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PHYSIOLOGY OF VEGETATIVE REPRODUCTION

**Roger RB Leakey
Agroforestry and Novel Crops Unit
School of Tropical Biology
James Cook University
PO Box 6811
Cairns
AUSTRALIA
QLD 4870**

roger.leakey@jcu.edu.au

INTRODUCTION

Vegetative regeneration is both a natural process and an artificial process (Leakey, 1985). The artificial process is used by agriculturalists, horticulturalists and foresters, to capture and multiply individual genotypes, and so to produce cultivars and clones (Mudge and Brennan, 1999), with clonal forestry in China having a history going back 800 years (Minghe and Ritchie, 1999a). Typically the process is used to develop superior planting stock, although there are also many applications in research, where clonal uniformity is a powerful tool in the separation and identification of physiological and other growth processes in plants (Longman *et al.*, 1980).

This chapter primarily examines the current state of physiological knowledge about vegetative regeneration using stem cuttings. It builds on former reviews (Leakey, 1985; Davis *et al.*, 1988; Mudge and Brennan, 1999) and the standard text (Hartmann *et al.*, 1997). The level of understanding has progressed enormously since the earlier generation of reviews (Krugman 1974) in the period when clonal forestry was just becoming a reality and the focus was primarily on temperate trees. Although the focus is primarily on the rooting of cuttings, some mention will also be made of related issues in air-layering/ marcotting, grafting/budding, and different *in vitro* propagation methods.

The review draws heavily on studies done with *Triplochiton scleroxylon*, a tropical hardwood of West Africa, because of the large number of relatively comparable experiments done using a similar type of material (single-node leafy cuttings) under similar environmental conditions, to seek some physiological principles of wider relevance. In addition, the review offers some suggestions on how future research should be implemented to enhance the identification of underlying physiological principles determining successful rooting of stem cuttings. The problem that needs to be overcome is the high level of interaction between the large number of pre-severance, post-severance and propagation environment factors.

ROOTING STEM CUTTINGS

Stem cuttings can come in many forms, but the two major groups are leafy softwood cuttings from relatively unligified, young shoots (Figure 1), and leafless hardwood cuttings from older and more lignified shoots which typically have already shed their leaves due to the onset of winter or a dry season. It is important to understand that the factors determining the rooting of these two types of cuttings are very different: leafy cuttings depend on current photosynthates produced while in the propagation bed, while hardwood cuttings depend on the hydrolysis and availability of carbohydrates stored within the stem tissues.

In leafy stem cuttings, Leakey *et al.* (1994) identified different situations where it was necessary to have a good physiological understanding of the factors influencing rooting ability:

(i) The propagation environment

The most important aspect of the propagation environment is that it encourages physiological activity (photosynthesis and transpiration) in the leaf to minimise the physiological stresses experienced by the tissues, from transpiration and respiration, and encourages meristematic activity (mitosis and cell differentiation) in the stem. The transport of assimilates and nutrients from the leaf to the base of the stem, and of water from the base of the stem to the leaf, are also important. Recent physiological measurements (Mesén *et al.*, 1997a) confirm general experience (Hartmann *et al.*, 1997) that the duration of physiological shock arising from severing a cutting from its stockplant and inserting it in a propagator can be minimized by controlling the propagation environment (. Minimizing this shock enhances rooting.

There are many different types of propagation systems for stem cuttings (Hartmann *et al.*, 1997), but the most common are (i) fogging systems, (ii) intermittent mist (Figure 2), controlled by a range of different sensors and (iii) air-tight, water-tight, high humidity, non-mist propagators (Figure 3). These are all very effective, but vary in their cost and sophistication. The non-mist propagators (Leakey *et al.*, 1990) have the advantage of being very low cost and low-tech and so are highly suitable for use in developing country or rural situations, where electricity and piped water are not available. The basic principles behind all these systems are that the cuttings are well supplied with water at the cutting base while the leaves are in a cool, shady environment with low vapour pressure deficit (VPD) to minimise water stress. Moist leaves also keep the cuttings cool, due to the evaporation of water from the leaf surfaces (Hartmann *et al.*, 1997). While shading is beneficial, leafy cuttings need enough light to photosynthesise. Interestingly, the highest values of photosynthesis in severed cuttings have been found at relatively low levels of irradiance.

Comparative studies between different propagation systems for cuttings (eg mist and non-mist) found that non-mist propagators provided as good an environment for rooting as mist, if not better, and found consistently higher air and leaf temperatures and VPD under mist (Newton and Jones, 1993a). There were also differences in the frequency of peaks in VPD associated with peaks in irradiance. Peaks of VPD can also occur as a result of misting frequency, and its spatial distribution on the propagation bed. In a second study, using cuttings of four tropical trees (two from the moist tropics and two from the dry tropics), significant water deficits did sometimes

develop in the leaves of the cuttings under both systems. The patterns of variation in relative water content (RWC) were similar to those of water potential and stomatal conductance (Newton and Jones, 1993b). Interestingly, the species differences in RWC and stomatal conductance seemed to be more closely related to leaf morphology, than to their environment of origin. Leaf morphology may also account for differences between the four species in optimum leaf areas (Newton *et al.*, 1992) indicating the need to report full details of as many of the long list of morphological and physiological pre- and post-severance factors affecting rooting as possible (eg. Hoad and Leakey, 1994, 1996; Mesén *et al.*, 1997b), if a good understanding of the vegetative propagation process is to be achieved.

