CARBON ECONOMY DURING ROOTING OF CUTTINGS OF *NAUCLEA DIDERRICHII* (DE. WILD. AND TH. DUR.) MERILL

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DECLARATION

I certify that this thesis is my work and has not been submitted for any degree other than that of Master of Philosophy in the University of Edinburgh

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ABSTRACT

The natural physiological changes occurring within the leafy single node stem cuttings during rooting of *Nauclea diderrichii* (De. Wild. and Th. Dur.) Merill, an important West African hardwood species, were investigated. Two main experiments were done to examine the relationship between carbohydrate economy and rooting ability. In the first experiment, indole-butyric acid (IBA) was used as the rooting hormone. In the second experiment, the stock plants were grown in two different light conditions: light (mean photon flux density 121 µmol $m^{-2}s^{-1}$) and shade (mean photon flux density 5.6 µmol $m^{-2}s^{-1}$) for 28 days. Cuttings were cultured in a rooting propagator placed inside a glasshouse. Morphological changes were noted, photosynthesis and respiration rates were measured, and the carbohydrate concentrations of leaf, stem, bud and roots were analysed separately at intervals during rooting.

In the first experiment, 100% rooting occurred after 3 and 5 weeks with or without IBA respectively, and IBA changed the pattern of callus formation; increased the percentage of cuttings forming buds or leaves; increased the number of roots per rooted cutting; affected the distribution of roots along the internode; and inhibited the root elongation. The initial rate of net photosynthesis was very low, sometimes not above zero, but after the formation of roots, there was an upward trend till the experiment finished. IBA did not enhance photosynthetic performance but it increased the concentrations of soluble carbohydrates in leaves and stems, probably by starch hydrolysis. A marked downward transport of carbohydrates was observed both in control and treatment. In the second experiment, 100% rooting occurred after 4 weeks irrespective of the light regime applied to the stock plants. Shade increased the amount of callus, average number of roots per rooted cutting, average number of new leaves per sprouting cutting and also the fresh weight. In contrast, it did not affect the length of roots, distribution of roots or stem surface and the percentage of cuttings forming buds and leaves. The rate of net photosynthesis was similar to the first experiment except, in shade, there was a somewhat higher rate of net photosynthesis both before and after root formation. The concentrations of extractable carbohydrates increased both in light and shade treatments, but was significantly higher in light. The new roots did not run short of carbohydrate at all, even when the stock plants had been heavily shaded. There was no tendency for 'starved' plants to die through a lack of assimilates as they exhibited a high rate of photosynthesis and very rapidly recovered high levels of carbohydrates.

CHAPTER 1

INTRODUCTION

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1.1 THE NEED FOR VEGETATIVE PROPAGATION

1.1.1 Timber production

The rate of destruction of tropical forest has increased dramatically in recent years, attaining an estimated rate of 20-50 ha per minute (Leakey, 1986a). According to Evans (1982), the main causes of destruction are: clearance for agriculture; intensive logging for veneer, sawtimber and, more currently, for chipwood; exploitation for charcoal and firewood; shifting cultivation on too short a cycle; urban and industrial expansions; overgrazing and gathering fodder for domestic animals; accidental or deliberate burning of forest and ravages of war. Apart from these, soil erosion, salanity, drought etc are also well known in some parts of the tropics. The consequences of the forest destruction and degradation in the tropics are the acute shortage of firewood; shortage of raw materials for paper, match and newsprint industries; shortage of wood for lumber and plywood industries, wood for building boats, ships and as building materials and timber as sawlogs for export, to earn vital foreign currency.

Moreover, world demand for forest products has a direct relationship to the trends in world population. Freijka (1973) suggested that world population will be doubled by the year 2000, if there is no major change in current habits. On the basis of this prediction, Wood (1976) concluded that:

(i) if per capita wood consumption remains constant, world demand will be approximately doubled;

(ii) if the poorer nations become less poor, their per capita wood consumption is likely to rise; and

(iii) the use of wood as fuel and industrial raw material is likely to increase as non-renewable resources become scarcer or more expensive.

We therefore expect a rapidly increasing demand for forest products in and from tropical countries. Recognising this, most third world countries believe that the only course open is to establish large scale plantations in the tropics, using selections of fast growing tree species.

TABLE 1.0: List of botanical names with authorities.

Abelmoschus esculentus Moench.
Albizzia falcataria (L.) Fosb.
Araucaria cunninghamii Sw.
Aucoumea klaineana Pierre.
Bombax ceiba L.
Chrysanthemum morifolium Ramat.
Dalbergia sisso Roxb.
Eranthemum tricolor Lucona.
Eucalyptus deglupta Blume
Eucalyptus grandis (Hill) Maiden
Eucalyptus tereticornis Sm.
Gmelina arborea Linn.
Hedera helix L.
Helianthus tuberosus L.
Ilex crenata Thunb. var.
Ipomoea fistulosa L.
Phaseolus vulgaris L.
Picea abies [L.] Karst
Picea sitchensis (Bong.) Carr
Pinus banksiana Lamb.
Pinus caribaea Morelet.
Pinus radiata D. Don.
Pinus sylvestris (L.)
Pisum sativum L. cv. Alaska
Populus euramericana (Dode) Guinier syn.
Populus nigra L.
Populus robusta Schneid.
Populus tremula L.
Prunus cersifera Ehrh. x *Prunus munsoniana* W. Wight & Hedr.
Pseudotsuga menziesii (Mirb.) Franco
Salix babylonica Linn.
Salix pierotti (Not found as the original paper was not available)
Salix undulata Eng.
Shorea macrophylla (De Vr.) Ashton
Solanum andigenum Juz. and Buk.
Swietenia macrophylla King.
Tectona grandis Linn. f.
Terminalia superba (Engl. & Diels.)
Triplochiton scleroxylon (K. Schum.)
Wrightia tinctoria R.Br.

1.1.2 Plantation forestry in the tropics

Although man-made forestry has been in progress since the sixteenth and seventeenth centuries when exploration and expansion of European influence took place, extensive tree plantations as an industrial resource was started only at the beginning of the current century (Evans, 1982). Wood (1976) noted the cause of acceptance of plantation forestry in the tropics is the inadequate and unreliable natural regeneration, an obstacle for sustaining or increasing the forest productivity. Leakey (1986a) concluded that plantations stocked with unselected seedlings of wild origin are considerably more productive than natural forest. Plantation forestry not only increases the yield per unit area but also can raise plantations in convenient sites of consumption, and produce predictable and uniform materials. However, as many species in the tropics show higher yields in plantations (Table 1.1), so in recent years there has been a rapid increase in plantation forestry throughout the tropics (Table 1.2).

TABLE 1.1: Some yields from tropical forest plantations.

Species	Countries	Yields ($m^3ha^{-1}y^{-1}$)	Sources
Aucoumea klaineana	Gabon	up to 24	Wood (1976)
Swietenia macrophylla	Indonesia	up to 30	"
Pinus caribaea	Malaysia, Fiji,	up to 40 Africa	"
Araucaria cunninghamii, *Pinus radiata* and other highland softwoods	"	up to 45	"
Eucalyptus grandis	East and Central Africa	sometimes exceeding 70	"
Gmelina arborea	Brazil	35	Evans (1982)
Albizzia falcataria	Philippines	28	"

TABLE 1.2: Areas of plantations and open and closed forests in the tropics by continents (includes both industrial and non-industrial species).

Regions	Plantations ('000 ha) 1950	1976	1980	1985	Natural woody vegetation ('000 ha) closed	open	sources
Tropical America (23 countries)	40	2,570	4,620	7,293	678,655	216,997	Lanly (1982)
Tropical Africa (37 countries)	110	1,314	1,780	2,411	216,634	486,445	
Tropical Asia (16 countries)	530	3,016	5,111	7,303	305,510	30,948	
Total (76 countries)	680	6,900	11,511	17,007	1,200,799	734,390	

At present, the total open and closed industrial and non-industrial natural woody vegetations in the tropics is 1,935,189,000 ha (Lanly, 1982). Therefore, the area of man-made plantations in the tropics in 1950 was .03% of the total tropical forest, became 0.4% in 1976, 0.6% in 1980 and 0.9% in 1985, with a total planting area of 17.0 million ha (calculated and estimated from Lanly, 1982). Assuming a conservative average yield of 15 $m^3ha^{-1}y^{-1}$, after five years (1990) this area could produce 255 million cubic metres of wood. However, if this planting process continues up to the year 2000 with a rate of 1 million ha per year, estimated for 1980-85, there will be an area of 32 million ha of man-made forest in the tropics, which is equivalent to 1.6% of total forest in the tropics. However, it is vital that the forests are based on a productive and sustainable silviculture.

1.1.3 Limitations of plantation forestry

Traditional method of planting by seed collected from existing plantations and natural stands are still going on for afforestation and reforestation programmes in the tropics. Unskilled villagers are the main seed collectors for the foresters. The seed collectors do not always collect seed at the best time to achieve optimal maturation state. In addition, unpredictability of seeding of most species; heavy or gregarious flowering every few years, insects and fungi attacking both flower and seed; formation of poor percentage of flowers into fruits, etc, are very common in tropical natural forests (Ibrahim, 1977). Improved seed sources from pre-selected desired genotypes grown in a seed orchard or 'seed stand' for large-scale plantation, is not yet widely available. Maintaining genetic diversity, both in exotic and indigenous species, may not be possible, especially when seeds are collected from isolated specimens or groups of closely related

trees. It is also reported that low seed viability and poor germination is present in some tropical species (Troup, 1921; Longman, 1976). Furthermore, plants may fail to produce seed or to breed true.

As a result of the above, problems, such as shortage of seed supply, poor germination percentage, retention of variability resulting in heterogenous products, non-uniformity in timber, slow growth and average low productivity in stands, are most common in the tropical forests.

This is not to say that seed available is *always* a limiting factor. Availability, finance, species selection, silvicultural techniques, fertilizer, insecticides, irrigation, shortage of technological know-how - all, at times, imposed profound restrictions on the progress of afforestation.

1.1.4 Introduction of clonal forestry by vegetative propagation

Under the above circumstances, the most appropriate approach is clonal forestry based upon vegetative propagation. If the genotypes are carefully selected, there may be appreciable genetic gain and greater uniformity of the product than is possible through seed generation. Under some situations, vegetative propagation presents the opportunity to speed up the application of results from tree breeding (Zobel and Talbert, 1984). In forestry, the technique of vegetative propagation has many uses:

1. preservation of genotypes through clone banks;
2. multiplication of desired genotypes for special uses, such as in a seed orchard or breeding orchard;
3. evaluation of genotypes and their interaction with the environment through clonal testing; and
4. capture of maximum genetic gains when used for regeneration in operational planting programmes (Zobel and Talbert, 1984).

Vegetative propagation has been employed in forestry for more than a hundred years (Zobel and Talbert, 1984), whereas the practice has been used for the genetic improvement of forest species only during the last half century (Hong, 1975).

In the tropics, grafting and budding are two important methods of vegetative propagation to preserve selected phenotypes and to multiply them to create clonal orchards to produce seed for progeny testing and large-scale plantation programmes

(Burnes and Burley, 1987). In fact, there are some problems with grafted seed-orchards: general shortage of reliable flower induction methods or a lack of synchrony in the flowering times of parent clones; the high cost of establishment and maintenance of orchards; the graft incompatability between scion and root stocks, especially serious if it shows a long delay after planting. Therefore, Libby (1974b) concluded that to start a new tree breeding programme with grafted seed orchards, as has occurred in many countries of the world, is neither wise nor prudent. Furthermore, many other factors have to be taken into consideration before orchard establishment. It is necessary to separate the clones in a seed orchard to prevent unwanted mating and the rate of inbreeding must be understood, although it varies greatly by species (Stern, 1959). Moreover, the number of clones in a seed orchard should be sufficient to provide a suitable genetic base for operational planting. Most first generation vegetative seed orchards established with 25 to 40 clones may be reduced to 20 or fewer after testing and roguing (Zobel and Talbert, 1984).

Another method that is newest and currently the focus of attention of many tree breeders, is tissue culture. It has great potential but it must be viewed realistically (Bonga, 1980). Individuals within clones are sometimes phenotypically dissimilar. It may also be difficult to grow and establish the plantlets in uncontrolled forest conditions, taking them from artificial controlled environment where they are formed. Tissue culture plantlets have been reported to be inferior in their relative growth and form as compared to other kinds of vegetative propagules and seedlings. Zobel and Talbert (1984) mentioned that using current methods, tissue culture plantlets are expensive because of the multiple handling that is now required. Therefore, the major task for tissue culturists should be to make the system cost effective. However, to conceive this approach in the developing tropics is not yet justified because of the practical difficulties experienced with many species, laboratory facilities, trained manpower and substantial recurring expenditures. Each species requires its own cultural technique and it may take 3-5 years to devise an optimal protocol, even for an 'easy' species. Meanwhile, the demand for wood is increasing day by day.

On the other hand, foresters and research workers now consider vegetative multiplication of young trees from rooted cuttings to be an economical way of obtaining planting stock, and in many countries millions of cuttings are being rooted in every year for reafforestation programmes (Libby, 1974b). Cuttings of some genera, such as willow (*Salix* L.), poplar (*Populus* L.) and Cryptomeria (*Cryptomeria japonica* (L.F.)Don) have been routinely rooted for many years (Chemlar, 1974; Toda, 1974), and notable success

has recently been achieved with *Eucalyptus* spp. in Congo and Brazil; *Gmelina arborea* in Sabah (Leakey, 1987); *Pinus radiata* in New Zealand and Australia (Thulin and Faulds, 1968; Arnold and Gleed, 1985); *Picea abies* in West Germany (Brix and Van den Driessche, 1977); *Picea mariana* [(Mill.)B.S.P.] in Ontario, Canada (Armson *et al.*, 1980). The operational production of rooted pine cuttings has also been under way in Texas for several years (Van Buijtenen *et al.*, 1975), and has encouraged the tree breeders to go ahead with rooted cuttings in large-scale operational clonal forestry. The use of rooted cuttings is advantageous particularly in the tropics, because it does not need expensive laboratory facilities, it is relatively simple in operation, unit cost is low, and there is no risk of the kinds occurring in grafting.

Furthermore, there are reports that rooted cuttings perform better than the seedlings in different characteristics. In a growth study of rooted cuttings and seedlings in a 40-year old plantation of Eastern White pine (*Pinus strobus* L), it was found that the trees from cuttings were taller, and had larger diameters and more growth than the trees derived from seedlings (Struve *et al.*, 1984). In *Pinus radiata*, it was found that the cuttings tend to grow more vertically than the seedlings; have thin bark at the base of the trunk in plantations aged 6 years and older; have fewer branches and a smaller cross-sectional area of branches per unit length of trunk than seedlings; and start producing ovulate strobili sooner than the seedlings (Fielding, 1970). Similar results were also reported with *Terminalia superba* in the Congo and significant differences between mean clone heights and stem circumferences were found after 3 years growth in most of the clones (Leakey, 1987).

In contrast, fears are sometimes voiced about clonal forestry. It may give rise to large, biologically uniform stands with risks from pests, diseases or other hazards; rooted cuttings may have root systems that are inferior to those of seedlings and, consequently, clonal plantings may be more susceptible to wind throw; and clonal forestry may produce non-erect or plagiotropic plants in stands (Leakey, 1987). To avoid the dangers of a narrow genetic base, Leakey (1987) suggested that tree improvement should be an on-going process and that selection procedures which are reducing the numbers of clones grown commercially at one end, should at the other be continually introducing new genotypes for testing. Libby (1982) has indicated that a mosaic of several unrelated clones in small monoclonal patches is a good strategy, particularly when through experience many of the hazards are known; mixtures of 7-25 unrelated clones are probably the optimal strategy. A mixture of large numbers of clones is as safe as a plantation of seedlings, but the genetic gains will not be as high as could be achieved by

fewer clones. The safest situation is likely to be a mixture of a relatively small number of unrelated clones of different species. This option, however, necessitates the selection of well-matched clones of compatible species. A mixture of 2-3 clones is probably the worst strategy, because of the increased risk that a larger proportion of the plantations will suffer damage that will leave unacceptably few survivors. Because of this, it is vitally important that short-term commercial pressures do not result in the use of very few 'super' clones.

It is wrong to assume that all mature trees grown from seed (as opposed to cuttings) have a tap root, but most tree species subsequently produce a plate of large horizontal roots radiating away from the trunk and many of those forming on bare-root transplants are in fact advantitious (Deans, 1981). The exent to which the tap root and vertical sinkers then develop on a root plate is very variable between species (Jenik, 1978). On the other hand, the study of rooted cuttings of *Picea mariana, Pinus banksiana* and *Larix* spp. showed that the physical dimensions of rooted cuttings were usually superior to those of similar-aged seedlings, especially if the root systems were larger and more fibrous (Fung, 1978).

Finally, Leakey (1987) suggested that non-erect or plagiotropic plants are the result of using inappropriate shoots as a source of cuttings and it is most common when cuttings are collected from the crowns of large trees. This problem can be avoided by efficient stockplant management and the use of only mainstem-type shoots as the source of cuttings.

1.1.5 Optimal conditions for successful rooting

Although clonal forestry has progressed rapidly and is establishing its position in production forestry in the tropics, success depends upon achieving satisfactory rooting percentage of cuttings. For example, for *Eucalyptus* in Aracruz, Brazil, a 75% rooting is considered minimal for use in the planting programmes (Campinhos and Ikemori, 1980). It is well known that rooting ability varies between tree species, between clones within species and among plants within clones (Leakey, 1985). Moreover, there are many indications that trees propagated from older individuals grow more slowly than those taken from younger trees (Talbert *et al.*, 1982).

Rooting success is dependent upon optimizing many endogenous and exogenous factors. The endogenous factor includes maturation, state of cutting, donor, type of cutting, physiological condition of the cuttings, preconditioning of the cutting or cutting

donor, and the season of collection. The exogenous factors include rooting medium, ambient temperature and humidity, photoperiod and light intensity, and hormone and chemical treatments. The mode of action of the above factors for rooting is directly dependent upon whether the individual species is an easy or difficult rooter. Some of the important factors for successful rooting are discussed in the next section of this chapter.

1.2 FACTORS REQUIRED FOR SUCCESSFUL ROOTING

There follows a general survey of the factors which influence rooting. Some of the relevant literature simply records practical techniques used in the glasshouse or nursery. Some of it, on the other hand, is more physiological in nature.

1.2.1 External factors

1.2.1.1 Rooting media

Rooting success in cuttings generally depends upon the proper balance of aeration, moisture, drainage, temperature, presence of mycorrhizas, pH etc, of the rooting media. For a long time, the standard rooting media have been medium-to-coarse sand with no silt or clay present (Hitchcock, 1928). In the late 1930s the use of well-decomposed peat of sedge origin, or peat and moss of sphagnum origin was introduced (Girouard, 1974). The ideal ratio of the mixture of sand and peat was 2:1 (Doran, 1952). Since the 1950s, fresh sphagnum moss mixed with sand (1:1) has been used as a popular rooting medium (Gathy, 1958). Recently, workers have been using peat, sand, perlite and vermiculite as a rooting medium, in various ratios (Kormanik and Brown, 1974; Davidson, 1974). More recently, the use of gravel-sand with continuous misting or stored water in the medium is becoming popular, especially for easy-to-root leafy softwood cuttings (Leakey, personal communication). However, sandy loam or sand, soil and farmyard manure with sufficient water supply has been used as a good rooting medium for some tropical easy-to-root species (Bhatnagar, 1974).

1.2.1.2 Temperature

Cuttings generally root best with cool, moist air surrounding the tops, and warmer temperatures around the basal zone for enhancing callus formation, root initiation and development in the cuttings (Watanaba *et al.*, 1968; Brix, 1974). This temperature gradient allows greater activity at the base of the cuttings and minimizes respiration and moisture stress at the top of the cuttings. High humidity in the air surrounding the cuttings

can reduce the stress caused by transpiration and thus promote rooting (Rauter, 1983). The ambient air temperature can influence the length of time from striking to rooting. As the temperature decreases, the length of the time required to root cuttings increases (Rauter, 1983). Brix (1974) proposed, with *Pseudotsuga menziesii*, that an optimum temperature is necessary for the production of root promoters in the top of the cuttings and their translocation to the base for root initiation and development.

Cuttings of tropical and sub-tropical plants require a higher temperature for their rooting than do cuttings of plants from temperate and cold countries (Shul'gin, 1905). A good rooting success was found in *Picea* spp. with an air temperature between 10 to 22°C with high relative humidity (65 to 95%) and not less than 13°C in the rooting medium (Girouard, 1974). Cameron and Rook (1974) reported that 20 to 25°C air temperature at day-time and 5 to 10°C at night, are advantageous for rapid rooting in cuttings of *Pinus radiata*. Leakey *et al.* (1982a) reported with tropical species (*Triplochiton scleroxylon*) that rooting increased when temperatures of the propagating beds were raised above 20°C, and 28°C was optimal for most clones, especially if treated with auxins, with the air temperature 25-30°C. However, good rooting was found in *Terminalia superba* stem cuttings with an air temperature of 20°C ± 2°C (Siaguru, 1986) and propagating beds at 30°C ± 1.5°C.

1.2.1.3 Light

Light is essential for photosynthesis, and in softwood cuttings an increased rooting can be realised by increasing the available light (Hess, 1971). Leafy softwood cuttings, with little or no auxin or carbohydrate storage, require light for food and auxin formation, and subsequent root production (Hartmann and Kester, 1975), whereas leafless hardwood cuttings, which often contain stores of previously manufactured carbohydrates and auxin, initiate roots best in darkness (Hartmann and Kester, 1975; Komissarov, 1964). The quality of light which favours optimum photosynthesis has generally given best results in rooting (Komissarov, 1964). Reports are also available that high irradiance given to stockplants inhibits rooting of cuttings, whereas low irradiance enhances rooting (Hansen and Eriksen, 1974; Stromquist and Hansen, 1980; Poulsen and Andersen, 1980). Illumination of the base of the cutting caused fewer roots to form (Eliasson, 1980; Stromquist and Eliasson, 1979). In etiolated tissues, rooting potential may be greatly increased as the etiolated tissues contain more free sugar, less starch and no lignification (Hess, 1971). Cameron and Rook (1974) reported rooting in cuttings has a strong correlation with photoperiod (season), since long photoperiod or continuous illumination is more effective in rooting than short days (Komissarov, 1964).

1.2.1.4 Water relations

The natural water supply to the leaf from the roots is generally cut off in cuttings, and thus water stress may cause an adverse effect on rooting or may cause material to die before root formation (Hartmann and Kester, 1975). Physical contact must be maintained between the cut surface and the water in the pores of the rooting medium, to achieve hydraulic continuity. Optimum water content in cuttings of different species may vary according to their biological features and age (Komissarov, 1964). Rooting has been shown to be reduced when leaf water potentials fall to about -0.8 to -1.0 MPa (Loach, 1977), below which there is a linear relationship between declining leaf water potential and decreased rooting.

Water losses are affected by vapour pressure deficits of the air, radiation levels and leaf resistances to water loss (Leakey, 1985). However, excessive water loss can be checked by manipulating the environmental conditions or by reducing the transpirational surfaces by leaf pruning or deep planting (Niensteadt *et al.*, 1958) or by applying antidesiccants to the cut ends (Howard *et al.*, 1983). The use of artificial mist for rooting leafy cuttings is a widely accepted method (Gardner, 1941), where a film of water on the leaves not only results in a high water vapour pressure surrounding the leaf, but also lowers the air and leaf temperature - both of these tending to reduce the transpiration rate (Hartmann and Kester, 1975).

1.2.1.5 Season of scion collection

Plant species vary markedly in their ability to root. Some root easily, others with difficulty and still some do not root even with auxin application. Rooting ability may vary with season. The effect appears to be correlated with seasonal changes of cambial activity during the annual cycle and the effectiveness of auxins varies with the seasons (Nanda *et al.*, 1968). Logically, the best time to set cuttings should be during the growth period when the cuttings can be expected to establish a good root system within short time. Hartmann and Kester (1975) reported for some deciduous species that good rooting is possible if the leafy softwood or semi-hardwood cuttings are collected during the growing season (spring) when the stock plants commence active growth. For hardwood species, rooting ability generally increases in the dormant season of winter (Hartmann and Kester, 1975). Some reports are also available from India suggesting that rooting shoot cuttings of several species showed a marked influence of seasonal variations. As for example: cuttings of *Tectona grandis* root only if taken during March to May

(Bhatnagar *et al.*, 1968), and best rootings were achieved in *Populus* in November to March (Mathur, 1972); willows in January to March; *Tamarix* in February to March; *Bombax ceiba* in March to April; *Dalbergia sisso* and *Eucalyptus territicornis* in August to September (Bhatnagar, 1974).

1.2.2 Nature of cuttings

1.2.2.1 Age of donor plant

The maturation state of the donor plant seems to have a great influence over the rooting ability of cuttings. This influence is believed to be greater in difficult-to-root species than easy-to-root species. The more juvenile the donor tree, the greater is the success in rooting (Gardner, 1929). In addition, rooting percentage, rooting speed, root number and length decrease with increasing age of the donor plants (Girouard, 1971). Kiang *et al.* (1974), moreover, reported that the formation of roots is controlled by the age of donor plants rather than the age of the wood in the cuttings.

However, the physiological juvenility is believed to decrease in older plants because of the decrease in co-factors (Hartmann and Kester, 1975). Paton *et al.* (1970) showed with *Eucalyptus* cuttings that there was a direct and quantitative association between such decreased rooting and the production of a rooting inhibitor in the tissues at the base of the cuttings. It is also reported by him that in easily rooted young seedling stems this inhibitor was absent, as it was absent in adult stem tissue of the easily rooted *Eucalyptus deglupta*. The reduction of rooting potential in adult plants may possibly be a result of lowering phenolic levels. The phenols are generally known as auxin co-factors or synergists in root-initiation. In *Hedera helix*, lower phenolic levels were noted in mature forms than in the juvenile forms (Girouard, 1969). Furthermore, Ali (1966) reported that pear species root better as juvenile than as adult cuttings and that juvenile tissues contain the same level of DNA but less RNA than adult tissues. Ali suggested that this implies the blockage of 'flowering' information transfer in juvenile forms.

Maturation can be arrested either by hedging the donor plant and not allowing upward growth (Libby and Hood, 1976); by serial propagation of rooted cuttings (Kleinschmit, 1978), or by using the basal epicormic shoots or coppice shoots, which develop in many hardwood species (Longman *et al.*, 1978). The main principle of all these methods is to improve the physiological state of the plant by shortening the internal transport system and improving the supply of water and nutrients to the periphery of the hedge (Fortanier and Jonkers, 1976). Therefore, it appears that the closer the plant tissues

are to the root system, the more they are juvenile in character and also there may be reduced concentrations of inhibitors in this zone (Paton *et al.*, 1981). However, the maturation state of donor plants not only affects the rooting percentage of cuttings, but also affects the speed, quality of rooting, subsequent growth rate, form and wood properties (Hood and Libby, 1978).

1.2.2.2 Crown position

Cuttings from lower lateral shoots root better than those from upper vertical ones (Girouard, 1971; Hartmann and Kester, 1975; Leakey, 1983). According to some workers, this is because the shoots of the upper crown are at a more advanced state of maturation than those of the lower crown (Rauter, 1983). Cuttings from seedlings and coppices differ markedly in their content of inhibitors (Vieitez and Vieitez, 1976); various growth regulators, nucleic acids and rooting co-factors (Paton *et al.*, 1981). Different parts of stock plants also exhibit some important differences in their capacity to root (Leakey, 1985). This may be due to differences in leaf age, internode length, extent of lignification, secondary thickening, and gradients in carbohydrate, nitrogen and auxin contents (Leakey, 1985). However, Hartmann and Kester (1975) reported that an abnormal plant will be produced if cuttings are collected from markedly horizontal branches; the desired vertical plants can be achieved when cuttings are collected from upright growing shoots. If cuttings are collected from drooping lateral branches, they will produce only branches spreading along the ground, the plagiotropic condition.

1.2.2.3 Size of cuttings

The practical length of the cutting generally depends upon the length of shoot internodes. If the internodes are long, the cuttings may be one internode; if they are short, each cutting may contain two, three or more internodes. The literature suggests a species-specific optimal size. Komissarov (1964) reported that if a large quantity of nursery stock plants is available, it is better to use large cuttings, as the growth of planting material can be considerably speeded up. This may result from the initial vigour as growth occurs from a greater reserve of carbohydrates, water, minerals and other essential substances for root formation (Grace, personal communication). Leakey (1983) noted that in *Triplochiton scleroxylon* cutting size, as determined by the normal pattern of internode lengths, was closely correlated with rooting success - the longer cuttings from the apical end of the stock plant rooting best. But when all the cuttings were cut to the same length,

basal cuttings rooted best, and hence the normal gradient in rooting ability was reversed (Leakey and Mohammad, 1985). Brix (1974) found best rooting in *Pseudotsuga menziesii* cuttings that were 6 to 12 cm long but not very sturdy, or longer with a diameter less than 2 mm. Grace and Farrar (1945) reported that in Norway spruce, long cuttings (15 to 25 cm) formed the greatest number of roots and shoots on those cuttings that rooted, with the additional benefit that they were the largest and most upright plants four years after rooting. But, cuttings of intermediate length (8 to 15 cm) rooted with the highest success rate. On the other hand, some species showed best rooting with longer cuttings. As for example, 20-25 cm for poplars; 90-120 cm for willows; 22-30 cm for mulberry; 40 cm for *Tamarix*; 15-30 cm for bamboo; and 20 cm for *Tectona grandis* (Bhatnagar, 1974; Bhatnagar *et al.*, 1968).

The idea of using full length cuttings to produce the largest plants possible within a given period appears good, but in practice it is not always best. For example, plants produced by full length cuttings vary considerably in weight and vigour. These variations are troublesome as they exert a substantial effect on subsequent performance and result in non-uniform planting stock (Girouard, 1974).

1.2.2.4 Leafiness

The extent of leaf area may be critical to the survival of the cutting, as there must be sufficient to produce much needed carbohydrates but not so much as to lead to excessive loss of water. The idea of an optimal leaf area has often been advanced, though the precise value of this area clearly will depend on the illumination and on the conditions for evaporation. A notional optimal area can be achieved by trimming the leaf, which has an additional benefit of saving space on the rooting bench.

The harmful effects of sub- or supra-optimal leaf areas seem to be greater in difficult-to-root species (Leakey, 1985). Leaves may be a source of nutrients to stem tissues (Komissarov, 1964) and thus the translocation of carbohydrate from leaves is enabled for root formation in cuttings (Hartmann and Kester, 1975). It is common that leafless summer cuttings do not root well while those in winter root well. The cause may be the greater stored reserved and/or endogenous co-factors in winter cuttings than summer cuttings. In a few cases, winter cuttings may have preformed root initials (Cheffins and Howard, 1982a,b). Reports are also available that winter cuttings are dependent on rapid emergence of new shoots to meet the decreasing carbohydrate reserves (Okoro and Grace, 1976). In contrast, summer cuttings are often dependent on current photosynthesis.

On the other hand, it has long been known that the presence of leaves on cuttings has a strong influence on root formation (Van Overbeek and Gregory, 1945). The rooting may be due to a specific root forming substance manufactured in leaves and moving downwards to the bases of the cuttings to induce rooting (Sachs, 1882). The nature of these substances is discussed in Section 1.2.3.

1.2.2.5 Wounding

Wounding at the bases of cuttings promotes root initiation (Bhella and Roberts, 1975) and it also releases a specific 'wound-hormone' responsible for initiation of cambium activity (Bhella and Roberts, 1975). Hartmann and Kester (1975) reported that wounded cuttings may absorb more water and exogenous hormone than the unwounded in addition to increasing callus and root formation along the margins of the wound. Wounding can also help outward penetration of the developing roots through the tough ring of fibre cells in the cortex of some difficult-to-root species (Davis, 1968). However, Komissarov (1964) concluded that wounding or bark injury may have some positive effect on some species, but in general it has no substantial effect on rooting cuttings.

1.2.2.6 Polarity

Polarity may be longitudinal or transverse but in both the cases root develops at the natural lower end, whereas shoot develops at the upper end (Komissarov, 1964). Polarity in cuttings was first studied by Vochting (1878) who reported that it is a certain structural property of the organism, fixed in the internal structure of the protoplasm of every cell and the intensity of polarity varies among the different parts of the plants: strong in stem, weaker in root and much weaker in leaves. Polarity has strong correlation with auxin movement during root differentiation (Warmke and Warmke, 1950) and the inherent polarity of the individual cell is probably responsible for the redistribution of auxin (Gautherent, 1946). However, Komissarov (1964) concluded that rooting rate may vary substantially in relation to the orientation of cuttings.

1.2.3 Physiological factors

The downward movement of sap during adventitious root formation in stem cuttings was first discussed by Duhamel du Moncean in 1758. Later, Sachs (1880, 1882) suggested the existence of a root-forming substance formed in the leaves which moved

with basipetal polarity to induce root formation. Plett (1921) added that not only leaves but also buds stimulate the rooting of cuttings. Subsequently, those ideas have been supported by other workers. Among them, the need for leaves has been confirmed by some workers (Cooper, 1938; Van Overbeek and Gregory, 1945; Van Overbeek *et al.*, 1946) and the need for buds has been confirmed by others (Fadl and Hartmann, 1967). Moreover, Vander Lek (1924) supported Plett's findings and added that there exists a high degree of correlation between the intensity of bud development and root formation. He also suggested that root formation in stem cuttings is regulated by one or more specific hormones of foliar origin moving basipetally through the phloem. Before the discovery of auxin production by leaves, Went (1929) noted that diffusates from leaves could induce the formation of adventitious roots in cuttings. Bouillenne and Went (1933) discovered the presence of 'rhizocaline' in cotyledons, leaves and buds which stimulated rooting of cuttings. However, in the course of time it has been proved that the changes occurring during rooting in stem cuttings are mostly due to the activity of the physiological factors, some of which will be discussed in the next section of this chapter.

