=leaf area in cm^2; x=product of length and width in cm^2). This equation was derived by regressing the product of length and width against actual area of the leaves. Forty five leaves were randomly sampled and measured from mixed clones of *S. leprosula* potted rooted cuttings grown in the nursery under 33% sunlight. Actual leaf area was determined using leaf area meter (Delta-T series, Taiwan). The results indicated that the two variables were strongly correlated ($r^2=0.98$) (Figure 7.10).

P_n was measured using a portable gas analyser (LCA-3, ADC, Hoddesdon, UK) on five top most fully developed leaves which were randomly chosen from five plants in each block. One leaf per treatment per block was measured at a time. Measurements were made from 08:00 to 15:00 hours after which the boxes were covered with black cloth to acclimatise the plants to a dark environment. Dark respiration was then measured starting at 18:00 hours onwards.

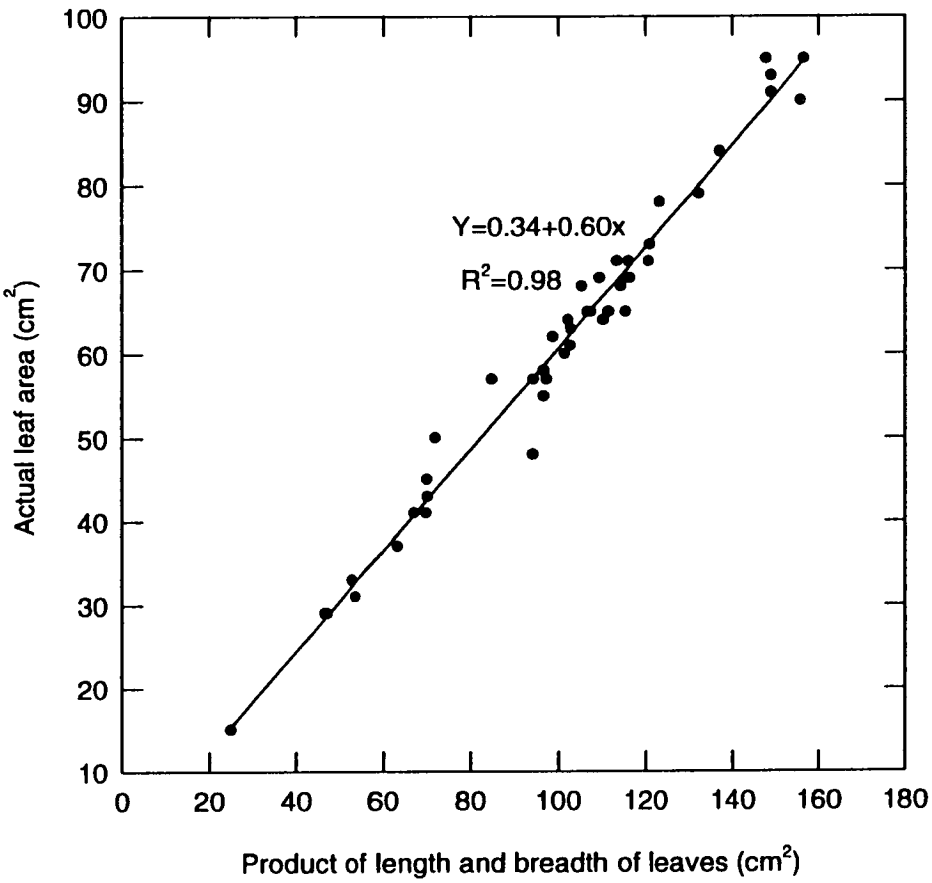


Figure 7.10 : Regression of actual leaf area and product of length and breadth of leaves taken from *S. leprosula* potted rooted cuttings grown under irradiance of 33% full sunlight (y=0.34+0.60x, r^2=0.98, n=45).

Analysis of variance was carried out on height, diameter, leaf area, node positions, P_n, g_s, R_d and PAR of experimental plants followed by Fisher's t test (LSD).

Rooting of stem cuttings

At the end of 30 weeks, cuttings from all the node positions with at least 30 cm^2 were harvested. The preparation of cuttings is as described in chapter 3. Initial diameter, length and node position of each cutting were recorded. The prepared cuttings were planted in a medium consisting of cleaned river sand. The treatments were arranged by randomly picking the stock plants. The number of stock plants varied between 4 to 7 per block per treatment. Node positions were held in sequential order on the rooting beds as they were on the stock plants. The lay-out of each cutting from the stock plants followed the same pattern as that in experiment 1 of this chapter. Each treatment consisted of 155 cuttings (125 and 30 cuttings for rooting and dry weight assessments respectively). These cuttings were randomly split into five blocks with 25 cuttings per block. Each block is a closed polythene propagator (1 m x 1 m x 0.8 m) with a misting unit in the centre. Details and illustrations of the propagation system used are as described in chapter 3.

Microclimate of propagator

Temperature, relative humidity and irradiance were recorded by a data logger (21X micrologger, Campbell Scientific, UK) using sensors as described in chapter 3. The sensors were placed in the centre of two blocks which were randomly chosen from the total of six blocks. The data logger was programmed to scan each sensor every 60 seconds, to calculate and store mean readings every 5 minutes. Data collection extended from day 16 to day 32 of the experiment.

Photosynthetic rate (P_n) and stomatal conductance (g_s)

P_n and g_s of cuttings prior to rooting were measured using a portable infrared gas analyser (LCA-3, ADC, Hoddesdon, UK). Four cuttings were randomly chosen per treatment per block and they were measured on days 1, 8, 14, 21 and 28 after planting in the rooting medium. P_n and g_s of rooted cuttings and cuttings that remained unrooted were measured on day 63. Four rooted and unrooted cuttings per treatment per block were randomly chosen. Measurements of P_n and g_s were made between 09:00 to 12:00 hours.

Dry weight of leaves and stems

Destructive samples of cuttings were harvested on day 0 at 17:00 hours after the experiment was laid out on the rooting beds. Six cuttings per treatment per block were randomly harvested giving a total of 30 cuttings per treatment. Dry weight of leaf and stem of each cutting was determined after drying in an oven (ULM 500 Memmert, Germany) at 40 °C for 48 hours.

Starch, sugar and nitrogen determinations

Since the amount of leaves and stems was little, the samples of every two blocks were combined for chemical analysis. Methods for determining the starch, sugar and nitrogen of leaf and stem are described in chapter 3.

Assessment of cuttings and statistical analyses

Assessment was made weekly until week sixteen, for rooted, unrooted and dead cuttings as well as number of roots on each cutting.

Analysis of deviance for stepwise regression in Genstat 5 (Payne *et al.* 1987) was used to determine which of the recorded variables were significantly

associated with rooting percentage. The method for graphical presentation of the association of rooting and their corresponding variables is described in experiment 1 of chapter 4. Analysis of variance followed by Fisher's t test (LSD) was used to test for significant effects of the treatments on mean accumulated number of roots per rooted cutting, leaf and stem dry weight, g_s , P_n and PAR. Significant difference in R_d between treatments was determined by t-test. Results in all the tests were considered significant when the probability level was less or equal to 5% ($P \leq 0.05$).

Results

Stock plants

The environmental data of the stock plants is as shown in Figures 7.11a,b,c,d,e. Irradiance level was 10% and 30% of the full sunlight for low and high irradiance treatments respectively.

Initial height of shoot did not differ significantly between treatments. Mean height was 1.0 cm and 0.9 cm for low and high irradiance treatments respectively. Measurements made at week 30 showed that plants grown under high irradiance were significantly taller, larger in diameter and had more nodes than those of low irradiance (Tables B60, B61, B62 and Figures 7.12a,b,c). Similar results were obtained with P_n, g_s, PAR as well as R_d measured at week 30 (Tables B63, B64, B65 and Figures 7.13a,b,c d). The P_n /PAR curve of stock plants grown in low and high irradiance is shown in Figures 7.14a,b.

No significant difference was obtained between treatments on leaf area. Mean leaf area was 68.7 and 79.6 cm^2 for stock plant under low and high irradiance respectively. Chi-square test indicated that there was no significant difference between treatments on the dead plants. The number of dead plants was four (10%) and seven (17.5%) for low and high irradiance treatments respectively.

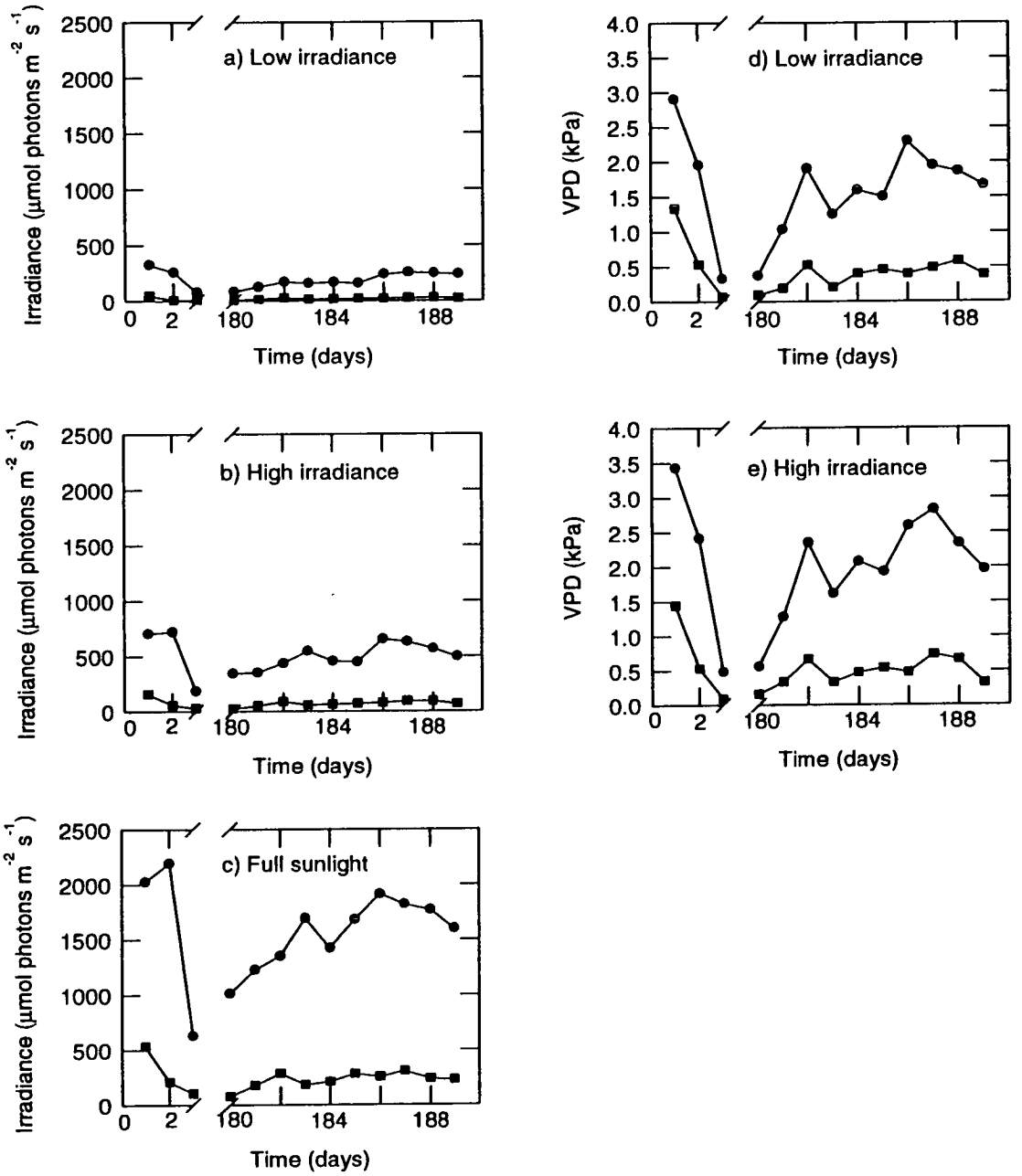


Figure 7.11 : Environmental data of *S. leprosula* potted stock plants grown under two irradiance levels measured on first 3 days after commencement of the experiment and later 10 days starting on day 180; a) Low irradiance level; b) High irradiance level; c) Full sunlight irradiance; d) VPD of low irradiance level; e) VPD of high irradiance level. Each data point per variable corresponds to the mean value of 2 blocks per treatment calculated as a 5 minute average. Mean values were calculated on 24 hours period daily (circle=maximum values; square=average values; minimum values are not displayed since they are zeros or closed to zeros).

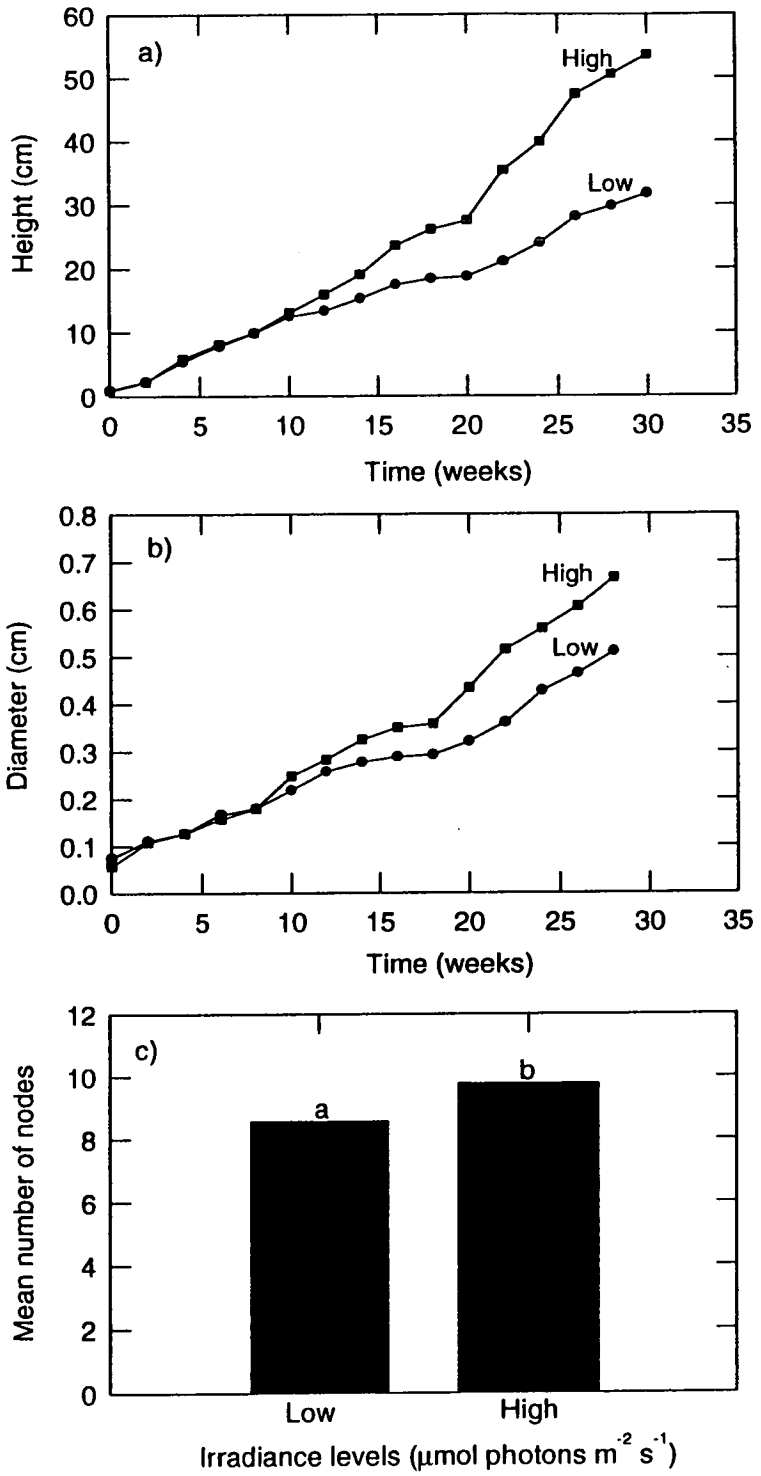


Figure 7.12 : Effect of two irradiance levels on mean a) Height; b) Diameter growth rate of *S. leprosula* potted stock plants raised from rooted cuttings (n=40 per treatment); c) Effect of two irradiance levels on mean number of nodes at week 30 (n=36 and 33 for stock plants from low and high irradiance respectively; 4 and 7 plants were dead in low and high irradiance levels respectively). Means with the same letters are not significantly different at $P \leq 0.05$.

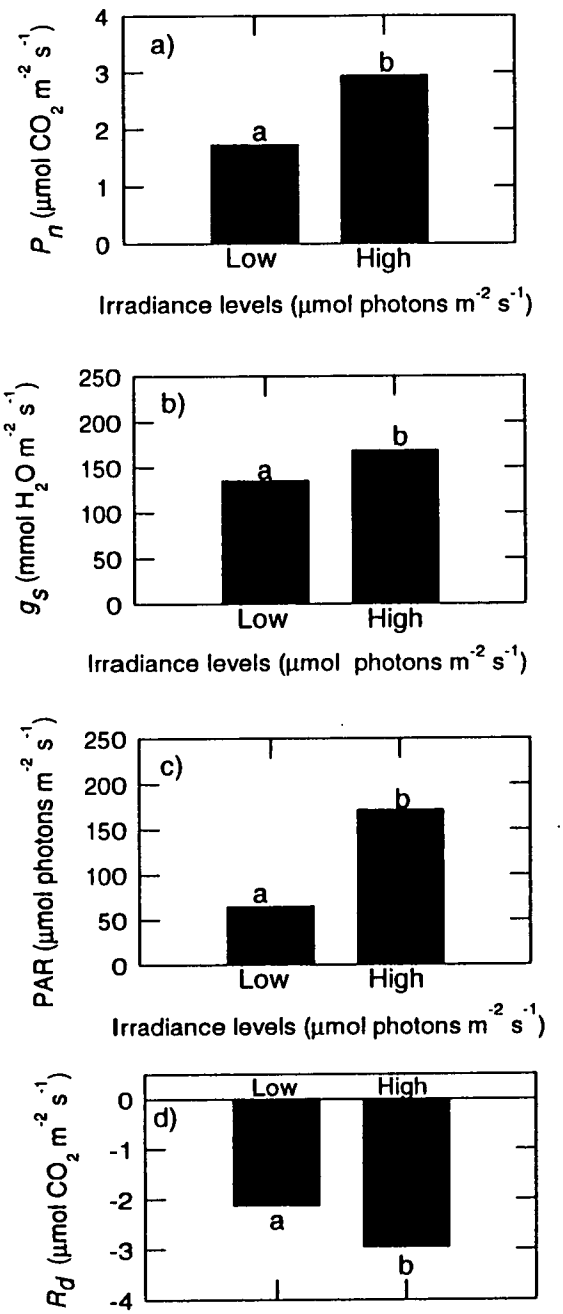


Figure 7.13 : Effect of two irradiance levels on mean a) P_n; b) g_s; c) PAR; d) R_d of *S. leprosula* potted stock plants measured on top most expanded leaf at week 30 (n=76 per treatment for P_n, g_s and PAR; n=12 per treatment for R_d). Mean of each variable with the same letters are not significantly different at $P \leq 0.05$.

