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Capture of genetic variation by vegetative propagation: processes determining success

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ABSTRACT

Vegetative propagation offers the opportunity to rapidly overcome the limitations to domestication imposed by long generation times, irregular fruiting/flowering and outbreeding. Vegetative propagation techniques are increasingly being applied to a wide range of tree species, of both moist and dry tropics. Identification of the critical factors determining adventitious root development is crucial to sustained, cost-effective propagation, even in species in which these factors are not currently limiting.

Over the past ten years, research at ITE has identified a wide range of factors which influence rooting, including the stockplant growth environment, cutting origin, post-severance treatments applied to cuttings, and the propagation environment. In order to understand the influence of these different factors and their interactions, an appreciation of the physiological, biochemical and cytological processes involved in rooting is required. The primary processes occurring in the leaf are net photosynthesis and transpiration, while those in the stem are starch hydrolysis, translocation of sugars, water and nutrients, respiration, mitosis and cell differentiation. Each of these processes is influenced by a number of environmental, morphological and physiological variables, such as leaf area and thickness, internode length, chlorophyll content, stomatal density, stem lignification, etc.

Suggestions are made for experimental approaches to improve further the understanding of the mechanisms of rooting of leafy stem cuttings. These approaches include the development of a mechanistic model of adventitious root development. The practical implications arising from a process-based approach to propagation are also discussed.

INTRODUCTION

In agricultural crops, substantial increases in productivity and quality of produce have been obtained by breeding and selection over many generations. In contrast, less progress has been made in the domestication of trees because of their (i) relatively long generation times, (ii) irregularity in flowering and fruiting, and (iii) high incidence of outbreeding, with consequent loss of genetic gain in subsequent generations. Vegetative propagation offers the opportunity rapidly to overcome these limitations, by circumventing the need for sexual reproduction and facilitating the capture of individual genotypes.

Vegetative propagation techniques are increasingly being applied to the domestication of tropical tree species (Leakey *et al.* 1990; Mesén, Boshier & Cornelius, pp249–255; Ladipo *et al.*, pp239–248). A range of approaches can be utilised (see Hartmann & Kester 1983; Leakey 1985), including grafting, stem cuttings (Table 1), hardwood cuttings, marcotting (air-layering), suckering, and *in vitro* techniques such as meristem proliferation, organogenesis and embryogenesis. Several of these techniques have the disadvantages of a low rate of multiplication, a high requirement for skilled labour, or the need for high capital investment. These problems have been overcome by recent advances in the development of a low-technology propagation system (Leakey *et al.* 1990; Newton *et al.* 1992; Newton & Jones 1993a), which has enabled the successful propagation of a wide range of species by leafy stem cuttings (see Table 1). This system utilises non-mist propagators constructed out of cheap and readily available materials, with no requirement for a piped water and electrical supply (Leakey 1991; see also Ladipo *et al.*, pp239–248).

Although many species can be propagated easily by leafy stem cuttings, some species are recalcitrant. For example, early attempts at the propagation of *Albizia falcataria* resulted in leaflet shedding and death of all the cuttings within ten days. Pre-conditioning the cuttings by intermittent droughting of the stockplants resulted in leaf retention and high rooting percentages, with more than 100 roots per cutting in some cases (R R B Leakey, unpublished data). In such situations, identification of the factors limiting rooting is critical to successful propagation. Enhanced rooting is important even in species which are propagated easily, as small gains in rooting percentage may be of considerable economic value when the species is mass propagated on a commercial scale. In some circumstances, the rooting ability of cuttings may decline with successive harvests of cuttings from a set of stockplants. This phenomenon can arise

when over-frequent harvesting of cuttings causes the depletion of the endogenous reserves of the stump, the death of the stockplant's fine root system, or a reduction in soil fertility. For all these reasons, an understanding of the factors which influence rooting is fundamental to successful and sustained vegetative propagation.

Another key reason for a better understanding of the rooting process is the accumulation of a body of apparently contradictory data in the scientific literature. For example, the role of carbohydrates in the rooting process is still the matter of great debate (see review by Veirskov 1988). Highly contrasting results have been obtained from the same species in different situations. Most propagation experiments usually consider only one or two of the many factors known to influence rooting, and fail to recognise their interactions. Many investigations also fail to measure and record sources of variation in rooting ability which can differ between experiments, such as propagation environment and stockplant growth conditions. Consequently, the mechanisms of root formation remain unclear.

In order to understand the interactions between the different factors influencing rooting, an appreciation of the physiological processes involved is required. In recent years, there have been a number of literature reviews on this topic (eg Leakey 1985; Andersen 1986; Haissig 1986; Davis, Haissig & Sankhla 1988; Thompson 1992). Consequently, this paper attempts to pull together data on these processes in leafy softwood cuttings obtained from a limited number of experiments with tropical tree species. In particular, it suggests how these processes may be influenced by the following factors: (i) the stockplant's growing environment; (ii) the cutting's position of origin within a shoot and the shoot's position within the canopy of the stockplant; (iii) the post-severance treatments applied to cuttings; and (iv) the environment of the propagating system. The implications of these conclusions for both research and the practical application of vegetative propagation techniques are discussed.

PROCESSES INVOLVED IN ADVENTITIOUS ROOT DEVELOPMENT

The key physiological, biochemical and cytological processes influencing adventitious root development in a leafy cutting are

Table 1. Tropical tree species vegetatively propagated at the Institute of Terrestrial Ecology, Edinburgh