1.2.3.1 Auxins

The use of synthetic growth regulators for rooting cuttings dates from the early 1930s (Komissarov, 1964). Several classes of growth regulators are known to plant physiologists: auxins and gibberellins - stimulate cell division; ethylene - stimulates the swelling or isodiametric growth of stem and root and the inhibitors inhibit growth and rooting in several ways (Leopold and Kriedemann, 1975). Proper combination and balance among them may cause tissue differentiation and organ formation (Skoog, 1944). Thimann and Went (1934) discovered auxin-B and the heteroxin effective in adventitious root formation. At that time synthetic indole acetic acid (IAA) was proved to be both root-forming and growth-promoting (Thimann and Keopfli, 1935). Then, study of the mode of action of the natural and synthetic stimulants on root formation began (Cooper, 1935). Later, it was reported that the rooting of cuttings is regulated by polarly transported auxin coming from the growing apex, leaves, or from growing buds (Van Overbeek *et al.*, 1945; Hess, 1971; Leopold and Kriedemann, 1975).

Rather little is known about the role of endogenous auxins in root formation in cuttings (Dore, 1965). In contrast, application of auxin or their derivatives promote rooting in cuttings (Eriksen and Mohammad, 1974; Mohammad and Ericksen, 1974; Hartmann and Kester, 1975). Among different synthetic auxins, indolebutyric acid

(IBA) is probably the best material for rooting cuttings due to its non-toxic character over a wide range of concentration (Hartmann and Kester, 1975). Mixtures of root promoting substances are occasionally more effective than either alone. Thus, a mixture of IBA and naphthaleneacetic acid (NAA), when used on a number of widely diverse species, induced a higher rooting percentage in cuttings and more roots per cutting than either compound alone (Evans, 1953). Haissig (1979) reported that on a molar basis, the phenylindole-3-acetic acid or the 3-hydroxyphenyl indole-3-acetic acid induced initiation of adventitious root primordia in bean cuttings at least 10 times greater than the IAA, whereas Siaguru (1986) did not obtain improved rooting with a group of synthetic aryl esters of indole-3-butyric acid on the rooting cuttings of *Terminalia superba.* The effect of auxins on rooting capacity sometimes depends upon the method of application. Common forms of auxin treatment include:

(i) a quick dip in relatively concentrated solution, in which a proportion of the solvent, if not all, is an alcohol; and

(ii) a soak in relatively weak aqueous solutions.

In both the cases, the amount of auxin absorbed is unknown. Sometimes auxins are applied with known weights to cuttings of known sizes and sensitivities (Bowen *et al.*, 1975; Leakey *et al.*, 1982a). Cuttings treated with IBA solution by quick dipping produced better rooting than those treated with IBA in talc powder (Bonaminio, 1984). The extent to which the auxin promotes rooting differs from species to species (Nanda *et al.*, 1970); within the species (Leakey *et al.*, 1982a); season of treatment (Eliasson *et al.*, 1977); and also with age and size of stock plants (Portlingis and Thelios, 1976).

1.2.3.2 Cytokinin

Although cytokinins are involved in cell growth and differentiation (Hartmann and Kestor, 1975), considerable evidence supports the view that they inhibit adventitious root formation in cuttings (Humphries, 1960; Harris and Hart, 1964). However, at very low concentrations cytokinins slightly promote adventitious root formation (Eriksen, 1974), especially in the presence of auxins (Heide, 1965). Skoog and Miller (1957) found that auxin and cytokinin interact to initiate cell division and the type of differentiation which occurs in a meristem depends on auxin-cytokinin proportion. Therefore, a high auxin-cytokinin ratio leads to root initiation whilst a high cytokinin-auxin ratio leads to shoot formation (Batten and Goodwin, 1978). In difficult-to-root species (*Populus tremula*) a higher level of endogenous cytokinins were found than in the easy-to-root species (*Populus* x *euramericana*) (Okoro and Grace, 1976).

1.2.3.3 Carbohydrate

The role of carbohydrate in controlling adventitious root formation is still unclear (Breen and Muaroka, 1974), although recently some workers have evaluated the relation between carbohydrate and adventitious rooting (Hansen and Eriksen, 1974; Altman and Wareing, 1975). Two schools of thought exist regarding the role of carbohydrates in rooting.

Kraus and Kraybill (1918) first showed a correlation between carbohydrate content and rooting ability in cuttings, and subsequently others have shown the same (Went and Thimann, 1937; Nanda *et al.*, 1971; Lovell *et al.*, 1972; Eliasson, 1978). Cuttings taken from dark-grown plants did not root due to a lack of carbohydrate (observation of Komissarov, 1964, on Galston's work, 1948, with asparagus), but when they were provided with favourable conditions for photosynthesis (Komissarov, 1964; Van Overbeek *et al.*, 1946), they rooted better. The level of extractable carbohydrate increased in leafy cuttings during the rooting period (Okoro and Grace, 1976) due to the activity of leaves. In the absence of leaves there was a marked decline in carbohydrates in *Populus* (Okoro and Grace, 1976). In fact, Van Overbeek *et al.* (1946) replaced the role of leaves in red *Hibiscus* with sugars and both organic and inorganic sources of nitrogen, and these substances accumulated at the base of leafy cuttings. Went and Thimann (1937) found a positive relationship between root initiation and the levels of several kinds of sugars in the pea plant. In addition, there are reports that root stimulation is possible with exogenously supplied glucose in etiolated *Populus nigra* (Nanda *et al.*, 1971); sucrose in *Eucalyptus* (Bachelard and Stowe, 1962).

On the other hand, there are many instances where carbohydrate-rich cuttings failed to root (Nanda and Ananda, 1970; Okoro and Grace, 1976) and rooting percentage, number of roots per cutting and root length, etc decreased with irradiance (Hansen and Eriksen, 1974; Van den Driessche, 1985).

1.2.3.4 Co-factors

As discussed above, it is now widely accepted that auxins play an important role in adventitious root formation to cuttings. But there are some difficult-to-root plants which fail to respond to auxins or to any other known root-promoting substances alone or in combination (Leopold and Plummer, 1961). It may be that auxin alone is unable to induce roots. Hess (1962) reported the existence of synergism between auxins and different phenolic compounds - responsible for high rooting capacity in cuttings and he suggests these substances as rooting co-factors. Later, this was supported by other

workers who found non-hormonal substances could be isolated from plants, which have a positive effect on adventitious root formation (Batten and Goodwin, 1978). Shibaoka *et al.* (1967) showed Heliangine, an inhibitor of stem elongation isolated from *Helianthus tuberosus* leaves, can promote adventitious root formation in leafy mung bean cuttings. It has also been reported that catechol, chlorogenic acid and pyogallol stimulate adventitious root formation in light-grown mung beans (Hess, 1964) and salicylic acid, gallic acid and P-hydroxybenzoic acid all stimulated adventitious root formation in leafy cuttings of *Eranthemum tricolor* when applied before treatment with various auxins (Basu *et al.*, 1969).

1.2.3.5 Inhibitors

The presence of rooting inhibitors was first noted by Spiegel (1954) in grape cuttings. It has been found that plants which are difficult-to-root often contain more rooting inhibitors and less root promoters in comparison to easy-to-root species (Paton *et al.*, 1970; Fadl and Hartmann, 1967). The physiologically dormant buds are reported to be the main source of such rooting inhibitors, whereas non-dormant buds promote rooting (Fadl and Hartmann, 1967). The adult scions of *Eucalyptus grandis* were found to contain high levels of inhibitors whereas juvenile cuttings of *Eucalyptus deglupta* contained no inhibitors at all (Paton *et al.*, 1970). This supports the idea that increased synthesis of a rooting inhibitor is involved in the ontogenetic age of plants (Muzik and Cruzado, 1958). However, reports are also available in some varieties of plants that inhibitor levels seem to be the same in both difficult- and easy-to-root species (Lee *et al.*, 1969).

1.2.3.6 Mineral nutrients

Reid (1924) reported that mineral nutrients influence rooting in cuttings when he was working with the cuttings of tomatoes soaked in nitrate solution. Relative amounts and proper balance of nutrients is also noted as an important factor for rooting cuttings (Pearse, 1943). Influence of mineral nutrition on rooting of cuttings has been investigated by several workers subsequently. Among the major elements, nitrogen seems to have the greatest effect. Doak (1940) reported 30 different organic and inorganic forms of nitrogen increased the rooting response of *Rhododendron* cuttings. A high carbohydrate-nitrogen ratio promotes, whereas a low carbohydrate-nitrogen ratio results in poor

rooting (Hyun, 1967). Thimann and Pontasse (1941) reported that adenine was the most effective source of organic nitrogen to stimulate root initiation in *Phaseolus vulgaris*, whereas in softwood cuttings a high level of nitrogen is generally detrimental as compared to a medium or low level of nitrogen (Preston *et al.* 1953). Among the micro-nutrients, boron was reported to promote root development very markedly (Hemburg, 1951).

Effect of nutrition of the stock plants upon the rooting ability of cuttings has also been studied by several workers (Kamp and Blum, 1950; Preston *et al.*, 1953). Cuttings taken from stock plants having plenty of nutrients rooted well (Kamp and Blum, 1950). This may be due to either the effect of mineral nutrient on carbohydrate accumulation, or increasing amount of endogenous auxin (Samish and Spiegel, 1957). However, when stock plants are grown at high levels of nutrient, the resulting soft growth is susceptible to rot in the cutting bench (Kamp and Blum, 1950), whereas cuttings from stock plants grown under nitrogen deficiency root better (Pearse, 1943). Unbalanced fertilizers, rich in nitrogen and low in phosphorus, potassium and other nutrients, might stimulate stem nitrogen and be successful in maintaining high rooting percentages from stock plants (de Souza and Felker, 1986).

1.2.3.7 Tissue characteristics

The extent of adventitious root formation in stem cuttings has been found to depend on the age of the stem tissue (Batten-Goodwin, 1978). Pericycle tissue has generally been reported as the site of adventitious root formation (Priestley and Swingle, 1929). The site gradually moves inwards with the age from the pericycle to the outer primary phloem parenchyma, inner primary phloem parenchyma, and finally to the inner secondary phloem parenchyma (Priestley and Swingle, 1929). Roots may occasionally arise from pith, primary xylem parenchyma, epidermis and callus (Kraus *et al.*, 1936). However, the variability in rooting of stem cuttings among clones and easy- or difficult-to-root species has been attributed to the anatomical structure of the primary phloem (Beakbane, 1969). In support of this theory, it is pointed out that difficult-to-root cuttings have an almost continuous cylinder of mature, thick-walled fibre cells encircling the secondary phloem, whereas in easy-to-root cuttings this sclerenchyma ring is not continuous and would permit the emergence of roots formed inside the ring. On the other hand, there are cases where rooting treatments cause considerable cell expansion and proliferation in the cortex, phloem and cambium, resulting in breaks in the continuous sclerenchyma ring

and thus this tissue is unlikely to be a primary cause of rooting difficulty (Priestley and Swingle, 1929). The presence of latent or preformed root initials is expected to enhance rooting but Carpenter (1961) observed that different species of *Citrus* have varying rooting ability, although all possess these latent roots.

1.2.4 Mechanism of physiological factors

It was postulated by Skoog (1944) from research using tissue culture techniques that cell differentiation and organ formation is most likely based on certain combinations and balances of naturally growth substances. Among them, it is now well accepted and has been subsequently confirmed many times by many workers (see Section 1.2.3.1) that auxins, natural or artificially applied, are a requirement for the initiation of adventitious roots on stems (Gautheret, 1969). There are many types of cutting with ample amounts of auxins in their tissues which do not therefore respond to added synthetic auxin. Conversely, in many other plants the native auxins may be in such slight amounts that the cuttings will show a definite response to added auxin, by an increase in the number of roots forming or by a reduced time of root development or both (Hartmann, 1969). The view generally held at present is that both exogenous and endogenous auxins in a freshly excised stem cutting move basipetally because of the polar transport of auxin which collects at the 'root tip' end of the cutting, where it causes the initiation of roots (see Section 1.2.2.6). This is supported by much classical work in the 1930s. It was found with a 15 mm segment cut from the fleshy taproot of chicory that soon after a cutting is excised, within a few days, auxin decreased at the shoot end and increased at the root end (Warmke and Warmke, 1950). However, Snow (1935) using seedlings and Söding (1936) using woody plants first demonstrated the case of apparent auxin control of cell division in the cambium. Root primordium initiation begins with dedifferentiation of cells, and these dedifferentiated cells attain the meristematic state and become the primordium initials. Primordium development occurs through division of the initial cells and of cells adjacent to the primordium. The division of the first root initial cells was dependent upon either applied or endogenous auxins (Haissig, 1972).

There is a general consensus that auxins enhance primordium development, not alone, possibly with auxin synergists or co-factors (see Section 1.2.3.4). Immature or embryonic leaves apparently synthesize the synergists which like IAA, undergo basipetal transport in cuttings. Somehow the synergist allows or enhances auxin-induced root primordium initiation and development. Thus the type of synergist seems to partially

determine whether cuttings initiate root primordia easily, with difficulty or not at all (Haissig, 1974). The chemical identities of endogenous synergists remain unclear but some exhibit the characters of phenolics. Haissig (1983), however, tried to examine the function of endogenous root forming component (ERS) which has an auxin component and non-auxin component. He demonstrated that the auxin component was required for development of callus in which root primordia were initiated, but for subsequent primordia development both auxin and non-auxin ERS were needed. On the other hand, Ermakov and Zhuravleva (1974) have demonstrated that protein synthesis was activated in the rooting zone in the period before callus formation, the content of amino acids and organic phosphates increased during the period of callus formation, and that the formation of meristematic root initials was accompanied by the utilization of organic phosphate compounds and active protein synthesis.

On the other hand, the action of auxins with other phytohormones are also important for callus formation and rooting. There are diverse opinions regarding the role of cytokinin in root initiation (see Section 1.2.3.2), but it is clear that cytokinin alone cannot initiate callus or root primordia. Reports are available that auxin and cytokinin interact to initiate cell division and the type of differentiation solely depend upon the auxin-cytokinin proportion. The recent information of regeneration of Calabrian pine from juvenile needles by in-vitro culture indicated that mixtures of cytokinins were more effective than separate cytokinins in producing buds on explants. The parenchyma cells in mesophyll layers after a period of activity and division became meristematic which later led to formation of bud primordia and subsequent rooting (64%) of shoots was achieved using a combination of two auxins and a low level of cytokinins (Abdullah and Grace, 1987).

Furthermore, reports of the role of gibberellins in root initiation as well as callus induction/proliferation are also contradictory. Most of the workers reported that GA_3 inhibited root formation (Nanda *et al.*, 1968); callus formation and organogenesis in explanted tissues (Kato and Hongo, 1974). On the contrary, GA_3 has been reported to be promotive in adventitious root formation in stem cuttings of *Ipomoea fistulosa* (Nanda *et al.*, 1972), *Abelmoschus esculentus* (Bhattacharya *et al.*, 1978) and in epicotyl cuttings of pea (Adhikari and Bajracharya, 1978). However, recently, Janardhanan and Lakshmanan (1982) reported that a mixture of gibberellins and auxins (GA_3, 5 mg/l + IBA, 5 mg/l) or auxins and gibberellins (IBA, 10 mg/l + GA_3, 5 mg/l) showed distinct and discrete callus masses on the eighth day which appeared in calli on the twelfth day on *Wrightia tinctoria* species. But the intensity of callusing was half that achieved with auxins alone.

Moreover, reports are also available that the effectiveness of an auxin in rooting stem segments is dependent upon nutritional factors. The ability of stem cuttings to root is determined by a proper balance between the nutritional factors and regulating substances. The most important action of auxins on carbohydrates in rooting cuttings is believed to be the maintenance of high concentrations of available carbohydrates, as low molecular weight sugar, which are used as energy in metabolism or to build new cells. Basal auxin treatment causes carbohydrates to be translocated to the base where it accumulates (Veierskov and Andersen, 1982) to produce root initials. The time of accumulation of carbohydrates at the bases of cuttings varies from species to species. For example, it took 4 days in *Pisum sativum* (Veierskov *et al.*, 1982), whereas the hormonal stimulation of assimilate movement has been observed within 4-6 hours in *Pisum sativum* and *Populus robusta* (Davies and Wareing, 1965) and within 6 hours in *Solanum andigona* (Booth *et al.*, 1962). However, the mechanism of assimilate transport and hence control (including hormones), is dependent upon the characteristics of the cellular pathway through which assimilate movement occurs (Patrick and Wareing, 1980). At the source, sucrose and possibly other phloem mobile assimilates, are loaded into the sieve-tubes from the apoplast against a steep concentration gradient by an energy-dependent, carrier-mediated transfer process (Geiger, 1975). Axial flow in the phloem is restricted to the sieve tubes whose ultrastructure is poorly understood (Parthyasarathy, 1975). Thus, interpretation of any observed phytohormone action, along the source to sink pathway, must remain tentative until phloem ultrastructure and mechanism is resolved. On the other hand, the pathway of assimilate unloading from the phloem channel would appear to depend upon sink type (Patrick and Wareing, 1980). Thus, accumulation in the stem involves unloading into the apoplast across a phloem membrane boundary with subsequent carrier-mediated transfer into ground tissue (Glasziou and Gayler, 1972). In contrast, transfer of assimilates from the phloem into nectarines (Gunning, 1976) and root tips (Dick and Rees, 1975) follows a symplastic route. However, whether the transfer is apoplastic or symplastic will dictate the form of hormonal control of assimilate movement from the phloem to the sink tissues (Patrick and Wareing, 1980).

Therefore, the mechanism of root initiation in stem cuttings is very complex and is regulated by a number of components, any one of which may limit the process. Auxins, phenolic compounds (synergists/co-factors), carbohydrates (nutrients), enzymes etc, are mainly involved in the process.

1.3 AIM OF PRESENT WORK

Forest systems throughout the world now aim to maximize production by afforestation and reforestation. Planting programmes need plenty of stock plants and vegetative propagation is a powerful tool for producing quality planting materials on a large scale. The method of vegetative propagation that is currently being developed most rapidly is rooting of cuttings (Zobel and Talbert, 1984). The main problem in rooting cuttings is the variability in rooting abilities between species and also within species.

It is often possible to manipulate the physiological and environmental factors to achieve enhanced success in rooting. Unfortunately, there is little information on the natural physiological changes occurring within the cuttings during rooting. Therefore, the present study was made to describe some of these changes during rooting cuttings of *Nauclea diderrichii* in controlled environmental conditions.

The thesis describes two main experiments which examined the relationship between carbohydrate economy and rootability. In both of them, rates of photosynthesis of the rooting cutting were recorded on a day-to-day basis whilst periodic determinations of carbohydrate levels were made. The overall aim was to investigate the hypothesis that rooting success may be limited by carbohydrate supply. In the first experiment, the treatment was an application of indole butyric acid (IBA) which is expected to improve rooting. In the second experiment the treatment was to subject the stock plants to a period of deep shade before taking cuttings. It was thought that this would deplete the levels of carbohydrates and thus alter the rooting success.

Nauclea diderrichii is a high density hardwood species in West Africa which regenerates very well both on cleared sites and also the verges of new roads. It was also recommended as a candidate for a tree improvement programme in the Republic of Cameroon by World Bank consultants in 1985 (Leakey, 1985).

CHAPTER 2

CULTURAL METHODS

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2.1 CULTURAL CONDITIONS

2.1.1 Construction of propagator

The propagator was rectangular in shape (3 x 1 m), constructed of wood and lined with black polythene sheets inside (Figure 2.1). The top was made of transparent polythene sheets tacked to wooden frames. The lid was removable to permit access to cuttings. The rooting medium was gravel and coarse sand. A filler pipe (2.5 cm diameter) was mounted along the length inside the rooting medium for watering, and another as an overflow pipe (2.5 cm diameter). The base was on a slight incline to facilitate drainage. Water level inside the propagator was checked via an inspection pot which, placed into the sand, enabled the water level to be seen. White plastic pots (13 x 7 cm) with holes at the bases were inserted into the top sand layer of the rooting medium. The lid of the propagator was closed as soon as the experiment was set up. The temperature of the rooting medium was thermostatically maintained at 30 $\pm$ 2°C both by day and by night. Heating cables were laid 20 mm apart in between the gravel and the sand layer of the medium. To ensure high humidity (near saturation), a hand spray was used two to three times a day, during very hot or sunny days. With this arrangement, humidity inside the propagator was maintained near saturation. The overflow pipe of the propagator was designed in such a way that a 10 cm reservoir of water was always maintained to supply water by capillary action. However, during very sunny days the water level was increased as an extra precaution. The lid did not provide a complete gas seal with the walls of the propagator so that carbon dioxide and water vapour concentrations inside the propagator were somewhat dependent on conditions prevailing in the glasshouse itself.

The propagator was inside a 6 x 4 m section of the glasshouse. Two ventilator fans were mounted on the wall near the roof of the glasshouse. Thermostatically-operated control was applied to the ventilators to minimise temperature fluctuations. There were three rooting benches: one (4 x 1 m) to the front, one (4 x 1 m) at the back and another (3 x 2 m) in the middle of the room. The propagator was positioned on the latter. The mean air temperature was about 20°C during the period of the experiments. Humidity was maintained at around 70% during the day by installing a humidifier near the propagator. At night it rose to 90%.

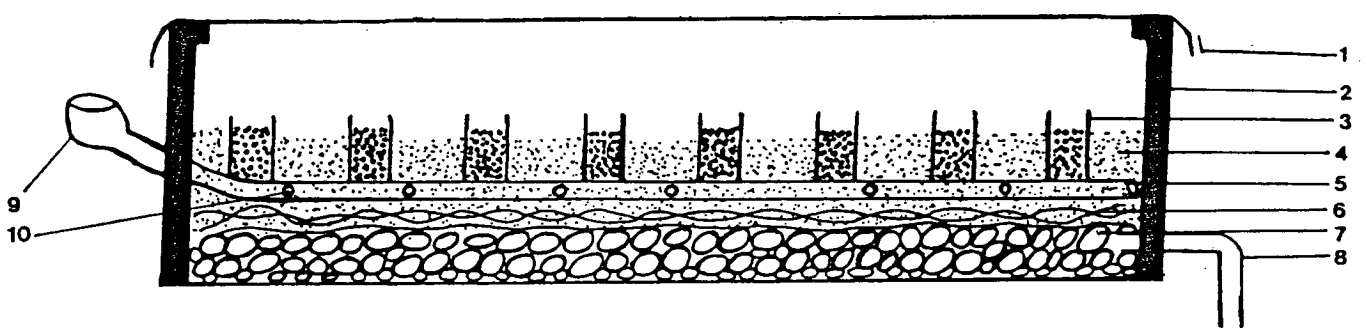

FIGURE 2.1: Diagram of a vertical section along the length of the rooting propagator.

1. polythene sheet on the top of the propagator
2. wooded frame
3. white plastic pots
4. coarse sand layer
5. filler pipe along the length of propagator
6. heating cables
7. gravel layer
8. overflow pipe
9. funnel of the filler pipe
10. holes of filler pipe

2.1.2 Typical conditions in summer

The experiments occurred during the summer with a day length of about 16 hr. Light, temperature and carbon dioxide were measured simultaneously with gas exchange. Environmental variables were as follows.

Light: Light was measured by a quantum sensor (Licor, Lincoln, Nebraska) mounted inside the glasshouse over the propagator, connected to a CR21 micrologger (Campbell Scientific, Utah). Sensors were scanned every 10 s by the datalogger and means calculated every 30 minutes. Mean photon flux density (day and night) in the first experiment was 167 μmol $m^{-2}s^{-1}$ while that in the second experiment was 121 μmol $m^{-2}s^{-1}$ (see Figures 2.2 and 2.3).

Temperature: Air temperatures both inside and outside the rooting propagator were measured by a thermistor (Campbell Scientific, Utah) connected to the micrologger noted above, and a 76 mm immersion mercury thermometer respectively. The immersion thermometer was exposed above the propagator and data were collected manually at about 1.00 pm every day. Mean air temperature inside the propagator during the first experiment was 24.9°C whereas in the second experiment it was 26.4°C (see Figures 2.2 and 2.3).

Carbon dioxide: Gas exchange of individual cuttings was studied in an open gas system incorporating an infrared CO_2 analyser (Binos, Leybold-Heraeus). Two signals from the analyser were scanned by the datalogger, the absolute CO_2 concentration of the air entering the chamber and the difference in CO_2 concentration between incoming and outgoing air (the mean absolute CO_2 concentration was 394.7 μmol mol^{-1} and in the second experiment it was 358 μmol mol^{-1} (see Figures 2.2 and 2.3). There were substantial differences between values by day and by night.

Humidity: The aim was to maintain a nearly water-saturated atmosphere inside the propagator, whilst minimising condensation on leaves and on the surface of the polythene film. A typical trace of temperature and humidity on a sunny day is shown in Figure 2.3a.

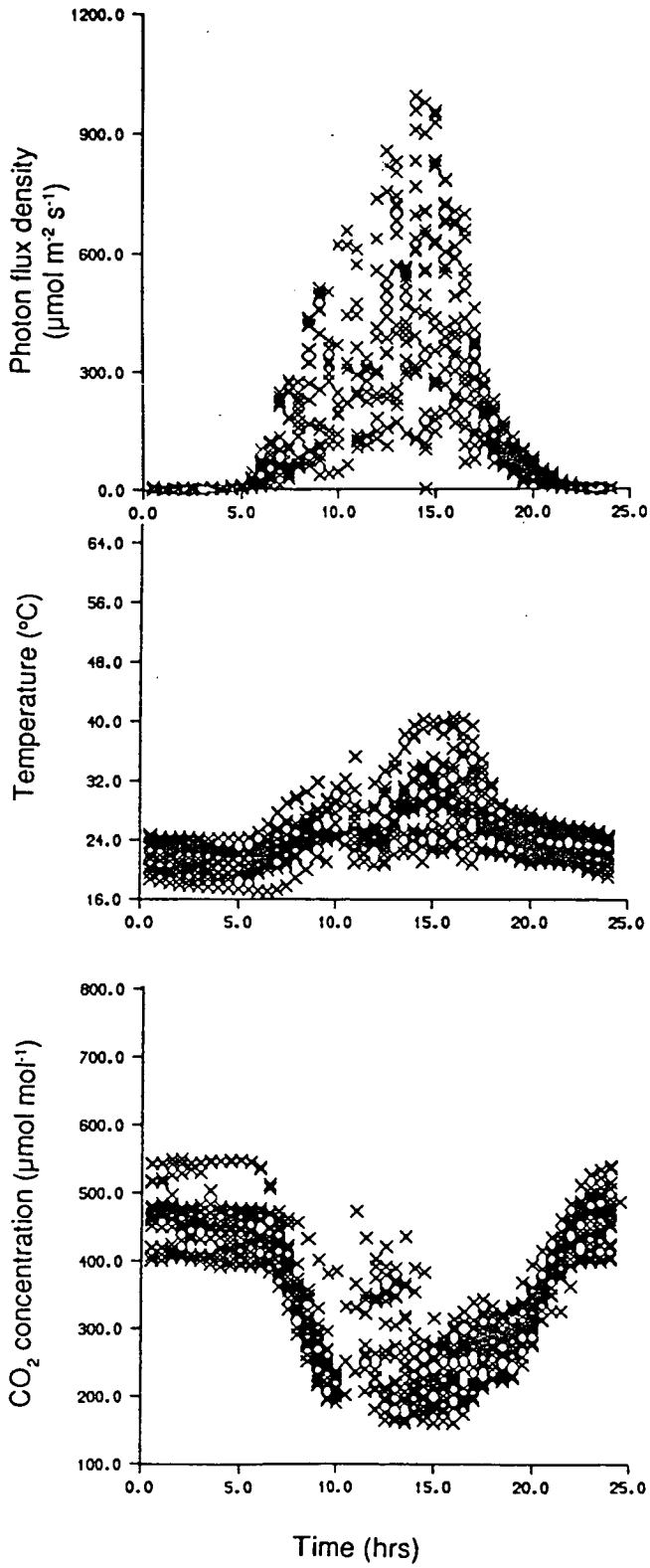

FIGURE 2.2: Environmental variables during the course of the first experiment, from 25 May to 28 June 1988. Discontinuities in the CO_2 record occur at 10.15 am each day because the zero setting of the differential mode was checked daily at that time.

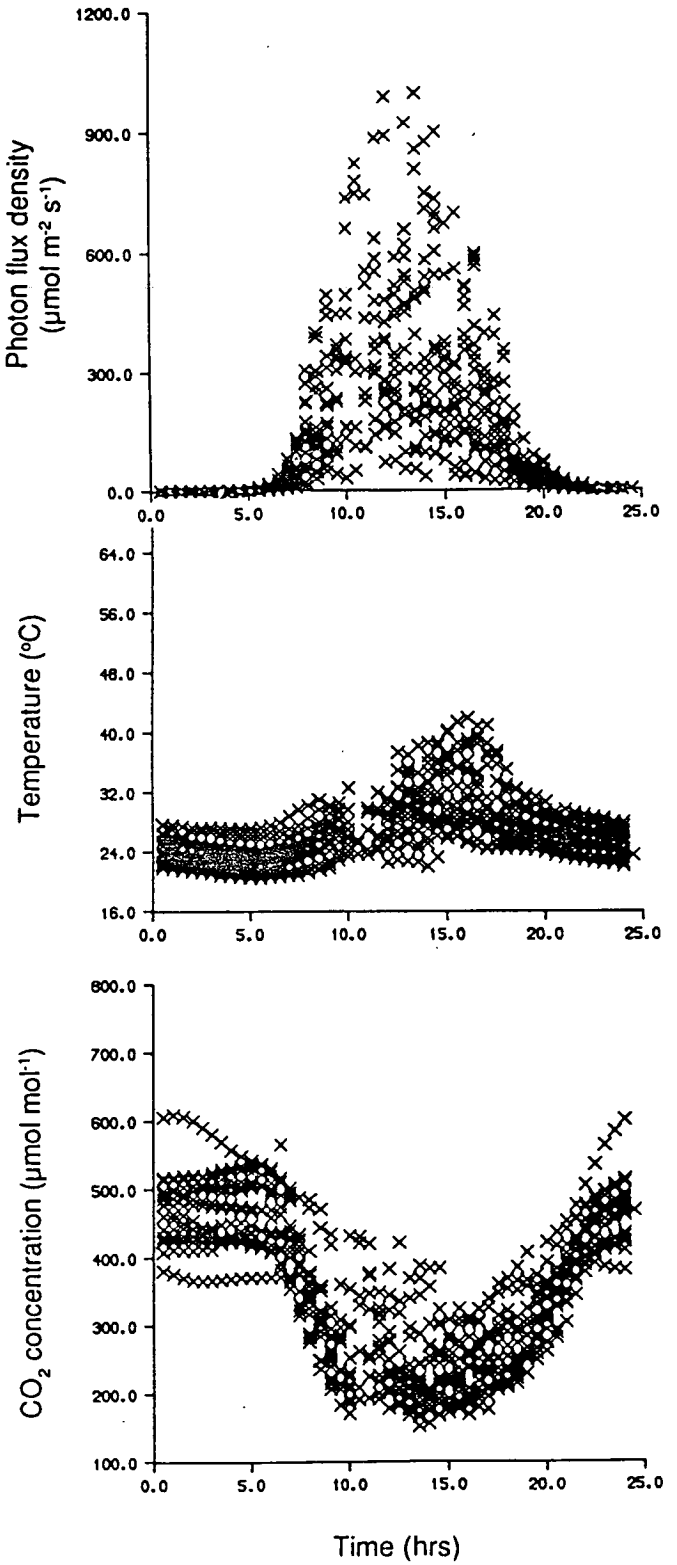

FIGURE 2.3: Environmental variables during the course of the second experiment, from 20 July to 23 August 1988. Discontinuities in the CO_2 record occur at 10.15 am each day because the zero setting of the differential mode was checked daily at that time.