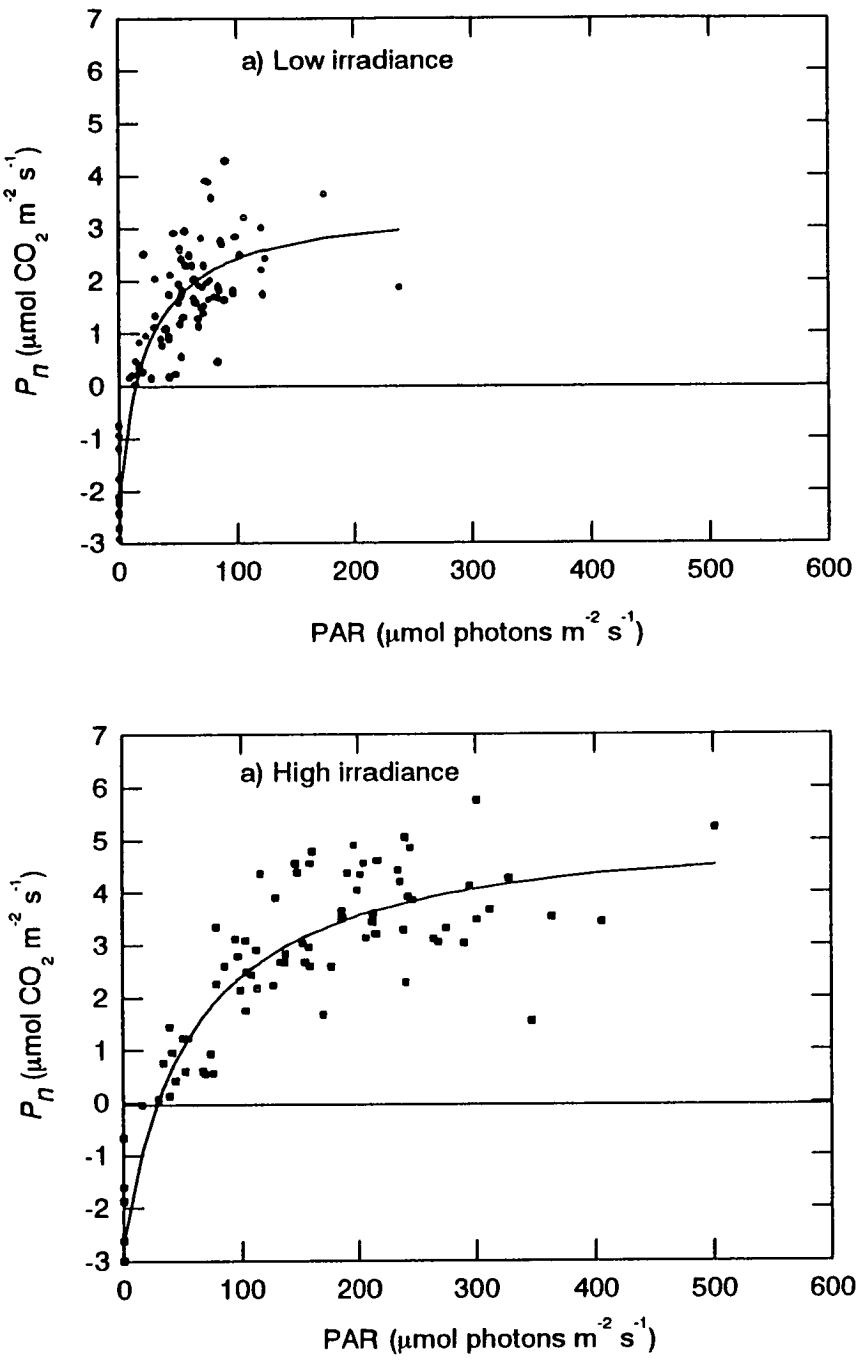


Figure 7.14 : P_n versus PAR curves of *S. leprosula* potted stock plants. P_n was measured on the top most expanded leaf at week 30; a) Plants grown under low irradiance; b) Plants grown under high irradiance (n=88 per treatment).

Stem cuttings

Environmental data on the propagation beds is shown in Figures 7.15a,b,c,d,e.

The initial length, diameter and volume of cuttings from high irradiance were significantly longer, larger in diameter and volume than those of low irradiance treatment (Tables B66, B67 and B68). Initial dry weight of leaf (which was trimmed to 30 cm^2 area) and stem was also significantly affected by treatments (Tables B69 and B70). Initial leaf and stem starch as well as total stem sugar were more in plants from high than that of low irradiance. However, total leaf sugar did not show much different between treatments. The component of sugar for each treatment is given in Table B71. Similarly not much different was obtained between the two irradiance treatments in the initial leaf and stem nitrogen. No statistical analysis was carried out on these variables since inadequate samples were available. Mean values of the above variables are given in Table 7.4.

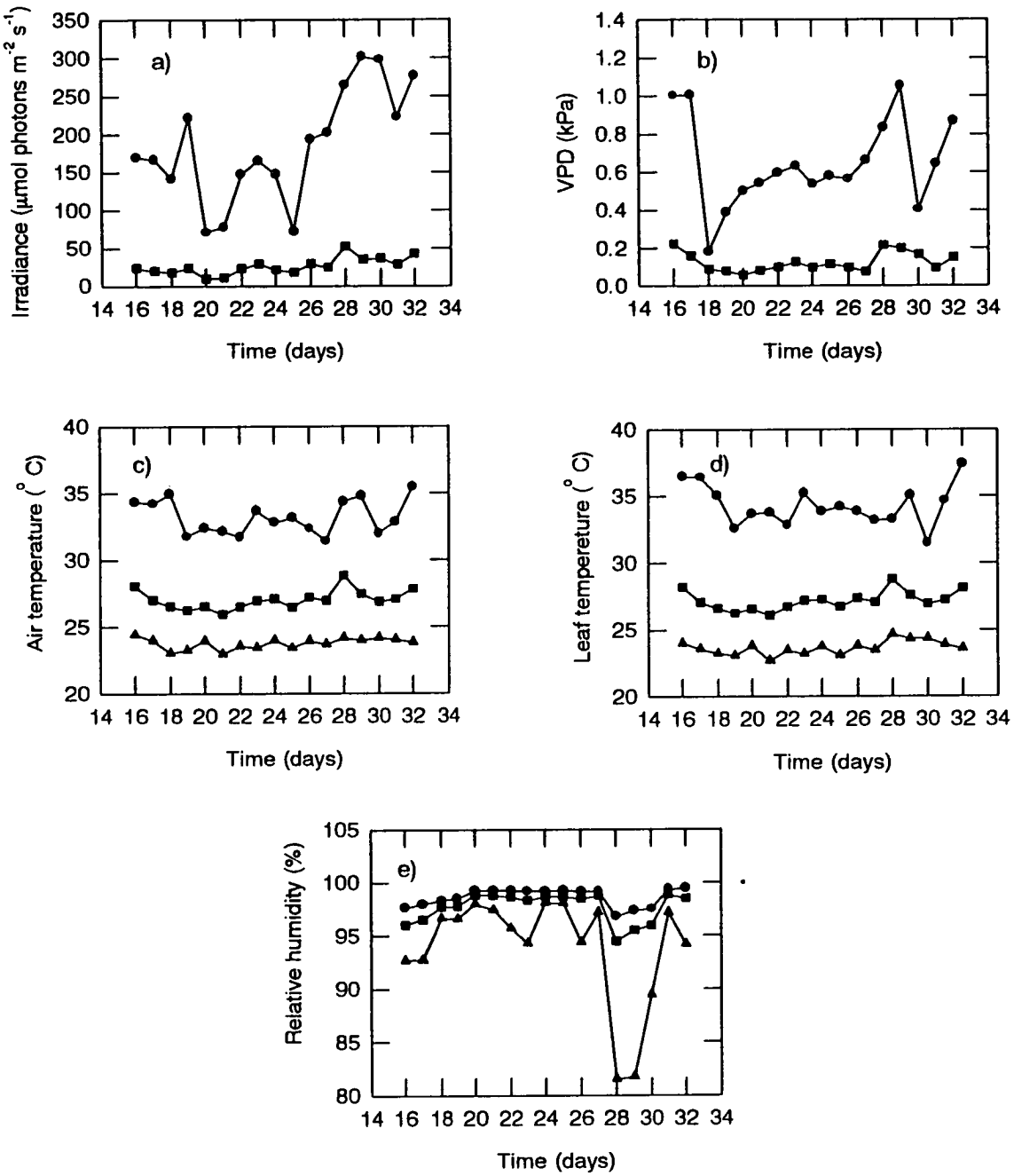


Figure 7.15 : Environmental data in enclosed mist propagators from day 14 to day 32 of the experiment. a) Irradiance; b) VPD; c) Air temperature; d) Leaf temperature; e) Relative humidity. Each data point per variable corresponds to mean value of 2 blocks calculated as a 5 minute average. Mean values were calculated on 24 hours period daily (circle=maximum values; square=average values; triangle=minimum values of each variable; minimum values of irradiance and VPD are not displayed since they are zeros or closed to zeros).

Table 7.3 : Mean values of initial length, diameter, volume of cuttings, initial dry weight, starch, total sugar and nitrogen of leaf and stem of *S. leprosula* cuttings taken from potted stock plants grown under low and high irradiance. Each cutting had a 30 cm^2 leaf area. Cuttings were harvested on day 0 at 17:00 hours after the experiment was laid-out. Low irradiance=0-325 µmol photons m^{-2} s^{-1}; High irradiance=0-722 µmol photons m^{-2} s^{-1}; ± satandard error of mean.

Variables	Low irradiance	High irradiance	Number of samples per treatment (n)
Length (cm)	4.06a	6.08b	125
Diameter (cm)	0.32a	0.49b	125
Volume (cm^3)	1.19a	0.38b	125
Leaf weight(g)	0.16a	0.21b	30
Stem weight (g)	0.10a	0.35b	30
Leaf starch (%)	8.65±0.94	10.30±1.32	3
Stem starch (%)	2.05±0.47	4.75±0.60	3
Leaf sugar (%)	2.93±0.90	2.79±0.36	#1
Stem sugar (%)	1.58±0.24	2.11±0.34	#1
Leaf nitrogen (%)	1.69±0.04	1.58±0.04	3
Stem nitrogen (%)	0.65	0.64±0.03	#2

Means followed by the same letters are not significantly different at $P \leq 0.05$

#1 : n=2 and 3 for low and high irradiance treatments respectively.

#2 : n=1 and 3 for low and high irradiance treatments respectively. No statistical analysis was carried out on the starch, sugar and nitrogen since inadequate samples were available.

Significantly higher rooting was obtained in cuttings from low than high irradiance (Table B72 and Figure 7.16a). Regression analysis shows that rooting was negatively correlated to volume of cuttings (Tables B72 and Figure 7.16b). The cuttings that remained unrooted were not significantly affected by treatments (Table B73 and Figure 7.17a) but they were significantly influenced by volume of cuttings and the relationship was positive (Table B73 and Figure 7.17b). The dead cuttings were 4% and 10% in cuttings from low and high irradiance treatments respectively. Chi-square showed that these dead cuttings were not significantly different between treatments.

Similar to rooting, number of roots was significantly more in low than high irradiance treatments (Table B74 and Figure 7.18). Number of roots produced was not significantly influenced by the morphological characteristics of cuttings.

There was no significant difference between treatments in P_n and g_s of cuttings prior to rooting. Mean P_n was 1.9 and 2.0 μmol CO_2 m^{-2} s^{-1} and mean g_s was 344 and 329 mmol H_2O m^{-2} s^{-1} for cuttings from low and high irradiance treatments respectively. No significant difference in PAR occurred when the measurements were made. Mean PAR was 145 and 143 μmol photons m^{-2} s^{-1} for cuttings from low and high irradiance respectively. There was significant difference in P_n, g_s and PAR between days of measurements (Tables B75, B76, B77 and Figures 7.19a,b,c).

P_n of rooted cuttings measured on day 63 was significantly higher than that of cuttings which remained unrooted (Table B78 and Figure 7.20a). A significant interaction was obtained between treatments and rooted/unrooted cuttings (Table B78). Figure 7.20b shows that P_n of unrooted cuttings was significantly higher in cuttings taken from high than low irradiance treatments and this was due to differences in PAR (Figure 7.20c). However, no different between treatments was observed in P_n of the rooted cuttings. No significant different was obtained in g_s and PAR either between treatments or between rooted/unrooted cuttings.

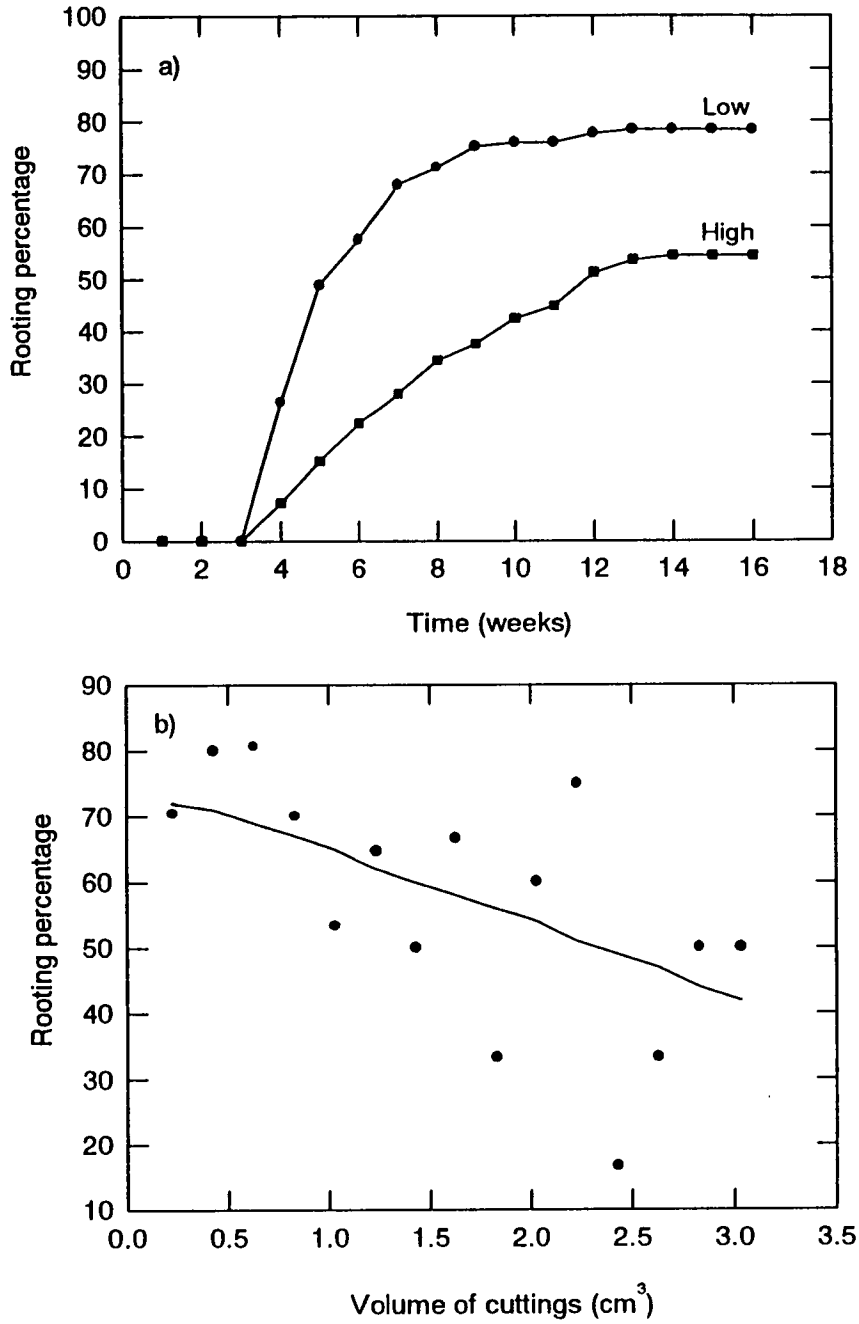


Figure 7.16 : Influence of irradiance levels to *S. leprosula* stock plants on a) Subsequent rooting rate of *S. leprosula* stem cuttings (n=125 per treatment); b) Relationship of rooting and cutting volume of *S. leprosula*. Scattered points are groups of observed data and line was drawn by connecting the predicted values computed from the multiple regression model.

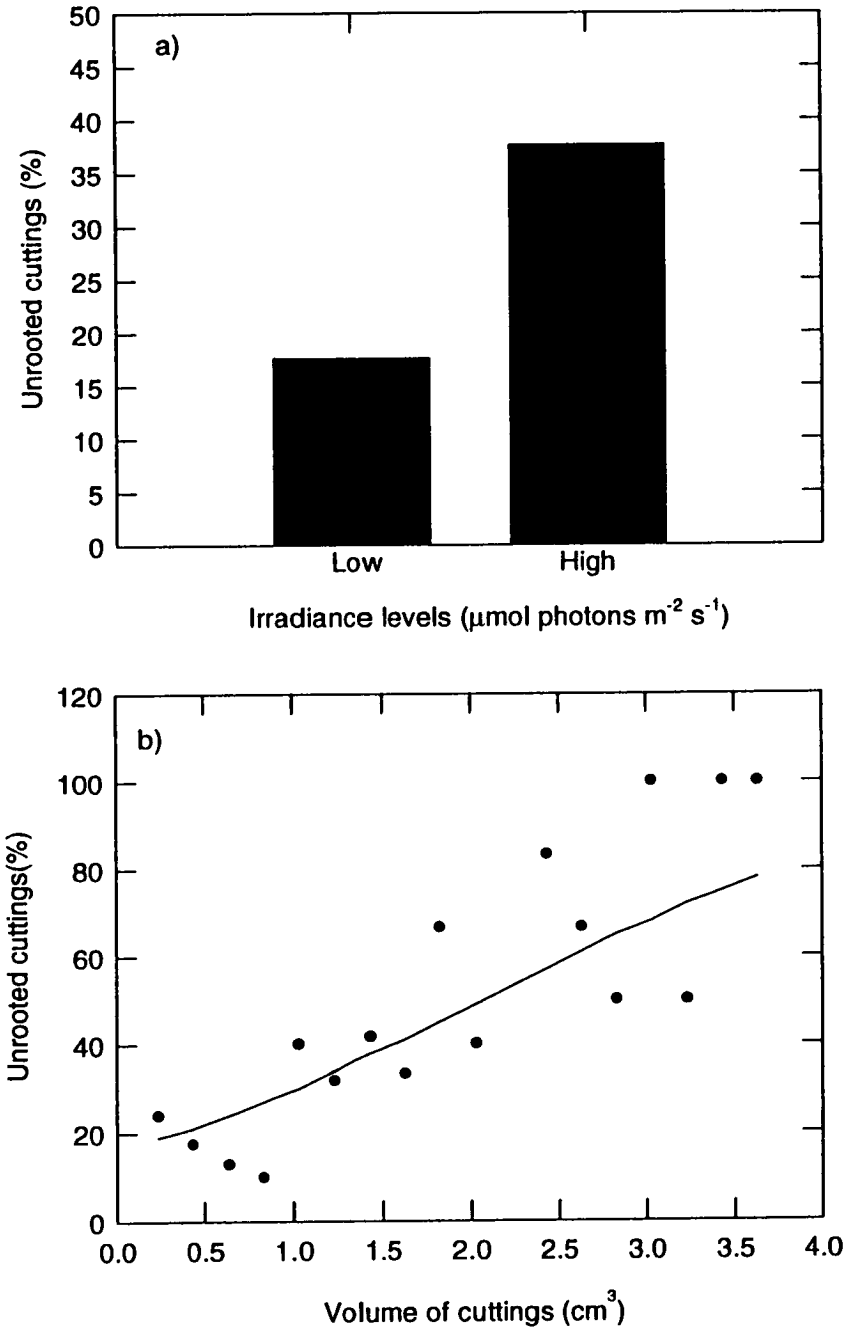


Figure 7.17 : Influence of irradiance treatments to *S. leprosula* stock plants on a) Subsequent stem cuttings that remained unrooted at week 16 (n=125 per treatment); b) Relationship of unrooted cuttings and cutting volume of *S. leprosula*. Scattered points are groups of observed data and line was drawn by connecting the predicted values computed from the multiple regression model.