West Africa	Central and South America	East and Central Africa	South Africa	SE Asia and Australasia
Afzelia africana	Albizia caribaea	Acacia nilotica	Acacia karoo	Acacia mangium
Afrostyrax lepidophyllus	Albizia guachapele	Acacia senegal	Colophospermum	Agathis australis
Baillonella toxisperma	Alnus acuminata	Acacia tortilis	mopane	Agathis dammara
Carapa procera	Annona muricata	Dalbergia melanoxylon	Triplochiton zambesiacus	Agathis macrophylla
Ceiba pentandra	Caesalpina spinosa	Faidherbia albida	Vangueria infausta	Agathis obtusa
Milicia (syn.	Casimiroa edulis	Maesopsis eminii		Agathis robusta
Chlorophora) excelsa	Cedrela odorata	Melia volkensii		Agathis vitiensis
Entandrophragma	Chrysophyllum cainito	Sesbania sesban var.		Albizia falcataria
angolense	Cordia alliodora	sesban		Anthocephalus chinensis
Khaya ivorensis	Eugenia jambos	Sesbania sesban var.		Araucaria hunsteinii
Khaya senegalensis	Ochroma pyramidale	nubica		Camposperma
Lovoa trichilioides	Prosopis cineraria	Terminalia brownii		brevipetiolata
Nauclea diderrichii	Prosopis juliflora	Terminalia prunioides		Citrus halimi
Ricinodendron heudelottii	Psidium guajava	Terminalia spinosa		Dipterocarpus turbinatus
Teclea verdoorniana	Swietenia macrophylla	Vateria seychellarum		Durio zibe thinus
Terminalia ivorensis	Swietenia mahagoni	Vitex keniensis		Gmelina arborea
Terminalia superba	Tipuana tipu			Shorea albida
Tetrapleura tetraptera	Vochysia hondurensis			Shorea contorta
Treculia africana				Shorea curtisii
Triplochiton scleroxylon				Shorea leprosula
				Shorea macrophylla
				Tamarindus indica
				Terminalia calamansanii
				Terminalia brassii
				Toona ciliata

photosynthesis, transpiration, respiration, starch hydrolysis, translocation of sugars, water and nutrients, mitosis, cell differentiation and elongation. While all of these processes may operate in different parts of leafy softwood cuttings, photosynthesis and transpiration primarily occur in the leaf, and mitosis and cell differentiation are generally of greatest importance in the cutting base. The stem is the primary organ of translocation for nutrients, carbohydrates and water between the leaf and the cutting base.

The interactions between these processes and the factors affecting rooting, mentioned earlier, are considered separately below, although, in reality, they are interlinked.

FACTORS INFLUENCING THE PROCESSES THAT DETERMINE ROOTING

Stockplant growth environment

The physiology and morphology of stockplants are influenced by the growth environment, which may include nutrients, water, temperature and light (both irradiance and spectral composition). In addition, the individual cuttings are influenced by the variation in microclimate within the canopy of the stockplant. The characteristics of the cutting (Figure 1) that are influenced by these environmental factors include:

- the area and thickness of the leaf;

- leaf photosynthetic capacity as affected by stomatal density, chlorophyll content, etc;

- internode length and stem diameter;

- leaf and stem starch, soluble carbohydrate and nutrient contents;

- pre-severance leaf and stem water potentials; and, probably,

- concentrations of endogenous growth substances.

Some influences of stockplant growth environment are illustrated by results from experiments with *Triplochiton scleroxylon*, in which rooting ability was found to be correlated with pre-severance net photosynthetic rate, which in turn was determined by irradiance and nutrient application treatments during growth. In stockplants grown under artificial lighting (6.3 red/far-red (R:FR) ratio) with an irradiance of 250 μmol m^{-2} s^{-1} and nutrient addition, photosynthetic rate varied between 0.003 and 0.009 mg CO_2 s^{-1} leaf^{-1}, and a mean rooting percentage of 33.3±5% was obtained. These figures compared with photosynthetic rates of 0.0005–0.002 mg CO_2 s^{-1} leaf^{-1} and an associated mean rooting percentage of 9.3±3.1% when stockplants were grown under an irradiance treatment of 650 μmol m^{-2} s^{-1} and without added nutrients. The low rooting percentages obtained under this high
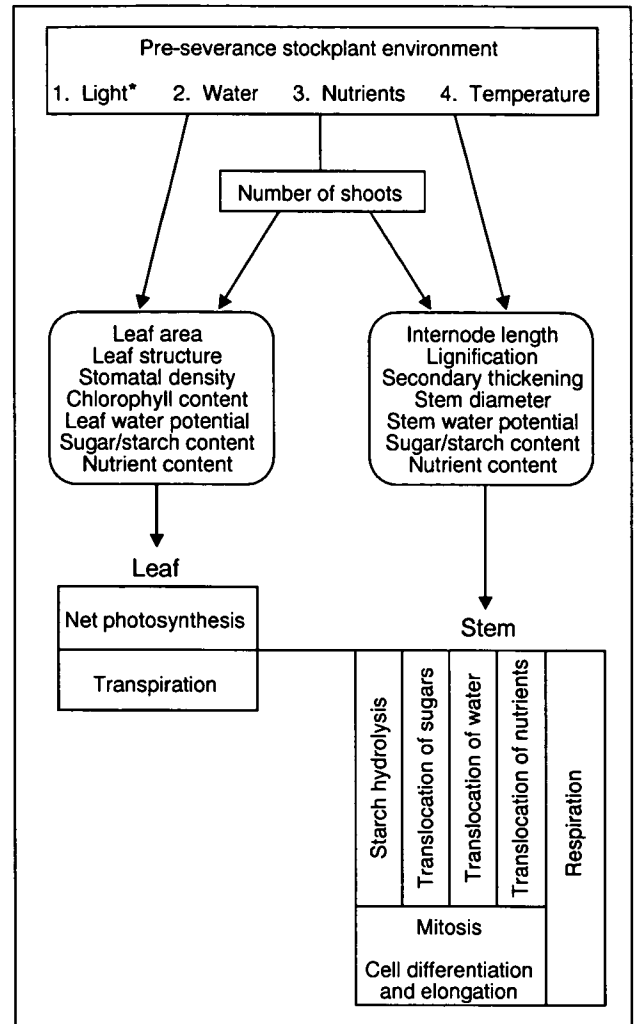

Figure 1. Schematic diagram of the effects of the pre-severance stockplant environment on the morphological and physiological factors affecting rooting ability in single-node, leafy cuttings *via* their influences on the biochemical processes of their leaf and stem (*irradiance and quality)

irradiance/low nutrient treatment were attributed to the accumulation of starch in the cuttings pre-severance (Leakey & Storeton-West 1992).