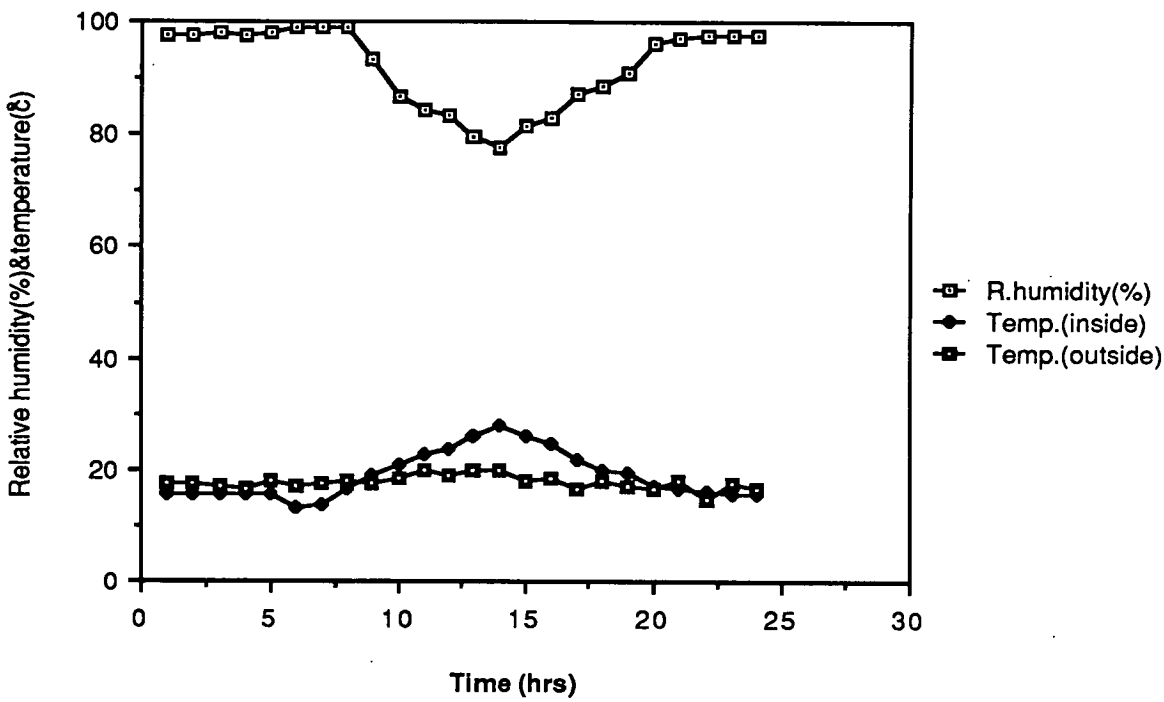

FIGURE 2.3a: A typical trace of temperature in and outside the rooting propagator and the relative humidity on a sunny day. Note that there were no cuttings inside the propagator.

2.2 DESIGN AND LAYOUT

2.2.1 Number of cuttings and blocks

The propagator was divided into four blocks. In the IBA experiment (first experiment), each block was divided into four columns and six rows where 24 white plastic pots were placed. For each block, 12 cuttings treated with IBA and another 12 without IBA were then planted in each pot, and pots were randomly distributed in each block. Six cuttings of each 12 were used for the measurement of photosynthesis and the other six cuttings were used to find out the morphological changes and the general rooting success.

In the second experiment, each block was divided into three columns and 10 rows, where 30 pots were randomly placed. A group of 15 cuttings was taken from the stock plants grown in the open light of the glasshouse and another group of 15 cuttings was taken from the stock plants grown in shade. Cuttings were then distributed randomly within each block. Eight cuttings from each group were used for the measurement of photosynthesis and subsequently harvested for carbohydrate analysis. Another seven cuttings from each group were used to find out the morphological changes and the general rooting behaviour. In both experiments, the respective planting pots were carefully labelled and filled with the rooting medium as discussed in Section 2.3.4.

2.3 TREATMENT OF PLANT MATERIALS

2.3.1 Origin of stock plant

The vegetatively propagated stock plants were presented by Dr R.R.B. Leakey, Institute of Terrestrial Ecology (ITE), Bush Estate, Penicuik. The plants were grown in plastic pots (13 x 12 cm) with a standard rooting medium (7:3:1 mixture of peat, sand and loam, with 4.2 g kg^{-1} 'Enmag', 2.6 g kg^{-1} John Innes base, and 0.3 g kg^{-1} of trace elements) within an automatically controlled tropicalized glasshouse at the ITE. The clones originated from seeds collected in Nigeria, West Africa.

Altogether 60 stock plants were collected from ITE in two lots. The first lot (40 full-grown stock plants) was collected on 22 April 1988, and the second lot (20 cut-back potted stumps) was collected on 5 May 1988. After collecting from ITE, the plants were grown and maintained in the glasshouse of the Department of Forestry and Natural Resources, University of Edinburgh, until the beginning of the experiments. The first experiment was set up on 25 May 1988, using the first lot of stock plants, and the second

experiment was set up on 20 July 1988 with the second lot of stock plants. The mean diameter at the base of the stem and the height of the stock plants were 4.8 mm and 790 mm, respectively. The stock plants of the first lot were branchy and grew at a medium rate, whereas the second lot had no branches and had excellent growth. The species has large opposite leaves of deep green colour and long internodes (see Figure 2.4a,b).

2.3.2 Treatment of stock plants

In the first experiment, stock plants were grown in the normal conditions of the glasshouse. In the second experiment, the stock plants were grown under normal 'light' and 'shade' conditions before cuttings were taken. The plants were sorted into two groups with 10 plants in each at 48 days after cutting back. One group was grown in the open and the other was grown inside a cuboid darkened enclosure (2 x 2 x 2 m; see Figure 2.5). The enclosure was constructed of wooden frames covered by black polythene sheets on each side with perforated hardboard on the top. Each plant inside and outside the chamber was placed in a circular plastic tray of water (size 20 cm diameter and 3 cm depth) to maintain an equal water regime. Watering was three times a day (9.00 am, 2.00 pm and 5.00 pm), except during sunny days. The plants were grown in the two different conditions for 28 days and cuttings were collected on the 29th day. The mean photon flux density was 5.6 µmol $m^{-2}s^{-1}$ inside the dark chamber and 121 µmol $m^{-2}s^{-1}$ outside the chamber in the open light. The mean air temperature was 21°C in the closed dark chamber and 20°C outside the chamber. The duration of treatment in the 'shade' was determined after cutting hand sections of leaf, root and stem, then staining for starch using the KI reagent. This stain demonstrated the presence of starch grains (Figure 2.7a-d; Section 2.3.8). After 28 days, the extent of the staining was appreciably diminished, so it was considered that the experiment could start.

2.3.3 Collection and preparation of cuttings

Cuttings were prepared from the stock plants by cutting the stems and the branches a little distance above each node. The plant is opposite leaved, so one leaf was excised and the other was trimmed to about 50 cm^2 with sharp scissors. Each cutting consisted of leaf and the subtending internodes. The cuttings were first excised by pruning shears and later trimmed by a sharp scalpel with a slanting cut at the basal end. As the number of stock plants was limited and the internodes were not always equal in length, the size of the cuttings could not be kept constant. The mean length of the cuttings was 10-15 cm

(see Figure 2.6) with a mean diameter of 7.5 mm. Three to six cuttings were made at a time and were treated with rooting hormones where applicable (see Section 2.3.5.1). The cuttings were then planted in the white plastic pots previously inserted into the rooting medium of the propagator (see Section 2.2.1).

2.3.4 Rooting medium

The rooting medium was washed coarse sand (3 mm particles). To avoid measuring gas exchange of micro-organisms in the sand, the sand for the plastic pots was also sterilized at 120°C for 30 minutes. Before the second experiment, the sand was removed and rewashed.

2.3.5 Treatment of cuttings

2.3.5.1 First experiment

A synthetic auxin, indole butyric acid (IBA) was used as the rooting hormone in the experiment. About 20 µg of IBA was applied in 10 µl droplets of methanol (Analar). A stock solution was made by dissolving 50 mg of IBA in 25 ml of absolute methanol. From the stock solution, 10 µl was applied to the clean base of each cutting using a precision 5 µl microsyringe. After the application of IBA, the surface was dried to evaporate the alcohol by exposure to the air for a few minutes. In the case of the control cuttings, 10 µl of alcohol alone was applied. All the operations were done inside the glasshouse and the cuttings were inserted into the rooting medium once the alcohol had evaporated from the cut surface.

2.3.5.2 Second experiment

In this experiment, no IBA was given to the cuttings.

2.3.6 Harvesting

Cuttings were examined every three days by carefully excavating them from the medium. When rooting occurred the following morphological changes were recorded:

1. formation of callus at the lower cut surface;
2. formation of root initials over the internodes;

3. differentiation of root initials on the circumference of the cut end;
4. formation of roots from wound;
5. formation of roots from etiolated area;
6. regreening of senescing leaves;
7. sprouting of buds;
8. formation of new leaves.

Three harvests were made for carbohydrate analysis (Table 2.1).

TABLE 2.1: Description of harvests of *Nauclea* cuttings for carbohydrate analysis.

Type of cutting	Harvests	Description	Time (days)
Leafy single node softwood cuttings	1	Freshly taken cuttings	0
	2	Cuttings with sufficient callus for analysis	8
	3	Cuttings with sufficient quantity of roots for analysis	25

2.3.7 Preparation of harvests

Cuttings were removed from the propagator and washed by tapwater. Leaves, roots and buds were separated, cut in smaller pieces where appropriate, and blotted. Then they were fixed by plunging into liquid nitrogen (collected from the Physics Department) for 15-20 s, storing briefly in polythene bags on ice, keeping in a deep freeze room (-20°C) before finally being freeze dried to constant weight. The dried samples were subsequently milled into powder to pass a 0.5 mm sieve, before storage in labelled airtight plastic pots at room temperature ready for carbohydrate analysis.

2.3.8 Photographs

FIGURE 2.4a: A representative of the first lot of full grown stock plants used in the first experiment.

FIGURE 2.4b: A representative of the second lot of stock plants used in the second experiment.

FIGURE 2.5: The second lot of stock plants under treatment in the dark chamber. Single row of full grown plants were positioned around them to minimise the edge effect of light.

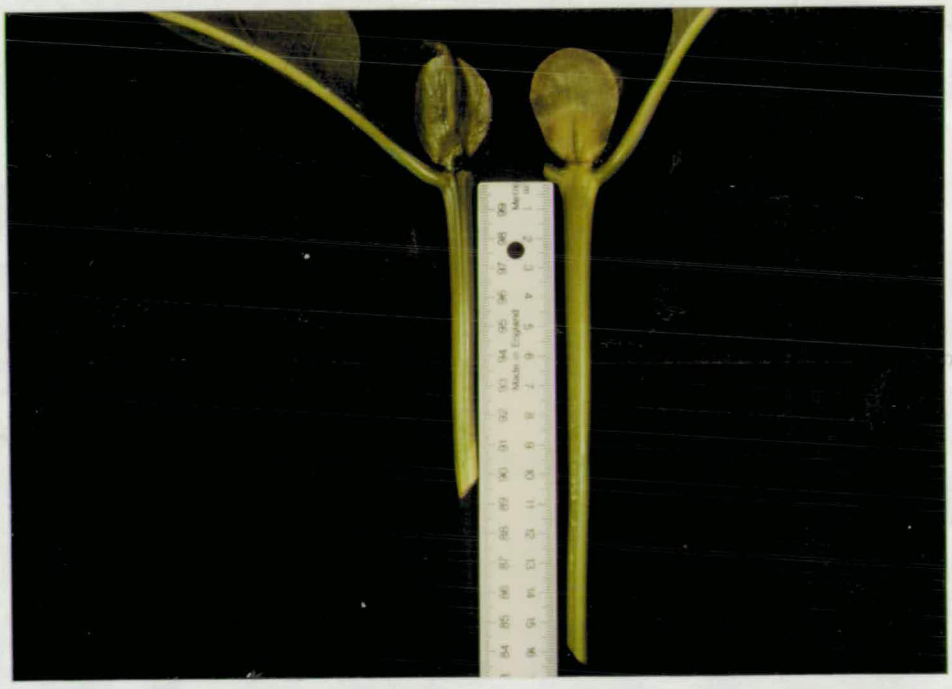

FIGURE 2.6: General appearance and size of cuttings, 10-15 cm.

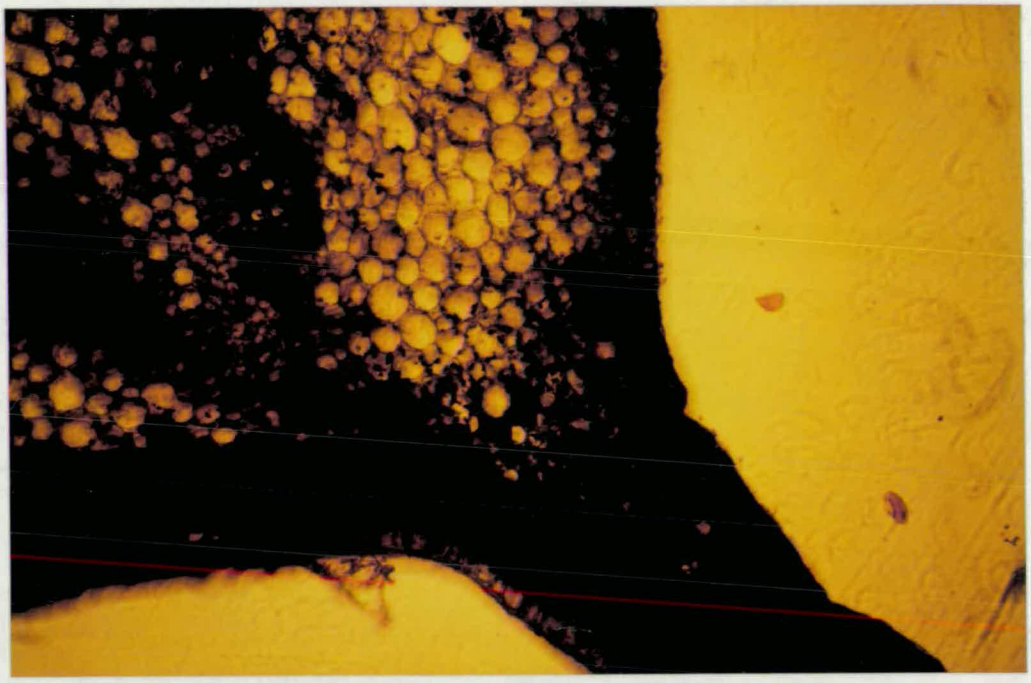

FIGURE 2.7a: Shows the microscopic view of a transverse section of a leaf of *Nauclea diderrichii* in open light condition. Black spots are the stored starch grains.

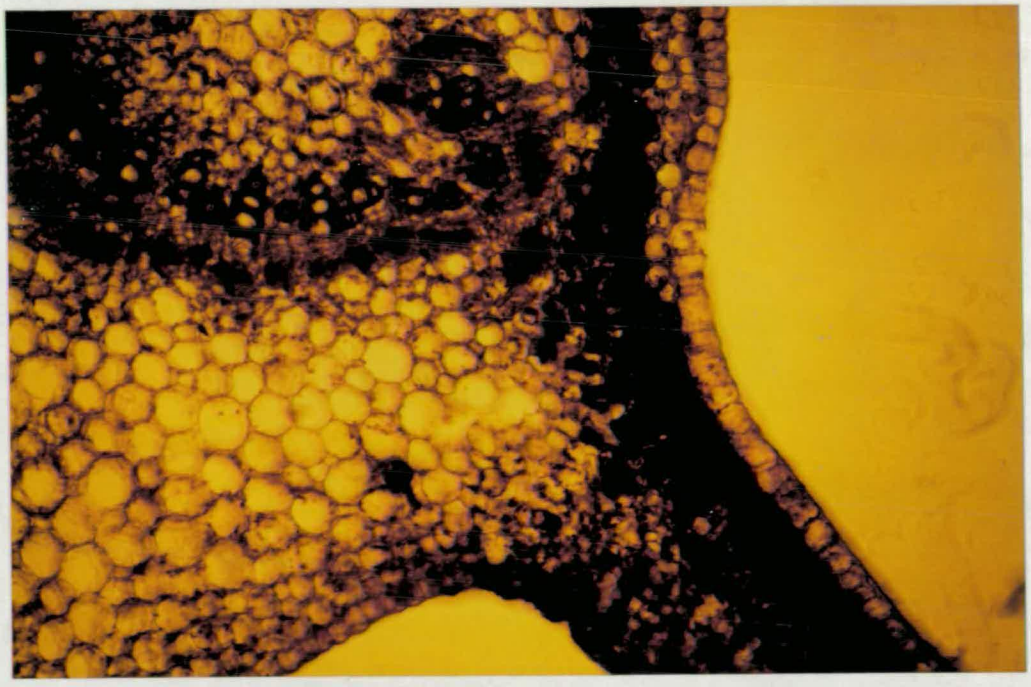

FIGURE 2.7b: Shows the microscopic view of a transverse section of a leaf of *Nauclea diderrichii* in dark condition. Black spots are the stored starch grains which are less here.

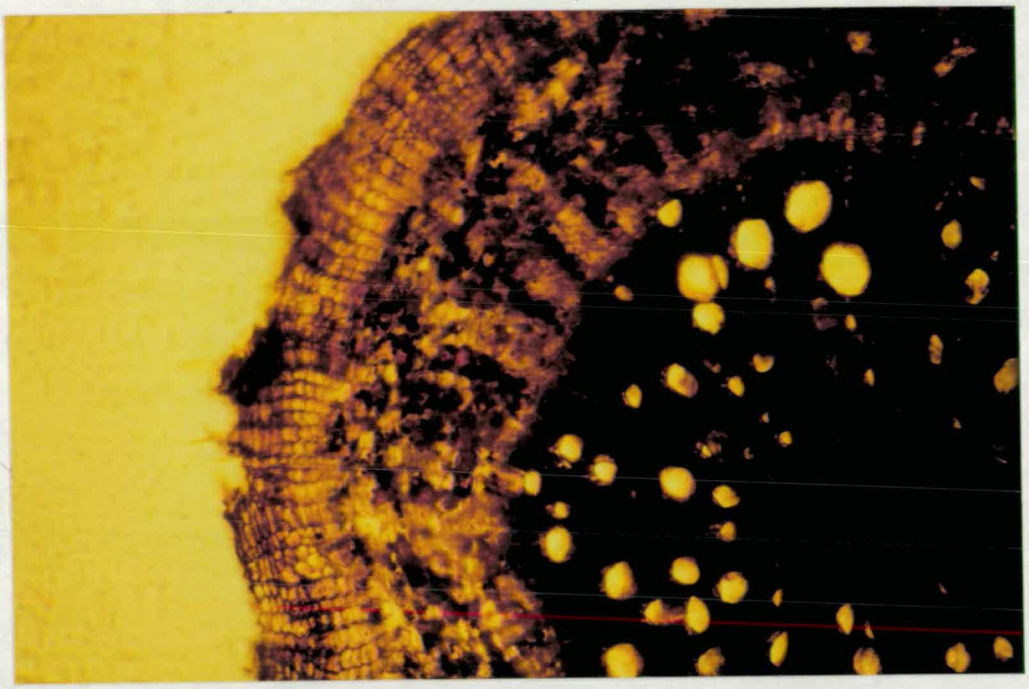

FIGURE 2.7c: Cross section of root of *Nauclea diderrichii* under light condition. Abundance of starch grains.

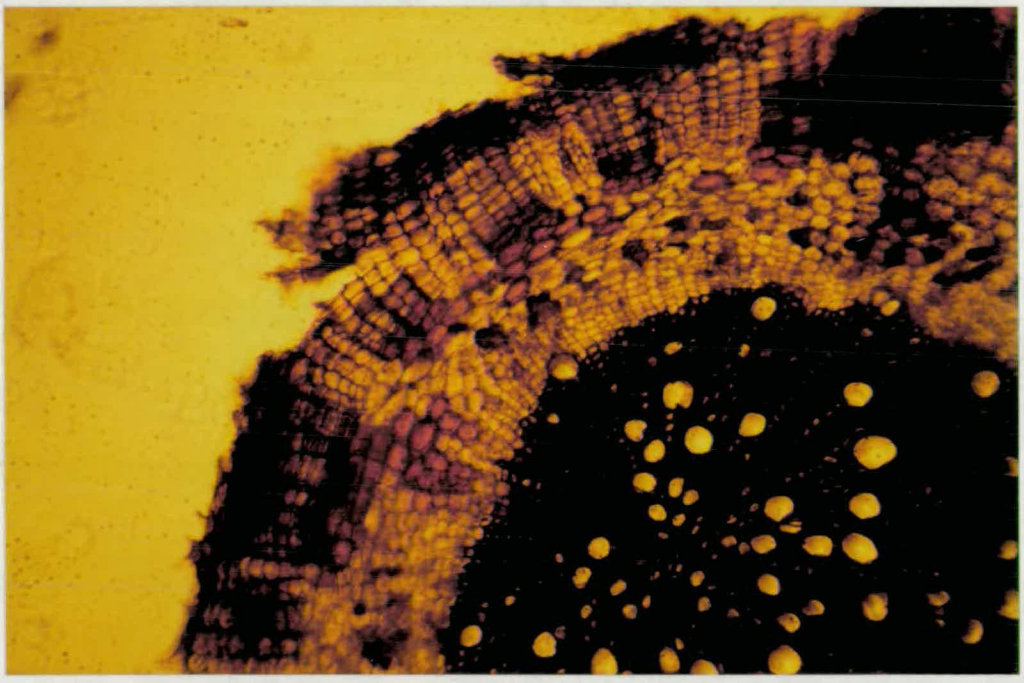

FIGURE 2.7d: Cross section of root of *Nauclea diderrichii* under dark condition. Less abundance of starch grains.

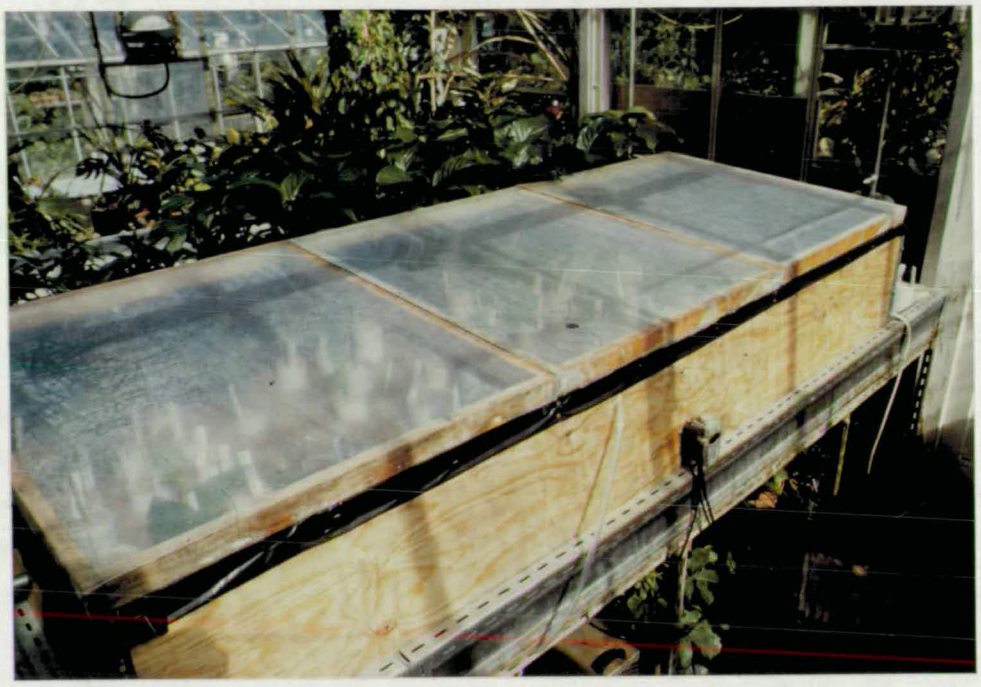

FIGURE 2.8: External view of the rooting propagator.

FIGURE 2.9: Internal view of the rooting propagator after the cuttings were planted in the bed.

CHAPTER 3

EXPERIMENT 1:

Influence of indole butyric acid (IBA) on rooting of leafy single node stem cuttings of *Nauclea diderrichii*

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3.1 INTRODUCTION

Cuttings of many species of tree have been stimulated to root by applications of synthetic growth substances (Thimann and Behnke-Rogers, 1950). In recent years, auxins have been at the centre of attention because of their potent influence on rooting in cuttings, originally shown by Went (1934) and Thimann and Went (1934). Later, among auxins, Napthalene acetic acid (NAA) and indole butyric acid (IBA) were reported as active adventitious root promoters by Thimann (1935) and Zimmermann and Wilcoxon (1935). IBA is probably the most potent and is non-toxic over a wide range of concentrations (Hartmann and Kester, 1975; Strömquist and Hansen, 1980; Eliasson and Arebland, 1984; Pythoud *et al.*, 1986). In tropical trees IBA is also effective in many instances (Lahiri, 1979; Pain and Roy, 1981; Leakey *et al*, 1982a; Negi and Tiwari, 1984). In contrast, in some species the overall percentage of rooting was found to be unaffected by IBA (Lo, 1985; Siaguru, 1986).

Many workers have found positive relationships between carbohydrate levels in cuttings and ability to initiate roots (see Chapter I, Section 1.2.3.3), although cuttings with high carbohydrate levels may not always initiate roots (see Chapter I, Section 1.2.3.3) because of overriding physiological factors. Starch is often the main carbohydrate in cuttings and most commonly, the starch content of the stems of cuttings rapidly declines during initiation of root primordia (Negisi and Satoo, 1956; Nanda *et al.*, 1970). The sugar content in the stems of cuttings often increases during the early stages of rooting because of starch hydrolysis and increased basipetal translocation (Stuart, 1938; Smith *et al.*, 1940), though it may increase in later stages with the development of new photosynthetic tissue (Okoro and Grace, 1976). However, sometimes it remains relatively constant throughout the rooting period (Stuart, 1938). The sugar pool declines if cuttings do not contain starch (Stuart and Marth, 1937) but exogenously supplied sugars supplant starch hydrolysis only in the absence of starch (Bausor, 1942) or under conditions of general shortage of carbohydrate (Nanda and Jain, 1971a; Nanda *et al.*, 1971; Nanda and Jain, 1972b). In contrast, applied sugars have no effect, or an inhibitory effect, when sugars are in short supply (Lovell *et al.*, 1971; Moore *et al.*, 1972). Auxin application in the presence of sufficient starch, causes enhanced starch depletion in tomato leaves

(Borthwick *et al.*, 1937) and this has been subsequently found for other species (Bausor, 1942; Hilton, 1966). In some species the sucrose levels also declined with auxin (IAA) application where starch was absent but no appreciable change was found in disaccharide levels (Hilton, 1966).

Furthermore, in addition to these changes occurring in the amount of carbohydrates in cuttings after planting, the photosynthetic and respiration rates can be expected to influence the food supply. There is little information in the literature regarding the trends in rates of net photosynthesis and respiration of detached shoots or cuttings. However, in most of the observations a declining rate was found immediately after planting, with rates remaining low throughout the period of callus formation and initiation of root primordia. But when the roots began to emerge the rate of net photosynthesis started rising (Negisi and Satoo, 1955, 1956; Cameron and Rook, 1974; Machida *et al.*, 1977; Eliasson and Brunes, 1980). However, reports are not available regarding the effect of plant growth regulators on the photosynthesis of cuttings or detached shoots. A few reports mentioned stimulation of photosynthesis of small seedlings by growth regulators (Marcelle and Oben, 1973; Treharne, 1978). The aims of the present work were to explore the relationship between the addition of IBA, the carbon balance of the cutting and its rootability. In particular, to answer these questions:

1. Does IBA increase the rate or extent of rooting?
2. If so, is this achieved through a more favourable supply of carbohydrate, caused by enhanced photosynthesis?

3.2 MATERIALS AND METHODS

3.2.1 Cultural techniques

Culture of the cuttings and the conditions during the experiment were explained in Chapter 2.

3.2.2 Carbohydrates

3.2.2.1 Reducing sugars

The plant materials were harvested at stages described in Section 2.3.6 and Table 2.1, and prepared, milled and stored as in Section 2.3.7, Chapter 2.

Preparation of extract: An accurately weighed (0.05-0.1 g) sample of dry powdered plant material (W) was extracted in 4 ml perchloric acid (50 mM) in a centrifuging tube, by boiling at 100°C for 10 minutes. It was cooled at room temperature and centrifuged. Pellets were washed with 1 ml perchloric acid (50 mM) and recentrifuged. The supernatants were made up to a standard volume V_1 (10 ml) with distilled water and the pH adjusted to 4.6.

Reducing sugars were determined using the Somogyi reagent as outlined in Appendix I.

3.2.2.2 Starch

Starch was determined as glucose after incubation of the extract with amyloglucosidase at pH 4.6 and 55°C for 1 hour. The progress of the reaction was monitored by testing small drops of the solution with 1% KI solution. The full details of the procedure is given in Appendix I.

3.2.2.3 Non-reducing sugar

The Somogyi reagent does not detect non-reducing sugars, and some of them (notably sucrose) are often a substantial component of the total carbohydrate within leaves and stems. Thus, paper chromatography was employed to see whether non-reducing sugars constitute a significant part of the carbohydrates in *Nauclea diderrichii*. The full details of the procedure for paper chromatography is given in Appendix 2.

3.2.3 Photosynthesis

Photosynthesis and dark respiration were measured over 24 hours on every alternate day in a plastic chamber within the propagating frame inside the glasshouse. The chamber, measuring 30 x 20 x 20 cm (Figure 3.1), was equipped with a small circulatory fan. One potted cutting which had been planted in autoclaved sterilised sand (Section 2.3.4) was randomly selected from each block on each alternate day. The pot was placed in a white polythene bag containing water to a depth of about 2 cm to maintain a proper water supply to the cut surface of the cutting. The upper end of the polythene bag was sealed carefully at the base of the cutting above the sand medium, leaving the upper part of the cutting open. This arrangement minimised any gas exchange between rooting medium and the

air. The potted cutting was sealed in the chamber by plastic film (''cling film wrap'', J. Sainsbury Plc, London). Typical conditions of light, temperature, carbon dioxide and humidity during the course of gas exchange were discussed in Section 2.1.2.

3.2.3.1 Gas flow system

Air from inside the propagator was pumped into the chamber using a diaphragm pump (Charles Austen Pumps Ltd, England), which maintained flows of 2 litre min^{-1}, the flow being monitored by a rotameter and controlled by a Flostat controller (GEC-Elliott Process Instruments Limited, England). As the humidity inside the propagator was near saturation, the air flow had to be first passed through a cold bath to condense most of the water. The air passed into a big glass bottle (10 L) to suppress fluctuations in the CO_2 concentration before dividing into two: one part going to the 'reference' and 'absolute' cells of the infra-red gas analyser (Binos, Leybold-Heraens), whilst the other went to the assimilation chamber. The air from the chamber then passed to the 'sample' cell of the IRGA (Figure 3.1).

Each day, at about 09.30 hours, the system was run with an empty chamber so that the instrument zero could be checked and adjusted if necessary.

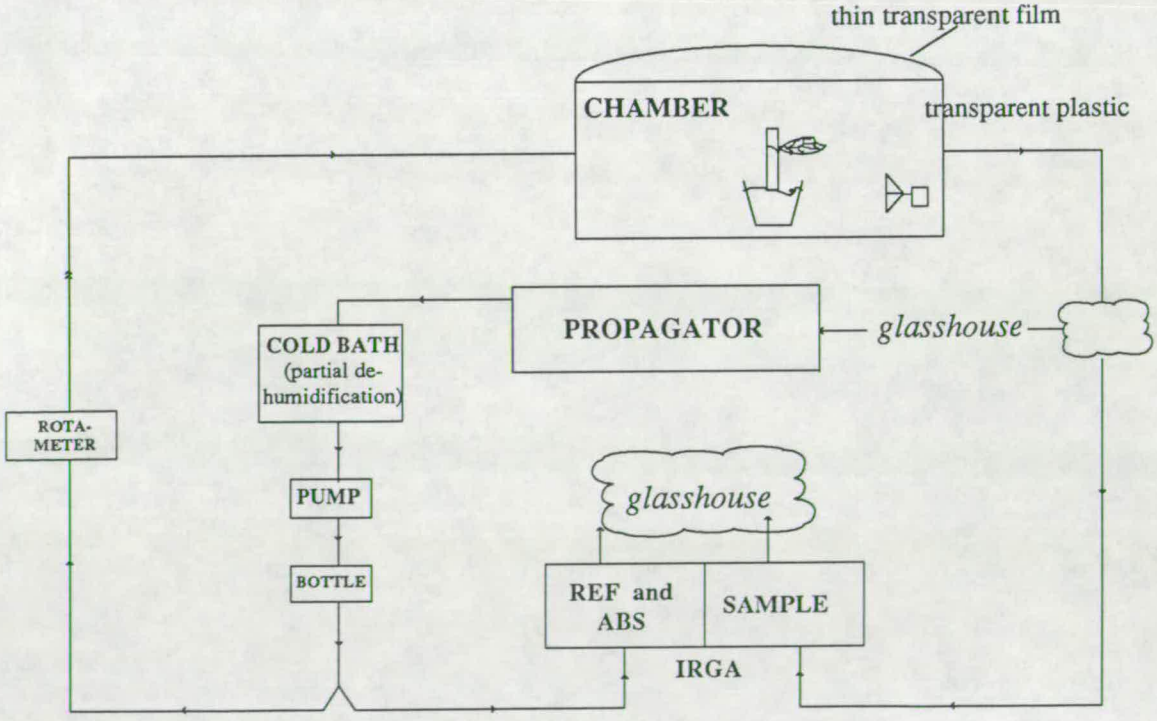

FIGURE 3.1: Gas flow system

3.2.3.2 Data acquisition

The infra-red gas analyser was connected to the CR21 micrologger mentioned in Section 3.2.3.1. Two signals from the IRGA were scanned every 10 s by the datalogger, the absolute CO_2 concentration of the air entering the chamber and the difference in CO_2 concentration between incoming and outgoing air. Every 30 minutes mean values were dumped to memory. The data from the datalogger were transferred to the mainframe computer. The rates of photosynthesis were calculated from the flow rates, leaf area and the difference in CO_2 concentration between incoming and outgoing air, using a FORTRAN program.