Few studies have been done on the effects on photosynthesis of the light environment during propagation, because of the difficulty of measuring gas exchange in cuttings with wet leaves, however, the use of a non-mist propagator has allowed measurements on the rates of photosynthesis, stomatal conductance and chlorophyll fluorescence ratio to be made on cuttings of *Cordia alliodora* with and without shade (Mesén *et al.*, 1997a). The study confirmed that photosynthesis does occur in severed cuttings on the propagation bed. Photosynthetic efficiency under these two levels of irradiance (24-53 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 106-159 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was found to be more closely related to rooting ability than photosynthetic rate, the latter being greatest at the higher irradiance, while rooting ability was either unaffected or reduced by irradiance, depending on the cuttings leaf area and presumably the balance between photosynthesis and transpiration. The number of roots produced was, however, closely related to photosynthetic rate. Further work is required to look at the interactions between propagation light environments and pre- and post-severance factors. The composition of the rooting medium, usually open compost or fine gravel, is often critical for rooting and can vary between species, and cultivars/clones. In addition to holding the cutting firm, it has to provide moisture and allow respiration from the tissues. Anoxia at the cutting base usually encourages rotting, so the optimal gas-filled pore space (air:water ratio) of the medium needs to be optimised by the use of various sized particles (sand and gravel) and a water-holding medium (perlite, peat or other organic products). The oxygen diffusion rate also needs to be adequate for respiration. In *Melicia excelsa*, the moisture content of the medium was positively related to the numbers of roots formed and negatively related to mortality and leaf abscission (Ofori *et al.*, 1997).

In temperate environments propagation beds, especially those for leafless hardwood cuttings, typically have some 'bottom-heat' provided by heating pipes or cables to promote meristematic activity at the cutting base, while the leaves remain cool. Sprouting by cuttings in the propagation bed often has negative impacts on rooting, presumably by the creation of a competing sink for assimilates. Consequently a lower air temperature than bed temperature can be advantageous. This differential, although more difficult to achieve, can also be important in the tropics.

To root cuttings have to have a positive carbon balance (ie. producing assimilates faster than they are losing them through respiration) (Mesén *et al.*, 1997a). However, very little is known about the overall respiration rates of cuttings under different environmental conditions, or about the ways in which cutting origin, leaf area, stem length/diameter affect respiration losses. Dick *et al.*, (1994) using an oxygen electrode to measure respiration in leafy, single-node *Prosopis juliflora* cuttings, found that

respiration rate per gram of dry matter decreased linearly down a shoot, as stem diameter increased. They attributed this to an increase in non-respiring lignified tissues, although the greater mass of cuttings with larger diameters, more than compensated for these losses and the larger cuttings had a greater overall rate of respiration. Perhaps more importantly, however, the respiration rate of the cutting base (1cm long section) was greater than that of the stem above it. This they suggest provides a concentration gradient which drives assimilate transport basipetally to the cutting base and is a key factor in the rooting process (see also Dick and Dewar, 1992).

(ii) Post-severance treatments

There is probably a larger body of literature on the effects of post-severance treatments, than on any other aspect of rooting cuttings. One characteristic of this literature is the apparent lack of consistent results between, and even within, species. This lack of any clear principles defining the rooting process, has led to a plethora of papers reporting apparently situation-specific results, rather than to enhanced understanding. To reverse this process it is important to seek the factors that create the variability in responses to different treatments and factors affecting rooting ability.

Probably the most important of the post-severance factors are: the application of auxins, leaf area, and cutting length/diameter. Consequently, to develop a practical rooting protocol for leafy stem cuttings of a previously unstudied species, it is first desirable to experimentally determine the optimal auxin application, optimal leaf area and optimal cutting length (Tchoundjeu and Leakey, 1996) and, if possible, to experimentally investigate their interactions within each propagation environment.

Auxin applications

The application of root promoting growth regulatory substances (auxins) is the most common treatment to enhance rooting in stem cuttings (Hartmann *et al.*, 1997). It is also probably the single most effective treatment to achieve successful propagation. In addition to effects on cell differentiation, auxins promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base (Das *et al.*, 1997), although increasing auxin concentrations did not result in respective increases in cutting dry mass (Mesen *et al.*, 1997b). However, behind this apparently 'cure-all' treatment there lies a considerable body of evidence showing that auxin applications are interactive with other treatments (Palanisamy and Kumar, 1997), types of material (Brennan and Mudge, 1998), and environmental variables (Fett-Neto *et al.*, 2001) affecting the rooting capacity of cuttings. This high degree of interaction is probably the reason that the literature is full of apparently contradictory statements about the precise physiological role of auxins in the rooting process, a situation that cannot be resolved when authors do not present definitive information about either the physiological condition of their material or the propagation environment used.