The importance of stockplant illumination is further illustrated by other experiments with constant irradiance but varying light quality. In *T. scleroxylon* under artificial lighting, higher rooting percentages (92%) were obtained at a R:FR ratio of 1.6, while lower rooting percentages (53%) were obtained at a R:FR ratio of 6.3 (Leakey & Storeton-West 1992). These results, together with those on the effects of irradiance, concur with results examining the varying rooting ability of cuttings from different shoots within the stockplant canopy (Leakey 1983). However, in common with many other vegetative propagation experiments in the scientific literature, no data were collected on the physiological activity of these various shoots. To hypothesise how these treatments affected the physiological processes and morphological condition of these shoots, it is necessary to examine a more extensive data set

from similar experiments with *Eucalyptus grandis* (Hoad & Leakey 1992) testing a wider range of R:FR ratios (including more natural ratios of R:FR, eg 0.4–1.3). In this more recent study, rooting ability was related to treatment-induced changes in leaf and stem morphology and to pre- and post-severance gas exchange and carbohydrate dynamics. In addition, pre-severance photosynthetic rate per unit leaf area, chlorophyll concentration, stomatal conductance and water use efficiency increased with an increase in the R:FR ratio. The photosynthetic rate per unit of chlorophyll was, however, greater at low R:FR ratios, and there was a positive linear relationship between the percentage of cuttings rooted and the pre-severance photosynthetic rate per leaf.

As mentioned above, there are important interactions between the light environment of the stockplant and its nutrient regime. These interactions have been demonstrated in *T. scleroxylon* as stockplant environment effects, using controlled conditions and shade trees above rows of stockplants in the field (Leakey & Storeton-West 1992). Similar interactions have also been demonstrated between the shoots of stockplants with and without fertilizers (Leakey 1983). In both instances, the highest rooting

percentages occurred in shaded shoots from plants with added fertilizers. The unrestricted application of fertilizers is, however, not recommended as, at very high fertilizer applications, plants of *Khaya ivorensis* yielded cuttings which suffered high mortalities. In this case, mortalities due to rotting were associated with increasing reducing sugar and declining starch contents of the leaves, during the period of propagation (Tchoundjeu 1989).

Cutting origin

The physiology, morphology and subsequent rooting potential of a cutting are also influenced by:

- the position within a stem from which the cutting originates (Leakey & Coutts 1989; Figure 2); and

- the position of the shoot within the stockplant canopy (Leakey 1983, 1985; Tchoundjeu 1989; Figure 3).

Cutting mortality can also be related to node position in the shoot (Leakey 1983), suggesting that the factors determining rooting and mortality are inversely related and form some kind of continuum.
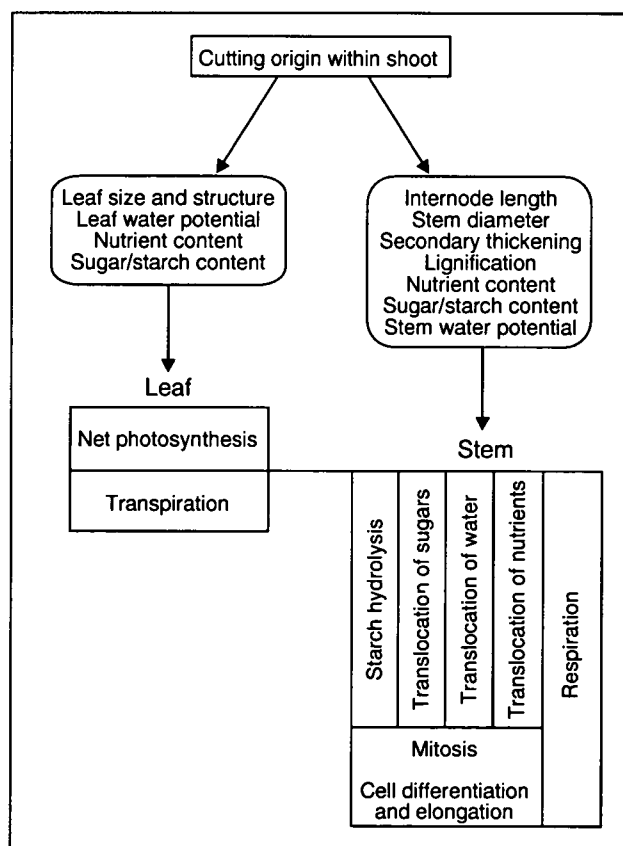

Figure 2. Schematic diagram of the effects of cutting origin within shoot on the factors affecting rooting ability in single-node, leafy cuttings *via* their influences on the biochemical processes of leaf and stem
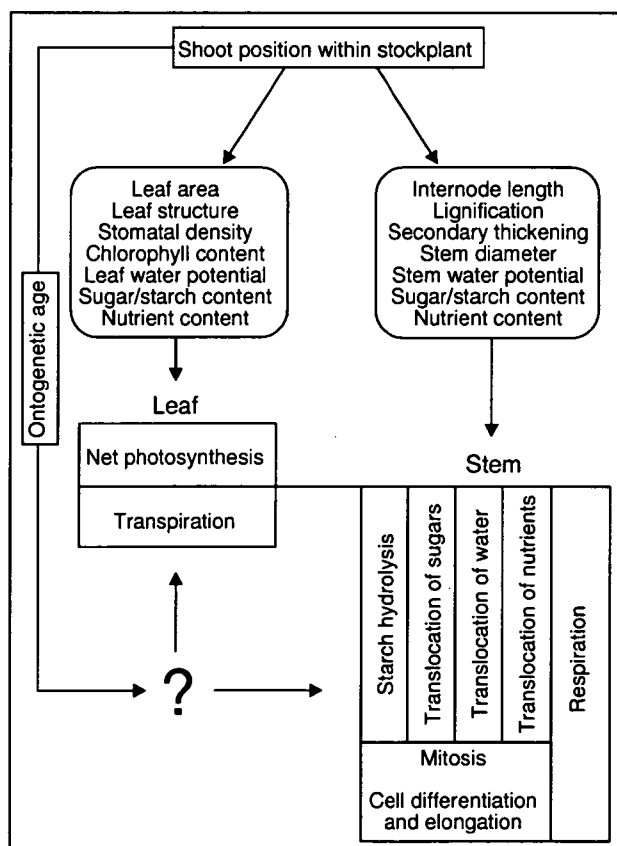