3.3 RESULTS

3.3.1 Rooting

Callus formation

Callus first appeared on the third day on cuttings treated with IBA and on the fifth day on cuttings without IBA (control). Significantly less callus occurred in controls and in some instances it did not even cover the whole cut surfaces (Figure 3.2a). The callus of treated cuttings became irregular especially at the cut surface and more generally around the base of the cuttings. Adjacent calli fused together forming an irregular tumour-like structure (Figure 3.2b). Calli seems to be originated from the complementary cells of lenticels making the lenticular pores swell up. Seventy-four and 53% of treated and control-cuttings, respectively, formed callus by the first week, whereas in the rest, callus formed at the beginning of the second week.

Formation of root initials

Formation of root initials took place more prominently in treated cuttings than controls, and root initials first appeared in the treated cuttings on the fifth day and in controls on the seventh day of planting (Figure 3.3b).

Roots from wound

Roots first appeared on the seventh day on treated cuttings and on the tenth day on controls. Roots were concentrated at the circumference of the obliquely cut surface of most cuttings in treated and control. Cuttings of the control showed thin masses of callus, poor root initials and subsequently less root development. Shoot tip cuttings showed quick

FIGURE 3.2a: Callus at the cut surface of 7-day old cuttings without IBA. Note that it is not covering the cut surface.

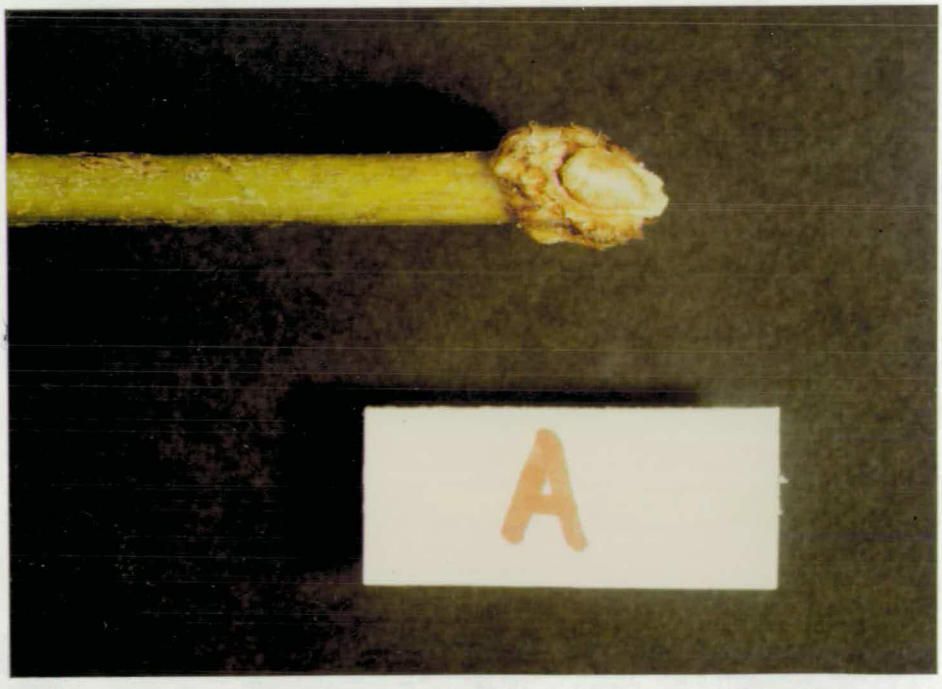

FIGURE 3.2b: Formation of abundant callus masses and swollen basal end of the cuttings treated with IBA (after 5 days).

FIGURE 3.3a: Root initials are seen along the whole internode of the IBA treated cuttings.

FIGURE 3.3b: Root initials and very young roots are emerging from the basal cut end of the untreated cuttings.

and more root production in both treated and control. After the first week, 32% of cuttings rooted in IBA with 1.5 roots per cutting whereas none rooted in controls. After the second week, 84% of cuttings rooted with 6.2 roots per cutting whereas in controls, 79% rooted with 2.2 roots (Table 3.1). All cuttings rooted after the third and fifth weeks in treated and controls respectively (Figure 3.4a). However, more roots were found per cutting between day 14 and day 21 in IBA and between day 21 and day 28 in the control. At the end of the experiment, on average, 18.0 and 9.3 roots were formed per cutting in IBA and control, respectively (Figure 3.4b), and the length of the longest roots were 7.0 and 5.6 cm in treated and control respectively (Table 3.2). The total number of roots in the treated cuttings was twice that of the control (Table 3.2).

Roots from the stem surface

About 25% cuttings in the treated and 15% cuttings in the control showed root initials along the whole internode. Roots appeared only from the pale zone 0-20 cm above the basal cut end. This is the zone from under the rooting medium (Figure 3.5). It seems that formation of these roots did not reduce the normal root production from the wound. The average number of roots per cutting was 5-7.

Bud formation

New axillary buds formed both in treated and control cuttings after 7 days. Treated cuttings showed significant increases in the percentage of cuttings forming buds throughout the experiment (Figure 3.7). There were no differences in the average number of buds per budded cutting (Table 3.2).

New leaves from buds

New leaves appeared on treated cuttings within the first week whereas in the control this did not happen until the second week. The percentage of cuttings forming leaves showed significant differences between the treatment and the control in different weeks and also at the end of the experiment (Figure 3.8a). No significant differences were found in average number of new leaves per budded cutting during the course of rooting (Figure 3.8b). About 10 and 15% of cuttings in treated and control, respectively, showed abscission among the old leaves. The colour of the leaves was pale green after planting but turned into deep green especially after the emergence of the roots.

TABLE 3.1: Rooting success and morphological changes on different characteristics of leafy single node stem cuttings of *Nauclea diderrichii* with or without IBA.

Time of observations		1st week	2nd week	3rd week	4th week	5th week
Month of cuttings planted		May				
Amount of auxin used, cutting^{-1}		20 µg				
No. of cuttings planted both + and - IBA		19				
No. of cuttings callus formed	+ IBA	14	19	19	19	19
	- IBA	10	19	19	19	19
No. of cuttings rooted	+ IBA	6	10	16	19	19
	- IBA	-	15	16	17	19
% of cuttings rooted	+ IBA	31.6	84.2	100	100	100
	- IBA	-	78.9	82.2	89.5	100
Average no. of roots formed per rooted cutting	+ IBA	1.5	6.2	11.9	17.0	18.0
	- IBA	-	2.2	5.3	9.0	9.3
Total no. of roots formed	+ IBA	9	99	227	324	343
	- IBA	-	33	85	153	176
Average length of the longest root per cutting (cm)	+ IBA	-	-	-	-	7.0
	- IBA	-	-	-	-	5.5
No. of cuttings bud formed	+ IBA	8	10	18	19	19
	- IBA	4	6	14	14	15
Total no. of buds formed	+ IBA	12	17	33	36	36
	- IBA	6	10	25	26	30
No. of cuttings leaves formed	+ IBA	3	6	12	14	14
	- IBA	-	3	7	7	8
Total no. of leaves formed	+ IBA	3	9	28	42	42
	- IBA	-	4	16	22	28
No. of cuttings leaves/ shoot dried	+ IBA	-	1	2	2	2
	- IBA	-	2	3	3	3
Mean fresh weights (g) of cuttings (including stem, leaves, buds and roots)	+ IBA	(9.9) 10.5	10.3	10.6	11.8	12.7
	- IBA	(8.7) 9.1	8.7	8.8	9.6	10.3

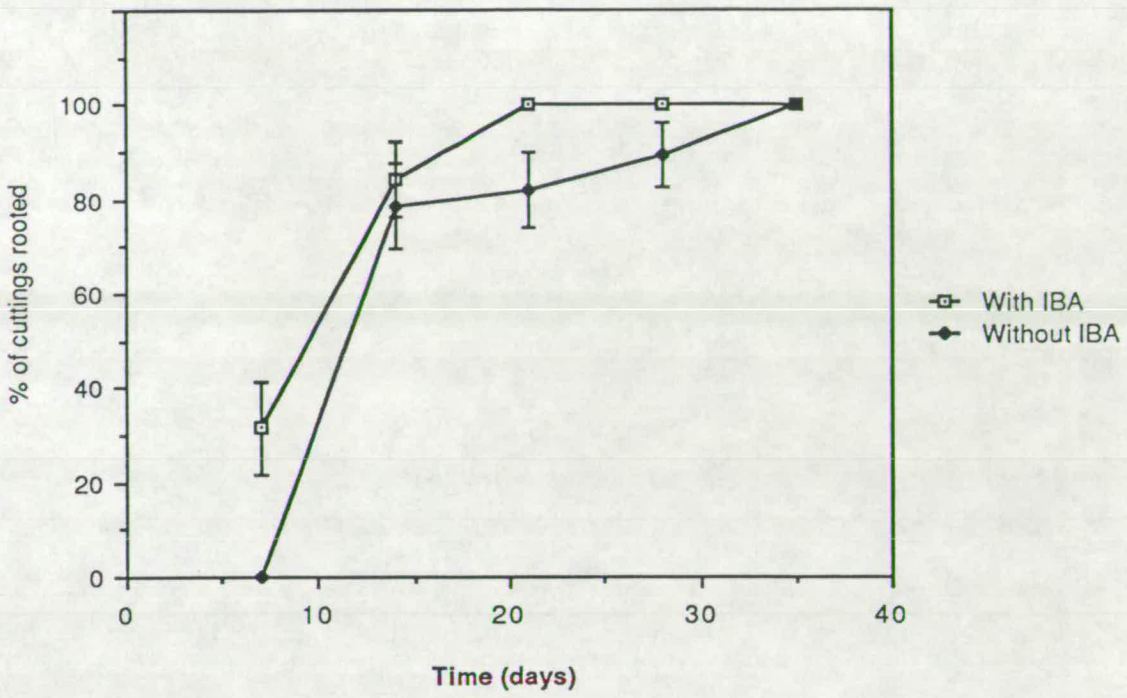

FIGURE 3.4a: Effects of IBA on rooting of leafy single-node cuttings of *Nauclea diderrichi* over a period of 35 days. Bars show standard error of means and the total number of cuttings was 19 both in treated and control.

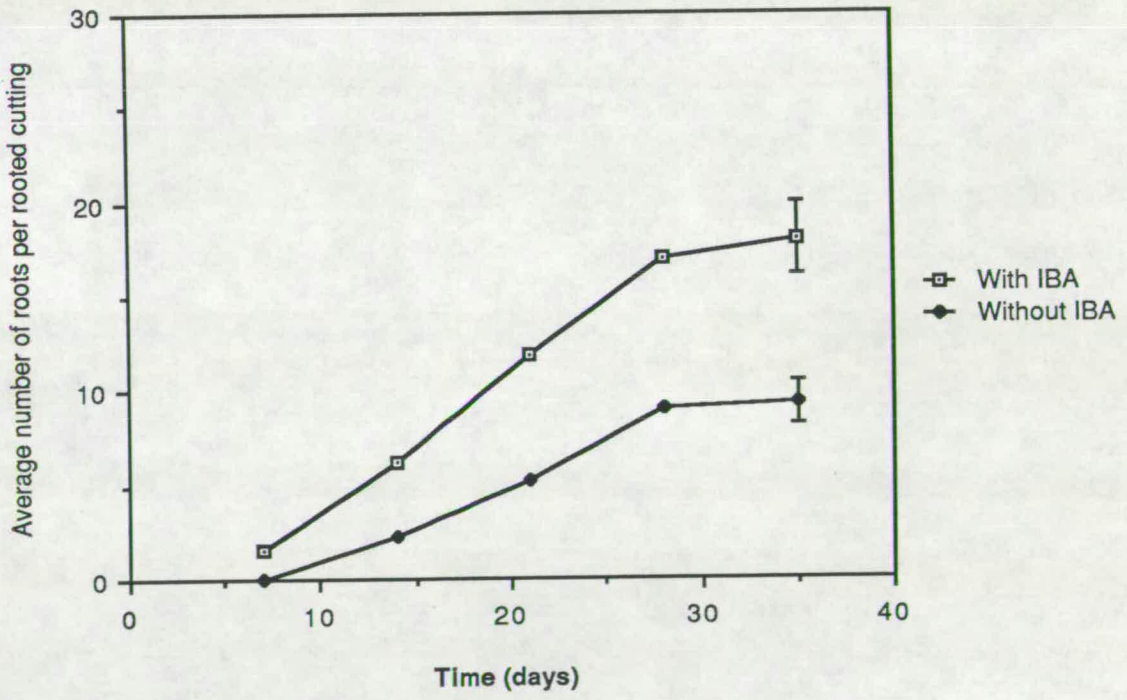

FIGURE 3.4b: Effects of IBA on the number of roots per rooted cutting of *Nauclea diderrichii* over the period of 35 days. Bars show standard error of means.

FIGURE 3.5: Roots emerged from the pale zone but not from the root initials along the whole internode.

FIGURE 3.6: The number of roots on IBA (A) treated cuttings are much more than on control cuttings (B). (At the end of the experiment - 5 weeks.)

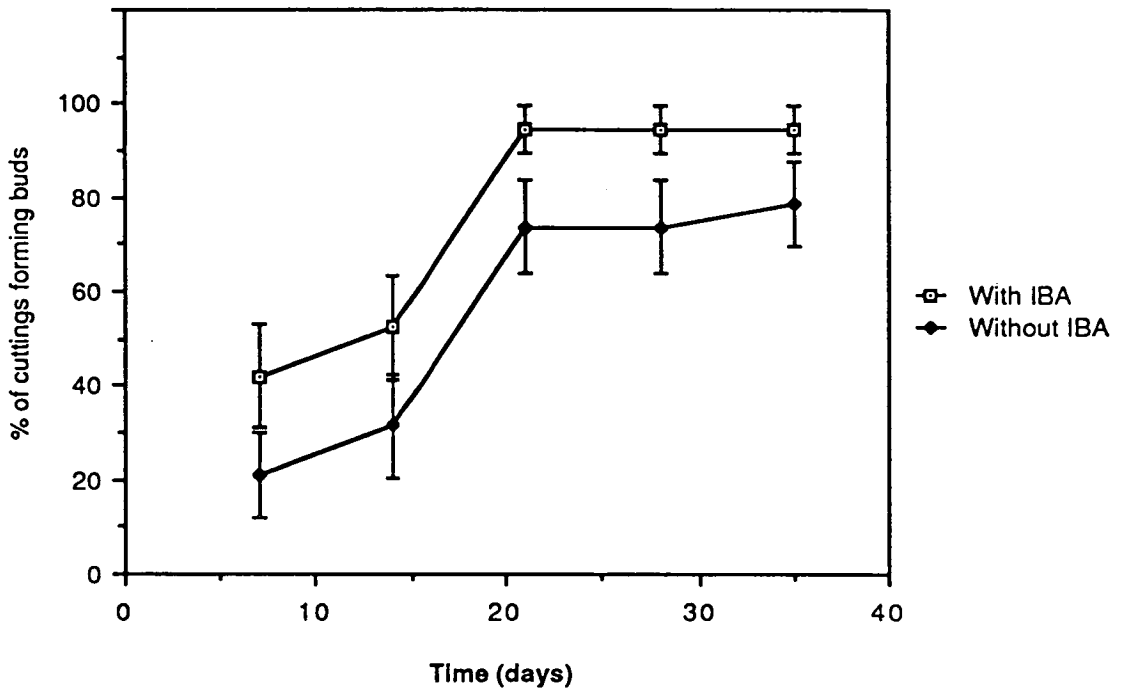

FIGURE 3.7: Effects of IBA on bud formation of leafy single-node cuttings of *Nauclea diderrichii* over the period of 35 days. Bars show standard error of means and the total number of cuttings was 19, both in the treated and control.

TABLE 3.2: Average number of buds formed per budded cutting.

Time (days)	With IBA	Without IBA
7	1.5	1.5
14	1.7	1.7
21	1.8	1.8
28	2.0	1.8
35	2.0	2.0

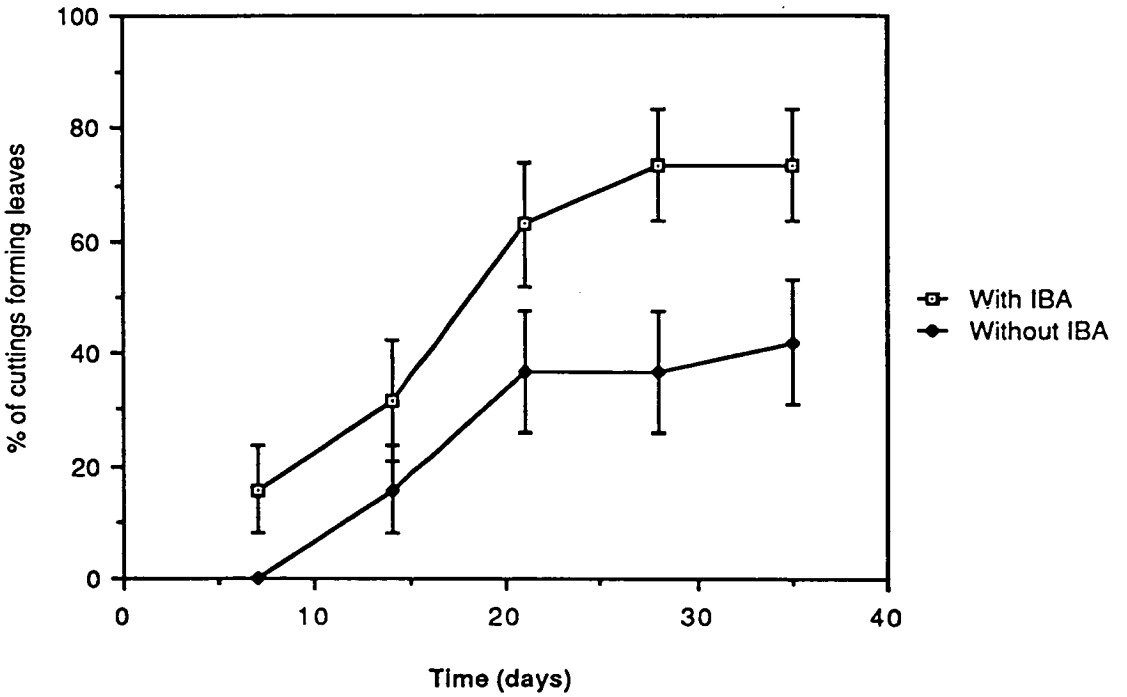

FIGURE 3.8a: Effects of IBA on new leaf formation of leafy single node cuttings of *Nauclea diderrichii* over the period of 35 days. Total number of cuttings was 19, both in treated and control. Bars show standard error of means.

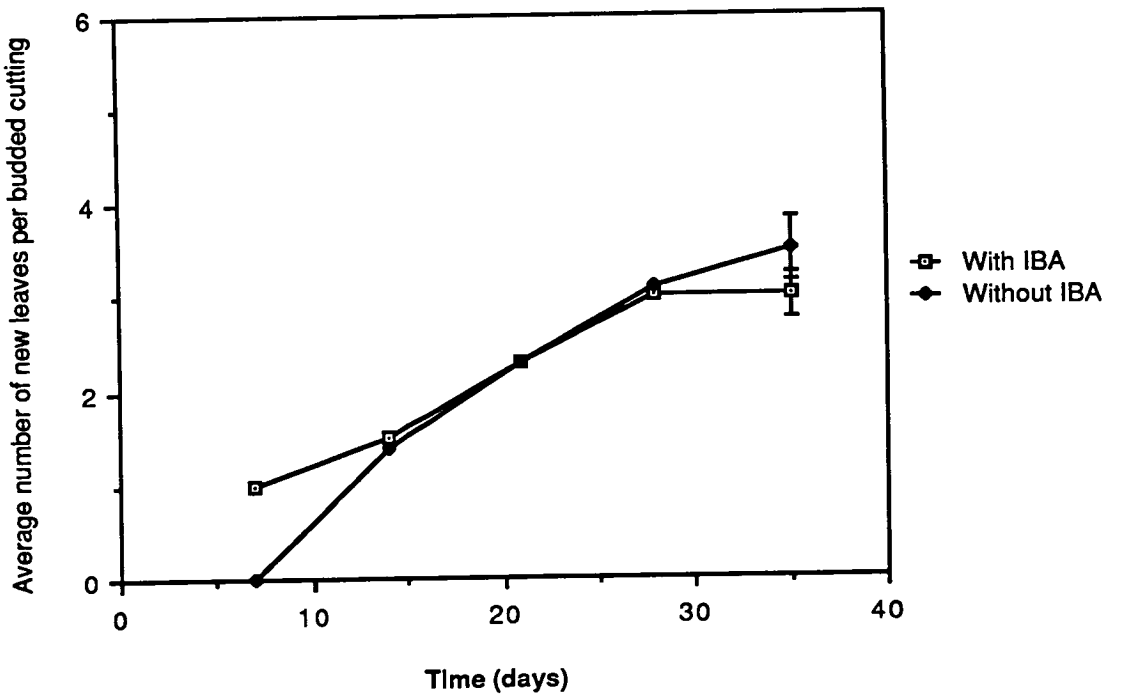

FIGURE 3.8b: Effects of IBA on the emergence of new leaves per budded cutting of *Nauclea diderrichii* over the period of 35 days. Bars show standard error of means.

Fresh weight (including leaf, buds and roots)

The mean fresh weight of cuttings during rooting was more or less constant, but after the third week an increase was observed (Figure 3.9).

3.3.2 Carbohydrates

The material was harvested at three developmental stages: (1) fresh cuttings; (2) formation of callus and root primordia; and (3) after root formation.

Total carbohydrate (without IBA)

In the leaves of the controls, there was a significant decrease in the total carbohydrate content at stage 2 and an increase after root formation at stage 3. In stems, the total carbohydrate content was more or less constant during the formation of callus and root initials, whereas a significant decrease occurred after root formation at stage 3.

However, in young buds a high level of total carbohydrate content was found at stage 3, with very high concentrations in the roots (Figure 3.10a).

Total carbohydrate (with IBA)

The general trends in the IBA-treated cuttings were like those in the controls except the IBA-treated individuals had a substantially lower concentration of carbohydrates in bud and root (18% versus 32% and 42% versus 47%; Figure 3.10b).

Starch content (Figure 3.11a)

Starch concentration was high in the leaf as well as in the stem at stage 1, but decreased significantly during the formation of callus and root initials at stage 2. No significant differences were found between treated and control cuttings in leaf and stem at this stage. But a gradual increase was found both in leaf and stem after root formation. In the leaf there was a significant difference between treated and control at this stage, whereas no difference was found in the stem. There was no difference either between second and third harvests of both treated and control in leaf tissue, whereas in stem a difference was found between second and third harvests only in treated cuttings.

Buds and roots of control plants always had high starch concentrations. In IBA-treated cuttings the starch concentration was reduced very substantially in the case of buds.

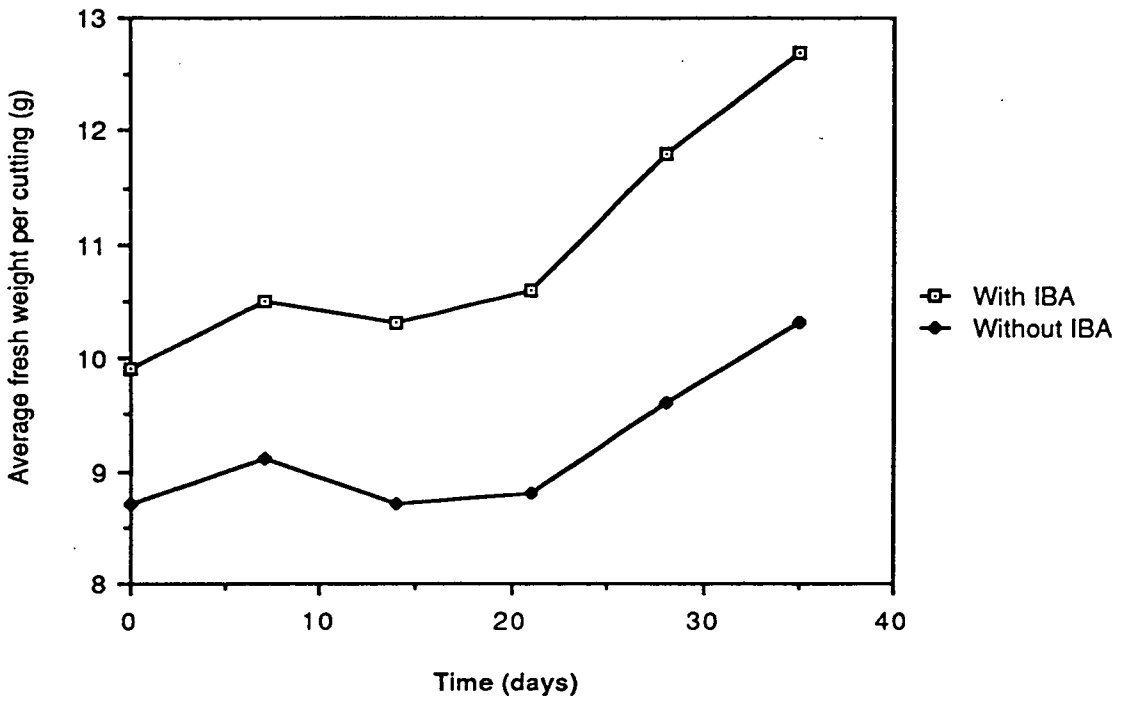

FIGURE 3.9: Mean effects of IBA on the trend of fresh weight of *Nauclea* cuttings during rooting over 35 days. Number of sample was 19, both in treated and control. Weighings were made using the same cuttings every week.

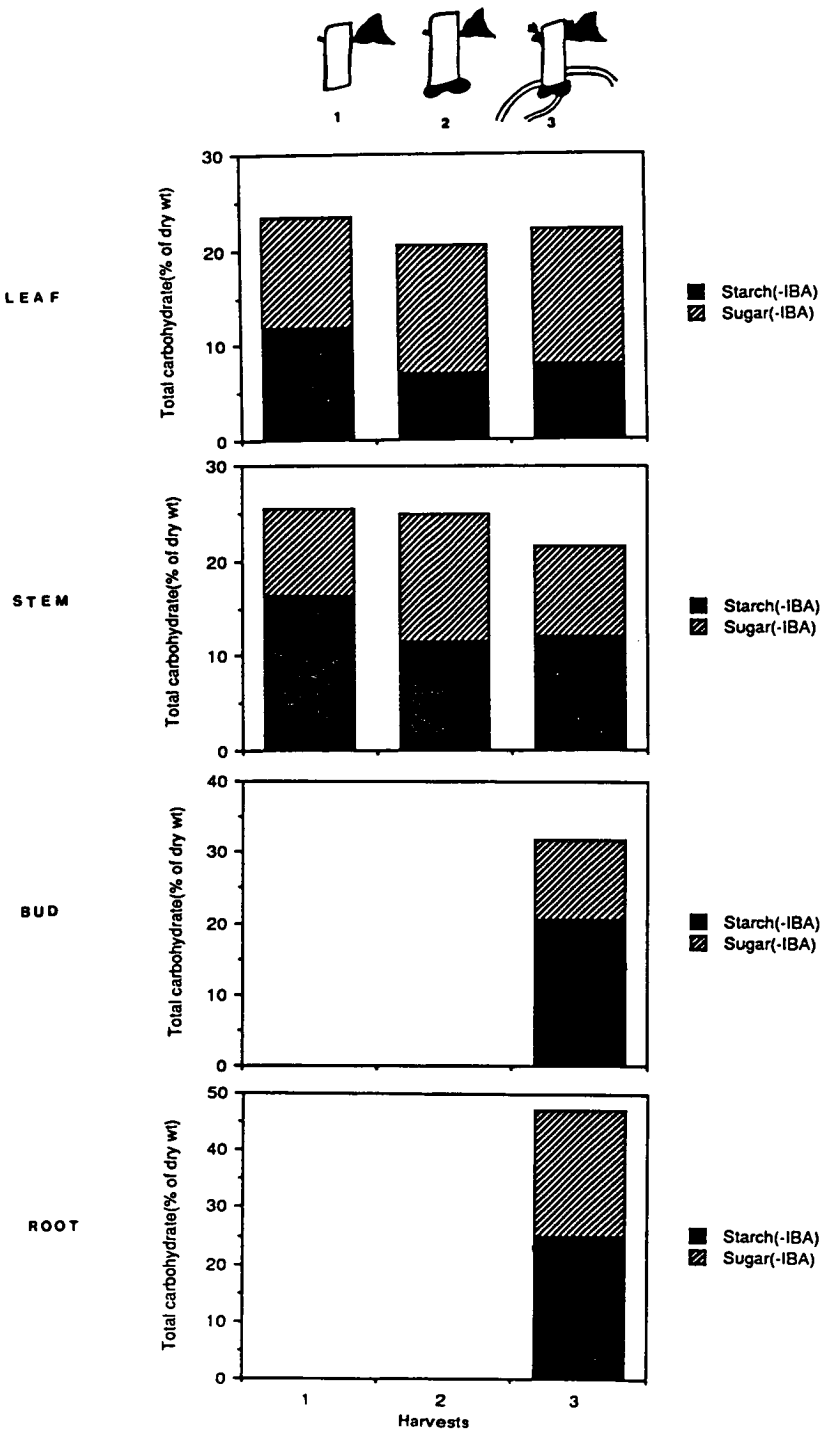

FIGURE 3.10a: Changing concentrations of total carbohydrate (% of dry weight) without IBA in leaf, stem, bud and root of leafy cuttings of *Nauclea diderrichii* during rooting.

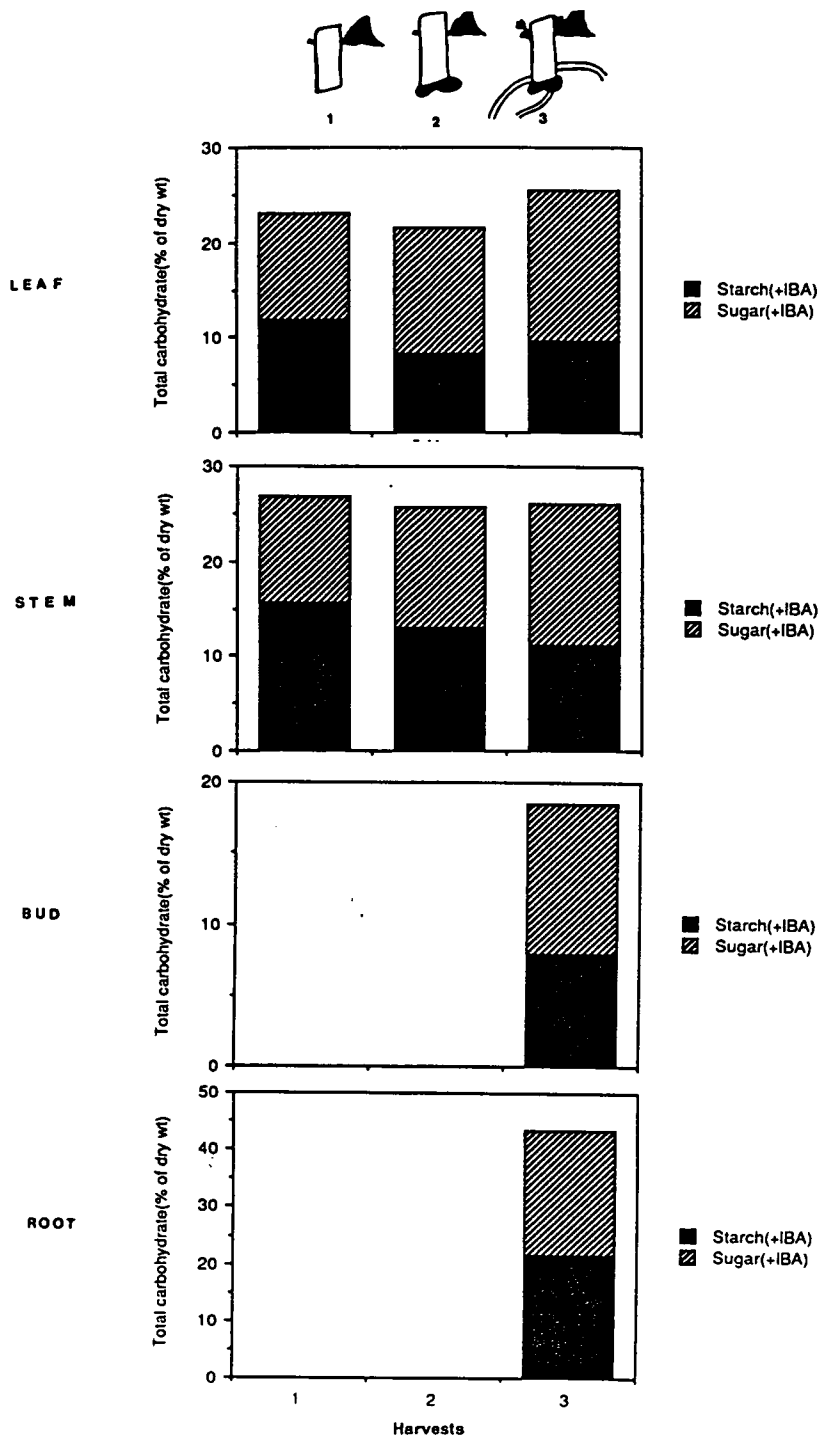

FIGURE 3.10b: Changing concentrations of total carbohydrate (% of dry weight) with IBA in leaf, stem, bud and root of leafy cuttings of *Nauclea diderrichii* during rooting.