Typically cuttings treated with auxins root more rapidly and produce more roots and usually with a higher percentage of cuttings rooted. Usually, indole-3-butyric acid (IBA) is found to be the most effective root promoting auxin, but occasionally α -naphthalene acetic acid (NAA) can be as effective, as in *Parkia biglobosa* (Teklehaimanot *et al.*, 1996). However, tree species and even clones can appear to respond differently to individual and mixed applications of auxin at differing concentrations, even when many other factors are constant. Interestingly, however,

clones of *Triplochiton scleroxylon*, which appeared to have different dose response curves, all rooted equally well at 40µg auxin cutting⁻¹ (Leakey *et al.*, 1982).

Leaf area

Usually the rooting of softwood cuttings is dependent on the presence of a leaf, and indeed in the physiological processes of this leaf. Cuttings without a leaf very quickly become moribund, while the most common reason for these cuttings failing to root is the death of the leaf due to rotting, necrosis, bleaching or to leaf abscission. All these causes of failure are due to either the use of inappropriate tissues (too old [senescent], photosynthetically inactive [below compensation point; water stressed; starch filled], diseased, pest infected), or to an undesirable rooting environment (too hot, too wet, too dry). The most common symptoms are leaf shedding, leaf rot, stem rot.

Studies of the role of the leaf have indicated that rooting ability is maximised when the severed cutting is photosynthetically active and producing assimilates for the development and elongation of the root primordia, and when the leaf is not suffering water stress (Mesén *et al.*, 2001)(Figure 4). Consequently, there often seems to be an optimum leaf area at which the balance between photosynthesis and transpiration is optimal (Aminah *et al.*, 1997). This varies between species and clones, depending on specific leaf area (leaf thickness), stomatal density, leaf morphology (waxiness, etc.) and the age of the leaf (node position). Optimising the balance between photosynthesis and transpiration seems to be particularly important in difficult-to-root material (; Leakey, 1985), with easy to root species not being very sensitive to leaf area (: Newton *et al.*, 1992). Cuttings with a leaf that is too small, rapidly decline in their carbohydrate (sugars and starch) contents, while those with an appropriate area increase in carbohydrate content (and hence dry matter), at least until the roots start to develop creating a 'sink' for assimilates (; Leakey and Coutts, 1989). One study has found that there is a relationship, which develops after severance, in cuttings with differing leaf areas, between rooting ability and the content of reflux-extracted soluble carbohydrates (Leakey and Storeton West, 1992), which suggests that rooting is promoted by the production of specific sugars during the period that the cuttings are in the propagator. Cuttings with an overly large leaf suffer from transpiratory water loss and stress, and close their stomata, thereby limiting their capacity to photosynthesise, and often triggering leaf abscission. As mentioned earlier, leaf area is an important variable interacting with the level of irradiance in the propagation environment. Consequently statements about the optimal leaf area for rooting have to be linked to statements about the specific propagation environment.

Despite the importance of current assimilates for rooting in leafy softwood cuttings, there is evidence that the level of dependency does vary between species, with some species also being able to utilise stored reserves (Magingo and Dick, 2001; Mesén *et al.*, 2001) in the same way as hardwood cuttings, perhaps reflecting differences in stem anatomy. In *Larix x europepsis* cuttings, for example, a dual ¹³C and ¹⁵N labelling approach, indicated that more than 80% of total carbon in the roots was newly assimilated carbon (Pellicer *et al.*, 2000).

Cutting length

Cuttings can either be cut to a constant length (in which case they will usually vary in the number of nodes present) or can be cut to the available internode length of a pre-determined number of nodes (Figure 5). The decision about which option to take will

have big impacts on the rooting ability, as long cuttings usually root best. From the practical point of view, the numbers of cuttings rooted is maximised by using a constant length, close to the optimum. This has the additional advantage that all the cuttings will penetrate the rooting medium to the same depth and that the leaf will be held the same height above the medium. From the research point of view, however, much can be learnt about the sources of variation by using a fixed number of nodes and utilizing the pattern of variation in internode length found within a stem (Figure 5 - see within shoot variation, below). In the latter case, it is important to remember that internode length varies sequentially down a stem and thus is not an independent variable.

(iii) Stockplant factors: cutting origin and environment

There are two major sources of variation attributable to the stockplant, these are attributable to: (a) Within shoot factors, and (b) Between shoot factors, both of which are strongly influenced by the stockplant environment. Additionally, there are other endogenously controlled growth changes, such as recurrent flushing (Tchoundjeu and Leakey, 2000), which can affect rooting ability.

Within-shoot factors

Within any shoot there are numerous gradients of variation. For example, from the top to the bottom, there is a gradient in age that affects the leaf size, leaf water potential, leaf carbon balance, leaf senescence, internode length, internode diameter, stem lignification, nutrient and stem carbohydrate content, respiration, etc. (Leakey and Mohammed, 1985)). These gradients mean that no two cuttings are physiologically identical and, hence, no two cuttings have the same rooting capacity. Consequently, it is possible to use these gradients node by node as a diagnostic tool for how physiological factors affect rooting (eg. Leakey *et al.*, 1994). For example, one of the unresolved issues that could be addressed is the importance of the relative concentrations of carbohydrates and nutrients in cutting tissues, which vary between node positions and over time. There is evidence of stored reserves being depleted early in the rooting process in *Larix* hybrid (Pellicer *et al.*, 2000), and in *Khaya ivorensis* (Tchoundjeu and Leakey, 2000).