Figure 3. Schematic diagram of the effects of the shoot position within stockplant on the morphological and physiological factors affecting rooting ability in single-node, leafy cuttings *via* their influences on the biochemical processes of leaf and stem

Within a stem, there are gradients in:

- succulence,
- leaf size, age and morphology,
- leaf water potential (Leakey 1983), and
- mutual leaf shading

which affect both the amount and quality of light received by the leaves (Leakey & Storeton-West 1992), and consequently net photosynthetic rate and stomatal conductance. In addition, there are gradients in:

- stem morphology, such as the extent of secondary thickening and lignification, and internode length (Leakey & Mohammed 1985), stem diameter and, consequently, stem volume (Leakey, Dick & Newton 1992);
- starch and sugar content (Leakey & Coutts 1989);
- nutrient content (Tchoundjeu & Leakey 1993); and, almost certainly,
- the content of plant growth regulators and rooting co-factors.

The most important of all these factors in *T. scleroxylon* seems to be the effect of cutting position on the internode length and diameter. Together, these two variables result in variation in cutting volume. From these results and those of other species (see, for example, Hoad & Leakey 1992; Dick, East & Leakey 1991), we have developed the hypothesis that cutting volume determines the capacity of a cutting to store assimilates produced both pre- and post-severance (cf Leakey & Storeton-West 1992). Cuttings with a small stem volume may become saturated with starch, which can inhibit photosynthesis and consequently rooting. However, an often overlooked aspect of the carbon budget is the stem respiration rate, which is influenced by the cutting's size and its environment (pre- and post-severance). To examine the mean respiration rate of internode tissue, a one cm section was taken from the base of *Prosopis juliflora* cuttings with a range of basal diameters (Dick, Blackburn & McBeath 1993). Using an oxygen electrode, the respiration rate per gram dry mass of tissue was found to decrease within sequentially more basal cuttings, possibly as a result of the increased lignification of older tissues. However, because of the larger mass of these basal cuttings, the total respiration for the whole 1 cm section of tissue (Figure 4) was greatest in the cuttings with the largest basal diameter. Thus, the larger mass of tissue in the larger cuttings more than compensated for their smaller respiration rate per unit mass. In addition, the larger diameter cuttings had a larger area of wound tissue which may also have contributed to their increased respiration. These results show that, compared with smaller diameter cuttings, larger diameter cuttings have
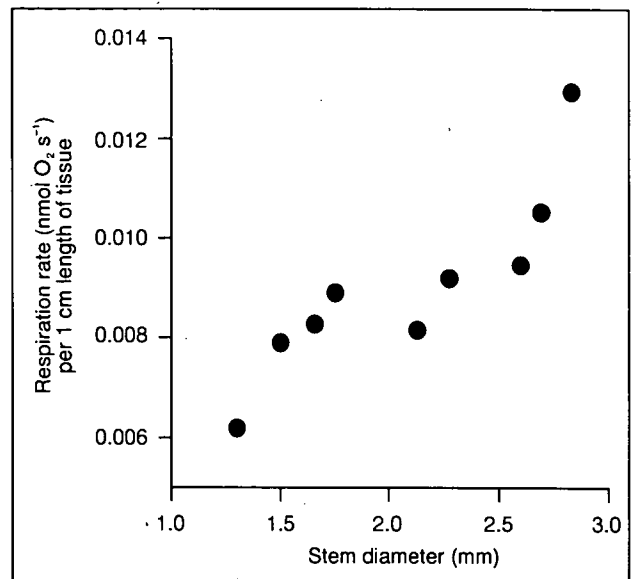

Figure 4. Total respiration rate of the basal 1 cm section of *Prosopis juliflora* cuttings measured at the time of severance, using an oxygen electrode (source: Dick *et al.* 1993)

greater total respiratory/metabolic activity at their basal (wounded) end. This feature may contribute to the increased rooting potential of large diameter cuttings (Dick, Dewar & Leakey 1992).

Between shoots there are variables that affect rooting which are similar to those environmental factors affecting the whole stockplant, except that they are associated with the microclimatic environment of the individual shoot. As in the whole plant, the interactions between light (quality and quantity) and nutrient availability seem to be particularly important (Leakey 1983), presumably mediated by effects on shoot morphology and gas exchange. Cuttings from basal shaded shoots of *T. scleroxylon* stockplants given added nutrients have a high rooting ability. In addition to these microclimatic effects, there is also inter-shoot competition, which is related to the number of shoots on the stockplant and to their position within the dominance hierarchy between shoots (Leakey 1983). In small *T. scleroxylon* stockplants (height 0.5 m), higher rooting percentages were associated with cuttings from the top shoot, and progressively declined with each shoot down the plant. In larger plants (ie 1.5 m), rooting percentages of cuttings from the upper shoots were low and those from basal shaded shoots were relatively high. In *Lovoa trichilioides*, another West African hardwood species, rooting percentages of cuttings from basal shoots were highest, even in small stockplants (Tchoundjeu 1989). The rooting ability of these cuttings from different shoots also depended on how many shoots there were per stockplant.