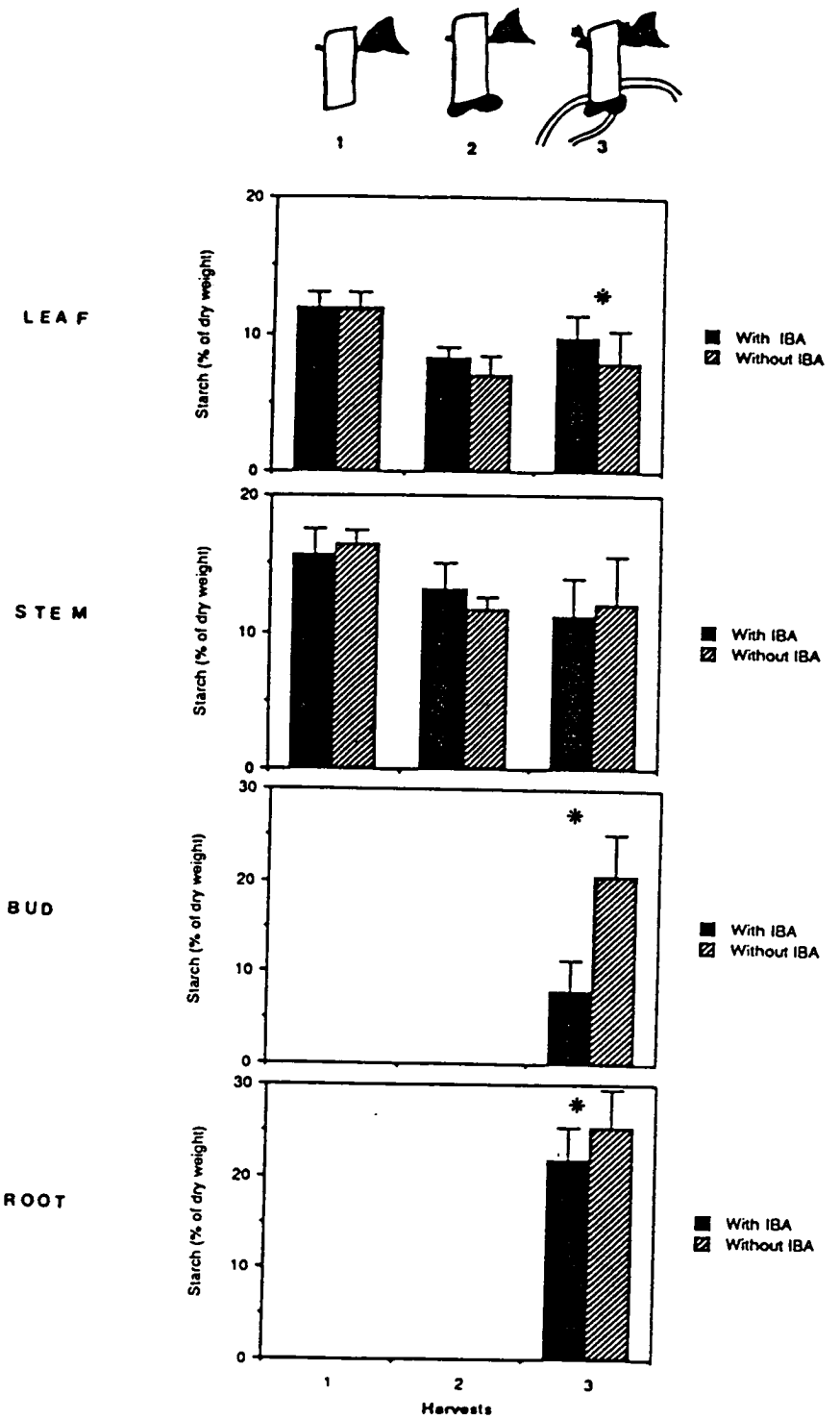

FIGURE 3.11a: Changing concentrations (% of dry weight) of starch with or without IBA in leaf, stem, bud and root of leafy cuttings of *Nauclea diderrichii* during root formation.
Bars indicate 95% confidence limit, and * signifies significant difference (p=0.05) as measured by the t-test.

Reducing sugar (Figure 3.11b)

In leaf, a gradual increase in reducing sugar concentrations was found in both cases over the entire period. But no differences were found between treated and control cuttings in any harvest. In the stem, a similar trend of concentrations was found in the treated cuttings, whereas in the control sugar increased significantly during the formation of callus and root initials then fell (harvest 3). No differences were found between treatment and control in the second harvest, whereas there were significant differences at the first and third harvests.

Buds displayed rather low concentrations of reducing sugars, but the young roots had much higher concentrations.

Non-reducing sugar

Inspection of the paper chromatogram revealed no sucrose in the extract of leaf, stem and root. Most of the total sugars appeared to be monosaccharide (Figure 3.11c). It was concluded that the quantitative analysis for reducing sugars and starch would be adequate for the present work, so no further analysis was carried out.

3.3.3 Photosynthesis

The relationship between photon flux density and rate of photosynthesis indicates considerable variation (as scatter Figure 3.12a). The rate of net photosynthesis increased with photon flux density in the range 0-200 μmol $m^{-2}s^{-1}$ (Figure 3.12a). There was considerable scatter in the relationship. All that can be said about the effect of the IBA treatment is that the respiration rates were somewhat more negative in IBA. As a result, the average light compensation point appears to be lower without IBA than with IBA. The diurnal trends in net photosynthesis are shown in Figure 3.12b. Rates become positive shortly after sunrise, and the variability is like that of photon flux density (Figure 2.2 and 2.3, Chapter 2). However, there is a suggestion of a reduction in the rate at about 14.00 hours. This might be caused by water stress at that time, but is more likely to be the result of CO_2 reduction, (Figure 2.2 and 2.3, Chapter 2).

An attempt to account for the variation in terms of CO_2 concentration and stage in development was not successful and is not described here. Instead, attention was concentrated on the daily totals of carbon gain, obtained by integration of the half-hourly rates.

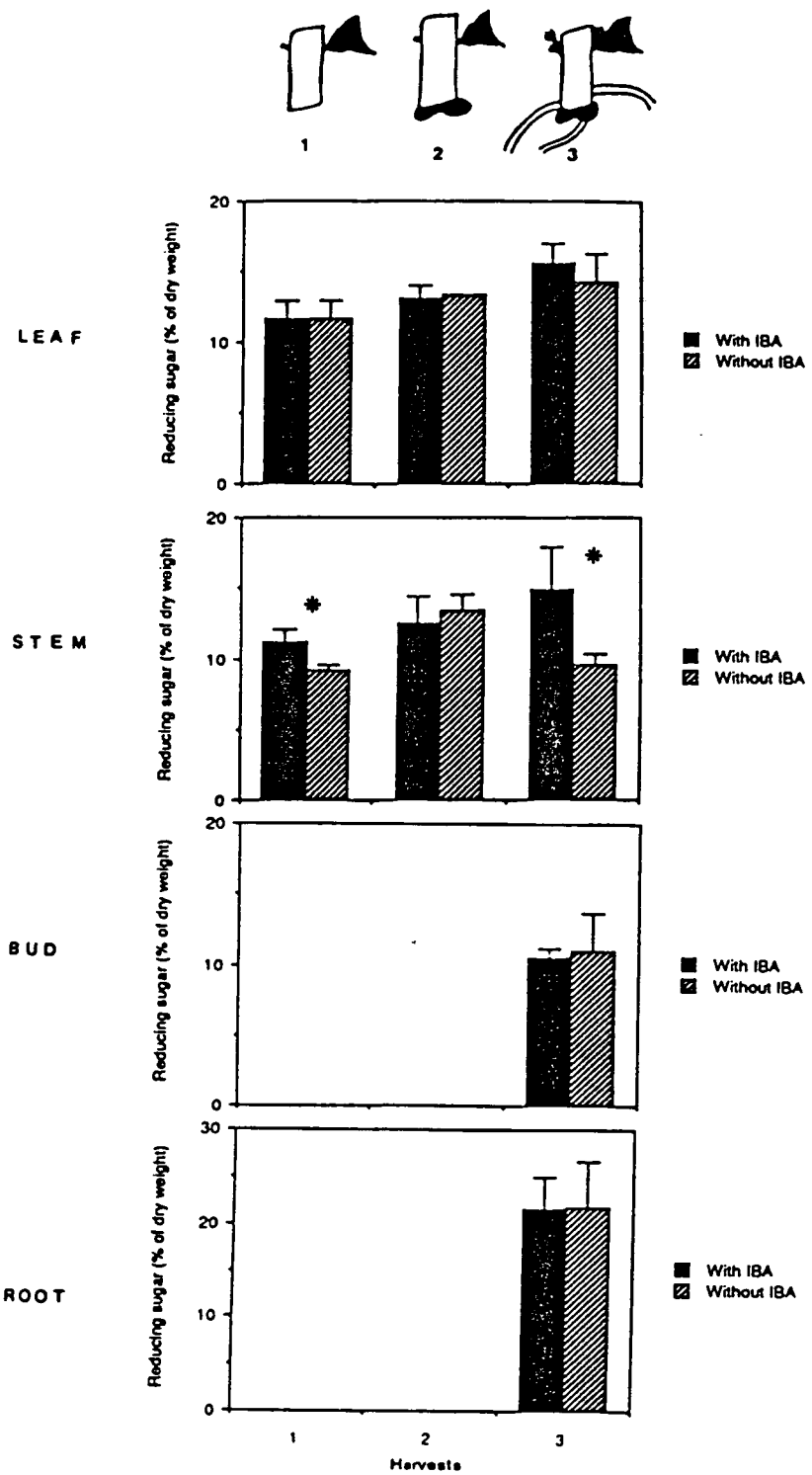

FIGURE 3.11b: Changing concentration (% of dry weight) of reducing sugar with or without IBA in leaf, stem, bud and root of leafy cuttings of *Nauclea diderrichii* during root formation.
Bars indicate 95% confidence limit, and * signifies significant difference (p=0.05) as measured by the t-test.

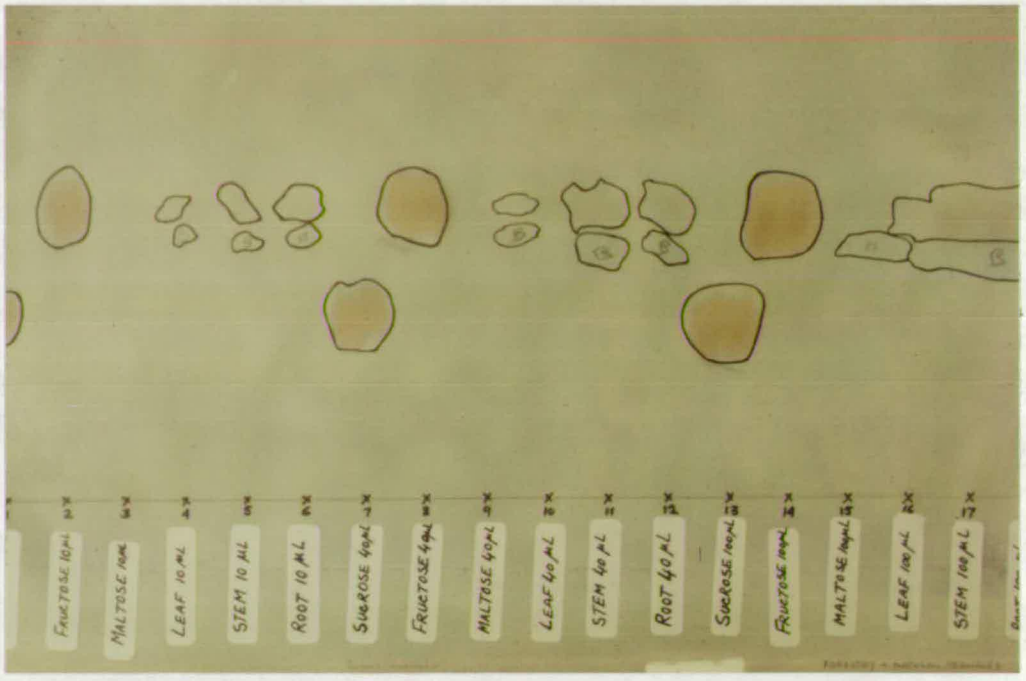

FIGURE 3.11c: Examination of the extract for sucrose, using paper chromatography. Most of the carbohydrate moves to the location of glucose/fructose, and sucrose is absent.

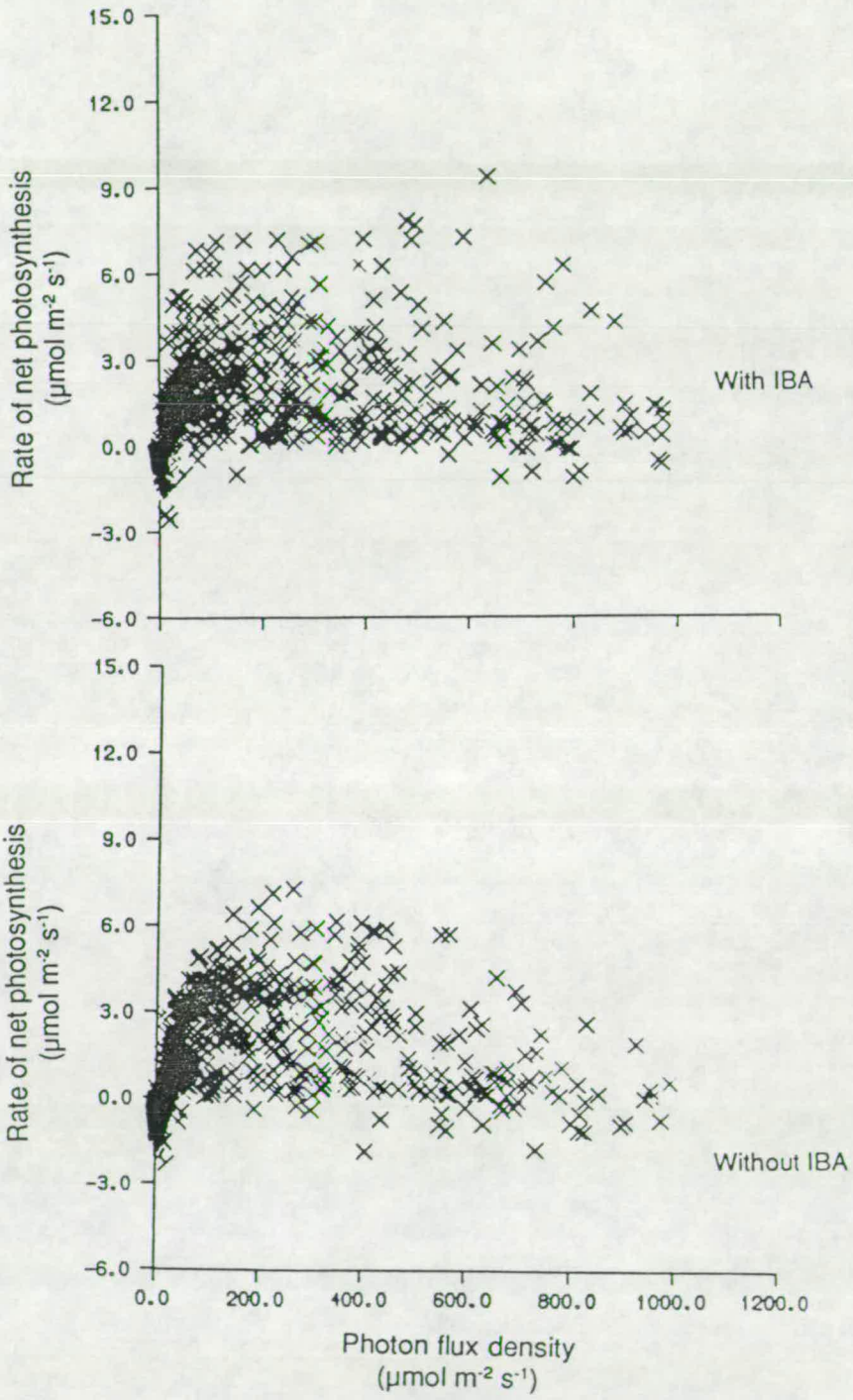

FIGURE 3.12a: Relationship between net photosynthesis and photon flux density, all hours of all days combined, treatments presented separately.

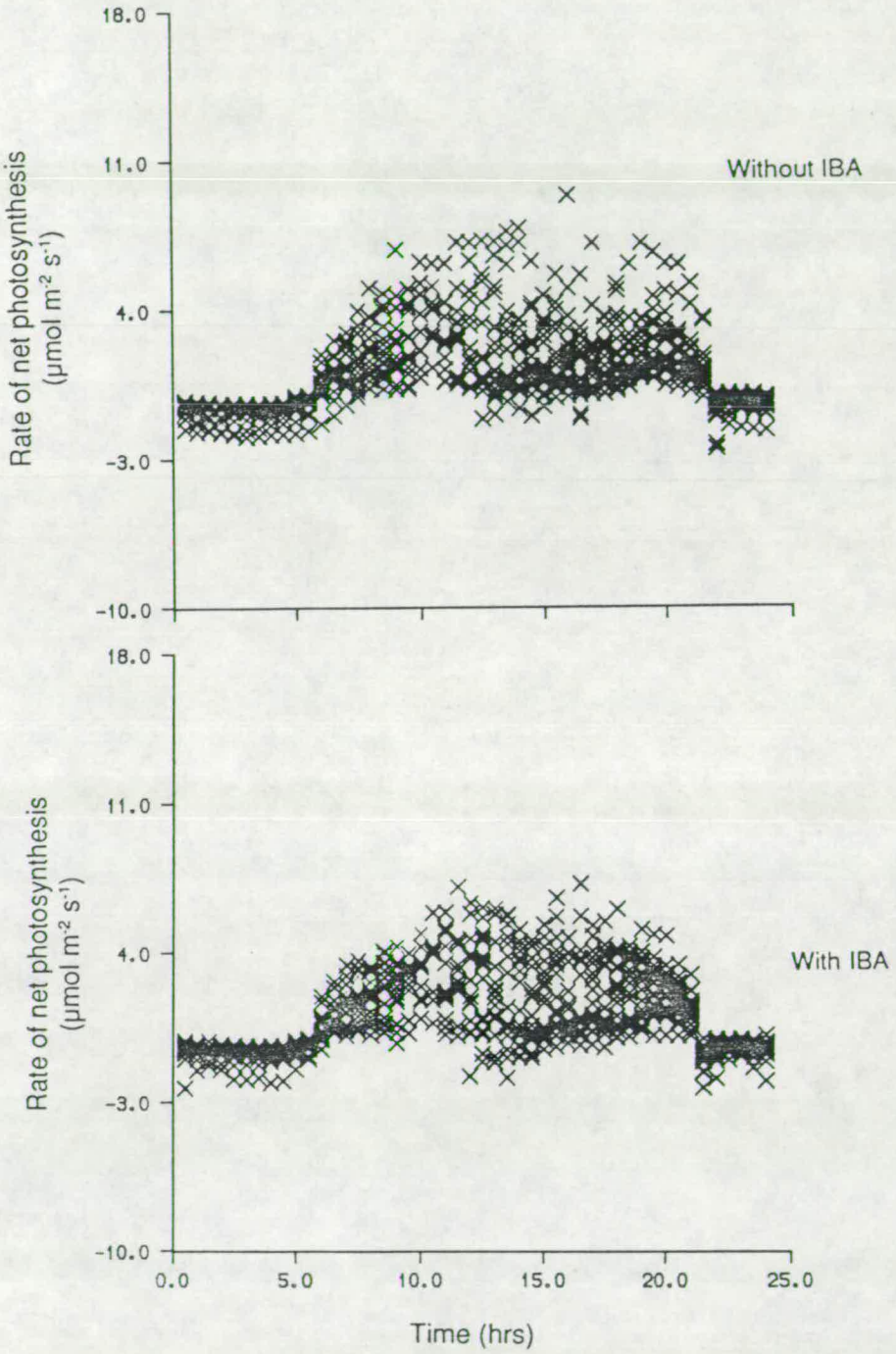

FIGURE 3.12b: The diurnal trends in net photosynthesis of the cuttings with or without IBA.

The daily totals of photosynthesis at the start of the experiment were low, sometimes not above zero (Figure 3.12c). There was an upward trend after twenty days, the trend resembling that of fresh weight (Figure 3.9), and this was associated with the development of a full complement of adventitious roots and new leaf area.

3.4 Discussion

It is clear that *Nauclea diderrichii* is easy to root. All cuttings rooted within 3 to 5 weeks under control conditions with or without IBA. The pattern of callus formation was considerably changed by applying IBA as has frequently been found for some other woody species: *Hedera helix* (Girouard, 1967); *Pinus radiata* (Cameron and Thomson, 1969); *Wrightia tinctoria* (Janardhanan and Lakshmanan, 1982). The callus seems to have originated from cortex and pith (Hartmann and Kester, 1975). The large amount of callus at the cut surface and the swollen basal end of the cuttings was probably accompanied by the accumulation of reserve nutrients, as suggested by the carbohydrate analysis where a significant increase of total carbohydrate was found in stems rather than leaves at harvest 2 (Figure 3.10a,b). This function of callus as storehouse of reserve nutrients was reported by Komissarov (1964) in common yew and Canadian hemlock. The percentage of cuttings rooted was considerably high in IBA-treated cuttings in different weeks, but at the end of the experiment, after 35 days, the overall percentage was similar to control. So the IBA treatment was not necessary for rooting in this type of easy to root species and no beneficial effect of the treatment on final rooting success could be established. Similar behaviour of IBA treatment has also been reported with some other tropical species, such as *Shorea macrophylla* (Lo, 1985) and *Terminalia superba* (Siaguru, 1986). However, number of roots per rooted cutting was increased and the rooting period was decreased by the application of IBA, also reported by others on different tropical species: *Triplochiton scleroxylon* (Leakey *et al.*, 1982a); *Dalbergia sisso* (Pain and Roy, 1981); *Tectona grandis* and *Tamarix* (Bhatnagar, 1974). The results of the present study broadly substantiate those obtained in *Nauclea* by Leakey (1989, in preparation) where 100% success in rooting was found within 2 to 4 weeks when different IBA concentrations and leaf areas were tested. Such treatments also affected the number of roots per cutting. The early rooting (2-4 versus 3-5 weeks) and the increased number of roots per cutting (29.1 versus 18) in his findings could be caused by slightly different conditions: he used intermittent mist.

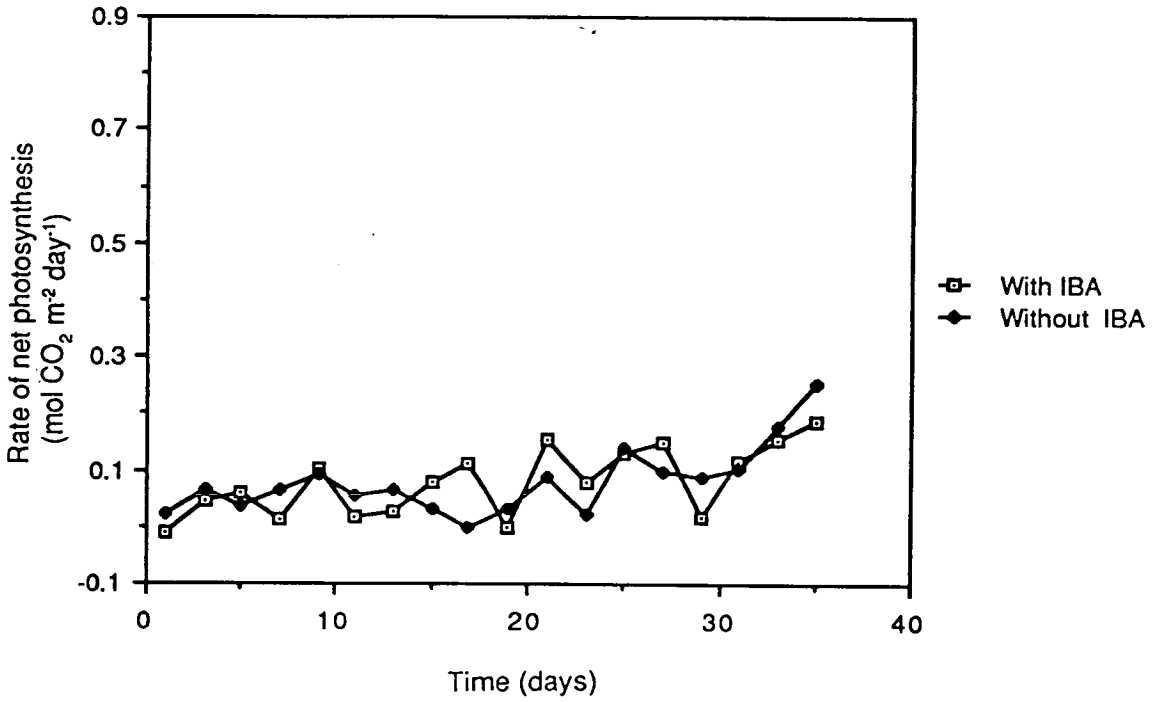

FIGURE 3.12c: Daily total rates of photosynthesis with or without IBA in rooting cuttings of a leafy soft wood cutting of *N. diderrichii.*

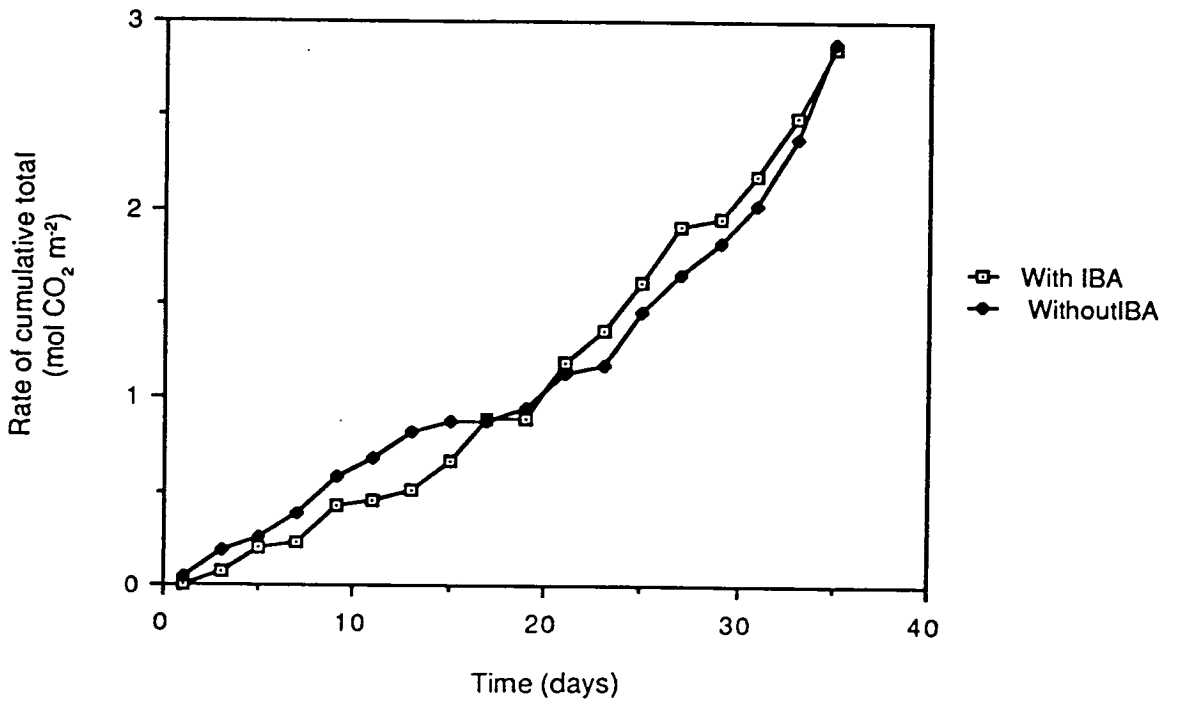

FIGURE 3.12d: The daily cumulative total of carbohydrates of *Nauclea* cuttings during rooting with or without IBA.

Light influences all aspects of growth, development and differentiation of plants (Furuya, 1968). Twenty-five and 15% cuttings showed root initials along the entire internodes of the treated and control individuals, respectively, but rooting took place only from the pale zone under the rooting medium. It might be that the levels of IAA and inhibitor ß are under light control and they may play an important role in the growth regulation of the stem (Tillberg, 1974). Moreover, the well-known inhibitory effect of auxin on root elongation was also observed in the present study (Aberg, 1957).

New buds and leaves began to emerge after one and two weeks in treated and control, respectively. As morphological changes occurred, physiological changes were soon observed. When the experiment started, the rate of net photosynthesis both in treated and control were very low, maintaining the carbon balance just above zero (Figure 3.12c). Negisi and Satoo (1955, 1956), Cameron and Rook (1974) and Machida *et al.* (1977) also observed this. The low rate could be partly caused by loss of chlorophyll (leaves became pale and a small percentage of leaves abscinded). However, the most probable cause of the initial low rate in the present study was water stress incurred as the potted plant was taken out from the propagator to be placed in the photosynthetic chamber. This was also found by Gay and Loach (1977) in some woody leaf cuttings, and Eliasson and Brunes (1980) in aspen and willow cuttings. In addition, detaching the shoot from the parent plant might cause a wound response, normally seen as enhanced respiration rates. An increased respiration rate would also result from mobilisation of reserves and formation of callus and initials. Thus, the net rate of photosynthesis would be expected to decline (Cameron and Rook, 1974).

However, the rates of net photosynthesis increased in both the treated and control as roots formed. This has been reported by Negisi and Satoo (1956), Humphries and Thorne (1964) and Okoro and Grace (1976). The increased rates after root formation in the current study was accompanied by slight regreening, associated with the emergence and expansion of new leaves from the new buds. Similar results have also been reported by Chibnall (1954): the development of a root system on detached bean leaves arrested the senescence phenomena of protein and chlorophyll degradation in the leaf lamina. Formation of roots help to absorb water to maintain a healthy water balance and replenish water lost in transpiration (Sands, 1984). Chibnall (1954) also reported that protein decreased in detached *Phaseolus* leaves but increased when roots form on the petioles. A positive correlation was found between the size of root system and the net photosynthesis with *Phaseolus vulgaris* (Humphries, 1963a). Furthermore, Sweet and Wareing (1966) reported with *Pinus radiata* that by removing the apices from shoots, the remaining leaves had a significantly lower rate of photosynthesis, but with observable

new growth occurring photosynthesis increased again. It is therefore clear that some internal factor in the root system is controlling the assimilation rate. Humphries (1963a) believed that the factor is the rate of translocation of carbohydrate from source to sink which in turn depends on growth rate of the root system. However, recently Wareing (1978) concluded that IAA and cytokinins promote both acropetal and basipetal transport by effects produced at the site of application, and the supply of assimilates to a sink involves their transfer from the phloem to the sink tissue via the apoplast (Glasziou and Gayler, 1972).

As the rate of net photosynthesis was fairly high after the formation of roots, so an accumulation of carbohydrate is expected. Both in the treated and control, the daily cumulative balance was positive and increased consistently (Figure 3.12d) with a final gain of carbohydrates. Table 3.3 shows the mean dry weight (g) per standard cutting in different harvests. In harvest 1, the mean dry weight was 2.5 g and 1.7 g in treated and control, respectively, whereas in the final harvest the weights were 2.8 g and 2.17 g in the treated and control, respectively. In both the initial and final harvests, the differences of weights between treated and control remain more or less the same (0.7 g and 0.6 g). The net gains of dry weights after 25 days (harvest 3) were 0.30 and 0.38 g in treated and controls, respectively. The mean rate of net photosynthesis at the end of experiment (35 days) was 0.08 mol $m^{-2}d^{-1}$ in both the treated and control (Figure 3.12c). By calculation from the rate, the net gain of the carbon intake was 0.44 g in both the treated and control (see calculations in Appendix 3). The cumulative value at the end of experiment (35 days) was 2.75 mol CO_2 m^{-2} which is equivalent to 0.6 g carbon intake. The increased values for rate and cumulative total from the dry weight is due to the fact that for dry weights, the plants were collected after 25 days (harvest 3), whereas in the rate and cumulative total the values were calculated after 35 days (Figure 3.12c,d).

However, the above calculation was made on the basis of old attached leaf (50 cm^2) but in fact there was an addition of new leaf areas on each cutting (on average 3 small leaves, Table 3.1) during the propagation. So an increased dry weight might have been expected but practically it did not happen. Presumably, the production of new photosynthates was contributed only by the old foliage and the contribution of the new foliage was used up for the terminal shoot growth, as has also been reported by Cameron and Rook (1974) with the cuttings of *Pinus radiata*. Generally speaking, young leaves do not become net contributors to the carbon balance until they have fully expanded.