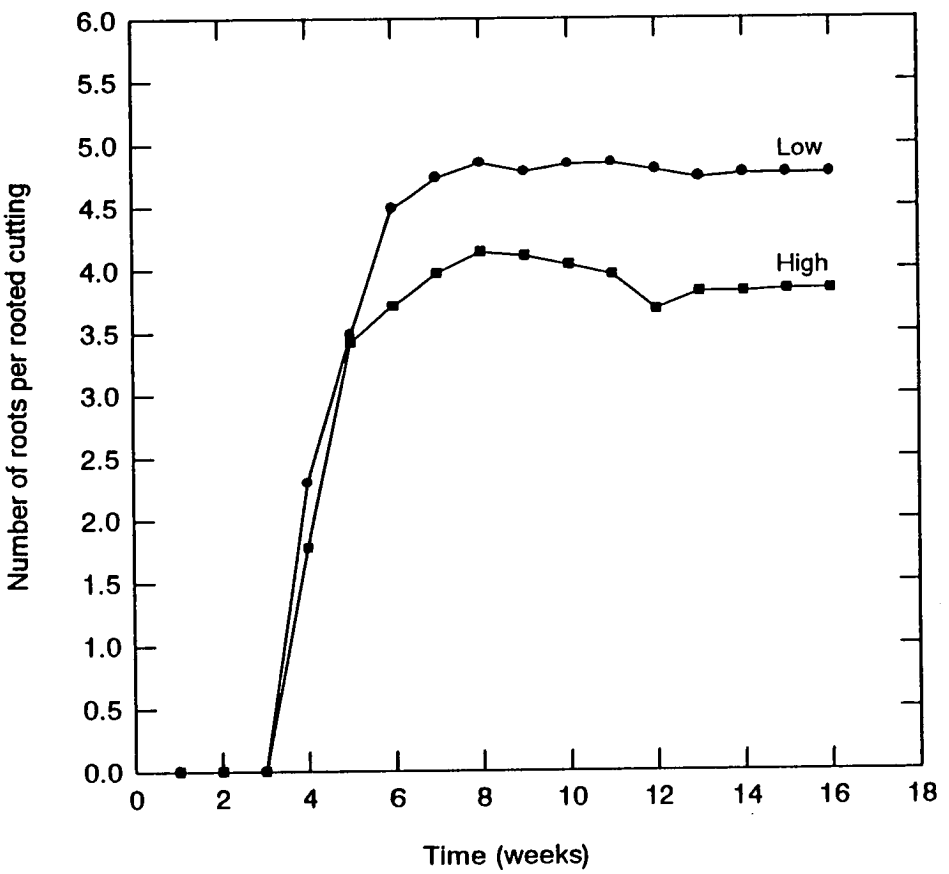


Figure 7.18 : Influence of irradiance levels to *S. leprosula* stock plants on rate of mean accumulated number of roots per rooted stem cutting of *S. leprosula* (n=125 per treatment).

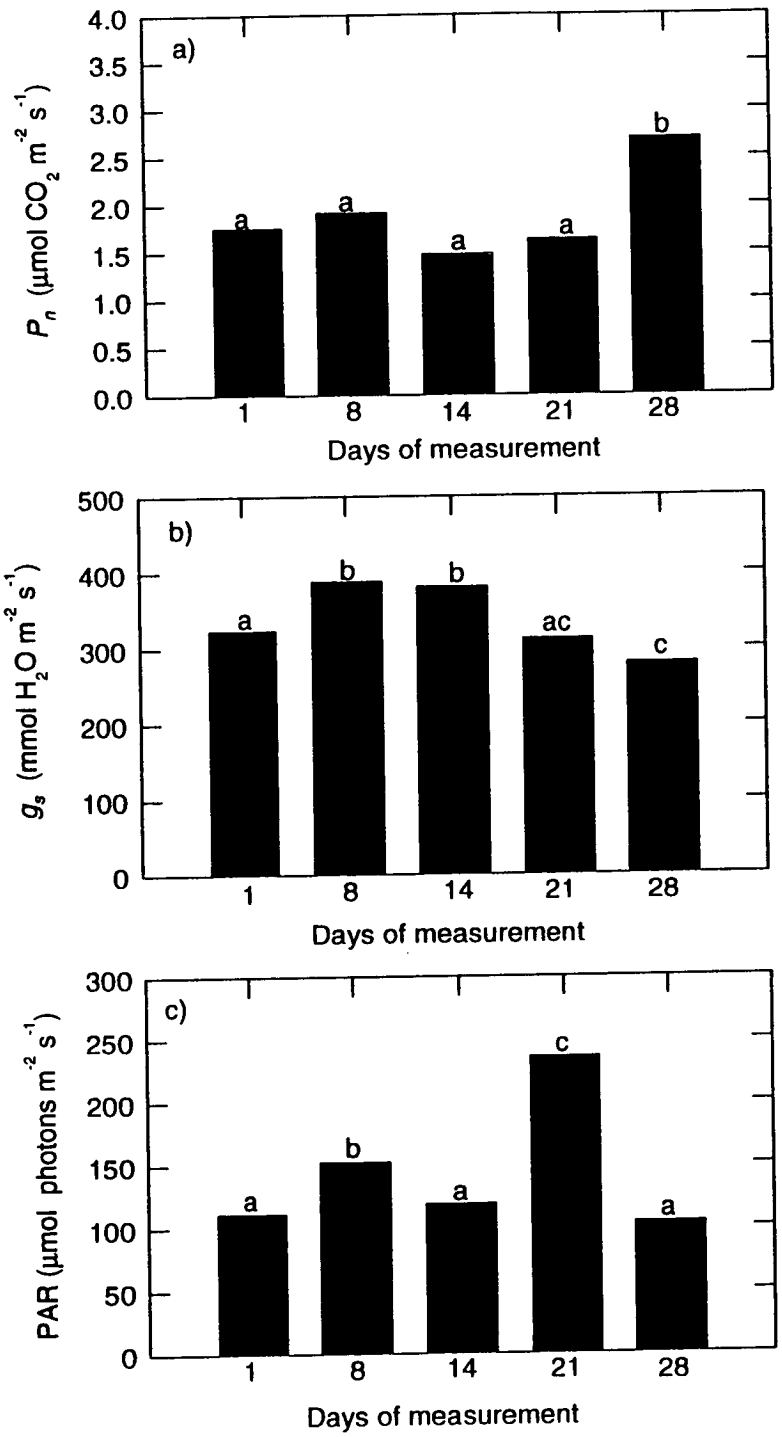


Figure 7.19 : Influence of days of measurement on a) Mean P_n; b) Mean g_s of *S. leprosula* stem cuttings prior to rooting; c) Mean PAR when measurements of P_n and g_s were made (n=40 per day; means with the same letters are not significantly different at $P \leq 0.05$).

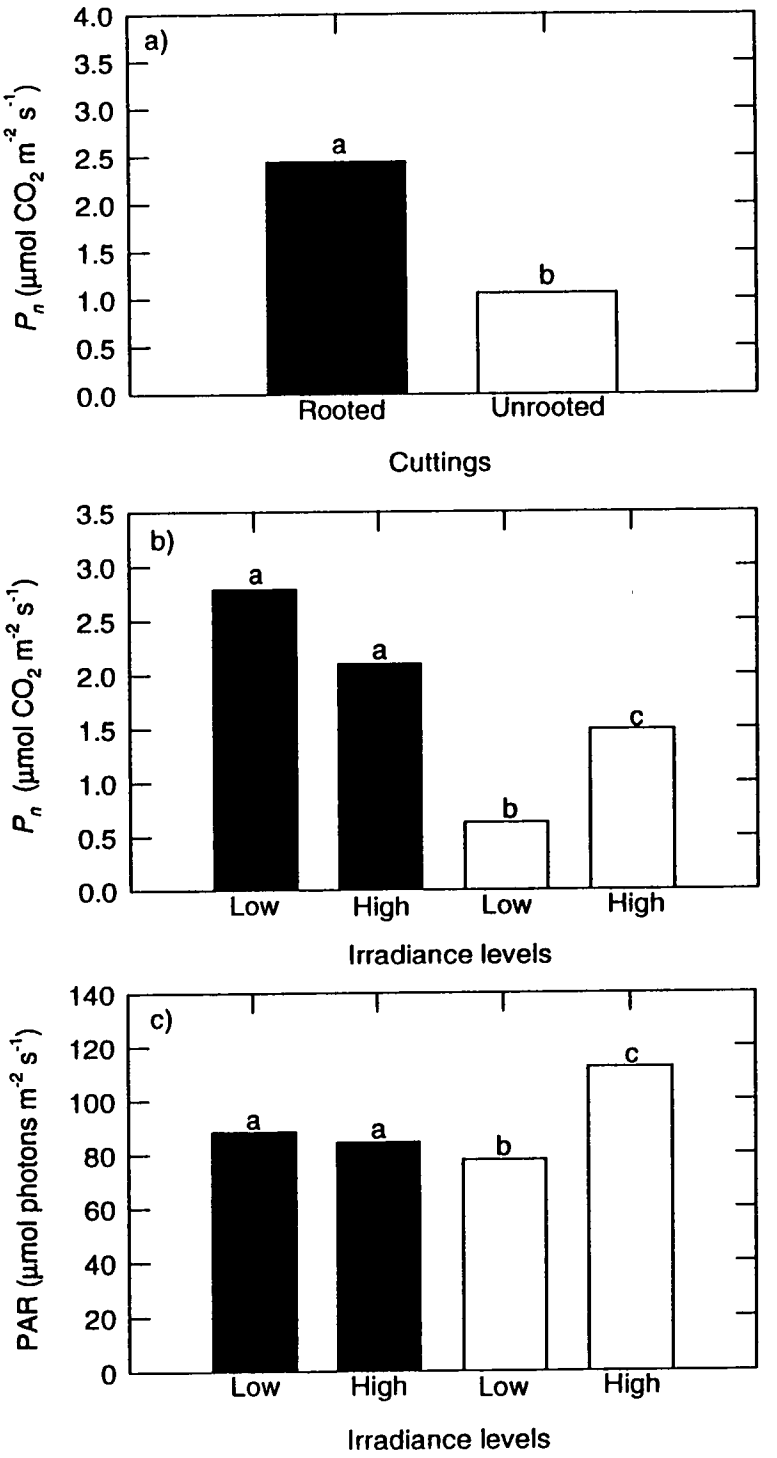


Figure 7.20 : Influence of irradiance levels to *S. leprosula* stock plants on a) Mean P_n of rooted cuttings and mean P_n of cuttings that remained unrooted of *S. leprosula*; b) Mean P_n of rooted cuttings and cuttings that remained unrooted for low and high irradiance levels; c) Mean PAR when the measurements of P_n and g_s were made for the respective treatments (measurements were made on day 63; n=40 for rooted and unrooted cuttings; solid bar=rooted cuttings; open bar=unrooted cuttings). Means of rooted/unrooted cuttings with the same letters of are not significantly different at $P \leq 0.05$.

Mean g_s ranged between 176 to 220 mmol H_2O m^{-2} s^{-1} whilst the range of mean PAR was 83 to 98 µmol photons m^{-2} s^{-1}.

Discussion and conclusions

In the present study, growth of *S. leprosula* stock plants was enhanced by high irradiance, probably influenced by the overall P_n, which was higher under high irradiance. Enhanced growth could also be linked with high R_d as fast growth requires rapid rates of energy metabolism associated with cell division (Riddoch *et al.* 1991). Attempt has been made to use the model described by Jarvis *et al.* (1985) for the diurnal P_n values but the data did not fit well with the model. This could be due to the low irradiance treatments used for the present experiment especially that of low irradiance level. Even in high irradiance level, very few data points were above 300 µmol photons m^{-2} s^{-1}.

Conversely, an increase in both rooting percentages and number of roots was obtained in cuttings taken from stock plants grown under low irradiance. These results were consistent with those reported by Hansen and Ericksen (1974); Hansen *et al.* (1978); Elliasson and Brunes (1980); Poulsen and Andersen (1980); Moe and Andersen (1988); Leakey and Storeton-West (1992) in a range of plant species. This effect has been associated with several conditions such as an increase in leaf auxin content, possible changes in rooting inhibitors and/or promoters, a beneficial change in internal structure of the stem where roots would form and an increase sensitivity of tissues to auxin (Hartmann and Kester 1983; Blazich 1988; Maynard and Bassuk 1988; Moe and Andersen 1988). However, actual mechanisms involved were poorly understood (Moe and Andersen 1988). On the other hand, an increase in irradiance has reduced subsequent rooting of *S. leprosula* stem cuttings, perhaps due to high concentration of carbohydrates as reflected in higher dry mass of stem and leaf. Besides that, there was an indication that the initial leaf and stem starch as well as total stem sugar of cuttings were higher in high than low irradiance, but these variables were not

statistically analysed due to inadequate samples available. The unfavourability of high initial carbohydrates content to rooting has been postulated by Hansen and Eriksen (1974) with cuttings of *Pisum sativum*. However, carbohydrate content in the present experiment may not be high enough to result in suppression of post-severance photosynthesis in cuttings as demonstrated by Leakey and Storeton-West (1992) in *Triplochiton scleroxylon* cuttings. Also, photodestruction of auxin, changes in water relations as well as concentration of rooting inhibitors and/or promoters may occur in stock plants grown under high irradiance (Moe and Andersen 1988).

Irradiance treatments to stock plants could also result in alteration of morphological characteristics of cuttings (Moe and Andersen 1988). Results of the present experiment showed that rooting decreased with the increase in volume of cuttings and a high percentage of cuttings with larger volume remained unrooted. These larger volume cuttings tend to have a larger diameter. Larger diameter cuttings had probably undergone secondary growth and thickening of lignin layer which may create physical barrier to root initiation (Hartmann *et al.* 1990; Liew 1992). These lignified cuttings were generally poor rooters and they either remained unrooted or died when the carbohydrate reserves were depleted. Negative correlation between rooting and volume of cuttings may indicate that reserved starch was not converted to sugar and so not available for cuttings. Also the relationship suggests that cuttings depended to a greater extent on current photosynthates rather than carbohydrate reserves (Veierskov 1988; Leakey and Coutts 1989). In the present experiment, P_n was found to increase at the stage of root formation on day 28 in spite of low PAR and g_s. This may indicate that plants were actively photosynthesising to produce assimilates for root formation. From the work on P_n of cuttings on the rooting bed, there is increasing evidence to indicate that photosynthesis occurs before rooting and contributes to rooting success of several tree species (Eliasson and Brunes 1980; Davis and Potter 1981; Smalley *et al.* 1991; Leakey and Storeton-West 1992; Newton *et al.* 1992; Hoad and Leakey 1993; Mesen 1993).

P_n of rooted cuttings was higher than that of cuttings which remained unrooted in all treatments. Similar results were obtained by Newton *et al.* (1992); Hoad and Leakey (1993). P_n of rooted cuttings may be enhanced by the presence of roots as sink for assimilates (Wareing *et al.* 1968; Okoro and Grace 1976; Elliasson and Brunes 1980). Also an increase in P_n after rooting may be due to roots supplying leaves with cytokinin which may increase the activity and/or amount of carboxylating enzymes (Okoro and Grace 1976). There was an interaction between treatments and rooted/unrooted cuttings. The higher P_n values of unrooted cuttings in high than low irradiance treatment was due to higher PAR occurred during the measurements, which happened despite randomisation of treatments.

S. leprosula stem cuttings from stock plants grown at high irradiance produced fewer roots compared to cuttings from low irradiance stock plants. Similar results have been obtained in *C. alliodora* (Mesen 1993); *T. scleroxylon* (Leakey and Storeton-West 1992) and *P. sativum* (Baadsmand and Andersen 1984). Baadsmand and Andersen (1984) suggested that high levels of stock plant irradiance enhanced auxin transport to the base of subsequently collected cuttings resulting in earlier establishment of dominance by the first formed roots which may suppress further root development. Hence fewer roots were obtained per cutting as happened in *P. sativum* (Baadsmand and Andersen 1984).

The work on stock plant irradiance and subsequent rooting in Dipterocarps has not been reported. However, Leppe and Smits (1988) reported that shading of stock plants is required to achieve good rooting percentages in cuttings of several Dipterocarp species. They grew their stock plants under canopies of trees, but the actual amount of light was not quantified (Leppe and Smits 1988). Kantarli (1993) grew stock plants of *Hopea odorata* under 50% black shade nets obtained 59% to 81% rooting depending on stump height of stock plants from the ground. In many reports of cutting experiments of Dipterocarps, no mention has been made on the history of stock plant irradiance (Momose 1978; Muckadell and

Malim 1978; Halle and Kamil 1981; Srivastava and Manggil 1981; Smits 1983; Lo 1985; Omon *et al.* 1989; Siagan *et al.* 1989; Noraini and Ling 1993; Moura-Costa and Lundoh 1994).

The data on microclimates showed that mean VPD in propagators could be kept close to zero. Periods of water deficit did occur as indicated by the maximum VPD which was more than the threshold level (0.5 kPa) suggested by Grange and Loach (1983a) for many broadleaved species. As in previous experiments, this temporary water deficit was not affecting the rooting of *S. leprosula* stem cuttings.