Creating inverse relationships between cutting length and node position (Leakey and Mohammed, 1985) cutting length (in reality probably cutting volume) has a major influence on rooting ability. It seems that this may be due to the need for storage capacity for current assimilates until the new roots form a sink for these carbohydrates. The importance of this was further shown by Leakey and Tchoundjeu (1996) who found a negative relationship between leaf area and cutting length, suggesting that short cuttings can't provide the storage capacity for assimilates coming from a large leaf.

Between shoot factors

Even in the simplest type of stockplant, a seedling that has been cut back once previously (Figure 6), there are considerable differences in rooting ability between the lateral shoots, with those from upper shoots being best (Leakey, 1983). In *T. scleroxylon*, the percentage of cuttings rooted declines as the height of the stockplant increases, there being a relationship between percentage rooting and the number of shoots per plant. The rooting ability of cuttings from lower shaded shoots can, however, be enhanced by the use of fertilizers, or by re-orienting the stockplant

(angled or horizontal) to alter the patterns of correlative inhibition. The rooting ability of cuttings from the upper shoots can be enhanced by removal of the lower shoots, implying that intershoot competition is a factor in the determination of rooting ability. However, under situations of low but equal competition, basal shoots have a higher rooting ability than upper shoots. However, if basal and upper shoots are under similar light environments their rooting ability becomes very similar (Leahey 1983) (Figure 7).

In more complex stockplants, cuttings from first-order lateral shoots of *Cryptomeria japonica* rooted better than those from second-order laterals (Jull *et al.*, 1994), indicating the need for better physiological and morphological understanding of such structural variation.

Stockplant environment pre-severance

As seen above, both nutrients and light have been confirmed to have major effects on rooting ability as preconditioning agents, a finding confirmed in *Albizia guachapele* (Mesén *et al.*, 2001). When this was tested experimentally in *T. scleroxylon*, it was found that there are complex interactions between nutrients and the quantity and quality of light, which affected photosynthesis and the carbohydrate status of cuttings (Leahey and Storeton-West, 1992). Photoinhibition and high starch concentrations in cuttings appeared to inhibit rooting, while active photosynthesis was associated with good rooting. Both low irradiance and low red:far-red ratios independently enhanced rooting ability, but in many natural systems these characteristics of shade occur together and probably have additive benefits. The mechanisms for this enhancement seem to be both morphological and physiological; cuttings from 'shaded' stockplants of *Eucalyptus grandis* have longer internodes, greater specific leaf area, greater co-dominance between shoots, lower rates of pre-severance net photosynthesis, lower chlorophyll concentration, but higher rates of net photosynthesis per unit of chlorophyll, and many other differences in gas exchange characters (Hoad and Leahey, 1994) (Figure 8). These characteristics of pre-severance physiology and morphology, subsequently enhanced the cuttings post-severance physiological status and promoted high rooting ability (Hoad and Leahey, 1996). In *T. scleroxylon*, analysis of deviance indicated that the effects of light quality on rooting were entirely attributable to increased internode length (Newton *et al.*, 1996). The changes in these stockplant factors are largely attributable to changes in the physiological condition of the shoots and are often related to vigour, thus they can be seen as a component of physiological 'ageing', as defined by Wareing and Frydman (1976). The effects of pre-severance light quality on rooting ability have now been demonstrated in a number of different taxa, but as expected, there are differences in stem and leaf morphology. Shoot etiolation under low irradiance and the pre-severance elimination of light from the area of the cutting base independently and additively enhance subsequent rooting ability, having histological effects on stem development and retarded sclereid development (Maynard and Bassuk, 1996). There is also some evidence of etiolation on levels of rooting 'co-factors' and sugar concentrations (Subramani *et al.*, 1996). This newer understanding of pre-severance factors is important as it involves easily overlooked differences in the ambient environment. This contrasts with the manipulative treatments like bark girdling, which have also been used to enhance rooting.

Stockplant management

The importance of all of the above stockplant factors clearly indicates the opportunity for enhancing the rooting ability of cuttings through stockplant management (especially a combination of pruning, fertilizer use and light management) to promote the appropriate morphological and physiological conditions of the shoots. Field trials in the tropics have indicated that using nitrogen-fixing species like *Leucaena leucocephala* to provide a shading canopy above a stockplant hedge can be very beneficial. Hedging approaches have been tested over long periods, and found to be robust with no loss of rooting ability with time, although rooting success is affected by the height of hedges and the types of shoots used (Copes, 1992).

Efforts to improve stockplant management in *Larix* have included the extension of the photoperiod with artificial illumination in autumn, but without success, (Pellicer *et al.*, 1998), and the use of cytokinins to promote the outgrowth of short shoots (Edson *et al.*, 1991). In *T. scleroxylon*, however, the injection of auxins pre-severance was found to enhance rooting ability (Leakey, 1992), with earlier injection dates enhancing the rooting ability of short cuttings.

Phase Change

One aspect of the impact of stockplant physiology on rooting ability is unresolved. As trees grow they develop a gradient towards reproductive maturity (ontogenetic ageing) and after a time reach a threshold above which the newly developing shoots have the capacity to fruit and flower, while those below the threshold are still juvenile (Figure 9). The transition from juvenility to maturity state is called Phase Change. There are large numbers of reports in the literature that cuttings from mature shoots are very much more difficult to root than those from juvenile (seedling or coppice) shoots, although increasingly there are reports of old mature trees being propagated by cuttings, with reasonable success (Schneck, 1997) especially in the early spring (Swamy *et al.*, 2002). However, a good understanding of the reasons why mature trees are difficult has never been achieved.