The changes that occurred in the rate of net photosynthesis were confirmed by the chemical analysis of the plant materials. The initial concentration of total carbohydrate

was more or less similar both in the leaf and stem of both treatment and control. In harvest 2, a significant depletion of total carbohydrate occurred as the respiration was high and rate was low. The decrease occurred in the starch part, not the sugar which was more or less constant. As the callus formation and root initiation require much energy, so there was high carbohydrate demand and starch probably acted as the prime and possibly the sole carbohydrate source for root primordia initiation and development. Therefore, no accumulation of reserve occurred. Moreover, there was mobilisation of starch (harvest 2, Figure 3.11a,b). A considerable body of literature supports the most common findings of starch depletion during root primordia initiation and development (Alexander, 1938; Stuart, 1938; Smith *et al.*, 1940; Negisi and Satoo, 1956; Nanda *et al.*, 1970).

TABLE 3.3: Mean dry weights (g) per standard cutting in different harvests and values estimated from CO_2 uptake (Figure 3.12d).

		From carbohydrate values		From CO_2 uptake	
Harvests	Time (days)	With IBA	Without IBA	With IBA	Without IBA
1	0	2.5006	1.7853	2.5006	1.7853
2	8	2.5198	1.7983	2.5906	1.8953
3	25	2.8020	2.1701	2.8526	2.1153

However, after the formation of roots (harvest 3) as the rate of net photosynthesis increased, the total carbohydrate increased more evidently in the leaves of both the treated and control. Most commonly, this increase occurred in the sugar part, perhaps due to starch hydrolysis or due to the addition of new photosynthesis by positive net photosynthesis (Figures 3.10a,b, 3.12c), as has also been reported by Stuart (1938) and Hilton (1966). In the present study, IBA seems to have stimulated the starch-sugar conversion (Figure 3.10a,b) as reported with tomato leaves by Borthwick *et al.* (1937). The total carbohydrate increased in the stem and decreased significantly in buds (18.5%) of the treated cuttings, whereas in controls it decreased in the stem (21.5%) but increased in the bud. This indicates one of the pronounced features, the marked downward transport of carbohydrate evident in the softwood cuttings and as accumulation in new roots as starch in both the treated and control. A similar instance of starch deposition in root caps of primordia of tomato cuttings resulted from the treatment with the IAA or ß Napthoxyacetic acid (Bausor, 1942). It seems that the translocated carbohydrate in the

treatment was imported from the newly formed buds and in the controls from the old leaves and stem. The strong basipetal transport of assimilates after root formation has also been reported with other species: in cotton plant (Mason and Maskell, 1928a,b); plum (*Prunus cersifera* x *Prunus munsoniana*) (Breen and Muraoka, 1974); *Populus euramericana* (Okoro and Grace, 1976). The sugar level was constant but starch decreased with small significant difference in those treated with IBA as roots developed.

In the final harvest (harvest 3), the higher accumulation of total carbohydrate in the roots of both the treated and control plant would have increased the natural C/N ratio at the base, a feature often associated with rooting activity (Kraus and Kraybill, 1918; Mahlstede and Haber, 1957; Hyun, 1967; Okoro and Grace, 1976).

CHAPTER 4

EXPERIMENT 2:

Influence of light to stock plants on rooting of leafy single node stem cuttings of *Nauclea diderrichii*

CHAPTER 4

EXPERIMENT 2: Influence of light to stock plants on rooting of leafy single node stem cuttings of *Nauclea diderrichii*

4.1 INTRODUCTION

It has been observed in many species that cuttings taken from stock plants grown at low irradiance produced more roots than those taken from the stock plants grown at high irradiance. Examples include: *Pisum sativum* (Hansen and Eriksen, 1974); *Hedera helix* (Poulson and Andersen, 1980), *Pinus sylvestris* (Stromquist and Hansen, 1980) and *Picea sitchensis* (Van den Driessche, 1985). In contrast, in *Chrysanthemum morifolium* (Fischer and Hansen, 1977) the number of roots increased with an increase of irradiance. In other cases, the presence of light not only to the stock plants but also to the stem part from which roots are formed, generally the bases of cuttings, caused fewer roots to form: *Picea abies* (Strömquist and Eliasson, 1979); *Pisum sativum* (Eliasson, 1980). Shorter photoperiod during stock plant growth resulted in an increase in the number of roots as well as the percentage of rooting, e.g. *Ilex crenata* (Kelley, 1965) and *Pinus sylvestris* (Hansen and Ernstsen, 1982). Moshkov and Kocherzhenko (1939) reported that among three *Salix* species, *Salix pierotti* cuttings showed the similar result with short day conditions whereas *Salix undulata* and *Salix babylonica* cuttings showed better rooting when cuttings were collected from stock plants under long days.

It is also reported that etiolation has enhanced the rooting potential of many plants (Smith, 1924; Gardner, 1937; Sachs *et al.*, 1964). Stooling, air-layering, trench layering, etc, all appear to work by this principle.

There is little information in the literature on the effect of light on stock plants or the basis of rooting of tropical trees during adventitious root formation. Leakey *et al.* (1982a) reported that in *Triplochiton scleroxylon*, leaves (50 cm^2) covered by aluminium foil rooted better than cuttings with fully exposed leaves. Boulay (1984) explained with *Eucalyptus* that a dark period is used during root initiation because it has a positive effect on root primordia formation.

The purpose of this experiment was to see whether the light régime given to the stock plants of *Nauclea diderrichii* affected the rooting success. The 'shade' treatment was designed to reduce the carbohydrate reserves to a 'starvation' level, to see whether the cutting was capable of recovering from this. Carbohydrate concentrations and rates of photosynthesis were measured during the rooting period.

4.2 MATERIALS AND METHODS

These were the same as in the previous chapter. The experiment was conducted in the period from 20 July to 23 August 1988.

Low and high irradiance treatments are subsequently referred to a 'shade' and 'light'.

4.3 RESULTS

4.3.1 Rooting

Callus formation: Callus first appeared on the 3rd day in the cuttings taken from plants grown both in light and shade. Callus formed normally at the cut end surface but in shade it was thicker and more extensive than in the light treatment (Figure 4.1b).

Formation of root initials: The visible root initials first appeared on the 5th and 7th day on the cuttings, taken from light and shade respectively (Figure 4.2a,b). Production of root initials was finally higher in shade than light (Figure 4.2b).

Roots from wound: First roots appeared after the first week on cuttings taken from plants grown in light whereas in shade, after the second week, roots originated from the margin of the obliquely cut surface of all the cuttings both in the light and shade. After the first week, 4% of cuttings rooted with one root per cutting in light whereas no roots formed on cuttings from shade at that time. After the second week, 46% of cuttings from light rooted with 6 roots per cutting whereas in shade 35% cuttings rooted with 5 roots per cutting. After the third week, the rooting of shade cuttings went up by 8% (Figure 4.4a). However, all cuttings rooted after the 4th week in both the cuttings from light and shade. More root formation took place per cutting in both the treatments between day 14 and day 21. After 5 weeks, at the end of the experiment, the average number of roots per cutting was 15.0 and 19.9 with an average length of the longest root 7.5 and 8.2 cm in light and shade respectively (Figure 4.4c). There were significant differences between the number of roots per rooted cutting (Figure 4.4b), but not in the length of the roots.

Roots from the stem surface: Four per cent of the cuttings from both the treatments shared root initials along the whole internode. Roots appeared only from the pale basal zone and this did not reduce the normal root production from the wound. An average of 3 or 4 roots were formed from the pale zone in the light or shade respectively.

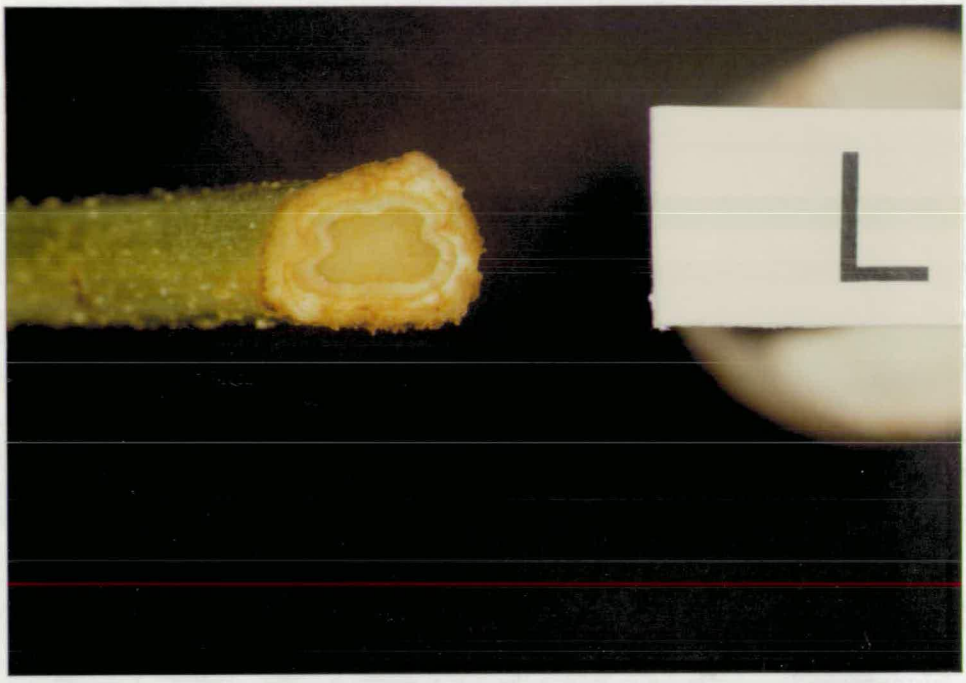

FIGURE 4.1a: Callus formation at the cut end surface of the *Nauclea* cuttings from plants grown in light.

FIGURE 4.1b: Callus formation at the cut end surface of the *Nauclea* cuttings from plants grown in shade.

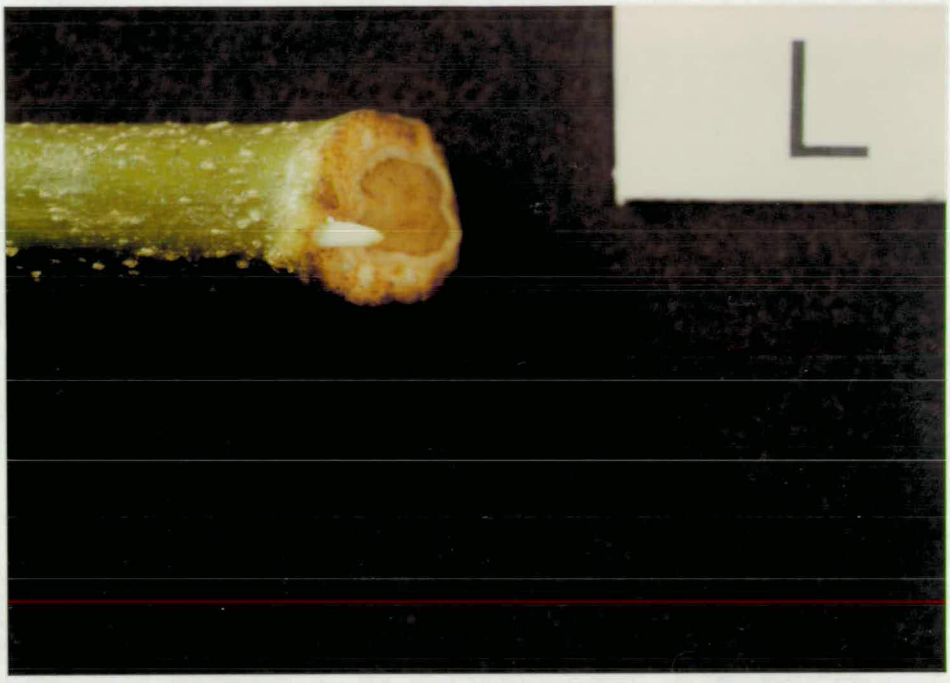

FIGURE 4.2a: Formation of root initials over the internodes and a young root from visible root initials on the circumference of the cut surface of the light originated cuttings (L).

FIGURE 4.2b: Formation of more young roots from more visible root initials on the circumference of the cut surface of the shade originated cuttings (S).

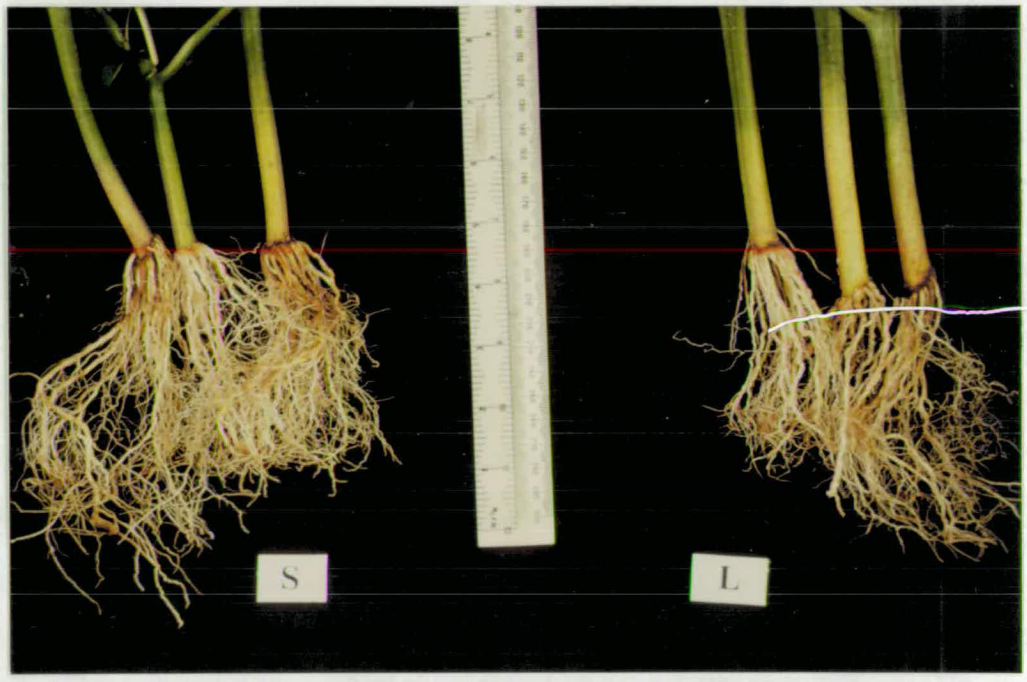

FIGURE 4.3: More root formation on the cuttings taken from plants grown in shade (S) than light (L). Note there was no significant difference between root lengths in light and shade.

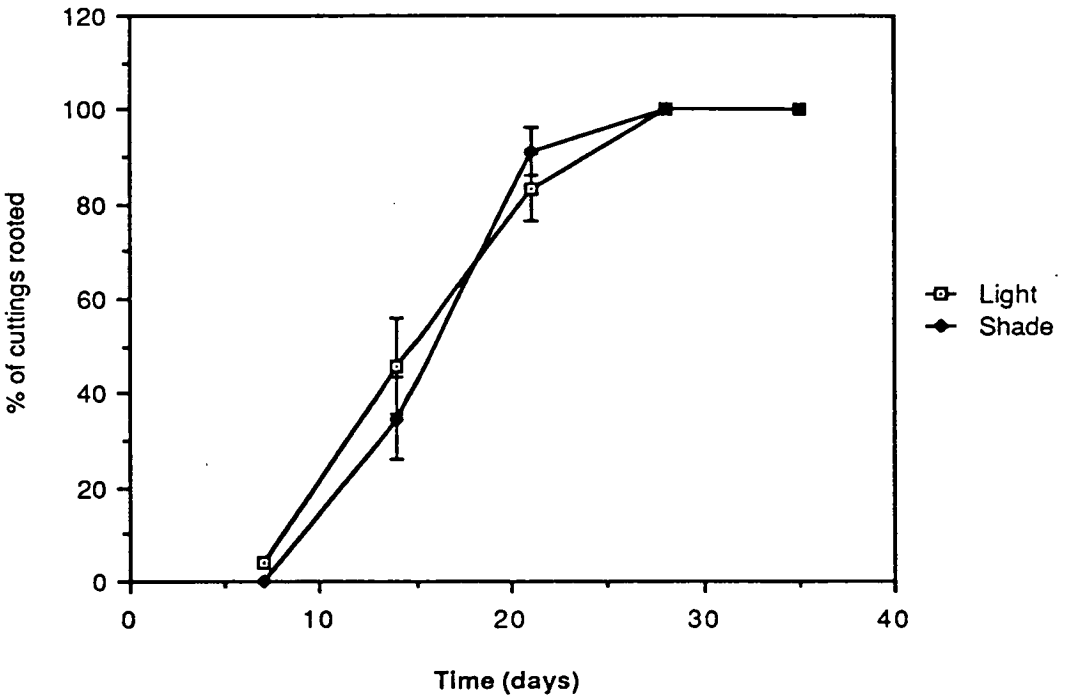

FIGURE 4.4a: Effects of light and shade on rooting of leafy single node cuttings of *Nauclea diderrichii* over a period of 35 days. Bars show standard error of the means. Total number of cuttings was 24 in light and 23 in shade.

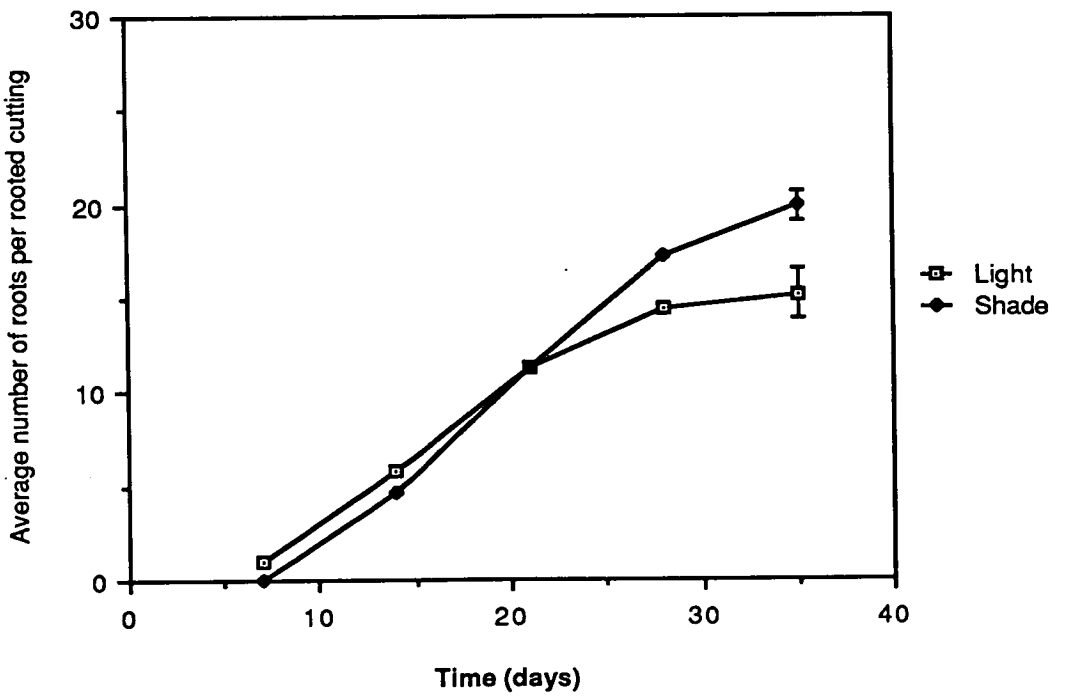

FIGURE 4.4b: Effects of light and shade on the number of roots per rooted cutting of *Nauclea diderrichii* over the period of 35 days. Bars show standard error of means.

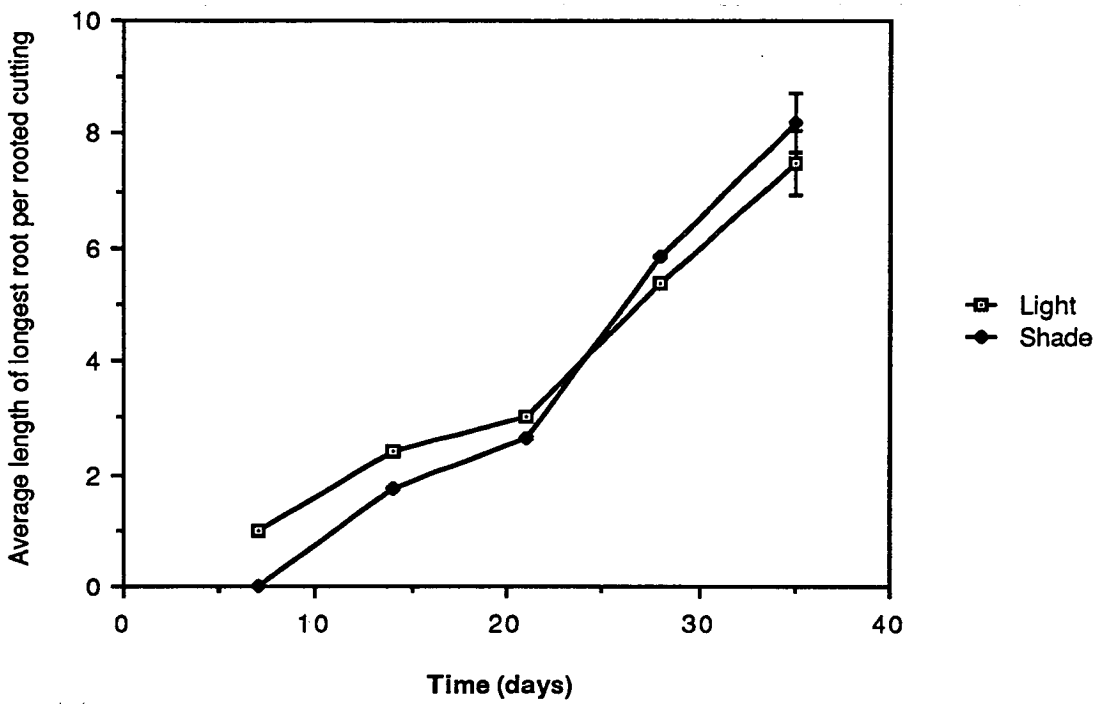

FIGURE 4.4c: Effect of light and shade on the length of root per rooted cutting of *Nauclea diderrichii*. Bars show standard error of means.

Bud formation: New axillary buds formed after the first week both in the light and shade. There was significant differences in the percentage of cuttings forming buds between light and shade in each week, but after the 4th week both the treatments showed 100% budding (Figure 4.5). However, no differences were found in the average number of buds formed on each budded cutting (Table 4.1).

New leaves from buds: New leaves first emerged in shade after the first week whereas in light after the second week. There was significant differences after the second week in the percentage of cuttings forming leaves whereas after 5 weeks no differences were found (Figure 4.6a). On the other hand, average number of new leaves per budded cutting showed significant differences in different weeks as well as at the end of the experiment (Figure 4.6b). About 4% cuttings both in light and shade showed abscission among the old leaves. The colour of the leaves in light was pale-green but after root formation became deep green whereas, in shade, leaves were deep green both before and after the root emergence.

Fresh weight: Mean fresh weight of the cuttings including leaves, roots and buds, were more or less constant but after the 3rd week an increase was observed (Figure 4.7).

4.3.2 Carbohydrates

The material was harvested at three development stages: 1, fresh cuttings (day-0); 2, formation of callus and root primordia (day-8); and 3, after root formation (day-25).

Total carbohydrate: (Figure 4.8a,b)

Leaf: A higher concentration of total carbohydrate was found initially both in leaf and stem of the cuttings taken from plants grown in light than in shade. The level increased significantly both in light and shade after the formation of root primordia (harvest 2) and was maintained up to the formation of new roots (harvest 3) in light, whereas it decreased significantly in shade.

Stem: There was also a higher initial concentration of total carbohydrate in light than shade. In light, the level was maintained more or less constant throughout the rooting period whereas, in shade, a gradual increase was observed after the formation of root primordia (harvest 2) and maintained to the formation of new roots (harvest 3).

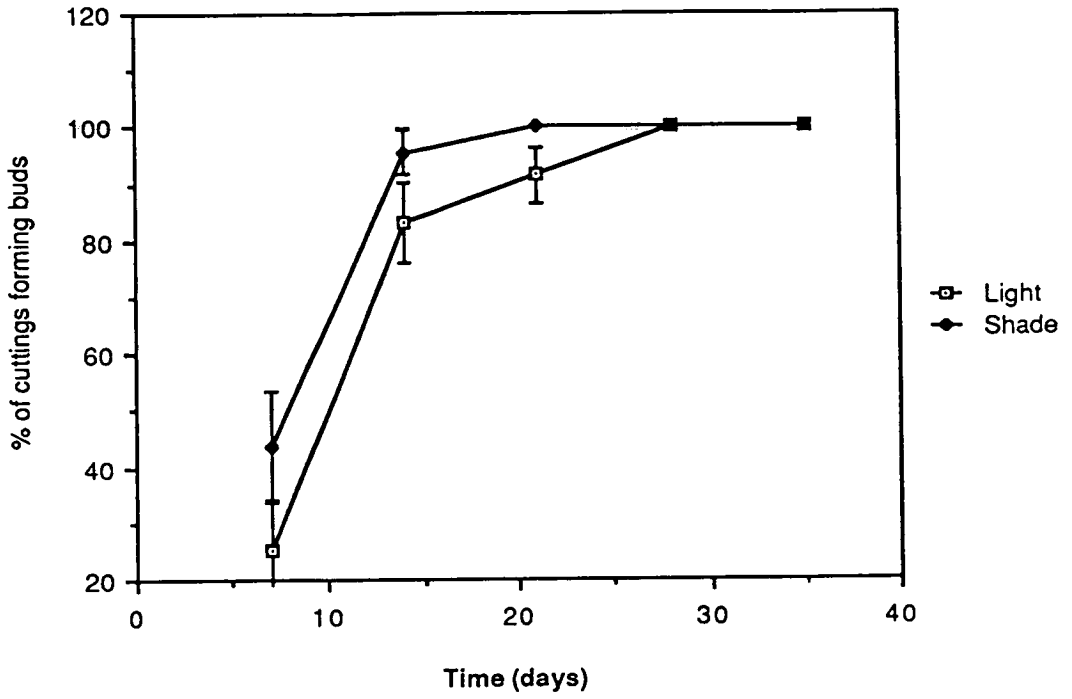

FIGURE 4.5: Effects of light and shade on bud formation of leafy single node cuttings of *N. diderrichii* over 35 days. Bars show standard error of means.

TABLE 4.1: Average number of buds formed per budded cutting.

Time (days)	Light	Shade
7	1.8	2.0
14	2.0	2.0
21	1.9	2.0
28	1.9	2.0
35	1.9	2.0

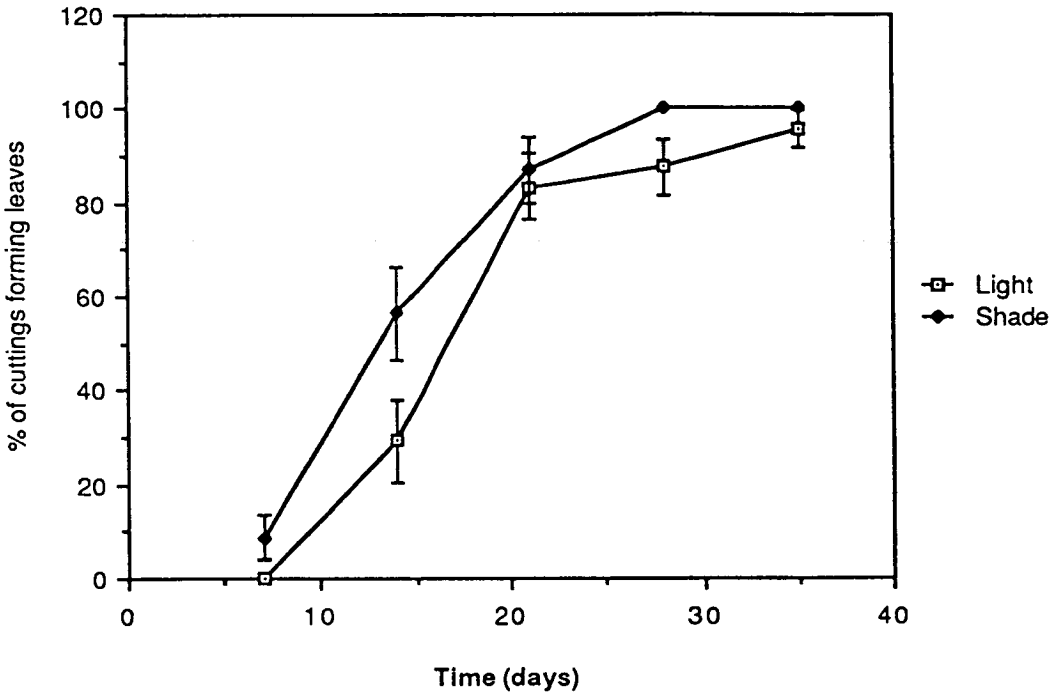

FIGURE 4.6a: Effects of light and shade on new leaf formation of leafy single node cuttings of *N. diderrichii* over 35 days. Bars show standard error of means.

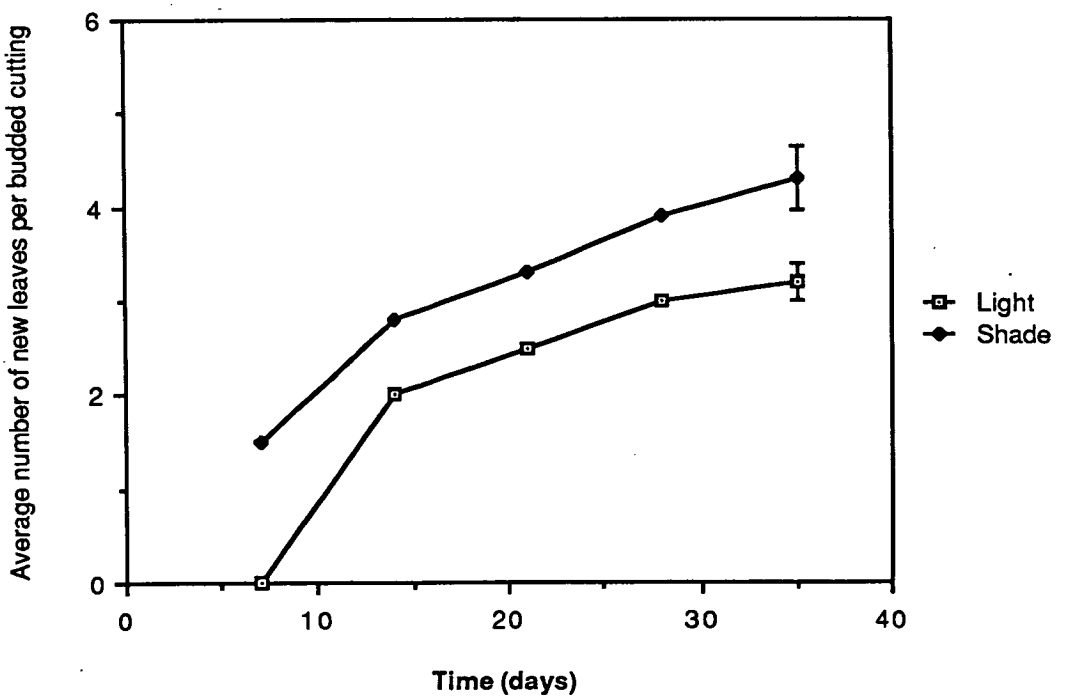

FIGURE 4.6b: Effects of light and shade on the emergence of new leaves per budded cutting of *N. diderrichii* over 35 days. Bars indicate standard error of means.

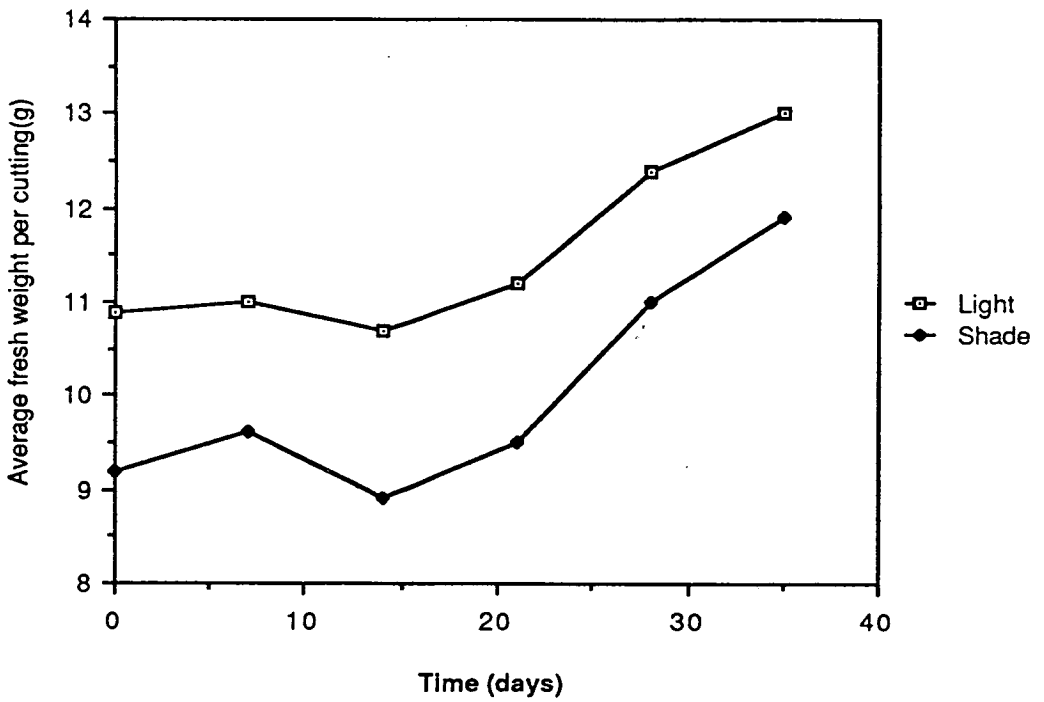

FIGURE 4.7: Mean effects of light and shade on the trend of fresh weight of *Nauclea* cuttings during rooting over 35 days. Number of cuttings was 24 and 23 in light and shade respectively. Weights were recorded using the same cuttings every week.