Compared to high irradiance of 0 to 722 μmol photons m^{-2} s^{-1} (nominally 30% full sunlight), low irradiance of 0 to 325 μmol photons m^{-2} s^{-1} (nominally 10% of full sunlight) was more suitable for production of cutting materials. However, many cuttings produced under this low irradiance had shorter internodes and were inconvenient for handling. It is anticipated that internode of subsequent cuttings could be lengthened for easier handling if intermediate irradiance between those tested above is used for raising the stock plants. Cuttings with longer internode have been reported to promote rooting in several plant species (Veierskov 1978; Poulsen and Andersen 1980; Leakey 1983; Leakey and Mohammed 1985; Dick *et al.* 1991a; Leakey and Storeton-West 1992; Hoad and Leakey 1993).

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

Stock plant growth

A knowledge of stock plant management is essential to obtain a sustained supply of cutting materials at suitable physiological state for propagation. Application of fertiliser to potted stock plants of *S. leprosula* is necessary for the production of cutting materials since without fertiliser, the potted stock plants grew very slowly and assumed a stunted appearance (Aminah, unpublished). Similar observations have been made by De Souza and Felker (1986) with *Prosopis alba*. In the present study, rooting of subsequent cuttings was not affected by fertiliser rates applied (0.5 g/1.5 g per stock plant per 2 weeks). Perhaps, the high rate of fertiliser applied to *S. leprosula* stock plants may not have been supraoptimal as no negative effect on subsequent rooting of cuttings was observed. However, in other species, unrestricted fertiliser applications to stock plants have reduced the rooting of subsequent collected cuttings (Moe and Andersen 1988; Tchoundjeu 1989; Leakey and Storeton-West 1992; Mesen 1993). High nutrients applied to stock plants may presumably reduce rooting through an effect on morphological and physiological characteristics of cuttings produced. For example, high nutrients to stock plants could result in leaves with low specific area (thick leaves); which may increase mutual shading of chloroplast and thus reduce the efficiency of gas exchange in cuttings (Hoad and Leakey 1993). The reduction of rooting due to low P_n activity has been reported by Mesen (1993) in cuttings of *Albizia guachepele* taken from stock plants treated with high nutrient and high irradiance. On the other hand, the low P_n activity may reduce rooting in cutting by reducing the basipetal transport of auxin (Scott and Briggs 1963; Kampula and

Potter 1984) and rooting cofactors (Salisbury and Ross 1985) which may originate from leaves. This aspect however needs further investigation.

Fertiliser tests like this, may not reveal the actual rate of nutrients required by plants, but at least they provide guideline for practitioners. A more appropriate way is to supply nutrients at a rate related to biomass production. This has been reported by Ingestad (1982) where the amount of nutrients required per unit of time increases with increasing biomass, hence the amounts to be supplied must increase correspondingly.

The effect of irradiance on the stock plants of *S. leprosula* was more pronounced than that of fertiliser. An enhanced P_n under high irradiance (0 to 722 μmol photons m^{-2} s^{-1}) may be the cause of the better growth rate in plants raised under high irradiance. Higher R_d could also be associated with faster growth since metabolic activity increased under high irradiance, and leaves may be expected to increase in P_n to meet the rapid phloem loading and transport of carbohydrates to growing tissues (Riddoch *et al.* 1990).

However, cuttings obtained from stock plants grown under low irradiance (0 to 325 μmol photons m^{-2} s^{-1}) were more inclined to root and produce more roots than those from the high irradiance. These results are consistent with those obtained by several authors for other plant species (Hansen *et al.* 1978; Elliasson and Brunes 1980; Poulsen and Andersen 1980; Moe and Andersen 1988; Leakey and Storeton-West 1992; Hoad and Leakey 1993). The high irradiance had also resulted in high leaf and stem starch as well as high total stem sugar being produced in cuttings in the present study which may be unfavourable for rooting, as reported by Lovell *et al.* (1972); Hansen *et al.* (1978); Loach and Whalley (1978); Poulsen and Andersen (1980). However, the high irradiance in the present study may not be high enough to result in supraoptimal carbohydrate production to suppress post-severance photosynthesis in cuttings as demonstrated

by Leakey and Storeton-West (1992) in their studies with cuttings of *Triplochiton scleroxylon.*

Rooting environment

Cuttings of *S. leprosula* could be successfully propagated both in the non-mist and mist propagation systems as long as a consistently low VPD can be maintained. In fact more roots were produced in cuttings planted in non-mist than in the mist system. This could perhaps be due to more efficient use of nutrients and auxin for root development in cuttings since leaching is less likely to occur in the non-mist than mist system. Leaching of mineral nutrients from cuttings while under mist has been demonstrated by Good and Tukey (1966); Blazich *et al.* (1983). However, this aspects should be further investigated. Care should be taken when using the non-mist system as shock response could occur soon after insertion. In the present study (chapter 4), rapid shedding of leaves occurring soon after insertion could have been caused by cuttings being water stressed during harvesting. The condition was worsened with the increase in VPD in the propagators which were initially not shaded as the experiment was set up during rainy season. This shock response soon after insertion also occurred in cuttings of *A. guachepele* and *Calliandra calothyrsus* planted in non-mist system (Newton and Jones 1993b; Dick *et al.* 1994b respectively). It is therefore important to keep consistently low VPD in the propagators which could be achieved by proper shading throughout the propagation period. Cuttings should also be kept moist by spraying during harvesting and preparation of the experiment. The shock response was felt less in the mist system since cuttings benefit from water covering the leaves within 5 to 10 minutes of severance and evaporative cooling reduces leaf temperature and leaf water vapour pressure (Loach 1988a).

In terms of VPD, although the threshold value of 0.5 kPa has been used as a reference (Grange and Loach 1983a), this is an arbitrary value which may be

more applicable to temperate species. In the tropics, an increase in irradiance at high temperature can easily result in a rise in VPD of more than 0.5 kPa as observed in the present study both in mist and non-mist propagation systems even though shading was provided to the propagators. In experiment 5 for example the high irradiance of 0 to 658 μmol m^{-2} s^{-1} can still produce 50% rooting even though maximum VPD of up to 3.6 kPa was recorded in the propagator. It appears however, that cuttings could tolerate this temporary rise in VPD, and could recover following the decreasing VPD which approached zero during night and early morning. This was indicated in the present study where *S. leprosula* cuttings showed high RWC and g_s in the morning despite experiencing a temporary water deficit at the high VPD which occur during mid-day and afternoon. Similar findings have been reported by Mesen (1993); Newton and Jones (1993b) for several tropical species. An extreme example was reported by Mesen (1993) where *C. alliodora* cuttings planted in unshaded non-mist propagator still survived, and a rooting success of 60% was obtained in spite of experiencing temporary maximum VPD which ranged from 4.91 to 9.85 kPa. In *Prosopis alba* cuttings, optimum temperature for rooting was 35 °C and cuttings were also found to survive and still root at 42 °C (Klass *et al.* 1985). For most species, the degree of water deficit that a cutting can withstand and still root has not yet been defined and needs further investigation (Newton and Jones 1993b). However, consistent exposure of cuttings to temperatures of more than 40 °C should not be practised because most leaves of tropical species may be damaged at about 42 °C (Turner and Newton 1990).

To reduce irradiance and VPD in the propagators, shading is required. However, very low irradiance resulted in poor rooting despite the low VPD being maintained in propagators. Poor rooting under low irradiance (0 to 5% full sunlight) in the present study corresponded with low P_n and cuttings were running into carbohydrate deficit. Therefore, shading should not be so much as to reduce P_n in cuttings while on rooting beds. The low irradiance of 0.3% and 8% full sunlight was also detrimental in rooting of *Populus tremula x tremuloides*

and *P. alba* respectively (Elliasson and Brunes 1980; Klass *et al.* 1985 respectively). Besides affecting the carbon budget of cuttings, low P_n activity may also indirectly reduce rooting by slowing down basipetal transport of auxin and other rooting cofactors which may originate from leaves and buds (Kampula and Potter 1984; Salisbury and Ross 1985). In the present study, 0-360 μmol m^{-2} s^{-1} (ca. 0-18% sunlight) is adequate for rooting cuttings of *S. leprosula*. High irradiance to cuttings of *S. leprosula* would be of no benefit since P_n seemed to saturate at 400 μmol photons m^{-2} s^{-1} and further increase in irradiance may only induce higher VPD in the propagator which may have a negative effect on rooting (Grange and Loach 1983a,b; Grange and Loach 1985; Loach 1988a; Mesen 1993; Newton and Jones 1993b). In high irradiance level, carbohydrate produced may have been used for maintenance respiration at high temperature rather than for root formation as in experiment 2 of chapter 5. Also high irradiance could result in photoinhibition and destruction of chlorophyll and auxin, changes in the concentration of rooting inhibitors/promoters (Moe and Andersen 1988).

In the mist system, misting frequency seemed to be important. In the present study, the effect of misting frequency could not be well demonstrated due to a problem encountered in the experimental layout. Due to low irradiance experienced by cuttings planted under misting intervals of 6 hours, rooting under 6 hours misting was similar to that of 1 hour misting. However, there was an indication that misting frequency of 3 hours may induce water deficit in cuttings as lower values of RWC and g_s were obtained with this treatment compared with those of 1 hour misting interval. The sensitivity of other *Shorea* species to misting frequency was demonstrated by Lo (1985) where *S. macrophylla* cuttings planted in an open mist units resulted in high mortality when the misters were off at night. Although no environmental data was presented, the author attributed that high mortality of cuttings to water deficit in the absence of misting.

Photosynthesis and rooting

In the present study, P_n of cuttings in the propagators prior to rooting was low (mean values of 0.34 to 2.3 µmol CO_2 m^{-2} s^{-1} depending on treatments) compared to P_n of stock plants (5 µmol CO_2 m^{-2} s^{-1}) in experiment 1 of chapter 7. P_n values of 25 mg CO_2 dm^{-2} $hour^{-1}$ (ca. 16 µmol CO_2 m^{-2} s^{-1}) was measured in the upper canopy of *S. leprosula* trees (Sasaki and Mori 1981). The low P_n in cuttings obtained in the present study may be due to low irradiance on the rooting beds. Also it may be a normal feature of unrooted cuttings which do not have roots to act as sink for assimilates. An increase in irradiance in the propagators to above 500 µmol photons m^{-2} s^{-1} (experiment 2 of chapter 5) did not result in any increase in P_n of *S. leprosula* cuttings and P_n seemed to saturate at irradiance of ca. 400 µmol photons m^{-2} s^{-1} (ca. 20% full sunlight). This irradiance level was lower than the irradiance (35,000 Lux) required to achieve maximum P_n of upper canopy of *S. leprosula* trees (Sasaki and Mori 1981).

P_n in cuttings of *Cordia alliodora* was also reported to saturate at an irradiance level of ca. 400 µmol photons m^{-2} s^{-1} Mesen (1993). A lower irradiance of 100 W m^{-2} (ca. 13% full sunlight) was found sufficient for rooting and P_n to occur in cuttings of several broadleaf species (Grange and Loach 1983a). On the other hand, a very low irradiance of 0 to 98 µmol m^{-2} s^{-1} (0 to 5% full sunlight) was detrimental to rooting of *S. leprosula* stem cuttings and this was related to low P_n values (experiment 2 of chapter 5). In another instance, number of roots was reduced when irradiance levels in propagators was between 0 to 185 µmol photons m^{-2} s^{-1} (0 to 9% full sunlight) and this also corresponded to a low P_n value (experiment 1 of chapter 5). These results may indicate that post-severance P_n is essential for successful rooting of *S. leprosula* stem cuttings. The occurrence of P_n prior to rooting has been reported by other researchers on cuttings of several plant species and in most cases P_n was positively related to rooting (Elliasson and Brunes 1980; Davis and Potter 1981; Smalley *et al.* 1991;

Leakey and Storeton-West 1992; Newton *et al.* 1992; Hoad and Leakey 1993; Mesen 1993).

In the present study, P_n of rooted cuttings was higher than in cuttings that remained unrooted as the primary water mechanism has been restored and this is normally associated with higher g_s values. Similar results were obtained by Newton *et al.* (1992); Hoad and Leakey (1993). P_n of rooted cuttings is enhanced by the presence of roots as sink for assimilates (Wareing *et al.* 1968; Okoro and Grace 1976; Elliasson and Brunes 1980). Also high P_n in rooted cuttings could presumably be due to cytokinin from the newly-formed roots which may increase the activity and/or amount of carboxylating enzymes (Okoro and Grace 1976). However, this possibility has not been critically evaluated.

Mean g_s values measured in *S. leprosula* stem cuttings while on the propagation beds was 80 to 344 mmol H_2O m^{-2} s^{-1} depending on the treatments. These values were higher than that recorded for *Cornus* and *Rhododendron* which was 40 mmol H_2O m^{-2} s^{-1} (Gay and Loach 1977). On the other hand, the g_s values obtained in the present study were comparable to those measured on cuttings of *C. alliodora*, *T. scleroxylon*, *A. guachepele* and *Terminalia spinosa* which ranged from 160 to 480 mmol H_2O m^{-2} s^{-1} (Newton and Jones 1993b). Stomatal conductance was also found to vary between canopies as measured in several tropical rainforest species in Amazon Basin where mean g_s was ca. 300 mmol H_2O m^{-2} s^{-1} in top canopy and ca. 40 mmol H_2O m^{-2} s^{-1} near the forest floor (Roberts *et al.* 1990). Values of g_s increase with radiation up to 600-700 W m^{-2} (75%-88% full sunlight) followed by a levelling off in response (Roberts *et al.* 1990).

Morphology of cuttings

In the present study, leaf area of 15 to 30 cm^2 is sufficient to yield good rooting of *S. leprosula* stem cuttings. Cuttings with 60 cm^2 leaf area seemed to suffer

water stress as indicated by greater leaf shedding and fewer rooted cuttings. This showed that there is a need to strike a balance between P_n and transpiration for optimal rooting of cuttings supporting the idea proposed by Leakey *et al.* (1982b); Leakey and Coutts (1989); Newton *et al.* (1992). Leafless cuttings of other tropical tree species were observed to root poorly or not at all (Leakey *et al.* 1982b; Aminah 1991b; Newton *et al.* 1992) which lends further support for a role of current assimilates in root primordia development. However, other factors such as rooting cofactors or auxin could be important for rooting and may originate from leaves (Hartmann *et al.* 1990). However, this aspect has also not been critically evaluated.

Length of cuttings within the tested range (1 to 18.5 cm) in the present study did not influence rooting of *S. leprosula* as reflected in the results of experiment 1 in chapter 6 and experiments in chapter 7. There was also no indication that leaves lying on the rooting medium were rotting. This is because the leaves of *S. leprosula* are waxy and sclerophyllous and much less susceptible to rotting. The leaves of *Eucalyptus grandis* and *Albizia guachepele* which are soft and may be more susceptible to rotting when lying on wet rooting medium (Hoad and Leakey 1993 and Mesen 1993 respectively).

In general, results of the present study showed that rooting decreased with increasing cutting volume (experiment 2 in chapter 4, experiments in chapter 5 and experiment 2 in chapter 7). This increase in volume could be associated with increasing in diameter since cuttings were cut to the same length as in experiment 1 in chapter 4 and experiments in chapter 5. The unfavourability of larger diameter/volume of cuttings was also indicated in the number of roots produced (experiments 2 in chapter 4 and experiment 1 in chapter 7). Cuttings with larger diameter/volume were liable to die. Larger diameter cuttings can be associated with increasing secondary growth and thickening of lignin layer which may create physical barrier to rooting and these lignified cuttings were generally poor rooters. A negative relationship between rooting and volume may also imply that initial

carbohydrate reserves is not important in rooting of *S. leprosula* cuttings or the starch was not converted to sugar and so not available for cutttings. Such relationship may reflect the fact that current assimilates may be sufficient to support rooting (Veierskov 1988). These results were similar to that of *Lovoa trichiliodes* where no significant difference was obtained between rooting of long and short cuttings with a thinner diameter (Tchoundjeu 1989). However, the results were contrasted to that obtained by Leakey and Mohammed (1985) where rooting of *T. scleroxylon* was positively correlated to cutting diameter when cuttings were cut to the same length. On the other hand, rooting of *C. alliodora* was not affected by diameter of cuttings but the number of roots was increased with cutting diameter (Mesen 1993). The author supposed that root initiation was hormonally controlled; and number of roots was more affected by the ability of cuttings to supply carbohydrates to the cutting base.

Rooting medium

Both water and oxygen are necessary for the development of adventitious roots in cuttings (Loach 1985; Hartmann *et al.* 1990; Smits *et al.* 1994). However, optimal conditions vary according to species (Loach 1985) and this has resulted in negative, positive or no effect at all on rooting in response to different components of rooting medium. In the present study, *S. leprosula* cuttings showed no preference for the media either with high or low water content such as sand, coconut fibre and the mixture of these two media. The results were consistent to that obtained with several Dipterocarp species which could be rooted in various types of media such as sand (Momose 1978; Lo 1985; Aminah 1991c; Moura-Costa and Lundoh 1994); coconut fibre and paddy husk (Noraini and Ling 1993); or even aerated water (Smits *et al.* 1994; Tolkamp and Aldrianto 1994). Other species which showed no preference for the rooting media were *Albizia guachepele* and *Gmelina arborea* (Leakey *et al.* 1990). In contrast, some species required certain media for optimum rooting for example cuttings of *Lovoa trichiliodes* are sensitive to medium with high water content (Tchoundjeu 1989).

Addition of sawdust to gravel or fine sand was detrimental to rooting of *Vochysia hondurensis* cuttings planted in non-mist system, probably due to water logging (Leakey *et al.* 1990). Cuttings of *Prosopis juliflora* rooted poorly in composted crop residue compared to perlite/vermiculite or coarse volcanic gravel media. Compost was found to be too dense and did not provide adequate drainage and aeration and remained water logged between misting (Wojtusik *et al.* 1994).

Auxin

Auxin is important for root initiation and development in cuttings. The beneficial effect of auxin could be seen in rooting and number of roots in cuttings (Darus 1988; Spethmann and Hamzah 1988; Leakey *et al.* 1982b; Kamis and Ng 1989; Leakey *et al.* 1990; Mesen 1993). Besides improving rooting and root system, auxin treated cuttings rooted faster than the untreated cuttings as indicated in the present study. Speeding up the process of rooting is important since the earlier the cuttings form root, the greater the chance for them to survive especially if the rooting environment is less than ideal. Rooted cuttings could be potted earlier, enabling the rooting bed to be released for the next batch. This aspect has often been neglected as assessment is normally carried out once at the end of experiment, which may lead to the false conclusion that auxin is not beneficial to rooting of cuttings. Therefore, it is necessary to check cuttings regularly to evaluate the effect of any treatment.

In conclusion *S. leprosula* stem cuttings can be successfully rooted as long as suitable cutting materials are used and proper rooting environments are provided. However, as indicated in the above discussion, rooting of cuttings is influenced by many interacting factors and there is a need to develop a process based model as a tool to understand and simulate the dynamics of physiological activities in rooting of cuttings. An example of such a model has been presented by Dick and Dewar (1992). This model provides a qualitative scheme how root development depends on properties such as leaf area, internode length and initial carbohydrates

of cuttings. The model has been verified using published data of single node cuttings of *Triplochyton scelroxylon* (Leakey and Coutts 1989). The results of the simulation were in agreement with those obtained in the rooting experiments by Leakey and Coutts (1989). Like any other mechanistic models, the above model makes various assumptions (Dick and Dewar 1992). So far, only one of the assumptions used in the model has been tested and found to be consistent with one of the predictions of the model; respiration rate at the base of the cuttings increases with time as the new roots and callus are forming (Dick *et al.* 1994a). Although in principle, this model may be applicable to other tropical species rooted from leafy cuttings, further testing is needed before it can be accepted as a predictive tool in vegetative propagation. Limitation of time does not allow this aspect to be investigated in the present thesis.