From this examination of the effects of cutting origin and stockplant environment, it is clear that the physiological and morphological condition of

the cuttings at the time of severance from the stockplant is very important, and that, even in small, 'juvenile' plants, rooting ability can range from 0–20% in unsuitable material to 80–100% in suitable material. It is also clear that, as stockplants get bigger and more complex, it becomes more difficult to obtain shoots in a physiological/morphological condition that confers good rooting ability. This loss of rooting ability is frequently attributed to the loss of juvenility (ie attainment of sexual maturity or ontogenetic ageing). Numerous experiments have demonstrated this loss of rooting ability, but they have all been confounded by the many other changes that occur as plants grow bigger and more complex (Leakey 1985). There is, therefore, no good evidence from in vivo studies that sexual maturation per se affects rooting ability, although there are currently some studies from in vitro culture that suggest that there are events in vitro which apparently 'rejuvenate' plant tissues (Jones & Webster 1989). As yet, there is no understanding of what these events are or how they are mediated. For the moment, therefore, it seems most appropriate to indicate (see Figure 3) that the effects of ontogenetic ageing on the rooting process are not known. What is clear is that physiological ageing, as manifested by the physiological and morphological condition of the cutting, does have major effects on the success of vegetative propagation programmes (Leakey et al. 1992).
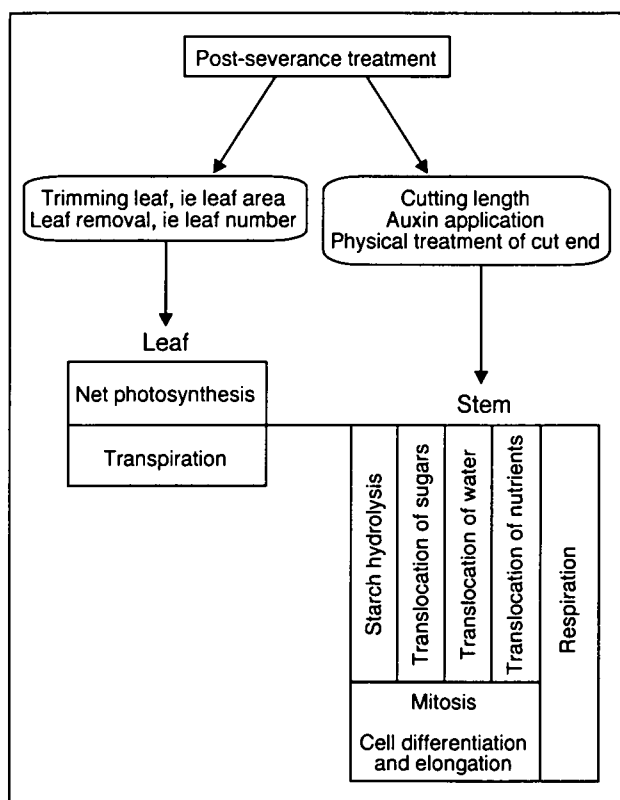

Figure 5. Schematic diagram of the effects of post-severance treatment on the morphological and physiological factors affecting rooting ability in single-node, leafy cuttings *via* their influences on the biochemical processes of leaf and stem

Post-severance treatments applied to the cuttings

The rooting of cuttings is influenced by the application of a number of post-severance treatments (Figure 5). The most common treatment is application of an auxin 'rooting hormone'. Auxins often hasten rooting, increase the percentage of cuttings rooted, and increase the number of roots formed. However, the results of even this most commonly applied treatment are subject to considerable variation, because of the interactions with the factors listed previously (Newton et al. 1992). Other post-severance factors which influence rooting include leaf area, which is often reduced to minimise water deficits. Evidence from a number of species suggests that there is an optimum leaf area, as a compromise between having a large enough leaf to produce assimilates and a small enough leaf to minimise water loss (Leakey & Coutts 1989). In T. scleroxylon and several other species, the optimal leaf area is about 50 cm^2 (Leakey 1985). However, in L. trichilioides and Khaya ivorensis, about 200 cm^2 and 20 cm^2 respectively have been shown to be optimal, under the conditions tested (Tchoundjeu 1989). In contrast, other species which root easily have displayed no tendency towards an optimum leaf area (Leakey 1990; Newton, Muthoka & Dick 1992). In small-leaved species, the optimal leaf area can perhaps only be achieved by retaining more than one leaf per cutting and thus more than one node, but there is some evidence that there are interactions between cutting length and the numbers of leaves and nodes.

Cutting length is another variable that can be manipulated at the time of severance. The effect of stockplant/shoot environment on cutting length and volume has already been discussed. However, it is not often realised how trimming a cutting to a standard length rather than accepting natural variation in internode length affects the cutting volume (Leakey et al. 1992). Standardising cutting length has the advantage that all cuttings can be inserted into the medium to the same depth and also have their leaf held at the same height above the cutting medium, which can be important for cutting survival (S P Hoad & R R B Leakey, unpublished). As cutting diameter usually increases down a shoot, taking constant-length cuttings usually confers an advantage on more basal cuttings as their stem volume is greater. In contrast, taking cuttings of a constant number of nodes may result in an advantage to apical cuttings, if their greater length exceeds the effect of cutting diameter on cutting volume (Leakey et al. 1992).

It may sometimes be necessary to store cuttings prior to insertion in the propagator, particularly if they are collected from the field at some distance from the propagation unit. Rooting ability

declines with increased storage time, and may also be influenced by storage temperature. In *T. scleroxylon*, storage for 24 hours in an insulated box (18–23°C) had no effect on rooting, but, after 72 hours' storage, rooting percentages were significantly decreased (Njoya 1991).

The propagation environment

Successful propagation of leafy cuttings is dependent on the maintenance of suitable air and leaf temperatures, irradiances and leaf/air vapour pressure deficits (VPDs) during propagation (see review by Loach 1988b). Leafy cuttings are vulnerable to desiccation during propagation, particularly prior to root development and when VPD is high. The primary effects of water deficits are lower leaf and stem water potentials and the consequent inhibition of physiological activity, and of photosynthetic rate in particular. The association of low leaf water potentials with low rooting percentages has been illustrated by Loach (1988a) and Newton and Jones (1993b).