TABLE 4.2: Rooting success and morphological changes on different characteristics of leafy single node stem cuttings of *Nauclea diderrichii* taken from stock plants grown in light and shade.

1.	Time of observations	1st week	2nd week	3rd week	4th week	5th week
2.	Month of cuttings planted	July				
3.	Amount of auxin used/cutting	-	-			
4.	No. of cuttings planted:					
	light	24	24	24	24	24
	shade	23	23	23	23	23
5.	No. of cuttings callus formed					
	light	24	24	24	24	24
	shade	23	23	23	23	23
6.	No. of cuttings rooted					
	light	1	11	20	24	24
	shade	-	8	21	23	23
7.	% of cuttings rooted					
	light	4.2	45.8	83.8	100	100
	shade	-	34.8	91.3	100	100
8.	Average no. of roots formed per rooted cutting					
	light	1	5.8	11.3	14.4	15.2
	shade	-	4.7	11.3	17.3	19.9
9.	Total no. of roots formed					
	light	1	64	226	346	366
	shade	-	38	239	398	457
10.	Av. length of the longest root per cutting (cm)					
	light	-				7.5
	shade	-				8.2
11.	No. of cuttings buds formed					
	light	6	20	22	24	24
	shade	10	22	23	23	23
12.	Total no. of buds formed					
	light	11	40	43	45	45
	shade	20	44	46	46	48
13.	No. of cuttings leaves formed					
	light	-	8	20	21	23
	shade	2	13	20	23	23
14.	Total no. of leaves formed					
	light	-	16	50	65	74
	shade	3	37	66	91	99
15.	No. of cuttings leaves/shoot dried					
	light	-	-	-	-	-
	shade	-	1	1	1	1
16.	Mean fresh weights (g) of cuttings (including stem, buds, leaves and roots)					
	light	(10.9) 11.0	10.7	11.2	12.4	13.0
	shade	(9.2) 9.6	8.9	9.5	11.0	11.9

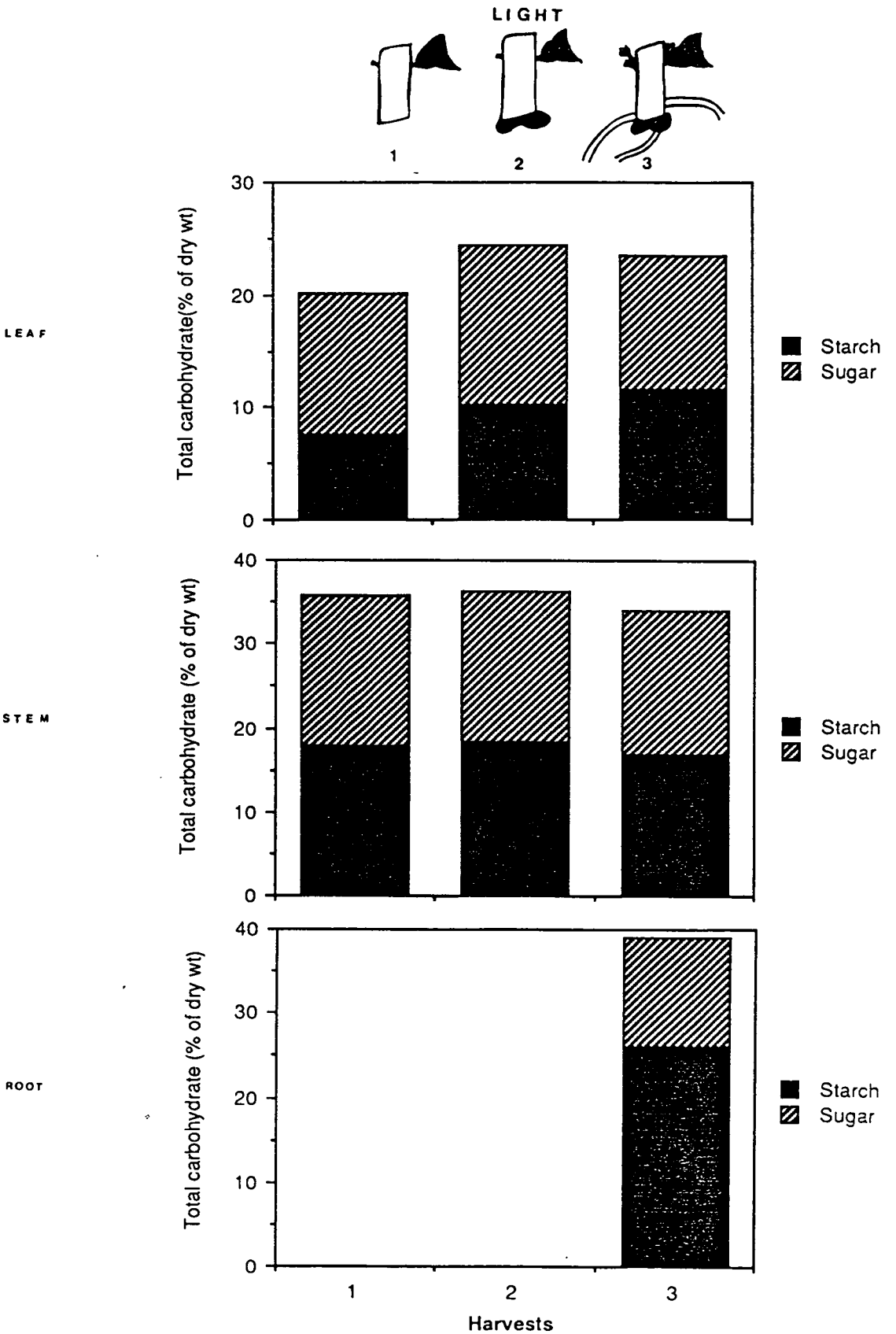

FIGURE 4.8a Changing concentrations of total carbohydrate (% of dry weight) in leaf, stem and root of the cuttings of *Nauclea* plants grown in light.

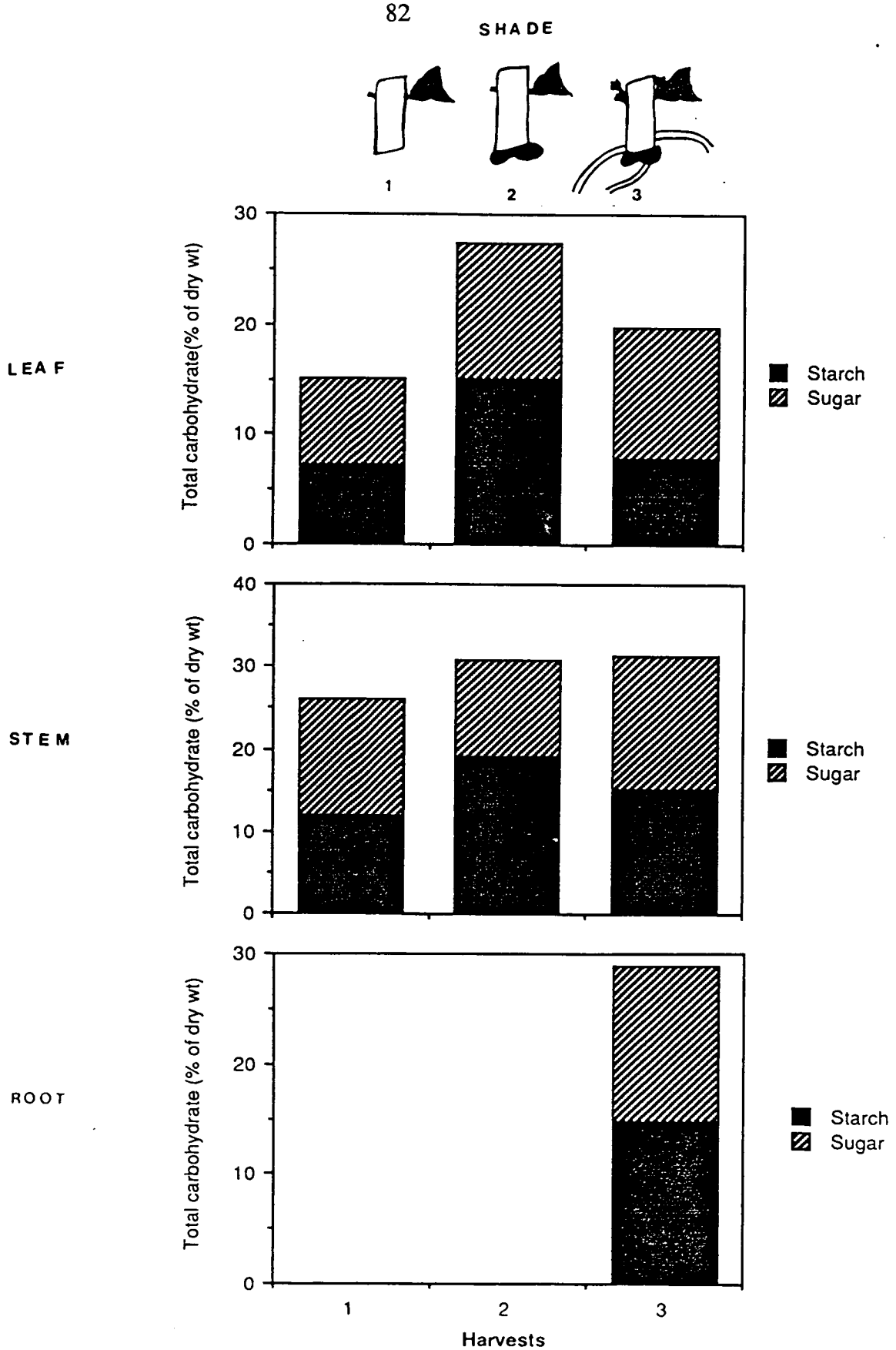

FIGURE 4.8b: Changing concentrations of total carbohydrate (% of dry weight) in leaf, stem and root of the cuttings of *Nauclea* plants grown in shade.

Root: In new roots, a high level of total carbohydrate content was found, especially as starch.

Starch: (Figure 4.9a).

Leaf: A gradual increase in starch level was observed over the entire rooting period in the cuttings from plants grown in light. In those from shade, a sharp increase was observed after the formation of root primordia (harvest 2) but declined later after new root formation (harvest 3). However, significant differences between the treatment and control were observed at harvests 2 and 3.

Stem: A higher starch content was found in the cuttings from plants grown in light and the concentration remained constant throughout the rooting period. In contrast, in shade, the changing pattern of starch concentration was very similar to that of leaf over different harvests. Significant differences were found between the treatment and control at harvests 2 and 3.

Roots: There were very high starch concentrations in the roots of cuttings from plants grown in light whereas the cuttings taken from plants grown in shade had only half as much.

Reducing sugar: (Figure 4.9b).

Leaf: A significantly higher initial level of reducing sugar was found in the cuttings taken from plants grown in light than shade and the concentrations remained more or less constant other than a slight rise after formation of root primordia. In shade, a gradual increase of reducing sugar concentrations was observed over the entire rooting period but significant differences were observed at harvests 1 and 2.

Stem: The initial concentrations were much higher in stem than leaf of both the light and shade treatments. In light, the concentration remained constant over the entire rooting period whereas in shade, there was significant depletion after the formation of root primordia and a rise after the formation of new roots (harvest 3). Significant differences between the treatments in both harvests 1 and 2 were observed.

Roots: New roots showed rather low concentrations of reducing sugar and no significant differences between light and shade.

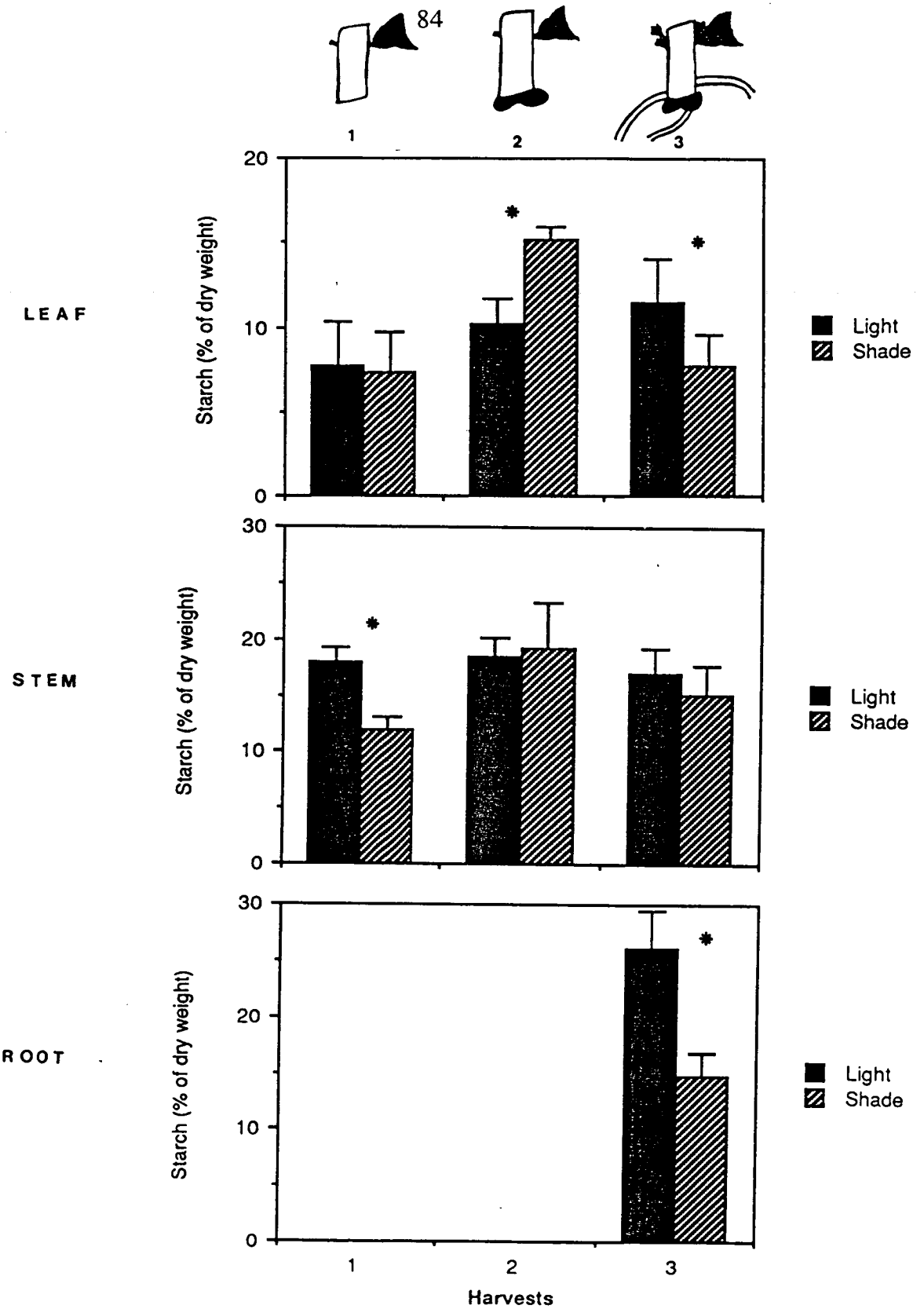

FIGURE 4.9a: Changing concentrations (% of dry weight) of starch in light and shade in leaf, stem and root of leafy softwood cuttings of *Nauclea diderrichii* during root formation. Bars indicate 95% confidence limit, and * signifies significant difference (P=0.05) as measured by the t-test.

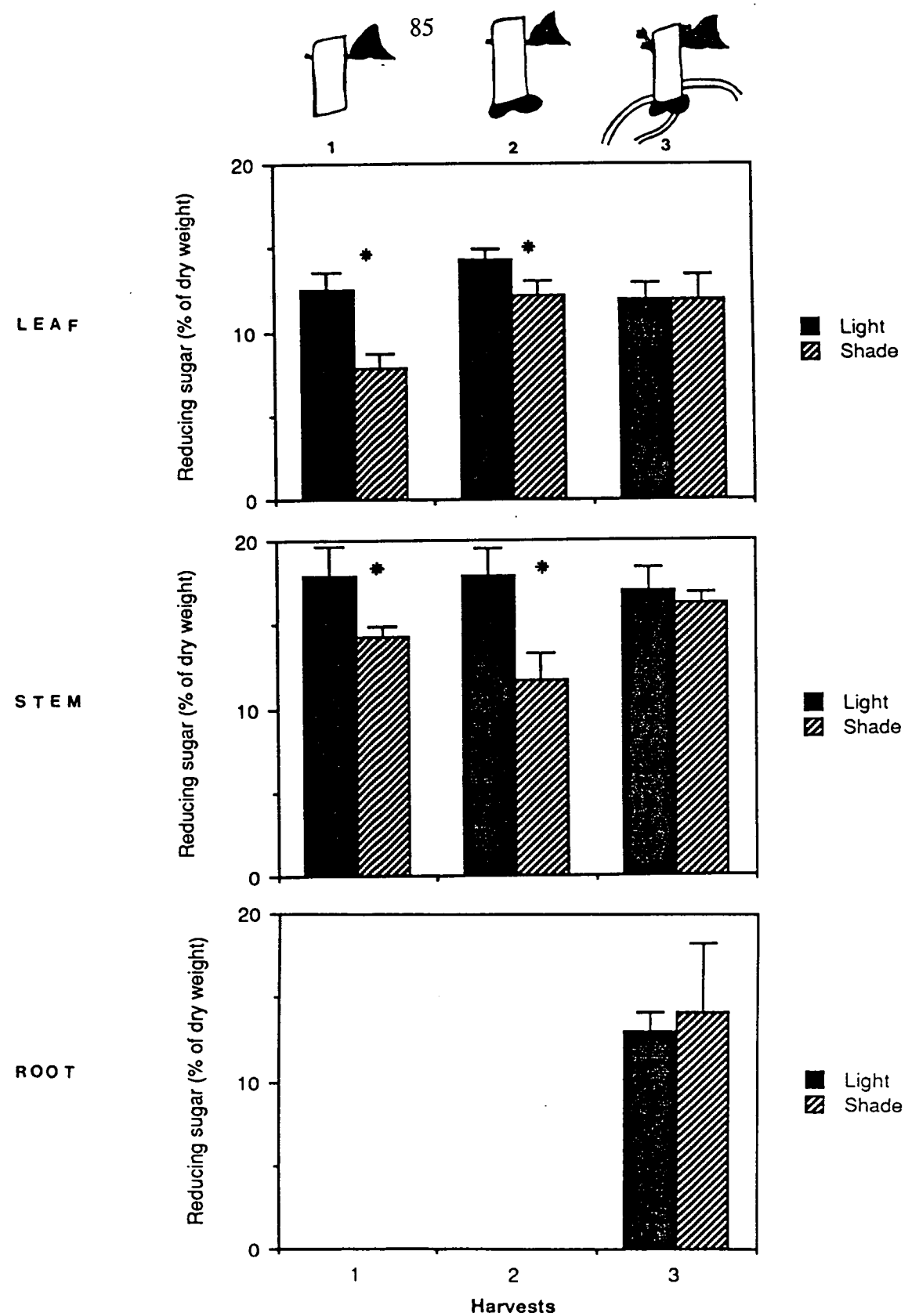

FIGURE 4.9b: Changing concentrations (% of dry weight) of reducing sugar in light and shade in leaf, stem and root of leafy softwood cuttings of *Nauclea diderrichii* during root formation. Bars indicate 95% confidence limit, and * signifies significant difference (P=0.05) as measured by the t-test.

Non-reducing sugar:

Inspection of the paper chromatogram revealed no sucrose in the extract of leaf, stem and root as in the previous experiment. Most of the total sugars appeared to be monosaccharide (see Figure 3.11c, Chapter 3). It was concluded that the quantitative analysis for reducing sugars and starch would be adequate for the present work, so no further analysis was carried out.

4.3.3 Photosynthesis

The relationship between photon flux density and rate of photosynthesis indicates considerable variation (Figure 4.10a). The diurnal patterns were more or less similar to the first experiment (Figure 4.10a,b).

As in the previous chapter an attempt to account for this variation in terms of CO_2 concentration and stage in development was not successful and is not described here. Instead, attention was concentrated on the daily totals of carbon gain.

The daily totals of photosynthesis in light, at the start of the experiment, were low and sometimes not above zero (Figure 4.10c), whereas in shade, the initial rate was fairly high but gradually declined to zero. There was an upward trend after twenty days, the trend resembling that of fresh weight (Figure 4.7), and this was associated with the development of maximal adventitious roots and new leaf area.

4.4 DISCUSSION

The stock plants were grown in the open light and under shade with the mean photon flux density of 121 µmol $m^{-2}s^{-1}$ and 5.6 µmol $m^{-2}s^{-1}$ respectively for 28 days prior to taking the cuttings. All cuttings rooted after 4 weeks, irrespective of irradiance to the stock plants. Shade treatment increased the amount of callus, average number of roots per rooted cutting, average number of new leaves per budded cutting and average fresh weight after the emergence of new roots and shoots. In contrast, it did not affect the length of roots, distribution of roots on the stem surface and the percentage of cuttings forming buds and leaves. The physiological role of callus in the life process of the cuttings has traditionally been a protective function, as well as a temporary storehouse of reserve nutrients and water, which can later be used for root development (Buryi, 1901; Komissarov, 1964). The higher amount of callus formation might be the source of higher

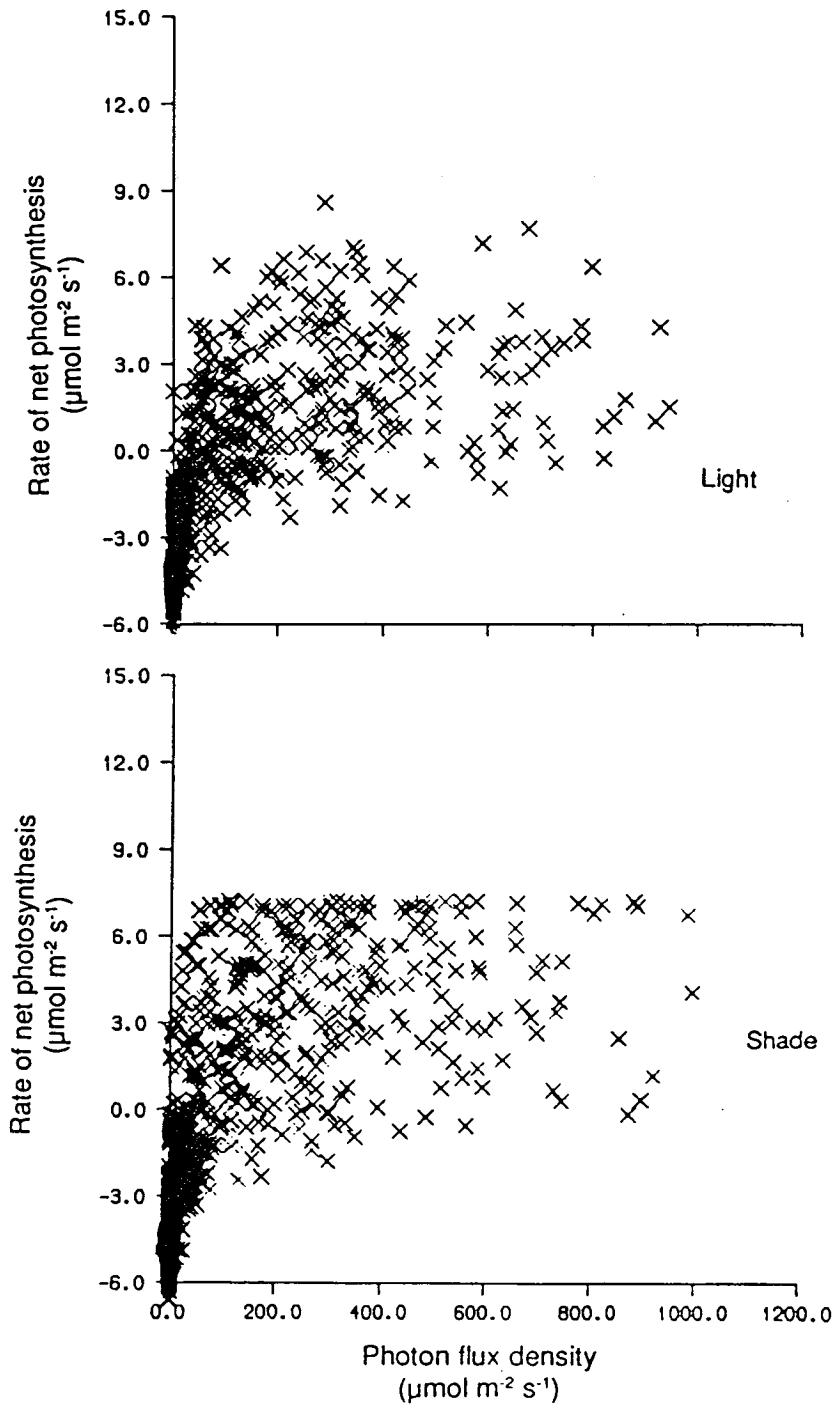

FIGURE 4.10a: Relationship between net photosynthesis and photon flux density, all hours of all days combined, treatments presented separately.

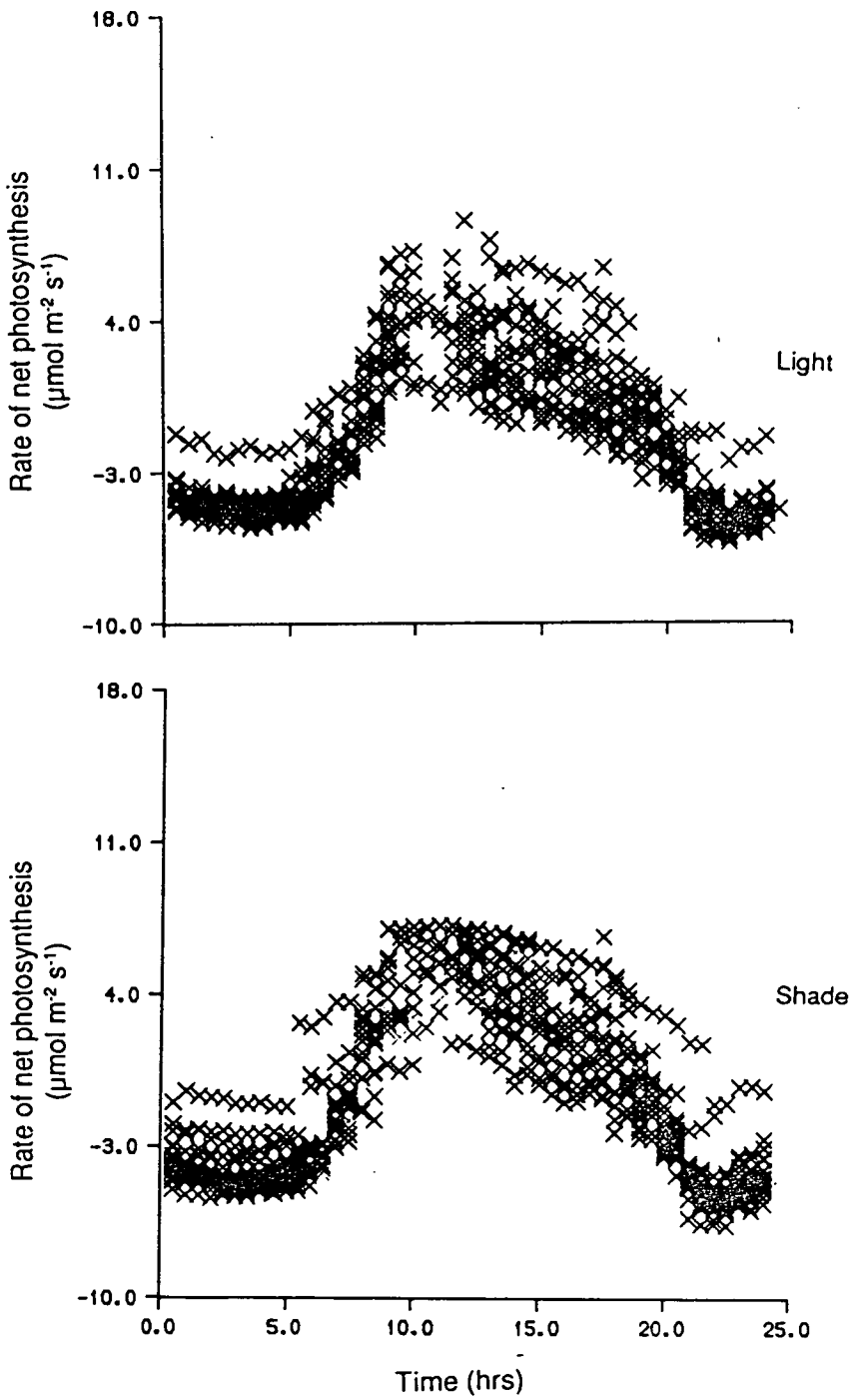

FIGURE 4.10b: Diurnal rate of net photosynthesis of a *Nauclea* cutting during rooting over 24 hours. Treatments are shown separately.

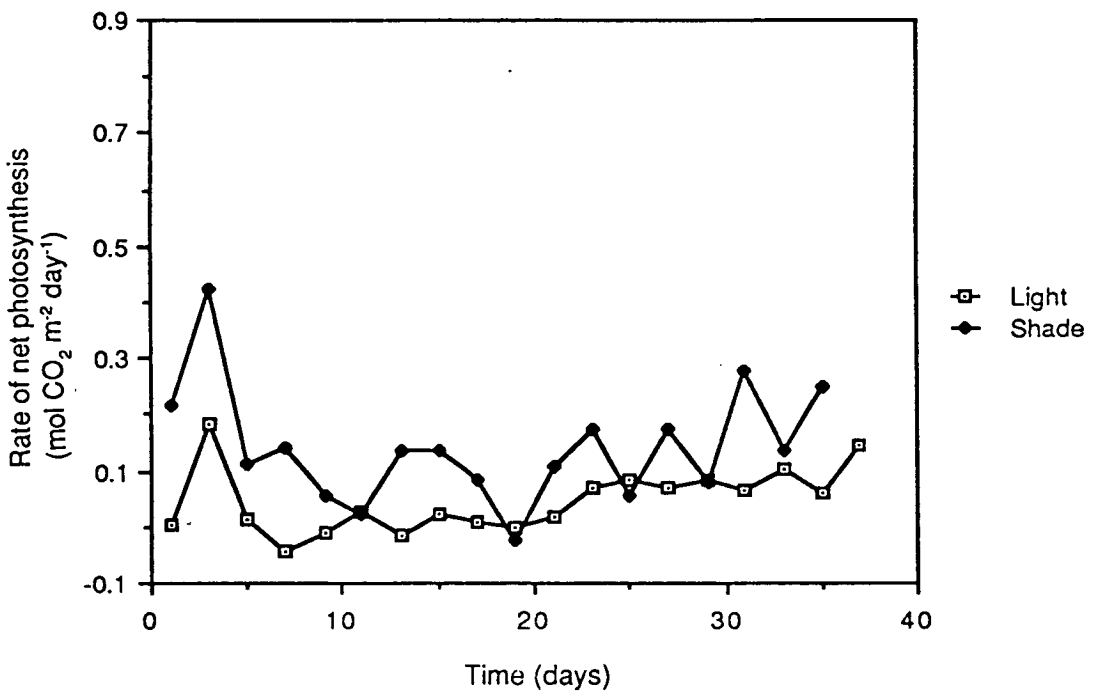

FIGURE 4.10c: The rate of daily net photosynthesis of an individual cutting of *N. diderrichii* during rooting over 35 days.

nutrient and water supply to the present cuttings. This might enable more root primordia and subsequently more rooting, as has also been reported with other species: *Hedera helix* (Girouard, 1967) and *Wrightia tinctoria* (Janardhanan and Lakshmanan, 1982).

The most important finding in this experiment was that root initiation in the cuttings was strongly affected by the irradiance under which the stock plants were grown. The highest number of roots per cutting, 19.9, was obtained in the cuttings from plants grown in shade, whereas the fewer number of roots per cutting, 15.2, was obtained in the cuttings from plants grown in light. But the overall rooting percentage was the same (Figure 4.4a). The influence of light and shade would seem to be at least two-fold (Figure 4.11a). Firstly, shade is apparently capable of activating the cells of the stem to divide. Thus cuttings from shade-grown plants produce more callus and more roots; and parts of the cutting that are screened from light are those that form callus and roots. It is not known whether this is a photomorphogenetic response, involving a photoreceptor (perhaps sensing the spectral nature of the shade) or if bright light simply breaks down a growth-promoting substance. Secondly, root initials, once formed, require a supply of carbohydrates for growth and this is only likely to occur where there is a large reserve or where the leaf is actually photosynthesizing. Other possible steps in the process of root formation are shown as (3), (4) and (5) in Figure 4.11a. It is presumed, from tissue culture studies, that whether or not callus proliferates or differentiates is under the control of growth substances and not particularly sensitive to light.