Practical implications

The following guidelines are offered for propagating stem cuttings of *S. leprosula*. The approach might serve as a model for propagating other Dipterocarp species.

1. The stock plants should be fertilised to provide sufficient supply of cutting materials especially if the plants are grown in pots. Commercial compound fertilisers such as NPK Blue (12%N: 12%P_2O_5: 12%K_2O: 2%MgO + Trace elements) applied at the rate of 0.5 g per plant every two weeks could be used. These plants were potted in 1 litre pot of forest top soil and sand (3:1). The 0.5 g dose of fertiliser was applied starting from the height of 4 cm to final height of 50 cm at week 22.

2. Shade should be provided to the stock plants for the production of suitable cutting materials. Although 10% full sunlight produced better cutting materials than 30% full sunlight, the cuttings produced have shorter internodes. Hence an intermediate irradiance between these two (15 to 20% full sunlight) could be tried

as an alternative. The stock plants can either be raised under artificial netting or tree canopies.

3. Both non-mist and mist propagation systems are suitable for propagating the cuttings of *S. leprosula*. The non-mist systems are adaptable for use in remote areas and villages, where there may be operated by local people in community projects. To minimise water stress, it is recommended that cuttings are kept moist during preparation for planting (by frequent spraying with water) and to shade the propagators from direct sunlight throughout the propagation period. In addition, cuttings should be sprayed with water every time the propagators are opened to avoid sudden change in humidity experienced by cuttings.

4. Rooting media such as sand, coconut fibre and a mixture of the two can be used as the rooting medium. In Malaysia, river sand is at present easier and cheaper to obtain than coconut fibre.

5. To speed up the rooting process and to increase the number of roots on cuttings, 20 μg IBA applied to each cutting is sufficient. Practically, speeding up rooting will increase survival and also increase the annual output of the propagation unit. With IBA treatment, more roots were produced per cutting and this will create better root system which is beneficial when these cuttings were planted in the field. Commercial IBA formulation such as "Seradix" can be used as an alternative for mass propagation of cuttings.

6. A misting frequency of once per hour with a one minute duration of the burst throughout day and night is sufficient for providing enough moisture to cuttings in an enclosed mist system.

7. An irradiance regime ranging from 0 to 360 μmol m^{-2} s^{-1} (ca. 0 to 18% full sunlight) is adequate to allow photosynthesis to occur. Range of VPD under this irradiance is 0-1.87 kPa.

8. A leaf area between 15 to 30 cm^2 retained on each cutting is sufficient for the rooting of cuttings. A cutting length of at least 3 cm is recommended for rooting and convenient handling. Thinner cuttings are better rooters while thick cuttings were liable to die. Recommended diameter is between 0.2 to 0.4 cm.

10. Rooting of cuttings may start as early as 3 weeks after the cuttings have been planted in the rooting medium, and rooting occurs until week 12, after which little rooting takes place. Therefore, a new batch of cuttings can be planted after every 12 weeks. Typically, a cutting of *S. leprosula* occupies an area of 50 cm^2. This figure may be adopted to provide an estimate of the number of rooted cuttings that are likely to be produced in a year for a given area of propagation bed.

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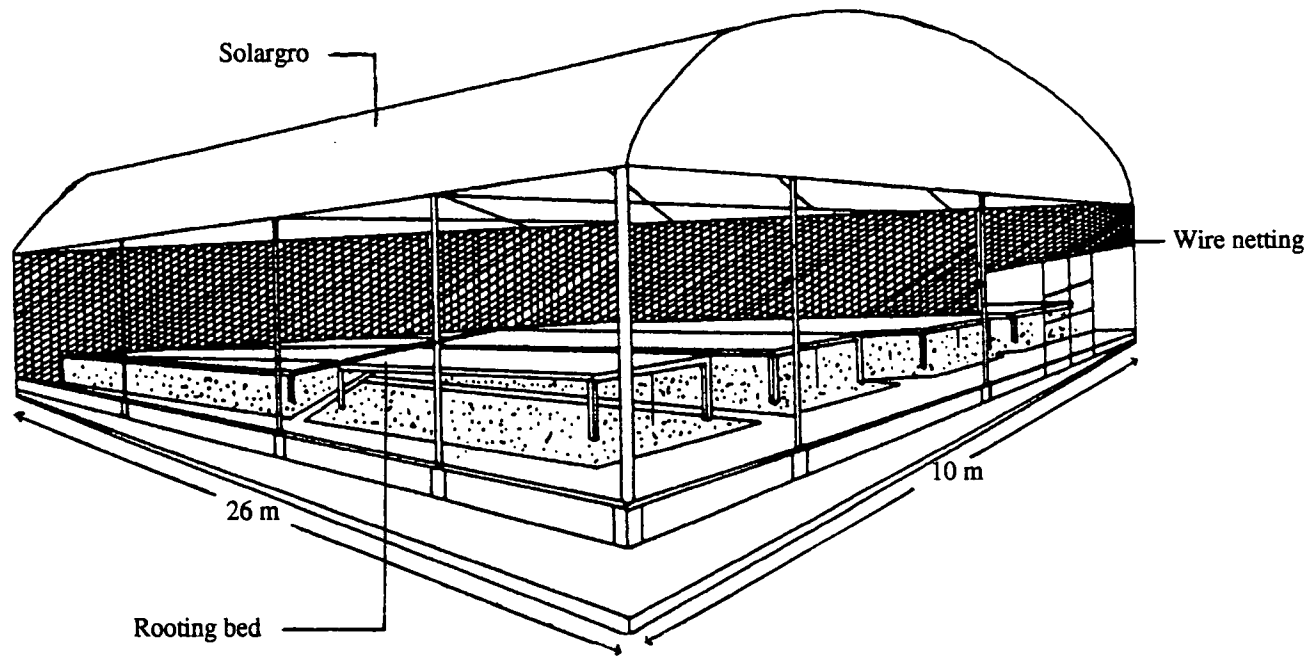

APPENDIX A : Cutting shed in the FRIM nursery

APPENDIX B

Table B1 : Analysis of deviance for a stepwise regression to determine the effect of different rooting media, propagation systems and morphological characteristics of *S. leprosula* stem cuttings on their rooting ability at week 16 (n=60 per medium per propagation system).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	5	12.09	2.42	2.05ns
Propagation systems	1	26.10	26.10	22.12****
Cutting volume	1	10.49	10.49	8.89**(-)
Residual	352	416.23	1.18	
Total	359	464.91		

Table B2 : Analysis of deviance for a stepwise regression to determine the effect of different rooting media, propagation systems and morphological characteristics on the dead stem cuttings of *S. leprosula* at week 16 (n=60 per medium per propagation system).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	5	10.88	2.18	1.82ns
Cutting volume	1	39.24	39.24	32.70****(+)
Propagation systems	1	24.82	24.82	20.68****
Residual	352	421.29	1.20	
Total	359	496.23		

ns : Not significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
**** : Significant at $P \leq 0.0001$
(+)/(-) : Regression coefficients

Table B3 : Analysis of deviance for a stepwise regression to determine the effect of different rooting media, propagation systems and morphological characteristics of *S. leprosula* stem cuttings that remained unrooted at week 16 (n=60 per medium per propagation system).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	5	4.85	0.97	1.02ns
Cutting volume	1	17.04	17.04	17.94****(-)
Residual	353	335.62	0.95	
Total	359	357.51		

Table B4 : Analysis of variance on the percentage of air component of the rooting media (n=24 per medium per propagation system).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	37.24	7.45	1.75ns
Propagation systems (Pr)	1	2764.59	2764.59	648.96****
Media (M)	2	9519.70	4759.85	1117.34****
Pr*M	2	519.83	259.92	61.01****
Residual	133	566.87	4.26	
Total	143	13408.23		

ns : Not significant at $P \leq 0.05$
**** : Significant at $P \leq 0.0001$
(-) : Regression coefficient

Table B5 : Analysis of variance on the percentage of water component of the rooting media (n=24 per medium per propagation system).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	3.92	0.78	1.30ns
Propagation systems (Pr)	1	7316.22	7316.22	12193.70****
Media (M)	2	23770.66	11885.33	19808.88****
Pr*M	2	608.21	304.11	506.85****
Residual	133	80.26	0.60	
Total	143	31779.27		

Table B6 : Analysis of variance on the percentage of solid component of the rooting media (n=24 per medium per propagation system).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	51.06	10.21	2.17ns
Propagation systems (Pr)	1	1086.36	1086.36	231.14****
Media (M)	2	63147.85	31573.93	6717.86****
Pr*M	2	71.16	35.58	7.57***
Residual	133	625.65	4.70	
Total	143	64982.08		

ns : Not significant at $P \leq 0.05$
* : Significant at $P \leq 0.05$
*** : Significant at $P \leq 0.001$
**** : Significant at $P \leq 0.0001$

Table B7 : Analysis of variance on the daily maximum VPD measured from day 1 to day 50 of the experiment in the non-mist and mist propagation systems planted with *S. leprosula* stem cuttings (n=50 per propagation system). Daily maximum VPD were mean values of 2 blocks calculated as 5 minutes average.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Propagation systems	1	0.80	0.80	20.00****
Residual	98	3.65	0.04	
Total	99	4.45		

Table B8 : Analysis of variance on P_n of *S. leprosula* stem cuttings prior to rooting as affected by propagation systems, media and days of measurements (P_n was measured on days 1, 8, 14, 21, 28; n=24 per treatment combination per day). Leaves were dropped during the periods of measurement, resulting in unequal n per day (n=144, 141, 138, 136, 136 for day 1,8,14,21 and 28 respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	6.69	1.34	2.31*
Propagation systems (Pr)	1	3.50	3.50	6.03*
Media (M)	2	0.39	0.20	0.34ns
Pr*M	2	0.92	0.46	0.79ns
Days (Dy)	4	8.18	2.05	3.53*
Pr*Dy	4	2.24	0.56	0. 97ns
M*Dy	8	1.42	0.18	0.31ns
Residual	668	390.54	0.58	
Total	694	413.88		

ns : Not significant at $P \leq 0.05$
* : Significant at $P \leq 0.05$
**** : Significant at $P \leq 0.0001$

Table B9 : Analysis of variance on PAR during measurement of P_n of *S. leprosula* stem cuttings prior to rooting as affected by propagation systems, media and days of measurements (P_n was measured on days 1, 8, 14, 21, 28; n=24 per treatment combination per day). Leaves were dropped during the periods of measurement, resulting in unequal n per day, (n=144, 141, 138, 136, 136 for day 1,8,14,21 and 28 respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	64416.98	12883.40	4.92***
Propagation systems (Pr)	1	12339.65	12339.65	4.71*
Media (M)	2	597.11	298.56	0.11ns
Pr*M	2	4048.12	2024.06	0.77ns
Days (Dy)	4	113701.43	28425.36	10.85****
Pr*Dy	4	16352.19	4088.05	1.56ns
M*Dy	8	6590.16	823.77	0.31ns
Residual	668	1750544.60	2620.58	
Total	694	1968590.24		

Table B10 : Analysis of variance for a stepwise regression on the effect of treatments and morphological characteristics on mean accumulated number of roots per rooted stem cuttings of *S. leprosula* at week 16 (n=54 and 56 for the non-mist and mist systems respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	0.39	0.39	0.20ns
Treatments	1	30.17	30.17	15.96***
Cutting volume	1	19.33	19.33	10.23**(-)
Residual	106	200.38	1.89	
Total	109	250.27		

ns : Not significant at $P \leq 0.05$
* : Significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
*** : Significant at $P \leq 0.001$
**** : Significant at $P \leq 0.0001$
(-) : Regression coefficient

Table B11 : Analysis of variance on the daily maximum VPD measured from day 1 to day 24 of the experiment in the non-mist and mist propagation systems (n=24 per treatment per block). Daily maximum VPD was calculated as 5 minutes average.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	5.767	5.767	50.15****
Treatments	1	0.472	0.472	4.10*
Residual	93	10.696	0.115	
Total	95	16.935		

Table B12 : Analysis of variance on daily average VPD measured from day 1 to day 24 of the experiment in the non-mist and mist propagation systems (n=24 per treatment per block). Average values were mean values of a 5 minute calculated over 24 hours period daily.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	1.14	1.14	114.00****
Treatments	1	0.14	0.14	14.00***
Residual	93	0.88	0.01	
Total	95	2.16		

* : Significantly different at $P \leq 0.05$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$

Table B13 : Analysis of variance on P_n of rooted cuttings and P_n of cuttings that remained unrooted of *S. leprosula* planted in the non-mist and mist propagation systems. Measurements of P_n were made on day 63 (n=20 per treatment of rooted and unrooted cuttings).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	0.13	0.13	0.25ns
Treatments (Trt)	1	0.04	0.04	0.08ns
Rooted/Unrooted (Rt)	1	25.81	25.81	48.70****
Trt*Rt	1	0.30	0.30	0.57ns
Residual	75	40.10	0.53	
Total	79	66.38		

Table B14 : Analysis of variance on g_s of rooted cuttings and g_s of cuttings that remained unrooted of *S. leprosula* planted in the non-mist and mist propagation systems. Measurements of g_s were made on day 63 (n=20 per treatment of rooted and unrooted cuttings).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	12652.97	12652.97	1.63ns
Treatments (Trt)	1	2290.87	2290.87	0.30ns
Rooted/Unrooted (Rt)	1	192285.86	192285.86	24.83****
Trt*Rt	1	3.16	3.16	0.004ns
Residual	75	580920.48	7745.61	
Total	79	788153.34		

ns : Not significantly different at $P \leq 0.05$
**** : Significantly different at $P \leq 0.0001$

Table B15 : Analysis of deviance for a stepwise regression to determine the effect of different treatments and morphological characteristics on the rooting ability of *S. leprosula* stem cuttings at week sixteen (n=60 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	2	1.38	0.69	0.53ns
Cutting volume	1	13.92	13.92	10.63**(-)
Residual	176	230.47	1.31	
Total	179	245.77		

Table B16 : Analysis of variance for a stepwise regression to determine the effect of different treatments and morphological characteristics on the mean accumulated number of roots per rooted stem cuttings of *S. leprosula* at week sixteen (n=38, 30 and 35 for 1, 3 and 6 hours misting frequencies respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	0.98	0.49	0.17ns
Treatments	2	26.97	13.49	4.77*
Residual	98	277.43	2.83	
Total	102	305.38		

ns : Not significant at $P \leq 0.05$
* : Significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
(-) : Regression coefficient

Table B17 : Analysis of deviance for a stepwise regression to determine the effect of different treatments and morphological characteristics on the dead stem cuttings of *S. leprosula* at week sixteen (n=60 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	2	0.68	0.34	0.54 ns
Cutting volume	1	9.52	9.52	15.11***(+)
Residual	176	111.16	0.63	
Total	179	121.36		

Table B18 : Analysis of variance on the daily maximum VPD measured from day 1 to day 28 of the experiment (n=28 per treatment). Daily maximum VPD was the mean value of a 5 minute average.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Treatments	2	3.72	1.86	4.89**
Residual	81	31.08	0.38	
Total	83	34.80		

Table B19 : Analysis of variance on the daily maximum irradiance measured from day 1 to day 28 of the experiment (n=28 per treatment). Daily maximum irradiance was calculated as a 5 minute average.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Treatments	2	12380.60	6190.30	9.00***
Residual	81	55697.32	687.62	
Total	83	68077.92		

ns : Not significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
*** : Significant at $P \leq 0.001$
(+) : Regression coefficient

Table B20 : Analysis of variance on the RWC of *S. leprosula* stem cuttings prior to rooting as affected by treatments, times of day and days of measurement (n=24 per treatment per time per day). Measurements were made on days 1,8,14,21.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	727.10	363.55	11.19****
Treatments (Trt)	2	1264.92	632.46	19.47****
Times (Tm)	2	9097.15	4548.57	140.00****
Trt*Tm	4	1175.15	293.79	9.04****
Days (Dy)	3	18920.33	6306.78	194.11****
Trt*Dy	6	724.04	120.67	3.71**
Tm*Dy	6	2686.26	447.71	13.78****
Residual	838	27227.56	32.49	
Total	863	61822.51		

Table B21 : Analysis of variance on the g_s of *S. leprosula* stem cuttings prior to rooting as affected by treatments, times of day and days of measurement (n=12 per treatment per time per day). Measurements were made on days 1,8,14,21.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	18912.96	9456.48	1.38ns
Treatments (Trt)	2	91353.24	45676.62	6.68**
Times (Tm)	2	1454828.24	727414.12	106.31****
Trt*Tm	4	3778.70	944.68	0.14ns
Days (Dy)	3	195865.51	65288.50	9.54****
Trt*Dy	6	30567.13	5094.52	0.74ns
Tm*Dy	6	140597.69	23432.95	3.42**
Residual	406	2777912.96	6842.15	
Total	431	4713816.43		

n s : Not significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
**** : Significant at $P \leq 0.0001$

Table B22 : Analysis of variance on the P_n of *S. leprosula* stem cuttings prior to rooting as affected by treatments, times of day and days of measurement (n=12 per treatment per time per day). Measurements were made on days 1,8,14,21.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	0.15	0.08	0.42ns
Treatments	2	1.16	0.58	3.05*
Times (Tm)	2	104.95	52.48	276.21****
Trt*Tm	4	1.85	0.46	2.42*
Days (Dy)	3	4.31	1.44	7.58***
Trt*Dy	6	1.60	0.27	1.42ns
Tm*Dy	6	6.14	1.02	5.37****
Residual	406	76.48	0.19	
Total	431	196.64		

Table B23 : Analysis of variance on the PAR during the P_n and g_s measurements as affected by treatments, times of day and days of measurement (n=12 per treatment per time per day). Measurements were made on days 1,8,14,21.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	2694.13	1347.07	1.88ns
Treatments (Trt)	2	14429.43	7214.72	10.08***
Times (Tm)	2	534512.89	267256.45	373.24****
Trt*Tm	4	25662.89	6415.72	8.96****
Days (Dy)	3	8363.30	2787.77	3.89**
Trt*Dy	6	3201.61	533.60	0.75ns
Tm*Dy	6	12149.31	2024.89	2.83**
Residual	406	290715.12	716.05	
Total	431	891728.68		

ns : Not significant at $P \leq 0.05$
* : Significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
*** : Significant at $P \leq 0.001$
**** : Significant at $P \leq 0.0001$

Table B24 : Sugar components found in the leaf and stem of *S. leprosula* cuttings subjected to three irradiance levels. Cuttings were harvested at 09:00 hours on day 1 (leaf area=30 cm^2 and stem=5 cm for each cutting; n=3 per treatment). These cuttings were taken from stock plants grown under 33% full sunlight (low irradiance=0-98 µmol photons m^{-2} s^{1}; medium=0-360 µmol photons m^{-2} s^{-1}; high=0-658 µmol photons m^{-2} s^{-1}).