The importance of physiological ageing in the rooting of cuttings from mature trees is illustrated by recent unpublished data in *Prunus avium* (Figure 10), which indicates that by comparison with leafy juvenile shoot cuttings, the rooting capacity of mature softwood cuttings was limited by the availability of stored reserves, while mature hardwood cuttings were constrained by leaf abscission. Other evidence that the poor rooting ability of 'mature' shoots can be attributed to 'physiological ageing' rather than to 'ontogenetic ageing' is seen in high rooting ability of ontogenetically mature and flowering plants formed when cuttings from mature tree crowns are successfully propagated and then used as stockplants (Figure 9). Although often plagiotropic, such plants have the vigour of juvenile seedlings and coppice shoots. To examine this experimentally within the crown of mature trees is difficult as it requires the formation of physiologically young shoots within an ontogenetically mature crown. This is not achieved by pollarding, but further research is needed to elucidate the relevance of 'phase change' to rooting.

Observations of leaf shape and other morphological characters indicate gradients as trees grow older and these changes have been examined in detail, often using ivy (*Hedera helix* as a model plant). This raises two questions: (i) are the changes in leaf shape relevant? (ii) do we need to have a maturity factor, or are the conditions in

mature cuttings, just a severe case of complex interactions arising from the sorts of stockplant variables examined above? Regarding leaf shape in ivy, there may be an error in the belief that the 'juvenile' leaf form converts to the 'mature' form as a result of ontogenetic ageing, as the 'juvenile' form is associated with the vine stems (the mainstem) and the 'mature' form is associated with free standing branches, which happen to be where ivy, like many other woody plants, flowers (Hallé and Oldeman, 1976: Figure 70C). Different leaf arrangements and shapes in mainstems and branches are very common in woody plants. It is not clear therefore whether the reported differences in genetic material (eg. Sanchez *et al.*, 1995), between these two tissue types is a result of ontogenetic phase change or to epigenetic differences between different tissues. In other species, there are changes in leaf morphology up the mainstem, as in *Acacia mangium* in which the pinnate true leaf on seedlings changes to a 'mature' phyllode. The unreliability of the phyllode as a marker of phase change, was shown in a rooting trial from coppice stumps, in which the rooting ability of cuttings with phyllodes was greater than that of cuttings with true leaves (Monteuuis *et al.*, 1995).

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The felling and subsequent coppicing of mature trees is generally regarded as the best way to return to the juvenile state. However, while this was successful in *Milicia excelsa*, the rooting ability of cuttings from coppice shoots was still negatively correlated with the age of the stump (Ofori *et al.*, 1997). Similarly in *Vochysia guatemalensis* increasing stump diameter also had a negative effect on rooting, with larger stumps produced more shoots, and inter-shoot competition perhaps reducing rooting success (Dick *et al.*, 1998).

This topic of how to propagate mature tissues is the major constraint to many tree improvement programmes focussing on cultivar development through vegetative regeneration. Some rejuvenation of *in vitro* cultures has been reported (eg. Amin and Jaiswal, 1993), but the mechanism remains unclear. *In vitro* micrografting has also been used to rejuvenate shoots (Ewald and Kretzschmar, 1996).

Genetic variation in rooting ability

Experience has suggested that there are genetic differences between species and even between provenances and clones within species (eg. ; Hartmann *et al.*, 1997; Puri and Swamy, 1999), which result in differences in rooting ability, although more and more

formerly ‘unrootable’ species and clones are now being rooted relatively easily. This suggests that as the within-clone factors affecting rooting ability are optimised the apparent genetic differences in rooting ability are actually attributable to genetic differences in the morphological and physiological factors that govern rooting ability. This view is supported by the use of stepwise regression to analyse data from rooting experiments (Dick *et al.*, 1999), which commonly finds that the factors explaining much of the variance are cutting length, leaf abscission, leaf area, etc., and that the genetic differences between clones etc., explain relatively little of the variance. Recent studies have sought to detect quantitative trait loci affecting vegetative propagation (Marques *et al.*, 1999) and indicate that the phenotypic variation has a meaningful genetic component. It is, nevertheless, hard to understand the evolutionary importance of a genetic trait for rooting ability in detached shoots.

Failure to root

To learn more about the processes affecting rooting, there is also a need to pay much more attention to the causes of rooting failure. Some cuttings, as stated earlier, display different symptoms of death from leaf abscission to different patterns of rotting, which can be attributed to water stress, photoinhibition, anoxia, negative carbon balance, etc. Other cuttings neither die nor root. More information needs to be collected on all these responses to attempted propagation.

INTEGRATION BY MODELLING

The complexity of the rooting process in leafy stem cuttings alone make it a very appropriate subject for modelling. Based on the *T. scleroxylon* data set, Dick and Dewar (1992) have developed a mechanistic model of carbohydrate dynamics during the rooting process that provides a framework for many of the other factors discussed above to be examined and compared with research results. Hopefully in future it will be possible to use this and other models (*eg.* Kubota and Kozai, 2001) to test differences in experimental methods and materials and so interpret the oftencontradictory experimental results.