Although many authors have suggested that current assimilates are important for rooting, there have been relatively few studies that have actually measured photosynthesis and transpiration during the rooting process (see review by Davis 1988). Recent studies have shown that many tropical tree species actively photosynthesise and transpire prior to root formation, supporting the suggestion that gas exchange during propagation is one of the critical factors influencing rooting (Leakey & Storeton-West 1992; Newton, Muthoka & Dick 1992; Newton *et al.* 1992; Newton & Jones 1993b; Hoad & Leakey 1992; Mesén, Leakey & Newton 1992; Mesén 1993). In *Terminalia spinosa*, mean photosynthetic rates of 2 μmol m^{-2} s^{-1} and a maximum rate of over 6 μmol m^{-2} s^{-1} were recorded in a non-mist propagator prior to root development. These photosynthetic rates were associated with rooting percentages of over 80%; the rate at any given time was found to be strongly dependent on stomatal conductance and irradiance (Newton *et al.* 1992b). Transpiration rates of 0.002–0.007 g H$_2$O cm^{-2} h^{-1} were recorded in cuttings of *Cordia alliodora*, depending on the rooting medium (Newton, Muthoka & Dick 1992; Newton *et al.* 1992), and stomatal conductances in the range 0.1–1.4 cm s^{-1} were recorded for four species in both mist and non-mist propagation systems (Newton & Jones 1993b). The contrasting effects of irradiance on VPD, photosynthetic activity and cutting water relations will determine the optimum leaf area of the cutting, and the shading regime to be employed during propagation (Newton & Jones 1993a, b).

Apart from influencing VPD, air and bed temperatures in the propagator also influence the rates of all the processes directly (Figure 6), particularly in metabolic processes such as starch mobilisation, respiration, mitosis and cell differentiation. Some species display clear temperature optima in terms of rooting. For example, from a range of 20–38°C, an optimum bed temperature of 29–31°C was identified in *T. scleroxylon*, at an air temperature of 25–30°C (Leakey, Chapman & Longman 1982). The relationship between air, leaf and bed temperatures is generally considered to influence the relative activity of the different parts of the cutting, and may account for shoot growth prior to root formation during propagation.

Application of nutrients during propagation may also facilitate root development (see review by Blazich 1988), by influencing photosynthetic rate and other metabolic processes. Nutrient application may be particularly beneficial in slow-rooting species under mist propagation systems, where nutrient leaching from the cutting can be a problem. Foliar nitrogen concentration is particularly influential in determining photosynthetic rate, and nitrogen is also important for starch mobilisation and other metabolic processes.

Variation in ambient CO_2 concentration of the air surrounding the cuttings may also influence rooting, primarily by its effect on photosynthetic rate and dry mass accumulation (Davis & Potter 1983). In non-mist propagators, CO_2 concentrations have been found to vary diurnally
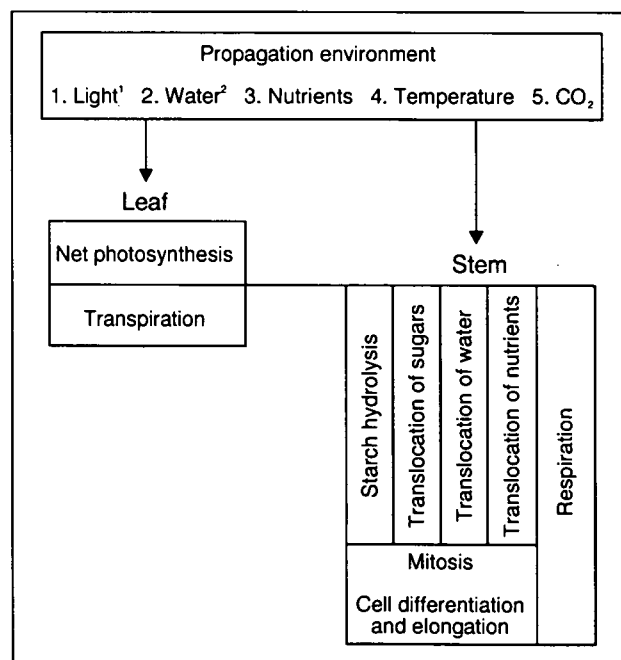
Figure 6. Schematic diagram of the effects of propagation environment on the rooting ability of single-node, leafy cuttings *via* their direct influences on the biochemical processes of leaf and stem ([1]irradiance and quality; [2]VPD, humidity, moisture content of medium, air/water ratio of medium)

between 150 and 550 µmol mol^{-1} (Leakey et al. 1990; Matin 1989; Newton & Jones 1993a), which may be expected to have a major effect on photosynthetic activity.

The rooting medium determines the amount of water and air available to the base of the cutting, which will influence the rates of transpiration and respiration respectively, as well as the other metabolic processes in the cutting base. The effect of different concentrations of indole-3-butyric acid (IBA) on rooting of *Bombacopsis quinata* and *Vochysia hondurensis* has been found to interact with the type of rooting medium utilised (Newton et al. 1992).

Genetic variation

Different species and clones vary in their physiological and morphological characteristics. It is likely, therefore, that much of the genetic variation in rooting success can be attributed to the interactions between the above processes and factors influencing rooting. In this connection, there is increasing evidence for the role of genes which encode enzymes involved in the biosynthesis and metabolism of the auxin indole-3-acetic acid (Blakesley & Chaldecott 1993).

SUGGESTIONS FOR EXPERIMENTAL APPROACHES TO UNDERSTAND THE MECHANISMS OF ROOTING

For research projects aimed at optimising rooting for a given species, it is necessary to conduct an experimental programme. Ideally, this programme should involve the following strategies, although it is recognised that the ideal situation is rarely achieved in practice.

Experimental analysis and design

All experiments should obviously follow standard experimental design practices and be well replicated. The minimum number of cuttings per treatment or clone/treatment interaction should be 40–60. The major difficulty with analysis is that either a cutting roots or it does not, and thus there are only two possible outcomes, giving a binomial distribution. With moderate numbers of replicates, it may be possible to use standard ANOVA or regression procedures on either the raw data or a transformed data set, but care is needed and the validity of various assumptions must be tested. Alternative approaches involve either the contingency table approach using χ^2 tests, although these results can be difficult to interpret, or use of a generalised linear model with its associated analysis of deviance, which requires more specialised computing software.