	Leaf sugar (%)			Stem sugar (%)		
Irradiance levels	High	Medium	Low	High	Medium	Low
Sucrose	1.3132	0.8013	0.6361	0.4833	0.4219	0.3865
Fructose	0.1956	0.1619	0.1198	0.0754	0.6256	0.1669
Glucose	0.5216	0.4803	0.3481	0.2137	0.0953	0.1918
Rhamnose	0.4093	0.0405	0.0420	0.3510	0.1814	0.2094
Fucose	0.0223	0.0607	0.0157	0.0166	0.0060	0.0021
Galactose	0.0379	0.3226	0.0247	0.0136	0.0764	-
Arabinose	0.1795	0.0132	-	0.0116	-	-
Xylose	0.0109	-	-	0.0020	-	-
Inositol	0.3837	0.5371	0.4421	0.1726	0.1461	0.1482
Mannitol	0.0246	0.0230	0.0204	0.0064	0.0187	0.0064

- : These sugar are not detected in the respective samples. Statistical analysis was not carried out on these sugar values since inadequate samples were available.

Table B25 : Analysis of deviance for a stepwise regression to determine the effect of treatments and morphological characteristics on rooting ability of *S. leprosula* stem cuttings at week sixteen (n=60 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	2	3.26	1.63	1.27ns
Treatments	2	12.86	6.43	5.02**
Cutting volume	1	10.60	10.60	8.28**(-)
Residual	174	222.25	1.28	
Total	179	248.97		

ns : Not significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
(-) : Regression coefficient

Table B26 : Analysis of deviance for a stepwise regression to determine the effect of treatments and morphological characteristics on dead stem cuttings of *S. leprosula* at week sixteen (n=60 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	2	0.86	0.43	0.34ns
Treatments	2	14.46	7.23	5.78**
Cutting volume	1	15.19	15.19	12.15***(+)
Residual	174	216.81	1.25	
Total	179	247.32		

Table B27 : Analysis of variance for a stepwise regression to determine the effect of treatments and morphological characteristics on the mean accumulated number of roots of *S. leprosula* stem cuttings at week sixteen (n=30, 37 and 18 for high, medium and low irradiance levels respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	31.98	15.99	2.51ns
Cutting volume	1	33.07	33.07	5.19*(-)
Residual	81	516.19	6.37	
Total	84	581.24		

Table B28 : Analysis of variance on the daily maximum VPD at different treatments measured from day 1 to day 25 of the experiment (n=25 per treatment). Daily maximum VPD data were values calculated as 5 minutes average.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Treatments	2	33.76	16.88	48.23****
Residuals	72	25.47	0.35	
Total	74	59.23		

ns : Not significant at $P \leq 0.05$
* : Significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
**** : Significant at $P \leq 0.0001$
(+)/(-) : Regression coefficients

Table B29 : Analysis of variance on the maximum PAR in different treatments measured from day 1 to day 25 of the experiment (n=25 per treatment). Daily maximum PAR were values calculated as 5 minutes average.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Treatments	2	2309608.57	1154804.29	596.50****
Residuals	72	139389.41	1935.96	
Total	74	2448997.98		

Table B30 : Analysis of variance on the average VPD in different treatments measured from day 1 to day 25 of the experiment. Daily average VPD were mean values of a 5 minute calculated over a 24 hour period.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Treatments	2	0.67	0.34	17.00****
Residuals	72	1.56	0.02	
Total	74	2.23		

Table B31 : Analysis of variance on the average PAR in different treatments measured from day 1 to day 25 of the experiment. Daily average PAR data were the mean values of a 5 minute calculated over a 24 hour period.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Treatments	2	55001.63	27500.82	194.89****
Residuals	72	10159.88	141.11	
Total	74	65161.51		

**** : Significant at $P \leq 0.0001$

Table B32 : Analysis of variance on the RWC of *S. leprosula* stem cuttings prior to rooting as affected by treatments, times of day and days of measurement (n=24 per treatment per time per day). Measurements were made on days 1,8,14,21.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	388.47	194.24	4.28**
Treatments (Trt)	2	1554.79	777.40	17.15****
Times (Tm)	2	2001.06	1000.53	22.07****
Trt*Tm	4	1160.04	290.01	6.40***
Days (Dy)	3	12460.14	4153.38	91.61****
Trt*Dy	6	1841.10	306.85	6.77****
Tm*Dy	6	9110.06	1518.34	33.49****
Residual	838	37992.76	45.34	
Total	863	66508.42		

Table B33 : Analysis of variance on the g_s of *S. leprosula* stem cuttings prior to rooting as affected by treatments, times of day and days of measurement (n=12 per treatment per time per day). Measurements were made under natural irradiance treatments given to the cuttings in the propagators. Measurements were made on days 1,8,14,21.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	25354.63	12677.32	0.84ns
Treatments (Trt)	2	31695.65	15847.83	1.05ns
Times (Tm)	2	2407076.25	1203538.13	79.56****
Trt*Tm	4	123383.67	30845.92	2.04ns
Days (Dy)	2	1106404.96	553202.48	36.57****
Trt*Dy	4	114473.16	28618.29	1.89ns
Tm*Dy	4	277840.76	69460.19	4.59**
Residual	303	4583393.21	15126.71	
Total	323	8669622.29		

ns : Not significantly different at $P \leq 0.05$
** : Significant at $P \leq 0.01$
*** : Significant at $P \leq 0.001$
**** : Significant at $P \leq 0.0001$

Table B34 : Analysis of variance on the P_n of *S. leprosula* stem cuttings as affected by treatments, times of day and days of measurement (n=12 per treatment per time per day). Measurements were made under natural irradiance treatments given to the cuttings in the propagators. Measurements were made on days 1,8,14,21.

Source of variations	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	0.56	0.28	0.70ns
Treatments (Trt)	2	64.45	32.23	80.58****
Times (Tm)	2	25.78	12.89	32.23****
Trt*Tm	4	5.28	1.32	3.30*
Days (Dy)	2	4.72	2.36	5.90**
Trt*Dy	4	1.25	0.31	0.78ns
Tm*Dy	4	10.24	2.56	6.40***
Residual	303	120.43	0.40	
Total	323	232.71		

Table B35 : Analysis of variance on the PAR during the measurements of P_n and g_s of *S. leprosula* stem cuttings as affected by treatments, times of day and days of measurement (n=12 per treatment per time per day). Measurements of P_n and g_s were made under natural irradiance treatments given to the cuttings in the propagators. These measurements were made on days 1,8,14,21.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	2	18698.72	9349.36	2.0ns
Treatments (Trt)	2	1070878.02	535439.01	114.35****
Time (Tm)	2	858081.50	429040.75	91.62****
Trt*Tm	4	422149.59	105537.40	22.54****
Day (Dy)	2	31262.39	15631.20	3.34*
Trt*Dy	4	23485.98	5871.50	1.25ns
Tm*Dy	4	107387.78	26846.95	5.73***
Residual	303	1418840.77	4682.64	
Total	323	3950784.75		

ns : Not significantly different at $P \leq 0.05$
* : Significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
*** : Significant at $P \leq 0.001$
**** : Significant at $P \leq 0.0001$

Table B36 : Combined curve analysis of variance (Ross 1981) for curves fitted using the theoretical model of Jarvis *et al.* (1985), to P_n of *S. leprosula* stem cuttings prior to rooting planted under the 3 irradiance levels in the enclosed mist propagators. Measurements of P_n were made for a period of 10 days after cuttings were planted on the rooting beds. Artificial tungsten light was given to the cuttings to increase the maximum irradiance level in each treatment (n=276 per treatment).

Curve comparisons	Degrees of freedom	Sum of square	Mean square	F-value
High versus medium irradiance	4	0.00	0.00	0.00ns
Residual	544	243.20	0.45	
High versus low irradiance	4	5.42	1.36	3.68**
Residual	544	198.60	0.37	
Medium versus low irradiance	4	15.42	3.86	9.41****
Residual	544	222.58	0.41	

Table B37 : Analysis of deviance for a stepwise regression to determine the influence of treatments and morphological characteristics on the rooting ability of *S. leprosula* stem cuttings at week sixteen (n=60 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	5	6.47	1.29	1.07ns
Treatments	2	10.49	5.25	4.34*
Residual	172	207.80	1.21	
Total	179	224.76		

ns : Not significantly different at $P \leq 0.05$
* : Significant at $P \leq 0.05$
** : Significant at $P \leq 0.01$
**** : Significant at $P \leq 0.0001$

Table B38 : Analysis of deviance for a stepwise regression to determine the influence of treatments and morphological characteristics on the *S. leprosula* stem cuttings that remained unrooted at week sixteen (n=60 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	5	7.41	1.48	1.44ns
Treatments	2	16.07	8.04	7.81***
Residual	172	176.74	1.03	
Total	179	200.21		

Table B39 : Analysis of variance on P_n per leaf of *S. leprosula* stem cuttings prior to rooting. P_n was measured on days 14, 21 and 28 (n=18 per treatment per day; one leaf was dropped on day 28 for 60 cm^2 leaf area giving the total n=161 instead of 162).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	0.0000440	0.0000088	0.65ns
Treatments (Trt)	2	0.0019000	0.0009500	69.85****
Day (Dy)	2	0.0000190	0.00000950	0.70ns
Trt*Dy	4	0.0000240	0.0000060	0.44ns
Residual	147	0.0020000	0.0000136	
Total	160	0.0039870		

ns : Not significantly different at $P \leq 0.05$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$

Table B40 : Analysis of variance on transpiration rates per leaf of *S. leprosula* stem cuttings prior to rooting. P_n was measured on days 14, 21 and 28 (n=18 per treatment per day; one leaf was dropped on day 28 for 60 cm^2 leaf area giving the total n=161 instead of 162).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	0.000150	0.000030	2.73*
Treatments (Trt)	2	0.004300	0.002150	195.45****
Days (Dy)	2	0.000190	0.000095	8.64***
Trt*Dy	4	0.000040	0.000010	0.91ns
Residual	147	0.001600	0.000011	
Total	160	0.006280		

Table B41 : Analysis of variance on P_n per unit leaf area of rooted cuttings and cuttings that remained unrooted of *S. leprosula*. (Measurements of P_n were made on day 63; n=54 for rooted and unrooted cuttings giving the total n=108).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	3.45	0.69	1.13ns
Treatments (Trt)	2	0.13	0.07	0.11ns
Rooted/unrooted (Rt)	1	47.52	47.52	77.90****
Trt*Rt	2	0.40	0.20	0.33ns
Residual	97	59.33	0.61	
Total	107	110.83		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$

Table B42 : Analysis of variance on g_s per unit leaf area of rooted cuttings and g_s of cuttings that remained unrooted of *S. leprosula*. (Measurements of g_s were made on day 63; n=54 for rooted and unrooted cuttings giving the total n=108).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	409718.36	81943.67	1.55ns
Treatments (Trt)	2	208931.46	104465.73	1.98ns
Rooted/unrooted (Rt)	1	253461.33	253461.33	4.80*
Trt*Rt	2	122093.72	61046.86	1.16ns
Residual	97	5119725.75	52780.68	
Total	107	6113930.62		

Table B43 : Analysis of variance on dry weight of leaf per unit leaf area (g) of *S. leprosula* stem cuttings on day 1 and day 28 (n=30 per treatment per day giving the total n=180). Samples of cuttings were taken at 09:00 hours on each day.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	0.00002301	0.00000460	2.82*
Treatments (Trt)	2	0.00017580	0.00008790	53.93****
Days (Dy)	1	0.00001085	0.00001085	6.66**
Trt*Dy	2	0.00000015	0.00000008	0.05ns
Residual	169	0.00027622	0.00000163	
Total	179	0.00048603		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
** : Significantly different at $P \leq 0.01$
**** : Significantly different at $P \leq 0.0001$

Table B44 : Analysis of variance on dry weight of leaf per leaf (g) of *S. leprosula* stem cuttings on day 1 and day 28 (n=30 per treatment per day giving the total n=180). Samples of cuttings were taken at 09:00 hours on each day.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	0.0286	0.0057	3.80**
Treatments (Trt)	2	2.1226	1.0613	707.53****
Days (Dy)	1	0.0142	0.0142	9.47**
Trt*Dy	2	0.0046	0.0023	1.53ns
Residual	169	0.2484	0.0015	
Total	179	2.4184		

ns : Not significantly different at $P \leq 0.05$
** : Significantly different at $P \leq 0.01$
**** : Significantly different at $P \leq 0.0001$

Table B45 : Mean sugar components found in the leaf and stem of *S. leprosula* cuttings subjected to three leaf area treatments. Cuttings were harvested at 09:00 hours on day 1 and day 28 of the experiment. Cuttings were taken from stock plants grown at 33 % full sunlight (n=3 for each treatment on day 1 and day 28; except for stem with 60 cm^2 leaf area on day 28 where n=2).

Day 1	Leaf sugar (%)			Stem sugar (%)		
Leaf areas	15 cm^2	30 cm^2	60 cm^2	15 cm^2	30 cm^2	60 cm^2
Sucrose	1.8569	2.2672	0.5789	0.5566	-	-
Fructose	0.6192	0.8773	1.3351	0.4469	0.3573	0.3329
Glucose	0.4969	0.6418	0.2630	0.2617	0.2143	0.2560
Rhamnose	0.1701	0.1481	0.2239	0.4420	0.3559	0.4847
Fucose	0.0337	0.0198	0.0255	0.0095	-	-
Galactose	0.0124	0.0265	-	0.0103	-	-
Xylose	0.0152	0.0038	0.3866	-	0.0274	-
Arabinose	-	-	-	-	-	-
Inositol	0.5433	0.8210	0.0288	0.0372	0.1083	-
Mannitol	0.0208	0.0232	0.2266	0.0084	-	-

Day 28	Leaf sugar (%)			Stem sugar (%)		
Leaf areas	15 cm^2	30 cm^2	60 cm^2	15 cm^2	30 cm^2	60 cm^2
Sucrose	0.5850	1.2819	0.1826	-	1.1809	1.4860
Fructose	1.0261	2.0191	1.4216	0.9848	0.3348	1.0585
Glucose	0.3677	0.5252	0.2988	0.3183	0.2470	0.4345
Rhamnose	0.2491	0.1715	0.3800	0.3805	0.2814	0.2859
Fucose	0.0049	0.0212	0.0174	-	0.0224	0.0170
Galactose	-	-	-	-	0.0161	-
Xylose	0.0664	0.0287	0.0669	-	-	0.0245
Arabinose	-	0.0025	-	-	-	0.0055
Inositol	0.1353	0.1966	0.4288	0.0417	0.4162	0.0112
Mannitol	0.0056	0.0183	0.0236	-	0.0175	0.0157

- These sugar components are not detected in the respective samples. Statistical analysis was not carried out on these sugar values since inadequate samples were available.

Table B46 : Analysis of variance on the mean initial diameter of *S. leprosula* stem cuttings (n=60 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-ratio
Blocks	5	2.042	0.408	102.00****
Treatments	4	0.048	0.012	3.00*
Residual	20	0.073	0.004	
Total	29	2.163		

Table B47 : Analysis of variance on the mean accumulated number of roots per rooted stem cutting of *S. leprosula* as affected by the IBA doses at week ten (number of cuttings rooted per treatment = 27, 42, 38, 35, 33 for 0, 20, 40, 60 and 80 µg IBA respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	14.10	2.82	1.93ns
Treatments	4	17.67	4.42	3.03*
Residual	20	29.19	1.46	
Total	29	60.96		

Table B48 : Analysis of variance on the initial height (cm) of *S. leprosula* potted stock plants treated with 0.5 g and 1.5 g fertiliser (n=42 per treatment). Plants were measured on day 0 after the experiment was laid-out.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	80.69	16.14	1.09ns
Treatments (Trt)	1	0.36	0.36	0.02ns
Clones (Cl)	6	600.70	100.12	6.75****
Trt*Cl	6	101.12	16.85	1.14ns
Residual	65	964.51	14.84	
Total	83	1747.38		

ns : Not significant at $P \leq 0.05$
* : Significant at $P \leq 0.05$
**** : Significant at $P \leq 0.0001$

Table B49 : Analysis of variance on the initial diameter (cm) of *S. leprosula* potted stock plants treated with 0.5 g and 1.5 g fertiliser (n=42 per treatment). Plants were measured on day 0 after the experiment was laid-out.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	0.0264	0.0053	0.78ns
Treatments (Trt)	1	0.0005	0.0005	0.07ns
Clones (Cl)	6	0.1920	0.0320	4.71***
Trt*Cl	6	0.0261	0.0044	0.65ns
Residual	65	0.4398	0.0068	
Total	83	0.6849		

Table B50 : Analysis of variance on the final height (cm) measured at week 22 of *S. leprosula* potted stock plants treated with 0.5 g and 1.5 g fertiliser (n=39 and 35 for 0.5 g and 1.5 g fertiliser respectively; 3 and 7 plants were dead per treatment respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	2602.38	520.48	4.14**
Treatments (Trt)	1	1355.21	1355.21	10.77**
Clones (Cl)	6	3272.15	545.36	4.33***
Trt*Cl	6	646.77	107.80	0.86ns
Residual	55	6919.95	125.82	
Total	73	14796.46		

ns : Not significantly different at $P \leq 0.05$
** : Significantly different at $P \leq 0.01$
*** : Significantly different at $P \leq 0.001$

Table B51 : Analysis of variance on the final diameter (cm) measured at week 22 of *S. leprosula* potted stock plants treated with 0.5 g and 1.5 g fertiliser (n=39 and 35 for 0.5 g and 1.5 g fertiliser respectively; 3 and 7 plants were dead per treatment respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	0.24	0.05	2.50*
Treatments (Trt)	1	0.15	0.15	7.50**
Clones (Cl)	6	0.97	0.16	8.00****
Trt*Cl	6	0.09	0.02	1.00ns
Residual	55	0.84	0.02	
Total	73	2.29		

Table B52 : Analysis of variance on initial length (cm) of *S. leprosula* stem cuttings taken from stock plants treated with 0.5 g and 1.5 g fertiliser (n=126 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	94.70	18.94	1.15ns
Treatments	1	78.33	78.33	4.77*
Residual	245	4024.94	16.43	
Total	251	4197.97		

Table B53 : Analysis of variance on initial diameter (cm) of *S. leprosula* stem cuttings taken from stock plants treated with 0.5 g and 1.5 g fertiliser (n=126 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	0.49	0.10	3.33**
Treatments	1	0.29	0.29	9.67**
Residual	245	6.96	0.03	
Total	251	7.74		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
** : Significantly different at $P \leq 0.01$
**** : Significantly different at $P \leq 0.0001$