APPARENT ABSENCE OF PRINCIPLES DETERMINING ROOTING SUCCESS

Recording Materials and Methods

On top of the highly interactive nature of the multitude of factors influencing the physiological condition of cuttings, there is an overarching methodological issue, which is that people taking cuttings don’t collect their cuttings in the same way, and more importantly, don’t report what they have done precisely. Because of the impacts of the interactive processes, two people propagating the same species under relatively similar conditions can obtain results that appear to be contradictory, while in fact their results are expressions of the different physiological and morphological condition of the tissues being propagated. Researchers will need to address these deficiencies in their techniques and to address the impacts of all the variables experimentally, before we can expect to identify the fundamental physiological principles determining the success of vegetative propagation.

Percentage rooting – a poor measure of success

To make matters worse, almost all research papers (my own included!) use the Percentage of Cuttings Rooted as the prime measure of rooting success. Taking a simple example, even if auxin application, leaf area and rooting environment are all optimised, when all the available cuttings are collected from a managed juvenile stockplant, they are likely to have a fairly low rooting percentage and hence create a relatively low number of new plants (Figure 11 a). This is because of the inclusion of all of the inherent variation in cutting size, shoot position, etc. If on the other hand, the same people go to an identical plant and because they know more about the species they are propagating and are aware that cuttings from the top two shoots are likely to have the greatest capacity to root, restrict their collection to these shoots (so maximising the use of their time and resources), they will root the same number of cuttings, but can claim an improved rooting percentage (Figure 11 b). In a third situation, where they have more understanding of stockplant management, and grow their stockplants under the shade of other plants and provide nutrients, the stockplant has greater co-dominance between shoots, producing more material to harvest, and the shoots are morphologically and physiologically in a better condition to root well. Now, more cuttings can be collected, and a third shoot can be included, resulting in a much larger number of cuttings rooted, but the percentage rooting may be lower than in Situation 2 (Figure 11 c). Which of these results is of greatest practical value, and how well does the measure of 'Percentage Cuttings Rooted' represent the overall rooting capacity of the cuttings collected? Clearly, if the understanding of vegetative propagation is to advance, we have to be more rigorous in describing the source of our cuttings, and we have to improve the way we quantify rooting capacity.

Many authors do use additional measures of rooting success; for example the number of roots/cutting, the mean or total length of roots produced, the mass of roots produced, and graphs often show differences in the rate of rooting. Interestingly, these measures of success are often unrelated to Rooting Percentage, and it is probably only when these different measures are well correlated that there is a good measure of rooting ability. These methods of quantifying rooting ability and their relationships, require further research.

OTHER PROPAGATION SYSTEMS

Grafting/budding, marcotting (Figure 12), root cuttings and *in vitro* culture (Figure 13) are all systems that are used to multiply ontogenetically mature shoots that are very difficult to propagate by cuttings. This is especially important for the propagation of fruit trees where ontogenetic maturity has to be retained,

Constraints on space in this review make it impossible to fully review progress in other propagation systems. *In vitro* systems, which potentially have much greater multiplication rates and allow for biotechnological developments through genetic manipulation, continue to be an area of rapid development (Mudge and Brennan, 1999), meriting an independent literature review. There is a need for better understanding of the use of these systems to promote easy propagation from a range of mature tissues. Evidence suggests that some physiological rejuvenation occurs after serial subculturing *in vitro* (with and without an assortment of culture medium supplements: eg. Hammatt and Grant, 1997), but the physiological processes involved are still unknown. Additionally, there is a need for more research on the role of pre-severance factors on the opportunities for more robust *in vitro* propagation. For example, light quality has been found to be important for root formation in *Betula*

shoot cultures *in vitro* (Pinker et al., 1989), while darkness was important for micrografting *Picea* shoots *in vitro* (Monteuuis, 1994). Against the advantages of *in vitro* systems are the facts that they are costly and require specialist facilities and staff, making them inappropriate for some developing country applications.

By contrast with *in vitro* systems there have been relatively few major advances in grafting and marcotting in recent years although they are still widely applied (Mudge and Brennan, 1999). Nevertheless, there is a need for improvements to make them more robust and reliable as low-tech options for mature tree propagation. Probably the most important area for future research is an investigation of how to use pre-severance treatments and environment to enhance the rate and success of both the propagation and the subsequent establishment and growth of the young propagule.

CONCLUSIONS

Within any of the numerous different types / systems of propagating trees, there are large numbers of factors that determine whether or not the propagule is in a good physiological condition, and will form a functional plant and grow. For example, when propagating from single-node, leafy cuttings from juvenile shoots, the factors that will determine the level of success are:-

Stockplant environment x stockplant management x topophytic variables x node position x nursery management x post-severance treatments x propagation environment

Each of these factors are themselves multi-faceted and influenced by the ambient environment (light quality and quantity, water, temperature, nutrients) of the stockplant garden, the nursery or the propagation bench. For example, in the stockplant garden the environment (light, water and nutrients) and the management of stockplants can have both short-term impacts on rooting ability by determining the levels of water or heat stress experienced by the tissues being propagated either before severance from the stockplant, or long-term impacts on rooting ability through their effects on the morphology or physiological condition of the shoots. Similarly once the cuttings have been severed from the stockplant the environment of the nursery and the handling of the severed cuttings before and after insertion in the propagation bed will also determine the levels of stress that the cuttings experience. In addition, the cuttings are also affected by the activities of the person doing the propagation and particularly the care taken by this person to minimise the levels of stress experienced by the cuttings (eg. maintenance in a cool, shady, moist environment; reduction of transpiration by leaf trimming, etc). The human element in this is what is commonly called having 'Green fingers' and reflects the person's sensitivity to the needs of the plant material.