Depending on the chosen technique of analysis, an appropriate experimental design should be adopted. Ideally, cuttings should be blocked in the propagator according to the spatial variation in the propagation environment, which may be pronounced (Newton & Jones 1993a). Treatments should be replicated across all blocks and randomised within a block, following standard practice. Other sources of variation should ideally also be randomised within each block, but frequently this is impracticable. For example, cuttings from a single shoot may be most easily laid out in node order rather than fully randomised with respect to node position.

A number of recent studies (Leakey & Storeton-West 1992; Hoad & Leakey 1992) have demonstrated that, if the experimental analysis is limited to the overall effect of treatments, a great deal of the variation in rooting may remain unexplained. By accounting for the variation attributable to node position, for example, more information on different processes is often obtained (Dick et al. 1991; Dick & East 1992). If the experiment also includes destructive harvests taken at different times during propagation, *i* additional information on the dynamics of different processes may be obtained. For example, studies of the carbohydrate dynamics in *T. scleroxylon* have demonstrated that there is no relationship between soluble carbohydrate content at insertion (day 0) and subsequent rooting, but a strong relationship between soluble carbohydrate content at day 28 and rooting at day 42 (Leakey & Coutts 1989). Destructive harvests of this sort obviously increase the numbers of cuttings required per treatment. These harvests can and should ideally be associated with non-destructive assessments of physiological variables (gas exchange, respiration, chlorophyll fluorescence, etc), as an understanding of the dynamics of these processes is fundamental to understanding the achievement of successful rooting.

Control of variation

In any given experiment, some variables will be manipulated, whereas others will either be controlled or simply measured. Any variables which cannot be controlled should be measured and reported. The variables which should be noted include all the factors which may influence rooting ability as outlined above, eg leaf area, stem length and diameter, node position, number of nodes, number of leaves, leaf shedding, changes in leaf colour, genotype, propagation environment, pre-severance environment, physiological age, chronological age, ontogenetic age, shoot number and position, nutritional status, etc. Although it is recognised that practical limitations will restrict which variables can be measured, experimenters should be aware of all the potential sources of variation in rooting ability, and should seek to control or record them as far as possible.

Modelling adventitious root development

As rooting is influenced by so many interacting factors, there is a need for process-based models as a tool to understanding. To date, a model has been constructed which describes the development of root structural dry matter from pre-formed initials, as well as the dynamics of sugar and starch pools within the cutting during the rooting process. This model (Dick & Dewar 1992) was constructed on the basis that the following previously mentioned factors were positively correlated with rooting: (i) respiration rate at the base of the cutting; (ii) labile sugar content, rather than total carbohydrates; and (iii) post-severance photosynthetic rate of the cutting.

The leafy softwood cutting is considered to have three structural compartments: leaf, internode and root, but can easily be adapted to include a growing shoot or to be a leafless hardwood cutting. A leafy softwood cutting is represented (see Figure 7) by boxes (i) and (ii) and defined by six dynamic variables: the soluble sugar pools (mg sugar) in the leaf (L_{sg}), internode (I_{sg}), and root (R_{sg}); the starch pools (mg starch) in the leaf (L_{st}) and internode (I_{st}); and the structural dry matter pool (mg structural dm)$^{-1}$ in the root (R_x). If, as in *Cordia alliodora* (Mesén 1993) or *Prosopis juliflora* (Dick *et al.* 1991), the cutting produces a new axillary shoot during propagation, this can be represented by the addition of an additional box (iii). In this case, the bud has a dry matter pool (B_x) which competes for sugars with the new root (R_x). In both these cases, the leaf and internode structural dry matter pools (L_x and I_x respectively) are held constant in the model. In the case of hardwood cuttings (which can be represented by box ii, with or without box iii; see Figure 7), the dynamic variables are the same as for the softwood cutting with a growing shoot, except that the leaf sugar and starch pools are omitted. Given the initial values of the dynamic variables at severance ($t=0$), the model simulates the development of root initials, and rooting is said to have occurred when the root structural dry mass reaches a threshold (x mg).

As explained earlier in this paper, many factors influence the processes that determine rooting. As an example of the situation when structural root growth is limited by the amount of sugar reaching the cutting base, the output of the model has been parameterised with published data for single-node cuttings of *Triplochiton scleroxylon* (Leakey & Coutts 1989), with leaf areas of 10, 50 or 100 cm^2.

To calibrate the model in the absence of measured rates of photosynthesis, respiration and sugar transport, physiologically reasonable parameter values were assumed (Table 2). These values were taken to be the same for cuttings
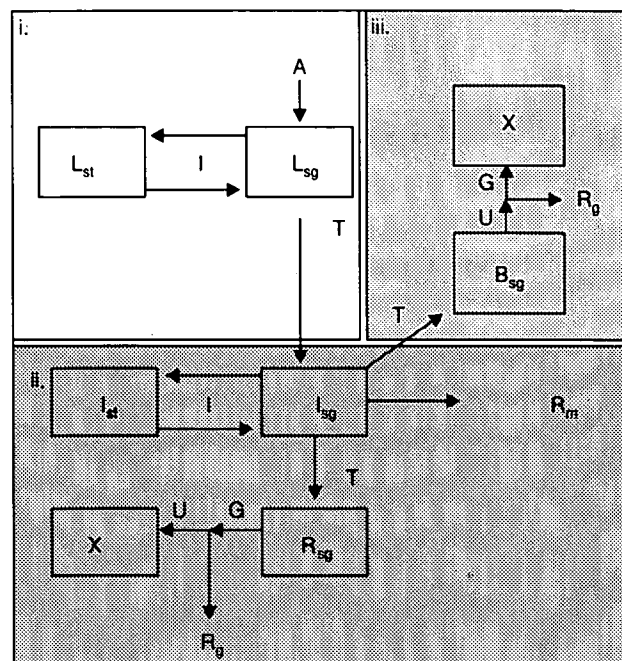

Figure 7. Schematic diagram of the rooting model. Box (i) represents the leaf, (ii) the internode and rooting zone, and (iii) a growing bud or shoot. Solid boxes represent the dynamic starch (st), sugar (sg) and structural dry matter (x) pools. Arrows represent fluxes of sugar assimilation (A), starch/sugar interconversion (I), sugar transport (T), root sugar utilisation (U), root structural growth (G), internode maintenance respiration (R_m) and root growth respiration (R_g)