Table B54 : Analysis of variance on initial volume (cm^3) of *S. leprosula* stem cuttings taken from stock plants treated with 0.5 g and 1.5 g fertiliser (n=126 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	33.55	6.71	5.16***
Treatments	1	36.21	36.21	27.85****
Residual	245	319.41	1.30	
Total	251	389.17		

Table B55 : Analysis of deviance for a stepwise regression on the effect of treatments and morphological characteristics on *S. leprosula* stem cuttings that remained unrooted at week 16 (n=126 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	5	11.18	2.24	2.67*
Treatments	1	4.49	4.49	5.35*
Residual	245	204.85	0.84	
Total	251	220.52		

Table B56 : Analysis of variance for a stepwise regression on effect of treatments and morphological characteristics on the mean accumulated number of roots per rooted stem cuttings of *S. leprosula* (n=92 and 88 for 0.5 g and 1.5 g fertilisers respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	34.84	6.97	2.60*
Diameter	1	36.08	36.08	13.46***(-)
Residual	173	463.28	2.68	
Total	179	534.20		

* : Significantly different at $P \leq 0.05$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$
(-) : Regression coefficient

Table B57 : Analysis of variance on P_n of *S. leprosula* stem cuttings prior to rooting. Cuttings were taken from stock plants treated with 0.5 g and 1.5 g fertiliser. P_n was measured on days 1,7, 14 and 28 (n=24 per treatment per day giving total n=192).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	20.90	4.18	2.61*
Treatments	1	0.15	0.15	0.09ns
Days	3	25.41	8.47	5.29**
Residual	182	291.40	1.60	
Total	191	337.86		

Table B58 : Analysis of variance on PAR taken during the measurements of P_n of *S. leprosula* stem cuttings prior to rooting. Cuttings were taken from stock plants treated with 0.5 g and 1.5 g fertiliser. P_n was measured on days 1,7, 14 and 28 (n=24 per treatment per day giving total n=192).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	5	121062.55	24212.51	5.93****
Treatments	1	11485.55	11485.55	2.81ns
Days	3	44433.52	14811.17	3.63*
Residual	182	743474.47	4085.02	
Total	191	920456.09		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
** : Significantly different at $P \leq 0.01$
**** : Significantly different at $P \leq 0.0001$

Table B59 : Analysis of variance on P_n of rooted cuttings and P_n of cuttings that remained unrooted of *S. leprosula*. Stem cuttings were taken from stock plants treated with 0.5 g and 1.5 g fertilisers. P_n was measured on day 63 (n=15 per treatment for rooted/unrooted cuttings). The measurements were taken in blocks 1,3,4,5 and 6 since inadequate samples of unrooted cuttings were available in block 2).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	5.02	1.26	1.16ns
Treatments (Trt)	1	0.01	0.01	0.01ns
Rooted/Unrooted (Trt)	1	27.20	27.20	24.95****
Trt*Rt	1	1.35	1.35	1.24ns
Residual	52	56.61	1.09	
Total	59	90.19		

Table B60 : Analysis of variance on the final height (cm) of *S. leprosula* potted stock plants grown under low and high irradiance levels at week 30 (n=36 and 33 for low and high irradiance treatments, 4 and 7 plants were dead in low and high irradiance treatments respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	164.86	164.86	0.45ns
Treatments	1	8234.53	8234.53	22.47****
Residual	66	24191.04	366.53	
Total	68	32590.43		

ns : Not significantly different at $P \leq 0.05$
**** : Significantly different at $P \leq 0.0001$

Table B61 : Analysis of variance on the final diameter (cm) of *S. leprosula* potted stock plants grown under low and high irradiance levels at week 30 (n=36 and 33 for low and high irradiance treatments, 4 and 7 plants were dead in low and high irradiance treatments respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	0.006	0.006	0.18ns
Treatments	1	0.421	0.421	12.38***
Residual	66	2.254	0.034	
Total	68	2.681		

Table B62 : Analysis of variance on number of nodes at week 30 of *S. leprosula* potted stock plants grown under low and high irradiance levels (n=36 and 33 for low and high irradiance treatments, 4 and 7 plants were dead in low and high irradiance treatments respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	0.07	0.07	0.01ns
Treatments	1	24.83	24.83	5.21*
Residual	66	314.88	4.77	
Total	68	339.78		

Table B63 : Analysis of variance on P_n measured at week 30 of *S. leprosula* potted stock plants grown under low and high irradiance levels. Measurements were made on the top most expanded leaf of 5 plants per treatment per block (n=76 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	0.06	0.06	0.04ns
Treatments	1	55.27	55.27	37.60****
Residual	149	219.23	1.47	
Total	151	274.56		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$

Table B64 : Analysis of variance on g_s measured at week 30 of *S. leprosula* potted stock plants grown under low and high irradiance levels. Measurements were made on the top most expanded leaf of 5 plants per treatment per block (n=76 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	22794.80	22794.80	6.33*
Treatments	1	42084.56	42084.56	11.68***
Residual	149	536920.96	3603.50	
Total	151	601800.32		

Table B65 : Analysis of variance on PAR when the P_n was measured on *S. leprosula* potted stock plants grown under low and high irradiance levels (at week 30; n=76 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	1	424.45	424.45	0.08ns
Treatments	1	431218.53	431218.53	78.99****
Residual	149	813431.87	5459.27	
Total	151	1245074.85		

Table B66 : Analysis of variance on initial length (cm) of *S. leprosula* stem cuttings taken from stock plants grown under low and high irradiance levels (n=125 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	14.92	3.73	0.42ns
Treatments	1	256.24	256.24	28.60****
Residual	244	2186.44	8.96	
Total	249	2457.60		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$

Table B67 : Analysis of variance on initial diameter (cm) of *S. leprosula* stem cuttings taken from stock plants grown under low and high irradiance levels on day 0 (n=125 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	0.04	0.01	0.50ns
Treatments	1	1.74	1.74	87.00****
Residual	244	4.87	0.02	
Total	249	6.65		

Table B68 : Analysis of variance on initial volume (cm^3) of *S. leprosula* stem cuttings taken from stock plants grown under low and high irradiance levels on day 0 (n=125 per treatment).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	0.92	0.23	0.52ns
Treatments	1	41.46	41.46	94.23****
Residual	244	107.11	0.44	
Total	249	149.49		

Table B69 : Analysis of variance on initial leaf weight (g) of *S. leprosula* stem cuttings taken from stock plants grown under low and high irradiance levels on day 0 after the experiment was laid-out (n=30 per treatment). Each cutting had a 30 cm^2 leaf area. Cuttings were sampled at 17:00 hours after experiment was laid-out.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	0.0033	0.0008	0.80ns
Treatments	1	0.0356	0.0356	35.60****
Residual	54	0.0526	0.0010	
Total	59	0.0915		

ns : Not significantly different at $P \leq 0.05$
**** : Significantly different at $P \leq 0.0001$

Table B70 : Analysis of variance on initial stem weight (g) of *S. leprosula* stem cuttings from stock plants grown under low and high irradiance levels (n=30 per treatment). Each cutting had a 30 cm^2 leaf area. Cuttings were sampled at 17:00 hours on day 0 after the experiment was laid-out.

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	0.05	0.01	0.20ns
Treatments	1	0.92	0.92	18.40***
Residual	54	2.57	0.05	
Total	59	3.54		

ns : Not significantly different at $P \leq 0.05$
*** : Significantly different at $P \leq 0.001$

Table B71 : Initial sugar components found in the leaf and stem of *S. leprosula* stem cuttings taken from stock plants grown under two irradiance levels. Cuttings were harvested at 17:00 hours after the experiment was laid-out on day 0. (leaf area=30 cm^2; n=2 and 3 for low high irradiance respectively; low irradiance=0-325 µmol photons m^{-2} s^{-1} ; high=0-722 µmol photons m^{-2} s^{-1}). Statistical analysis was not carried out on these sugar values since inadequate samples were available.

	Leaf sugar (%)		Stem sugar (%)	
Irradiance levels	Low	High	Low	High
Sucrose	1.4782	0.8587	0.3694	0.7933
Fructose	0.4675	0.3665	0.0373	0.1277
Glucose	0.4825	0.3055	0.0502	0.1314
Rhamnose	0.0693	0.3211	0.0792	0.1558
Fucose	0.0078	0.0299	0.0337	0.0172
Galactose	0.0452	0.0338	0.0688	0.0096
Arabinose	0.0037	0.3049	0.1460	0.2764
Xylose	0.0201	0.1745	0.1928	0.1650
Inositol	0.3377	0.3720	0.5385	0.4142
Mannitol	0.0185	0.0214	0.0600	0.0179

Table B72 : Analysis of deviance for a stepwise regression on the effect of treatments and morphological characteristics on the rooting ability of *S. leprosula* stem cuttings at week 16 (n=125 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	4	12.63	3.16	2.70ns
Cutting volume	1	18.09	18.09	15.46*** (-)
Treatments	1	5.12	5.12	4.38*
Residual	243	283.33	1.17	
Total	249	319.17		

Table B73 : Analysis of deviance for a stepwise regression on the effect of treatments and morphological characteristics of *S. leprosula* stem cuttings that remained unrooted at week 16 (n=125 per treatment).

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio
Blocks	4	2.74	0.69	0.62ns
Cutting volume	1	20.60	20.60	18.56****(+)
Residual	244	271.29	1.11	
Total	249	294.63		

Table B74 : Analysis of variance for a stepwise regression on effect of treatments and morphological characteristics on the mean accumulated number of roots per rooted stem cuttings of *S. leprosula* at week 16 (n=98 and 66 per treatment for cuttings taken from low and high irradiance respectively).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	16.43	4.11	0.93ns
Treatments	1	36.99	36.99	8.37**
Cutting volume	1	15.41	15.41	3.49ns
Residual	159	702.71	4.42	
Total	165	771.55		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
** : Significantly different at $P \leq 0.01$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$
(+) : Regression coefficient

Table B75 : Analysis of variance on P_n of *S. leprosula* stem cuttings prior to rooting. Cuttings were taken from stock plants grown under low and high irradiance treatments. P_n was measured on days 1,8,14,21 and 28 (n=20 per treatment per day).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	12.42	3.11	2.45*
Treatments (Trt)	1	1.13	1.13	0.89ns
Days (Dy)	4	35.68	8.92	7.02****
Trt*Dy	4	3.96	0.99	0.78ns
Residual	186	235.80	1.27	
Total	199	288.99		

Table B76 : Analysis of variance on g_s of *S. leprosula* stem cuttings prior to rooting. Cuttings were taken from stock plants grown under low and high irradiance treatments; g_s was measured on days 1,8,14,21 and 28 (n=20 per treatment per day).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	58856.13	14714.03	1.61ns
Treatments (Trt)	1	12119.69	12119.69	1.33ns
Days (Dy)	4	345190.38	86297.60	9.44****
Trt*Dy	4	37057.76	9264.44	1.01ns
Residual	186	1699482.34	9137.00	
Total	199	2152706.30		

ns : Not significantly different at $P \leq 0.05$
* : Significantly different at $P \leq 0.05$
**** : Significantly different at $P \leq 0.0001$

Table B77 : Analysis of variance on PAR during the measurements of P_n and g_s of *S. leprosula* stem cuttings prior to rooting. Cuttings were taken from stock plants grown under low and high irradiance treatments. Measurements were made on days 1,8,14,21 and 28 (n=20 per treatment per day).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	89957.33	22489.33	5.13***
Treatments (Trt)	1	259.92	259.92	0.06ns
Days (Dy)	4	474200:03	118550.01	27.06****
Trt*Dy	4	8179.73	2044.93	0.47ns
Residual	186	814925.07	4381.32	
Total	199	1387522.08		

Table B78 : Analysis of variance on P_n of rooted cuttings and P_n of cuttings that remained unrooted of *S. leprosula* from stock plants grown under low and high irradiance treatments (measurements were made on day 63; n=20 per treatment of rooted and unrooted cuttings).

Source	Degrees of freedom	Sum of square	Mean square	F-value
Blocks	4	7.05	1.76	1.22ns
Treatments (Trt)	1	0.16	0.16	0.11ns
Rooted/Unrooted (Rt)	1	38.16	38.16	26.50****
Trt*Rt	1	11.93	11.93	8.28**
Residual	72	103.87	1.44	
Total	79	161.17		

ns : Not significantly different at $P \leq 0.05$
** : Significantly different at $P \leq 0.01$
*** : Significantly different at $P \leq 0.001$
**** : Significantly different at $P \leq 0.0001$

APPENDIX C : Lay-out of each cutting in each block on the propagation bed for experiment 1 of chapter 7

Block 2

	T2n7	T2n3	T1n6	T1(549)n2	T2n5
T1n5	T2n6	T2(549)n2	T1n5	T2n8	T2n4
T1n4	T2n5	T1n8	T1n4	T2n7	T2(587)n3
T1n3	T2n4	T1n7	T1(549)n3	T2n6	T1n8
T1(590)n2	T2n3	T1n6 •	T2n8	T2n5	T1n7
T2n6	T2(590)n2	T1n5	T2n7	T2n4	T1n6
T2n5	T1n9	T1n4	T2n6	T2(559)n3	T1n5
T2n4	T1n8	T1(559)n3	T2n5	T1n9	T1n4
T2(550)n3	T1n7	T2n8	T2n4	T1n8	T1n3

Block 3

T2n5	T2(590)n3	T1n3	T2n6	T2n6	T2n7
T2n4	T1n6	T1(525)n2	T2n5	T2n5	T2n6
T2n3	T1n5	T2n10	T2n4	T2n4	T2n5
T2(549)n2	T1n4	T2n9	T2(550)n3	T2(581)n3	T2(525)n3
T1n7	T1n3	T2n8 •	T1n8	T1n7	T1n6
T1n6	T1(549)n2	T2n7	T1n7	T1n6	T1n5
T1n5	T2n10	T2n6	T1n6	T1n5	T1n4
T1n4	T2n8	T2n5	T1n5	T1n4	T1n3
T1(550)n3	T2n6	T2n4	T1n4	T1(587)n3	T1(581)n2

Block 6

T2n5	T1n4	T2n6	T2n5	T2(581)n2	T1n7
T2n4	T1(549)n3	T2n5	T2n4	T1n6	T1n6
T2(559)n3	T2n7	T2n4	T2n3	T1n5	T1n5
T1n8	T2n6	T2(590)n3	T2(525)n2	T1n4	T1n4
T1n7	T2n5	T1n9 •	T1n6	T1n3	T1(590)n3
T1n6	T2n4	T1n8	T1n5	T1(587)n2	T2n7
T1n5	T2(550)n3	T1n7	T1n4	T2n8	T2n5
T1n4	T2n7	T1n6	T1(559)n3	T2n7	T2n4
T1(550)n3	T2n6	T1n5	T2n8	T2n6	T2n3

Block 4

	T1n4	T1(550)n3	T2n7	T2n4	T1n6
T1n5	T1n3	T2n8	T2n6	T2n3	T1n5
T1n4	T1(587)n2	T2n7	T2n5	T2(554)n2	T1n4
T1n3	T2n6	T2n6	T2n4	T1n7	T1n3
T1(590)n2	T2n5	T2n5	T2(587)n3	T1n6	T1(581)n2
T2n6	T2n4	T2n4	T1n7	T1n5	T2n8
T2n5	T2n3	T2(581)n3	T1n6	T1n4	T2n7
T2n4	T2(549)n2	T1n6	T1n5	T1n3	T2n6
T2(559)n3	T1n7	T1n5	T1n4	T1(525)n2	T2n5

Block 1

T2n3	T1n5	T1(590)n2	T2n6	T2(559)n2	T1n8
T2(590)n2	T1n4	T2n6	T2n4	T1n7	T1n7
T1n9	T1(587)n3	T2n5	T2(587)n3	T1n6	T1n6
T1n8	T2n9	T2n4	T1n8	T1n5	T1n5
T1n7	T2n8	T2(581)n3	T1n7	T1n4	T1n4
T1n6	T2n7	T1n9	T1n6	T1n3	T1(525)n3
T1n5	T2n6	T1n8	T1n5	T1(550)n2	T2n5
T1n4	T2n5	T1n7	T1n4	T2n9	T2n4
T1(559)n3	T2n4	T1n6	T1n3	T2n7	T2n3

Block 5

T2(590)n3	T1n8	T2n5	T1(581)n3	T1(525)n2	T1n5
T1n5	T1n7	T2n4	T1n6	T2n8	T1n4
T1n4	T1n6	T2n3	T1n5	T2n5	T1n3
T1(550)n3	T1n5	T2(549)n2	T1n4	T2n4	T1(550)n2
T2n7	T1n4	T1n7	T1(581)n3	T2n3	T1n7
T2n6	T1(525)n3	T1n6	T2n9	T2(525)n2	T1n6
T2n5	T2n6	T1n5	T2n8	T1n6	T1n5
T2n4	T2n5	T1n4	T2n7	T1n5	T1n4
T2(581)n3	T2n4	T1(550)n3	T2n6	T1n4	T1n3

T1 : 0.5g fertiliser treatment
T2 : 1.5g fertiliser treatment
( ): Numbers in brackets are the clone number; each clone number in every block represents one stock plant
n : Node position on the stock plant
o : Misting unit in each propagator (block)

Arrangement of blocks on propagation bed

Block2	Block3	Block6	Block4	Block1	Block5

APPENDIX D : A Published paper produced during period of this postgraduate programme.

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Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*

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Abstract

The application of auxin (indole butyric acid, IBA) significantly increased the rate of root emergence in single node leafy stem cuttings of *Shorea leprosula* taken from 10-month-old potted seedlings. A range of IBA doses (0, 20, 40, 60 and 80 μg IBA per cutting) were tested and 20 μg per cutting was found to be the best with 70% of cuttings rooted within 12 weeks. Higher doses resulted in less rooting success. IBA application also enhanced the number of roots developed on each cutting. The mean accumulated number of roots per rooted cutting in Week 10 on cuttings treated with 20, 40, 60, and 80 μg IBA was 5.05, 5.26, 4.82 and 4.80 respectively compared with 3.11 for cuttings treated with only a 50% ethanol and water mixture.