FURTHER READING

Amin, MN and Jaiswal, VS (1993). In vitro response of apical bud explants from mature trees of jackfruit (*Artocarpus heterophyllus*). *Plant Cell Tissue and Organ Culture* **33**, 59-65.

Aminah, H, Dick, JMcP and Grace, J (1997). Rooting of *Shorea leprosula* stem cuttings decreases with increasing leaf area. *Forest Ecology and Management* **91**, 247-254.

- Brennan, EB and Mudge, KW (1998). Vegetative propagation of *Inga feuillei* from shoot cuttings and air layering. *New Forests* **15**, 37-51.
- Copes, DL (1992). Effects of long-term pruning, meristem origin, and branch order on the rooting of Douglas-fir stem cuttings. *Canadian Journal of Forest Research* **22**, 1888-1894.
- Das, P, Basak, UC and Das, AB (1997). Metabolic changes during rooting in pre-girdled stem cuttings and air-layers of *Heritiera*. *Botanical Bulletin of Academia Sinica (Taipei)* **38**, 91-95.
- Davis, TD, Haissig, BE and Sankhla, N (1988). *Adventitious Root Formation in Cuttings*, Dioscorides Press, Portland, Oregon, USA, 315p.
- Dick, JMcP and Dewar, RC (1992). A mechanistic model of carbohydrate dynamics during the adventitious root development in leafy cuttings. *Annals of Botany (London)* **70**, 371-377.
- Dick, JMcP, Blackburn, DG and McBeath, C (1994). Stem respiration in leafy stem cuttings of *Prosopis juliflora* during the rooting process. *New Forests* **8**, 179-184.
- Dick, JMcP, Bisset, H and McBeath, C (1996). Provenance variation in rooting ability of *Calliandra calothyrsus*. *Forest Ecology and Management* **87**, 175-184.
- Dick, JMcP, Zuniga, G, Cornelius, JP and Watt, AD (1998). Genetic variation in the number of cuttings harvestable and rooted from *Vochysia guatemalensis* coppiced shoots. *Forest Ecology and Management* **111**, 225-230.
- Dick, JMcP, Magingo, F, Smith, RI and McBeath, C (1999). Rooting ability of *Leucaena leucocephala* stem cuttings. *Agroforestry Systems* **42**, 149-157.
- Edson, JL, Wenny, DL and Fins, L (1991). Inducing long-shoot growth for vegetative propagation of Western larch. *New Forests* **5**, 51-60.
- Ewald, D and Kretzschmar, U (1996). The influence of micrografting *in vitro* on tissue culture behavior and vegetative propagation of old European larch trees. *Plant Cell Tissue and Organ Culture* **44**, 249-252.
- Fett-Neto, AG, Fett, J, Goulart, LWV, Pasquali, G., Termignoni, RR and Ferreira, AG (2001). Distinct effects of auxin and light on adventitious root development in *Eucalyptus saligna* and *Eucalyptus globulus*. *Tree Physiology* **21**, 457-464.
- Hallé, F, Oldeman, RAA and Tomlinson, PB (1976). *Tropical Trees and Forests: An Architectural Analysis*, Springer-Verlag, New York, 441p
- Hammatt, N and Grant, NJ (1997). Micropropagation of mature British wild cherry. *Plant Cell Tissue and Organ Culture*, **47**, 103-110.
- Hartmann, HT, Kester, DE, Davis, FT and Geneve, RL (1997). *Plant Propagation: Principles and Practices* (6th Edition), Prentice-Hall Inc, Upper Saddle River, New Jersey, USA, 770p.
- Hoad, SP and Leakey, RRB (1994). Effects of light quality on gas exchange and dry matter partitioning in *Eucalyptus grandis* W. Hill ex Maiden. *Forest Ecology and Management* **70**, 265-273. ????
- Hoad, SP and Leakey, RRB (1996). Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex Maiden: Cutting morphology, gas exchange and carbohydrate status during rooting. *Trees* **10**, 317-324.
- Jull, LG, Warren, SL and Blazich, FA (1994). Rooting 'Yoshino' cryptomeria stem cuttings as influenced by growth stage, branch order, and IBA treatment. *Hortscience* **29**, 1532-1535.
- Krugman, S.L. (1974). Special Issue on Vegetative Propagation, *New Zealand Journal of Forestry Science* **4**: 1-458.