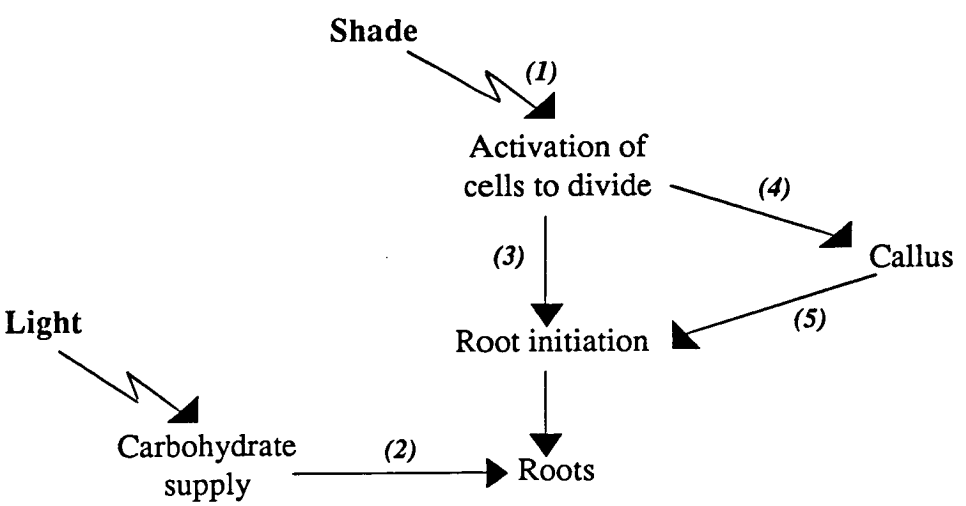

FIGURE 4.11a: Influence of light regime on the supply and demand of assimilates for root growth.

The higher initial rate of net photosynthesis in the shade treatment and later the decline might be due to the growth of new leaf area after planting. This has also been reported by others with different species: apple (Barden, 1977) and *Betula pendula* Roth and *B. pubescens* Shrh (Nygren and Kellomaki, 1983). The lower initial rate, on the other hand, in the light treatment might be due to water stress (Bielorai and Mendel, 1969, showed this with citrus seedlings, and Eliasson and Brunes, 1980, with aspen and willow), enhanced respiration rates during detaching the shoots from the parent plant (Cameron and Rook, 1974) or some other cause like adverse environmental conditions in rooting bench (discussed in Chapter 3, Section 3.4). Another important finding was that the cuttings taken from shade-grown stock plants exhibited higher rates of net photosynthesis, and they rapidly made up for their initial lower levels of carbohydrates. Thus, there is no suggestion of failure caused by carbohydrate starvation in shade-grown material. By the second harvest, these cuttings exhibited carbohydrate concentrations exceeding those of the light-grown stock plants. However, the rate of net photosynthesis further increased after the formation of new roots both in light and shade (Figure 4.10c). This has also been reported by Negishi and Satoo (1956) and Okoro and Grace (1976). The higher rate in the shade treatment might be due to more root formation at this low irradiance. There is a large literature about the relationship between photosynthetic rate and the level of carbohydrates. It seems that the rate of photosynthesis is often controlled by the 'need'. Thus, Humphries (1963a) got a positive correlation between the size of root system and the net photosynthesis with *Phaseolus vulgaris*. Kurssanow (1934) showed with green pea fruits that detaching or shading the leaves increased the rate of assimilation of CO_2 by the green pod.

In the light treatment, there was higher concentration of extractable carbohydrates but rooting was poorer. It might be that high irradiance causes a high carbohydrate content in the cuttings at the time of excision and so, as a consequence of this, the carbohydrate level during rooting is supraoptimal for the rooting process. Similar instances were reported in other species: *Pisum sativum* (Hansen and Ericksen, 1974; Veierskov, 1976), *Chrysanthemum* (Fischer and Hansen, 1977). In addition, there are reports that exogenously supplied sucrose promotes root formation only in the etiolated cuttings (Nanda and Jain, 1971) or when stock plants are grown at a very low irradiance (Eliasson, 1978). Moreover, Nanda *et al.* (1971) reported that auxin effects on rooting *Populus nigra* are influenced by the nutritional status of stem cutting and a proper balance between them was necessary for root development. This effect was concentration dependent and the optimal concentration was 0.5% glucose. Otherwise, light can increase auxin production

(Gordon, 1954) but the action, either promoting or inhibiting, depends on the percentage of glucose present in the medium. Lovell *et al.* (1972) found that a 2% solution of sucrose inhibited root initiation of excised cotyledons of *Sinapsis alba* L. and *Raphanus sativus* L. The inhibition was less when rooting was in darkness or after the inhibition of photosynthesis which reduced the endogenous sugar level.

However, during the next 35 days the growth of the cuttings, both in light and shade, showed increased content of extractable carbohydrates irrespective of irradiance (Figure 4.8a,b). It is interesting because in most cases, during callus or root primordia formation the concentration of extractable carbohydrate decrease with the decrease of net photosynthesis (see Chapter 3, Section 3.4), but in the present study due to irradiance treatment, the results were opposite. In both the cases, the total extractable carbohydrates increased despite a low rate of net photosynthesis and over the period of callus or root primordia initiation. This increase may be due to making the plants into cuttings since with *Pisum sativum* no such increase happened during subsequent days of growth in intact plants (Veierskov *et al.*, 1982). In addition, Hansen *et al.* (1978) mentioned with *Pinus* cuttings that this increase in carbohydrate content may be due to the absence of a sink, i.e. roots. A carbohydrate build-up can be expected although photosynthesis declined in all cuttings regardless of the irradiance pre-treatments (Brunes, unpublished results). Occurrence of instances where the level of extractable carbohydrates increased in leafy cuttings during the rooting period have already been reported by other workers (Moore *et al.*, 1974; Okoro and Grace, 1976; Hansen *et al.*, 1978; Veierskov *et al.*, 1982).

At the final harvest (harvest 3), there was a considerable accumulation of extractable carbohdyrate in the roots of both the cuttings taken from light and shade (Figure 4.8a,b). But in light, it was significantly higher and the increase occurred in the starch fraction of the total carbohydrate (Figure 4.8a,b). Thus the stock plants yielding the highest C/N ratio gave the fewest roots as cuttings. Therefore, the present study supports the general findings that the physiological status of the stock plants at the time of collection of cuttings is critical/important for the subsequent rooting process (Hansen and Eriksen, 1974; Hansen, 1976; Veierskov *et al.*, 1976; Hansen *et al.*, 1978).

An attempt was made to reconcile the accumulated rates of photosynthesis with the increase in dry weight of the cutting. To facilitate the calculation it is necessary to consider a cutting of standard initial weight, and then, knowing the carbohydrate percentage, to calculate the dry weight that the cutting would have had at each harvest. This calculation is quite independent of the fresh weights determined by repeatedly weighing the cuttings. The accumulated CO_2 uptake was then examined (Figure 4.11b), and a conversion

between mol CO_2 and dry weight was assumed (Appendix 3). The weight increase to be expected from the CO_2 uptake data was then calculated by interpolating Figure 4.11b at days 8 and 25. The comparison is shown in Table 4.3. In the case of 'light' the two methods produce similar weight gains. In the case of 'shade' there does not seem to have been a weight gain commensurate with the CO_2 uptake (i.e. much CO_2 uptake is not accounted for as weight gain). Where did the 0.34 g of assimilate go to?

One suggestion is that the 'starved' leaves started the experiment not only short of carbohydrates but short of other biochemical constituents (proteins, structural carbohydrates, lipids etc). Just as the cuttings 'rebuilt' the carbohydrate reserve, so they increased these other constituents. Of course, these were not counted in the carbohydrate analysis, hence the discrepancy.

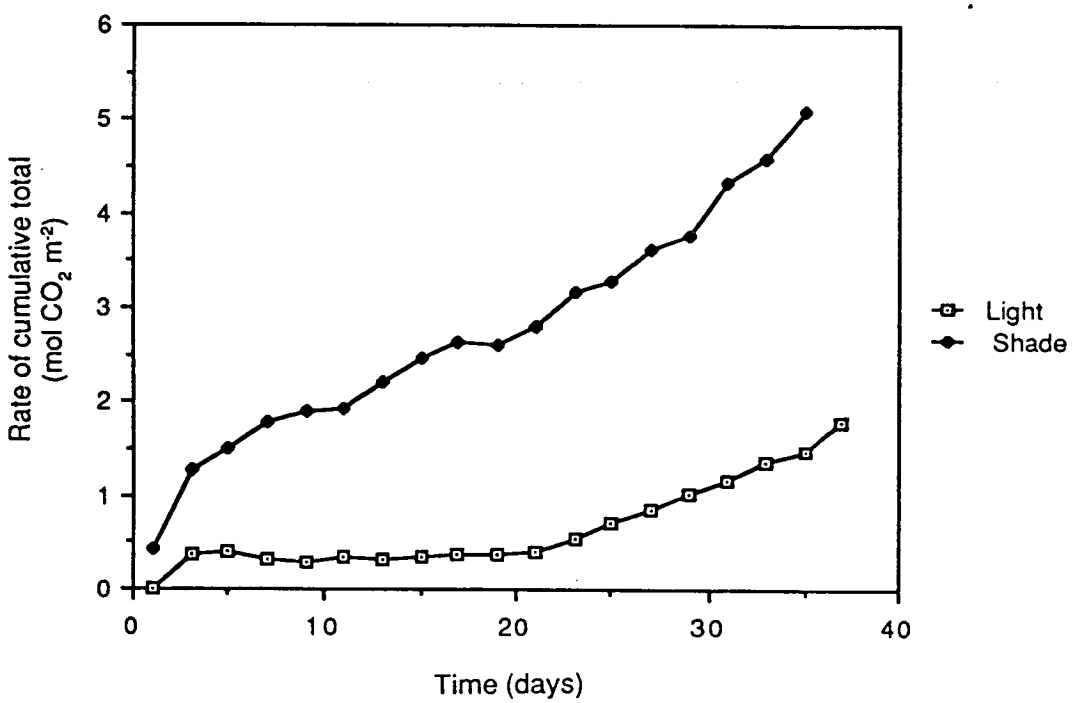

FIGURE 4.11b: Daily cumulative total of carbon balance of a *Nauclea* cutting during rooting over 35 days under light and shade.

TABLE 4.3 Mean dry weight (g) per standard cutting in different harvests, and values estimated from CO_2 uptake (Figure 4.11b).

		From carbohydrate values		From CO_2 uptake	
Harvest	Time(days)	Light	Shade	Light	Shade
1	Day-0	1.3189	1.0758	1.3189	1.0758
2	Day-8	1.3066	1.0630	1.3489	1.3710
3	Day-25	1.5008	1.2508	1.4419	1.5996

CHAPTER 5

GENERAL DISCUSSION

CHAPTER 5

GENERAL DISCUSSION

5.1 THE LIFE HISTORY OF A CUTTING

The fresh weight changes over time are consistent for all treatments (Figure 5.1). Between day 7 and day 14 a loss of weight associated with the appearance of roots is observed. This period is characterised by a low rate of net photosynthesis (except those treated previously by shade). This decline in weight is presumably the consequences of metabolic changes and a response to wounding, both of which are associated normally with high rates of respiration. This pattern of an initial loss of weight and then gradual increases due to production of carbohydrate with the formation of new leaves and root is a common phenomenon in detached leafy cuttings (Negisi and Satoo, 1955; Okoro, 1974; Machida *et al.*, 1977). However, at around 14 days there was a yellowing of leaves and some abscission. Similar senescence is also usual in detached leafy cuttings (Chibnall, 1954; Okoro and Grace, 1976).

When rooting exceeds about 80% a series of changes occur: the maximal photosynthetic rate increases in all the treatments, attaining rates similar to those recorded in attached leaves of other tropical trees like *Terminalia superba* and *Triplochiton scleroxylon* (Kwesiga *et al.*, 1986). The carbon balance became positive in all cases but the accumulated totals were not always consistent with the net gain measured by dry weight (a big discrepancy in those pretreated by shade). The fresh weight of all treatments increases in a parallel manner. In addition, maximal shoots/leaves are produced after root formation. There was enough reserve carbohdyrates in the stem parts of all treatments after root formation as observed from the carbohydrate analysis of both experiments (Figure 4.8a ,b), and this reserve carbohydrate was presumably available and used in the growth of stem and leaves (Eliasson, 1978). Moreover, regreening of all foliage occurred after the root formation, probably another usual feature in leafy cuttings (Chibnall, 1954; Okora and Grace, 1976).

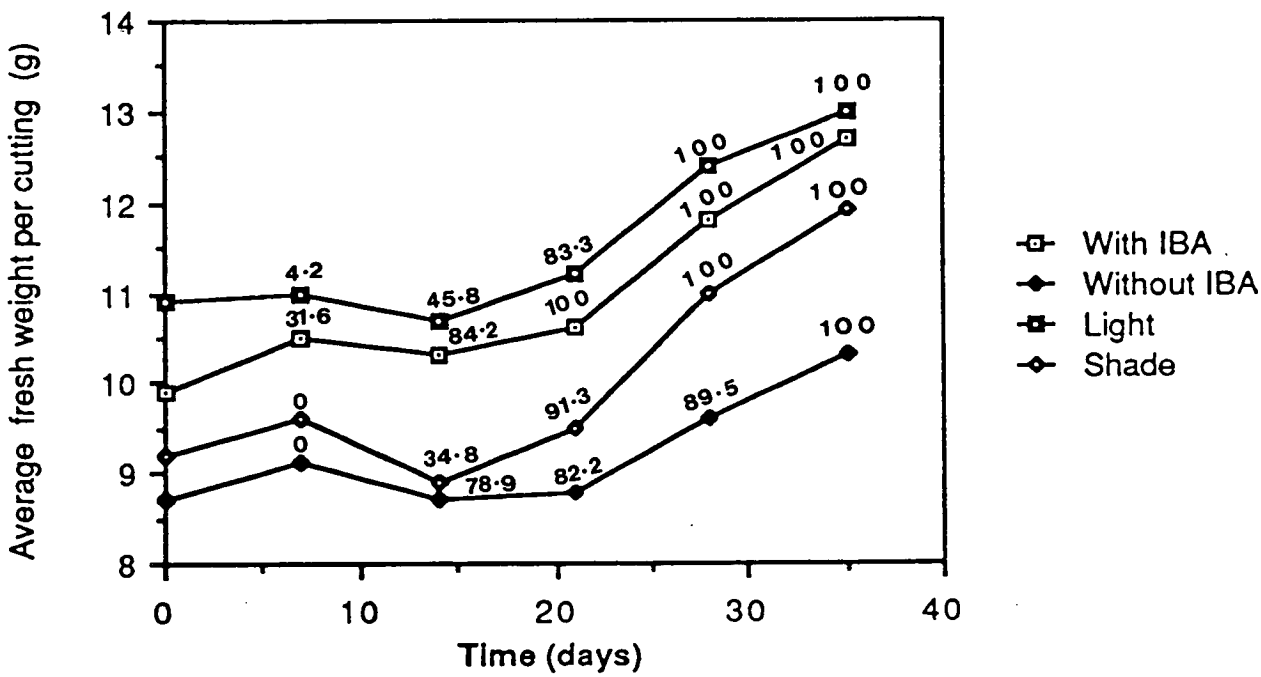

FIGURE 5.1: The average fresh weight per cutting of different treatments show a relative consistency among them during the entire rooting period.

5.2 INFLUENCE OF SHADE

The treatment of the stock plants with shade, although it did reduce the levels of carbohydrates, had very little effect on rooting. Certainly, there was no tendency for 'starved' plants to die through a lack of assimilates because these 'starved' plants exhibited high rates of photosynthesis and very rapidly recovered high levels of carbohydrates.

The conclusion of this part of the work for practical rooting procedures is that the light treatment of the stock plants is not important. However, it is possible that other variables, not studied here, for instance light quality, could be very important.

Another point emerging (from the light response curves) is that the light compensation point was very variable (5-1000 μmol $m^{-2}s^{-1}$). The present work is one of the first to explore photosynthetic rates in relation to the rooting environment. It would seem that more studies are required to elucidate the importance of the very low CO_2 concentrations which occur in the day time. Commercial propagating frames should perhaps incorporate CO_2 regulation to avoid a mid-day starvation of CO_2 to the cuttings (see Figures 2.2 and 2.3, Chapter 2).

5.3 INFLUENCE OF IBA

The species chosen turned out to be a very prolific rooter, and even without IBA the rooting was good. IBA simply made it occur sooner. In easy-to-root leafy softwood cuttings, the use of IBA is not beneficial, probably there is ample endogenous auxin in their tissue, and they do not respond to added synthetic auxin by root initiation (Hartmann, 1969). There is a suggestion that IBA increases the concentrations of carbohydrates in leaves and stem, this may be by an increase in the rate of starch breakdown, or IBA may indirectly activate root initials, which themselves send a 'message' (another growth substance) to the stem and roots to mobilize carbohydrate reserves. The well known inhibitory effect of auxin on root elongation is also observed (Aberg, 1957). IBA shows a positive effect on the rates of both callus formation and root initiation since it substantially increases movement of photosynthates to the site of root initiation at the base of the cutting (Cameron, 1970). IBA did not however have much effect on photosynthesis, although auxins have been reported to stimulate CO_2 assimilation of leaves and leaf cells, and this was attributed to stimulation of photophosphorylation (Higgins and Jacobson, 1978). The IBA treated cuttings showed no particularly large gain in fresh weight in comparison to the rest of the treatments (Figure 5.1).

5.4 CONCLUSION IN RELATION TO ORIGINAL AIMS

The original questions posed were

1. Does IBA increase the rate or extent of rooting?
2. If so, is this achieved through a more favourable supply of carbohydrate, caused by enhanced photosynthesis?

The conclusions to be drawn are that in *Nauclea diderrichii*, IBA does not increase the final rooting percentage but does have a stimulatory effect on the initial rate of production of roots and on the number of roots per rooted cutting. Secondly, IBA does not enhance the photosynthetic performance and the roots do not run short of carbohydrates at all, even when the stock plant has been heavily shaded.

5.5 SUGGESTIONS FOR FURTHER WORK

Further work is needed to investigate the physiological stress of cutting during rooting with especial reference to water relations and CO_2 intake in relation to rooting environment. A study is also necessary on the influence of light quality rather than light quantity in both the environment of the stock plant and the cutting.

In addition, a similar study with a closely-related difficult-to-root species could add some information about whether difficulty in rooting is anatomical or physiological.

5.6 PRACTICAL IMPLICATION

Nauclea diderrichii is a very easily rooted species, even by comparison with other related West African hardwoods like *Triplochiton scleoxylon* (Leakey *et al.*, 1982a; Leakey, 1983) and *Terminalia superba* (Siaguru, 1986). There is wide scope for this species in clonal forestry for large scale reforestation programmes for the following reasons.

(i) One hundred per cent rooting success in leafy single node stem cuttings is possible within 3-5 weeks, even without growth hormones.

(ii) It is a fast-growing species.

(iii) It needs a very simple rooting propagator which can be made by polythene sheet supported by wooden frames, very near to the plantation area and there is no need for any intermittent mist. Initial watering and irrigation 2-3 times a day with a hand spray may be enough for good rooting.

(iv) A micropropagation technique has been developed for this species (Leakey, personal communication).

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APPENDIX I:

The following methods for the determination of starch and reducing sugar in plant tissues were developed during the course of this work.

1. Plant material

It is best to fix the plant material by plunging into liquid nitrogen then freeze drying. If the material is put into an oven to dry, carbohydrate yield will be reduced due to respiratory losses as the material is gradually killed.

Dry material must be ground to a fine powder. For the analysis that follows, about 0.1 g is required.

2. Extraction

Accurately weigh out 0.05-0.1 g dry material (W) into a glass centrifuge tube. Add 4 ml perchloric acid (50 mM). Boil for 10 minutes using a boiling water bath, using marbles on the tubes to reduce evaporative losses. Cool in a cold water bath at room temperature. Centrifuge 10 min at 2000 rpm. Decant supernatant. Wash the pellet with 1 ml 50 mM perchloric acid and centrifuge again, adding supernatant to the previous volume of supernatant. Make up the volume to 10 ml with distilled water. Adjust the pH to 4.6 using a few drops of 2 M NaOH followed by acetate buffer (see Section 7). Record volume V_1. This pH is required so that the amyloglucosidase (Section 4) will work properly. *

3. Determination of reducing sugars

The classical method Somogyi (1945) works well and detects reducing sugars in the range 50-500 µg in a 2 ml sample. Calibration solutions should be run every time alongside the unknown solutions, to ensure that the technique has been standardised in every run. The reagents are cheap and stable at room temperature.

A. Somogyi regent; per litre we need:

28 g	anhydrous disodium phosphate
100 ml	1N NaOH
40 g	sodium potassium tartrate (Rochelle Salt)
8 g	crystalline cupric sulphate
180 g	anhydrous sodium sulphate

B. Nelson's (1944) arsenomolybdate reagent:

25 g	ammonium molybdate $(NH_4)_6\ MO_7O_{24}.4H_2O$)
21 ml	conc. sulphuric acid (NB: add slowly stirring continuously)
3 g	disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) {Sodium arsenate}

* "It is recognised that the acid extraction may degrade the sugars present in the extract; results should therefore be expressed as glucose equivalents."

2 ml of Somogyi reagent and 2 ml (or volume V_1) of sugar solution containing 0-1000 µg glucose are mixed. The 2 ml of 'sugar solution' may be made of 0.2 ml extract plus 1.8 ml distilled water, or some other proportion of extract and water depending on the amount of sugar in the extract. In the calculation, the volume of extract used is V_1. The tubes are covered with marbles, and immersed in a boiling water bath for 10 minutes. After cooling, 2 ml Nelson's reagent is added, the volume is made up to 25 ml, and the optical density at 540 nm is recorded. A blank tube using water instead of sugar solution must be included alongside, as well as several standard solutions of glucose. A typical calibration is shown (Figure 1). The calculations are shown in Figure 2.

4. Digestion of starch using amyloglucosidase

The enzyme breaks down starch to glucose units. Several preparations of amyloglucosidase are available from Boehringer and Sigma. The most expensive preparations are pure and more active. Cheaper preparations have traces of glucose in them. Very cheap preparations contain starch and should be avoided. Lyophilised preparations (=freeze dried) have to be dissolved in buffer.

The reaction is conveniently carried out in a tapered graduated centrifuge tube, using a small volume of the extract (V_2) which can be 0.2 ml, together with 0.2 ml buffer (pH 4.6) and 0.2 ml of the enzyme solution. The tubes are incubated at 55°C for 1 h. This incubation time can be varied according to the activity of the enzyme preparation (tests should be made at the outset). It is wise to confirm that all starch has been converted to glucose by taking drops from the tube and (on a spotting tile) testing for starch using iodine solution. This may be especially important with enzyme which has been around for a long time, or new batches which have not been tried yet. It is wise to test the procedure at the outset using pure starch.

The glucose is then measured using the Somogyi reagent as before. The extract will have had glucose in it as well as starch, so the mass of glucose obtained from Section 3 has to be subtracted from the result to obtain the starch. The formulae required to make all the calculations are summarised in Figure 2.

5. Comments

There are 'starch kits' available commercially but using these, each determination costs over £1. The method described above is very cheap.

Total carbohydrates can be determined with the Anthrone reagent (Yemm and Willis, 1954), but this involves hot concentrated sulphuric acid and it is not easy to exclude interference. Moreover, different carbohydrates have a somewhat different colour development.

Glucose may be determined with glucose oxidase (Boehringer, 1986), indeed this is used in the 'starch kit'. That method is probably more sensitive, but not as cheap or easy.

Perchloric acid is a powerful oxidizing agent and must be handled with great care, not being allowed to come into contact with wooden surfaces. An alternative extractant is hot water (Macrae and Armstrong, 1968) / chloral (Harcus and MacWilliam, 1954) / dimethyl sulphoxide (Jenner, 1968).

Leaves and stems contain carbohydrates other than starch and glucose. If there is much sucrose, invertase may be used to break down the sucrose to glucose. Chromatography should be used at the outset to see which carbohydrates are present. Starch may be determined colourimetrically using iodine solution (Jenner, 1968; MacWilliam, 1956; Merrit and Walker, 1969). This technique is fairly straightforward and very cheap. However, the colour development depends on the ratio of amylose to amylopectin and this is usually unknown for the species and may change with growing conditions. So this technique cannot be recommended.

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6. Items required

Centrifuge
Spectrophotometer and cuvettes
Fume cupboard
15 or 10 ml graduated tapered centrifuge tubes (pack of 50)
Test tubes
Marbles to act as stoppers for the tubes
Perchloric acid, 50 mM
NaOH, 2M
pH meter with small electrode
Iodine in KI
Somogyi reagents
Pipettes (preferably automatic) for 0.2, 0.5, 2 ml
Water bath at 55°C
Boiling water bath
1 litre volumetric flasks
Spotting tile

7. Recipe for acetate buffer

Sodium acetate-acetic acid buffer solutions, pH 3.7-5.6

Sodium acetate trihydrate, $CH_3COONa\text{-}3H_2O$, M. wt 136.09; 0.2M-solution contains 27.22 g/l.

x ml 0.2M-NaOAc and *y* ml 0.2M-HOAc mixed.

pH, 18°C	*x* ml 0.2M-NaOAc	*y* ml 0.2M-HOAc
3.7	10.0	90.0
3.8	12.0	88.0
4.0	18.0	82.0
4.2	26.5	73.5
4.4	37.0	63.0
4.6	49.0	51.0
4.8	59.0	41.0
5.0	70.0	30.0
5.2	79.0	21.0
5.4	86.0	14.0
5.6	91.0	9.0

8. Optical density at 540 nm

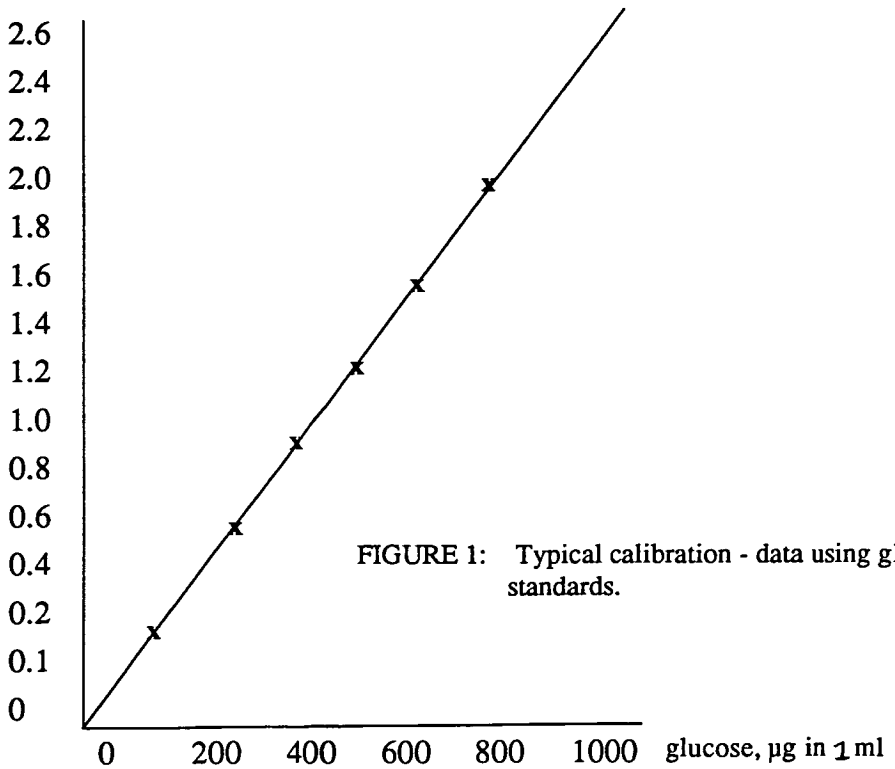
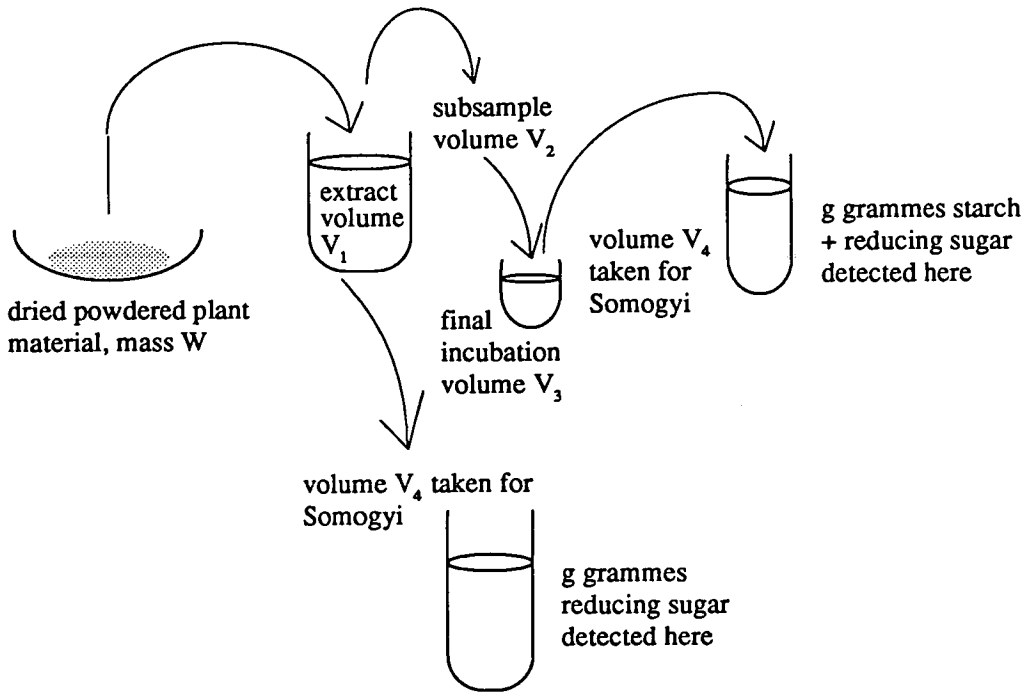


FIGURE 1: Typical calibration - data using glucose standards.



Recommended weights and volumes

- W 0.05-0.1 g
- V_1 10 ml
- V_2 0.2 ml
- V_3 0.2 ml
- V_4 0.2 ml for starch + sugar scheme
  0.5 ml for reducing sugar scheme

Reducing sugar, mass in extract $= m_1 = \frac{V_1 g}{V_4}$

Mass of starch + sugar $= m_2 = \frac{g V_3 V_1}{V_2 V_4}$

Reducing sugar % $= \frac{m_1 \times 100}{W}$

Starch % $= \frac{(m_2 - m_1) \times 100}{W}$

FIGURE 2: Calculations

APPENDIX 2:

Purpose: To test the presence of various non-reducing sugars (in particular sucrose) in plant tissues.

Equipment and reagents:
Large chromatography glass tank
Chromatography paper (approx. 60 cm x 45 cm)
Standard solutions of various sugar markers
Plant samples (perchloric acid extracts)
Solvent *
Stain **

Method

An original line was drawn 9 cm in from the short side of the filter paper and marked for placing the samples and markers at 2.5 cm intervals. Two markers, sucrose and fructose (1%) were used with three concentrations: 10 µl, 40 µl and 100 µl. The plant samples were also used with the same concentrations as markers. Ten µl of sample was placed as a 'spot' by a micropipette on the marks of the paper. A hairdrier was used to dry the spots. The other short end of the paper was made serrated to facilitate flow of the solvent. Finally, the paper was allowed to dry in room temperature overnight. The paper was then put in the chromatography tank, held in place by a glass rod. A second antisiphon rod was used to ensure even distribution of the solvent, which was held in the glass trough. The paper was dried after the solvent has travelled along the length of the paper and finally the stain for sucrose was sprayed over it and dried at 100°C for 5 min. The coloured spots were marked (see Figure 3.11c).

* Solvent 1: Ethyl acetate - pyridine - water (8 : 2 : 1)

or

Solvent 2: Butan-1-ol - Acetic acid - water (12 : 3 : 5)

** Stain: 200 ml 0.2% Naphthoresorcinol (Sigma) in absolute alcohol. Just before use add 20 ml H_3PO_4 (S.G. 1.85), spray the paper and dry at 100°C for 5-10 minutes and detect below the fructose and oligossacharide (e.g. sucrose, raffinose, etc.).

APPENDIX 3:

Calculation of carbohydrate gain from the rate of photosynthesis:

Leaf area = 50 cm^2 = 0.005 m^2 $cutting^{-1}$

M.W. of CO_2 = 12 + 32 = 44 g

For rate 0.1 mol $m^{-2} d^{-1}$ = 4.4 g $m^{-2}d^{-1}$

= 4.4 x 5 x 10^{-3} g d^{-1} $cutting^{-1}$

= 22.0 x 10^{-3}

= 0.022 g d^{-1}

After 25 days = 0.022 x 25 = 0.55g

Thus (i) at the rate of 0.08 mol m^{-2} day^{-1} = 0.44g (expected)

(ii) at the rate of 0.05 mol m^{-2} day^{-1} = 0.27 g (expected)

Calculation for cumulative values:

After 35 days = 2.75 mol m^{-2}

= 2.75 x 44 x 5 x 10^{-3} g $cutting^{-1}$

= 0.605 g (expected)