Keywords: *Shorea leprosula*; Indole butyric acid; Auxin; Stem cutting; Rooting

1. Introduction

Shorea leprosula Miq. belongs to the family Dipterocarpaceae. It is generally a large tree and can reach the height of 60 m and 3 m in girth (Symington, 1974). The growth rate of the species is relatively fast and it is estimated that the tree can reach the harvestable size of 50–60 cm diameter at breast height in 40 years (Azman et al., 1991). Among the Dipterocarps, the timber from the genus *Shorea* is one of the most commercially important and is marketed under the trade name meranti both locally and overseas. *Shorea leprosula* is one of the main sources of light red meranti timber and is commonly used for joinery, panelling, furniture, plywood manufacture and light construction work (Choo and Lim, 1983). To sustain the supply of harvestable timber from this species, effort has been made to plant the species in the deforested areas (Borhan and Rahman, 1987; Azman et al., 1991). However, planting programmes are often hindered by inadequate and irregular supply of planting stocks. This is because the supply of viable seeds is irregular and inadequate (Tang, 1971; Tamari, 1976; Sasaki, 1980). To overcome the problem, many attempts have been made to propagate the species by stem cuttings (Dick and Aminah, 1994). A number of studies have reported that *S. leprosula* can be propagated by

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leafy stem cuttings (Muckadell and Malim, 1978; Halle and Kamil, 1981; Leakey et al., 1982a; Alias, 1984; Srivastava et al., 1986; Kamis and Ng, 1989; Siagan et al., 1989; Liew, 1992; Smits et al., 1993). While many of these studies have applied auxin to the base of the cuttings, none has critically evaluated the influence of auxin on root initiation. Studies by Srivastava and Manggil (1981) and Halle and Kamil (1981) showed that rooting of *S. leprosula* cuttings was not improved by auxin application. The present study was carried out to determine the suitable auxin dose for rooting the stem cuttings of *S. leprosula*.

2. Materials and methods

The cuttings were taken from the stock plants raised from seeds collected from three trees in the Forest Reserve Ulu Teranum in the state of Pahang Malaysia. The seeds were sown in seed beds at the nursery of Forest Research Institute Malaysia. When the germinated seedlings were approximately 7 cm tall, they were transplanted into black perforated polythene bags (9 cm diameter × 17 cm height). The potting medium used was forest top-soil and sand in the ratio of 3:1 by volume. To every cubic meter of the medium, 1.2 kg triple superphosphate (46% P_2O_5) and 1.6 kg ground magnesium limestone (33% CaO) were added. The potted seedlings were kept on the transplanting beds shaded with plastic netting. The average mid-day irradiance on a sunny day under this shade was 770 μmol photons m^{-2} s^{-1} (38% of full sunlight) measured with a SKP 215/200 light sensor (Skye Instruments, UK). Granular compound commercial fertiliser 'NPK Blue' (12 N:12 P_2O_5:17 K_2O:2 MgO + Trace element) was applied at the rate of 1 g per seedling per month. Potted seedlings were watered to field capacity twice a day in the morning and late afternoon. Weeding, insecticide and fungicide applications were carried out whenever necessary. In January 1992 when the seedlings were 10 months old, 60 seedlings were randomly selected for the experiment. The average height of the selected seedlings was 61.75 ± 0.96 cm.

Single node cuttings were taken from these stock plants from the second to sixth node down the stem. The apical undeveloped shoots were discarded as they were not suitable for cuttings. Five cuttings were taken from one stock plant and each node was systematically allocated to each of the five indole-3 butyric acid (IBA) treatments so that each node position was equally represented in each treatment. This was to lessen morphological variation as the cutting material was taken from seedlings which were genetically heterogenous. The length of the cuttings was 5 cm and the leaf area retained on each cutting was 30 cm^2. The leaf area was cut using a 30 cm^2 template made of graph paper which was measured with leaf area meter (Delta-T Series, Taiwan). The base of the cuttings was cut at right angles and treated with one of five IBA doses: 0, 20, 40, 60 and 80 μg. The IBA formulation was prepared in liquid form using absolute ethyl alcohol which was diluted to 50% with distilled water. The control, without IBA, was 50% ethyl alcohol. The IBA was applied to each cutting using a micropipette (Model F10, Gilson Medical Electronic, France). The alcohol was immediately evaporated in a stream of air from a fan. These treated cuttings were inserted into the rooting medium of river sand (60% with 2 mm or less and 40% with ≥ 2–5 mm diameter of sand particle). The sand was cleaned with water to remove plant material and big stones, before it was placed into the rooting beds. Each treatment consisted of 60 cuttings and they were laid out in six blocks. Each block consisted of five plots with ten cuttings of each treatment per plot. The treatments were randomly allocated to plots within a block. The node was held on the rooting beds in sequential order of its position on the plants in order to simplify the lay-out. Unfortunately this resulted in node and cutting position on the rooting bed being confounded and therefore could not be statistically analysed. Ideally, the node positions should have been randomised. The initial diameter of each cutting base was recorded. Diameter of cuttings was measured using digimatic caliper (Model CD-6, Mitutoyo Corporation Japan). The planted cuttings were covered with transparent plastic enclosures supported by alumin-

ium frames to maintain a high air humidity. The plastic enclosures were then shaded with black plastic netting. The average mid-day photosynthetically active irradiance under this shade on a sunny day was 275 μmol photons $m^{-2} s^{-1}$ (14% of full sunlight). The rooting beds were kept moist by an automatic mist sprinkler system. The Macpennys atomiser jet (No. 1) was used to give fine misting to the planted cuttings. The misting frequency was every hour and each duration of spray was 1 min. An assessment was carried out on cuttings every 2 weeks starting 2 weeks after planting. At each assessment, the variables measured were number of rooted, unrooted, dead cuttings and number of roots on each cutting. A cutting was scored as rooted when it produced a root of 1 cm length or more and the cutting was considered dead when the whole stem turned brown. After the observation, the cuttings were replanted into the rooting bed and reassessed until the tenth week by which time the rooted cuttings were potted. The remaining unrooted cuttings and the cuttings with root primordia were replanted for further observation on rooting at Week 12. To ensure minimal stress to cuttings during assessment, the cuttings were sprayed with water before digging and after replanting. Repeated assessments on the same cuttings were made because of the limited cutting material available, this is a standard technique for this type of experiment (Leakey and Mohammed, 1985). The experiment was terminated 12 weeks after planting. By this time the leaves of unrooted cuttings were yellowing and the cuttings were dying. The mean accumulated root number was calculated by dividing the total number of roots produced by the total number of rooted cuttings at each assessment.

Table 1
The time taken for 50% of rooting response to occur for *S. leprosula* stem cuttings treated with five different IBA treatments

Treatments	Weeks
0 μg IBA	7.31
20 μg IBA	4.92
40 μg IBA	4.93
60 μg IBA	5.13
80 μg IBA	5.69

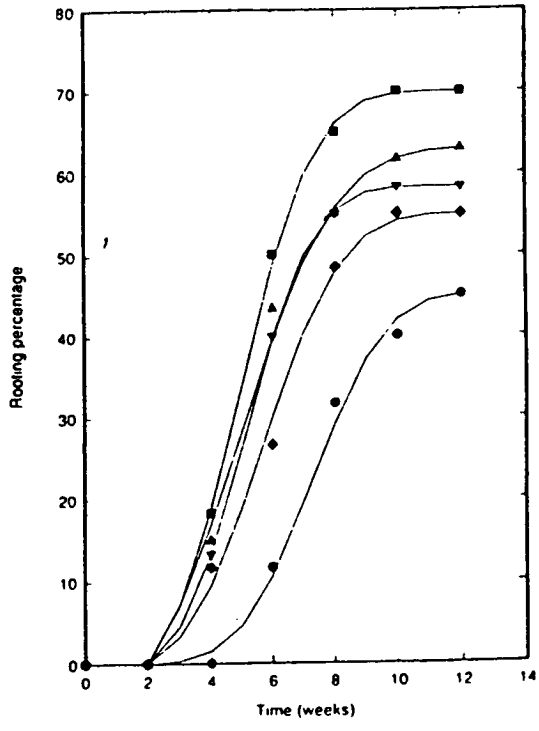

Fig. 1. Fitted cumulative distribution function of rooting response of *Shorea leprosula* stem cuttings at five different doses (●, 0 μg IBA; ■, 20 μg IBA; ▲, 40 μg IBA; ▼, 60 μg IBA; ◇, 80 μg IBA).

The number of cuttings rooted at each assessment was analysed using a modification of the cumulative distribution analysis formally presented by Hunter et al. (1984) and developed by Brain and Butler (1988). The problems with these types of data are that there is serial correlation between values at successive recording times and that the counts are not normally distributed. However, the underlying variable (the time to rooting) can be analysed by fitting its cumulative distribution function to the empirical cumulative distribution derived from the observed data. A maximum likelihood analysis was performed using Genstat 5 (Payne et al., 1987). The time to rooting in all cases appeared to be normally distributed with no transformation of the time axis. If time of recording is z, the cumulative distribution (F) is:

Table 2
Analysis of deviance to determine the influence of IBA doses on the rooting ability of *S. leprosula* stem cuttings at Week 12 (number of cuttings per treatment, 60)

Source	Degrees of freedom	Deviance	Mean deviance	Mean deviance ratio
Block	5	2.87	0.57	1.10 ns
Treatment	4	4.55	1.14	2.19 ns
Residual	20	10.49	0.52	
Total	29			

ns, not significant at 5% level of probability.

Table 3
Analysis of variance on the accumulated number of roots of *S. leprosula* stem cuttings as affected by the IBA doses at Week 10 (number of cuttings per treatment, 60)

Source of variations	Degrees of freedom	Sum of square	Mean square	*F* value
Block	5	14.10	2.82	1.93 ns
Treatment	4	17.67	4.42	3.03*
Residual	20	29.19	1.46	
Total	29			

*Significant at 5% level of probability.
ns, not significant at 5% level of probability.

$$F(z) = N(b(z-m))$$

where m is the mean time to rooting, b is the inverse of the standard deviation of time to rooting and N is the cumulative normal distribution function with zero mean and unit variance. The significance of differences between treatments was determined using the Chi-square test.

Analysis of deviance (Payne et al., 1987) was carried out at the final week of assessment to determine the significant influence of treatments on rooting percentage. Analysis of variance (Payne et al., 1987) was carried out to determine treatment differences in initial stem diameter and number of roots. The results in all tests were considered significant when the probability level was less than or equal to 5% ($P \leq 0.05$).

3. Results

Analysis of variance showed that there was significant difference between treatments in the initial diameter of cuttings used. Diameter of cuttings assigned to the 0 μg IBA treatment (3.217 mm) were significantly smaller compared with that of cuttings used for the 40 μg (3.328 mm) and 60 μg (3.315 mm) IBA treatments. However, no significant difference was found between initial diameter of cuttings used for 0 μg IBA and those used for 20 μg (3.258 mm) and 80 μg (3.278 mm) IBA. No significant difference was also found in initial diameter between IBA treated cuttings.

The Chi-square analysis showed that IBA treatments significantly accelerated rooting of *S. leprosula* stem cuttings ($\chi^2 = 15.5$, df = 8, $P \leq 0.05$). The predicted time taken for 50% rooting to occur was significantly shorter in cuttings with IBA treatments compared with untreated cuttings (Table 1). Fig. 1 presents the fitted cumulative distribution function for rooting response to occur for each of five IBA treatments. Hence predicted rooting percentage could be estimated with time. From Fig. 1, it can be seen that stem cuttings of *S. leprosula* treated with IBA initiated rooting earlier than the untreated cuttings. The onset of rooting was staggered over

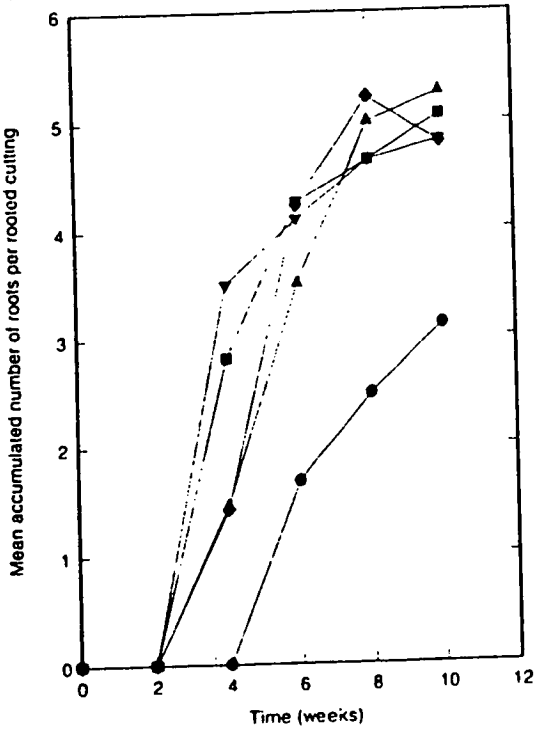

Fig. 2. Effect of IBA doses on the mean accumulated number of roots of *Shorea leprosula* stem cuttings at Week 10 (●, 0 μg IBA; ■, 20 μg IBA; ▲, 40 μg IBA; ▼, 60 μg IBA; ◇, 80 μg IBA).

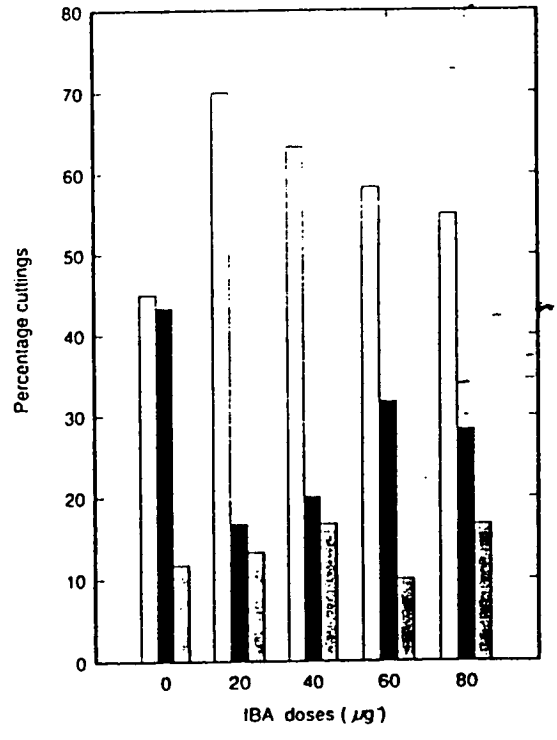

Fig. 3. Effect of IBA doses on the rooting of *Shorea leprosula* stem cuttings at Week 12 (open bar, rooted cuttings; solid bar, unrooted cuttings; hatched bar, dead cuttings).

several weeks until Week 10, after which little new rooting was obtained.

Analysis of deviance carried out on rooting of Week 12 revealed that treatment with IBA did not significantly influence the final rooting percentage of *S. leprosula* stem cuttings (Table 2). The accumulated rooting percentage at Week 12 for cuttings with IBA treatments of 20, 40, 60 and 80 μg was 70%, 63%, 58% and 55%, while the rooting percentage of the untreated cuttings was 45%.

Analysis of variance showed that IBA treated cuttings produced significantly more roots than the untreated cuttings throughout the assessment period. Table 3 shows the analysis of variance on the number of roots at Week 10. The mean accumulated number of roots per rooted cutting at Week 10 for 20, 40, 60 and 80 μg IBA was 5.05, 5.26, 4.82 and 4.80 respectively compared with 3.11 for cuttings without IBA treatment. Fig. 2 shows that IBA treated cuttings produced more roots than the untreated cuttings throughout the assessment period.

Analysis of deviance indicated that there was no significant difference between treatments in accumulated unrooted and dead cuttings at Week 12. It can be seen that a high percentage of cuttings remained unrooted (43%) in untreated cuttings (0 μg IBA). Among the IBA treatments, a higher percentage of cuttings remained unrooted when treated with 60 and 80 μg IBA compared with those receiving 20 and 40 μg IBA. The percentage of dead cuttings ranged from 10 to 17% (Fig. 3).

4. Discussion and conclusions

External hormone application to the base of cuttings has been shown to improve rooting of many difficult-to-root species (Morsink and

Smith, 1974; Leakey et al., 1982b; Darus, 1988; Kamis and Ng, 1989; Siagan et al., 1989). Injecting the hormone into the xylem of the stock plants of *Triplochiton scleroxylon* also improved the rooting performance of cuttings taken from these stock plants (Leakey, 1993).

The results of the present experiment showed that the final rooting percentage (Week 12) was not significantly affected by the treatments. These results were in agreement with those obtained in previous trials with *S. leprosula* (Halle and Kamil, 1981; Srivastava and Manggil, 1981) where no improvement in final rooting of cuttings treated with IBA was achieved. However, with more detailed assessments, the present experiment has demonstrated the significant response in the rate of rooting of cuttings treated with IBA. Similar results were reported by Lo (1985) where auxin application also enhanced the rate of rooting of *Shorea macrophylla* although the final rooting was not affected by the auxin application. The application of IBA may have an indirect influence by enhancing the speed of translocation and movement of sugar to the base of cuttings and consequently stimulate rooting (Haissig, 1974, 1982). Practically, speeding up the process of adventitious root formation is considered an advantage, as the earlier the cuttings were able to form roots, the greater the chances for them to survive. This aspect of rate of rooting has often been neglected in auxin experiments. A single assessment made at the end of an experiment cannot reveal the effect of auxin in accelerating rooting of cuttings (Muckadell and Malim, 1978; Halle and Kamil, 1981; Srivastava and Manggil, 1981; Siagan et al., 1989; Kamis and Ng, 1989; Noraini and Ling, 1993). Application of IBA also enhanced the number of roots developed on each rooted cutting of *S. leprosula* as indicated by the greater number of roots produced compared with the untreated cuttings (Fig. 2). This may have an advantage by enhancing good anchorage when planted in the field. Similar results have been observed in other studies (Kamis and Ng, 1989; Siagan et al., 1989). Besides the effect of IBA, the diameter of cuttings may have influenced the development of the number of roots on the cuttings since the initial diameter of cuttings used for 0 μg IBA were smaller compared with those used for IBA treatments. Bigger diameter cuttings may have more stored reserves for root development compared with smaller diameter cuttings. It has been shown by Mesen (1993) that an increase in cutting diameter of *Cordia alliodora* had resulted in significant increase in the number of roots. The importance of storage capacity of cuttings for root development had also been observed (Veierskov and Andersen, 1982; Veierskov et al., 1982). However, differences in cutting diameter had no effect on the rooting percentage of *C. alliodora* cuttings (Mesen, 1993).

Higher doses of IBA in the present experiment were not detrimental to cuttings as indicated by the mortality which was 10–17% among all the IBA doses used. The detrimental effect of IBA was also not observed on juvenile cuttings of *S. macrophylla* where a higher concentration of 10 800 ppm IBA resulted in only an average of 11% mortality (Lo, 1985). Hartmann and Kester (1983) stated that IBA could be used in a wide range of concentrations without giving toxic effect to the cuttings. But the higher levels of 60 and 80 μg IBA used in the present experiment may be less suitable for rooting the juvenile cuttings of *S. leprosula* because many cuttings remained unrooted even 12 weeks after planting in the rooting bed. A higher auxin dose (200 μg per cutting) had been shown to inhibit rooting in cuttings of certain clones of *T. scleroxylon* (Leakey et al., 1982b).

From the trend of the results obtained in the present experiment and from the economic point of view, 20 μg IBA are recommended to be applied for rooting of juvenile stem cuttings of *S. leprosula*. A lower dose of 5–10 μg IBA could be further tested to confirm the results. In other species, higher doses of IBA such as 100 μg and 150 μg were reported to significantly improve rooting of *Shorea acuminata* and *Shorea parvifolia*. However, a lower range of IBA doses were not tested in their experiments (Noraini and Ling, 1993).

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