Table 2. Parameter values and initial values of state variables used to simulate the root development of single-node leafy cuttings of T. scleroxylon, for three different leaf areas (source: Dick & Dewar 1992)

		Leaf area (cm^2)		
		10	50	100
Parameters				
g_{li}	Leaf/internode conductance (mg structural dm d^{-1})	150	150	150
g_{ir}	Internode-root conductance	150	150	150
i_x	Internode structural dm (mg)	300	300	300
k_1	Starch/sugar conversion coefficients (d^{-1})	0.0001	0.0001	0.0001
k_2	" "	0.03	0.03	0.03
k_3	" "	0.0001	0.0001	0.0001
k_4	" "	0.05	0.05	0.05
Y	Root growth efficiency	0.5	0.5	0.5
μ	Utilisation constant (d^{-1})	10.0	10.0	10.0
σ_i	Specific internode respiration rate [10^{-3} mg sugar d^{-1} (mg structural dm)$^{-1}$]	0.0	0.0	0.0
σ_l	Specific leaf photosynthetic activity [10^{-3} mg sugar d^{-1}(mg structural dm)$^{-1}$]	8.0	9.4	4.1
l_x	Leaf structural dry matter (mg)	300	500	700
Initial values of state variables (mg)				
i_{sg}	Internode sugar	9.9	9.9	9.9
i_{st}	Internode starch	10.8	0.8	10.8
l_{sg}	Leaf sugar	3.9	6.5	9.1
l_{st}	Leaf starch	0.3	0.5	0.7
r_{sg}	Root sugar	0.033	0.033	0.033
r_x	Root structural dry matter	1.0	1.0	1.0

with 10, 50 and 100 cm^2, except for the specific leaf photosynthetic activity (σ_l) which was derived, such that 50% rooting occurred after 24, 19 and 28 days for cuttings with 10, 50 and 100 cm^2 leaf areas respectively, as in Leakey and Coutts (1989). The results of this simulation suggest that the 10 cm^2 leaves were predominantly area-limited, while the 100 cm^2 leaves were predominantly limited by photosynthetic rate per unit area. This finding is consistent with the observation by Leakey and Coutts (1989) that 50 cm^2 was optimal for rooting and that assimilation rates were limited by low leaf water potential in cuttings with leaf areas of 100 cm^2.

This example serves to show that the model provides a framework for examining the role of factors known to affect rooting. As a further illustration, if an increasing range of specific internode respiration rates is used in the model, rooting in a cutting with a leaf area of 50 cm^2 is progressively delayed until the root sugar pool declines to zero, at which point further increases in respiration rate result in cutting mortality (Table 3).

Table 3. Predicted time of rooting or death of *T. scleroxylon* cuttings with leaf area 50 cm^2, for various values of the internode respiration constant, σ_i. All other parameters and initial values of state variables are as given in Table 2 (source: Dick & Dewar 1992)

Internode respiration constant [10^{-3} mg sugar d^{-1} (mg structural dm)$^{-1}$]	Days to rooting	Days to death
0	19	–
5	26	–
10	46	–
13	137	–
14	576	–
14.3	–	35
14.4	–	19
14.5	–	13
15	–	5
20	–	2

As already mentioned, the model, like all mechanistic models, makes various assumptions. So far, the only assumption tested is that the rate of respiration at the base of the cutting (rooting zone) is higher than that of the stem above it (Dick *et al.* 1993) This study confirmed this assumption and suggests that a concentration gradient is created which drives sugar transport basipetally. The measurements also supported one of the predictions of the model: during the formation of callus and new roots, the respiration rate at the base of the cutting increases with time (Dick *et al.* 1993).

In summary, the model provides a potentially useful framework for: (i) the design and interpretation of rooting experiments on a whole-cutting basis, and (ii) understanding the mechanistic control of adventitious rooting. With further development and a better understanding of the interactions between carbon, nutrients and water, we hope that it will be possible to use the model to improve stockplant management and achieve more predictable and sustained rooting during mass propagation. However, it is already clear, from the experience of developing the model, that there may be several contrasting sets of conditions that will give acceptable protocols for commercial vegetative propagation, depending on whether the stockplants and cuttings are managed in a way that makes them dependent on stored reserves or current assimilates.

PRACTICAL IMPLICATIONS

In situations where a full-scale research programme is inappropriate, the propagation of an undomesticated species may be approached by considering a limited number of key factors. These include leaf area, cutting length, auxin and propagating conditions (eg Tchoundjeu & Leakey 1993). As a starting point, cuttings should be 5–10 cm in length, with a leaf area reduced by 50% and an applied auxin concentration of 0.2–0.4% IBA in a solution of alcohol, or in the form of a commercial rooting powder. Cuttings should be inserted to a depth of 15–25 mm in the rooting medium, ensuring that the leaf does not touch the medium surface, and that the medium at the cutting base is not waterlogged. The rooting medium should consist of an inert, well-aerated moisture-retaining substrate, such as mixtures of fine gravel, sand or rotted sawdust. The propagator should be shaded to about 25% of full sunlight in order to keep the propagators as cool and as humid as possible and yet provide enough light for the promotion of physiological activity.

In any propagation programme, the reasons for lack of success may be difficult to ascertain, as so many factors influence rooting. From the above, it should be clear that the provision of a good rooting environment and the application of standard post-severance treatments are relatively easy and likely to result in a good measure of success. However, the commonest problems associated with vegetative propagation are those caused by inappropriate stockplant management or the unsuitable physiological state of the material to be propagated. To sustain good rooting over many cutting harvests and several years will require (i) much greater understanding of stockplant management in order to maintain stockplants in a good physiological condition, and (ii) a knowledge of how to manipulate rooting ability by pre-severance conditioning. We hope that, in due course, the mechanistic model

will be of practical use for this task. This need to sustain physiological youth, which is often confused with the retention of juvenility, is perhaps the most difficult aspect of vegetative propagation. It is important to improve our understanding of these ageing phenomena (Leakey et al. 1992).

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