- Kubota, C and Kozai, T (2001). Mathematical models for planning vegetative propagation under controlled environments. *Hortscience* **36**, 15-19.
- Leakey, RRB (1983). Stockplant factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum., an indigenous hardwood of West Africa. *Journal of Horticultural Science* **58**, 277-290.
- Leakey, RRB (1985). The capacity for vegetative propagation in trees. In: Cannell MGR and Jackson JE (eds.) *Attributes of Trees as Crop Plants*, pp 110-133. Institute of Terrestrial Ecology, Monks Wood, UK.
- Leakey, RRB (1992). Enhancement of rooting ability in *Triplochiton scleroxylon* by injecting stockplants with auxins. *Forest Ecology and Management* **54**, 305-313.
- Leakey, RRB and Coutts, MP (1989). The dynamics of rooting in *Triplochiton scleroxylon* cuttings: their relation to leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. *Tree Physiology* **5**, 135-146.
- Leakey, RRB and Mohammed, HRS (1985). The effects of stem length on root initiation in sequential single-node cuttings of *Triplochiton scleroxylon* K. Schum. *Journal of Horticultural Science* **60**, 431-437.
- Leakey, RRB and Storeton-West, R (1992). The rooting ability of *Triplochiton scleroxylon* cuttings: the interaction between stockplant irradiance, light quality, and nutrients. *Forest Ecology and Management* **49**, 133-150.
- Leakey, RRB and Simons, AJ (2000). When does vegetative propagation provide a viable alternative to propagation by seed in forestry and agroforestry in the tropics and sub-tropics? In: Wolf H and Arbrecht J (eds.) *Problem of Forestry in Tropical and Sub-tropical Countries - The Procurement of Forestry Seed - The Example of Kenya*, pp 67-81. Ulmer Verlag, Germany.
- Leakey, RRB, Newton, AC and Dick, JMcP (1994). Capture of genetic variation by vegetative propagation: processes determining success. In: Leakey RRB and Newton AC (eds.) *Tropical Trees: The Potential for Domestication and the Rebuilding of Forest Resources*, pp 72-83. HMSO, London, UK.
- Leakey, RRB, Chapman, VR and Longman, KA (1982). Physiological studies for tropical tree improvement and conservation – some factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum. *Forest Ecology and Management* **4**, 53-66.
- Leakey, RRB, Ferguson, NR and Longman, KA (1981). Precocious flowering and reproductive biology of *Triplochiton scleroxylon* K. Schum. *Commonwealth Forestry Review* **60** 117-126.
- Leakey, RRB, Mézen, F, Tchoundjeu, Z, Longman, KA, Dick, JMcP, Newton, AC, Matin, A, Grace, J, Munro, and Muthoka, PN (1990). Low-technology techniques for the vegetative propagation of tropical trees. *Commonwealth Forestry Review* **69**, 247-257.
- Longman, K.A., Manurung, R.M. and Leakey, R.R.B. 1980. Use of small, clonal plants for experiments on factors affecting flowering in tropical trees. In: Bawa KS and Hadley M (eds.) *Reproductive Ecology of Tropical Forest Plants*, Man and the Biosphere Series Vol 7, 389-399. UNESCO, Paris and Parthenon Publishing Group, Carnforth, UK.
- Magingo, F and Dick, JMcP (2001). Propagation of two miombo woodland trees by leafy stem cuttings obtained from seedlings. *Agroforestry Systems* **51**, 49-55.
- Marques, CM, Vasques-Kool, J, Carocha, VJ, Ferreira, FG, O'Malley, DM, Liu, B-H and Sederoff, R (1999). Genetic dissection of vegetative propagation traits in

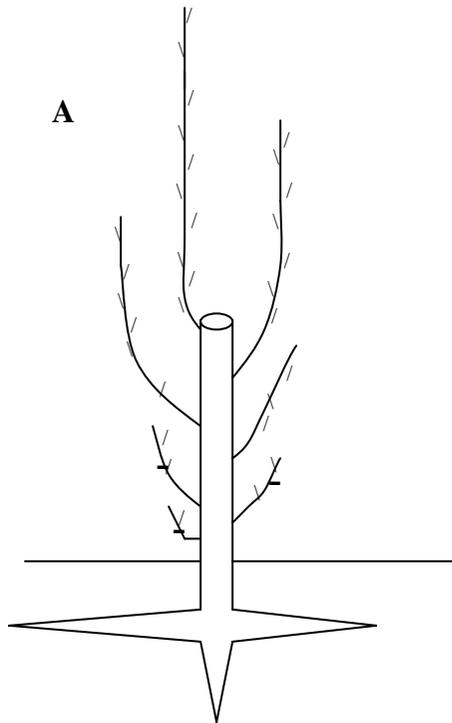
Eucalyptus tereticornis and *E. globulus*. *Theoretical and Applied Genetics* **99** 936-946.

- Maynard, BK and Bassuk, NL (1996). Effects of stock plant etiolation, shading, banding and shoot development on histology and cutting propagation of *Carpinus betulus* L. 'Fastigiata'. *Journal of the American Society for Horticultural Science* **121**, 853-860.
- Mesén, F, Newton, AC and Leakey, RRB (1997a). The effects of propagation environment and foliar areas on the rooting physiology of *Cordia alliodora* (Ruiz & Pavon) Oken cuttings. *Trees* **11**, 401-411.
- Mesén, F, Newton, AC and Leakey, RRB (1997b). Vegetative propagation of *Cordia alliodora* (Ruiz & Pavon) Oken: the effects of IBA concentration, propagation medium and cutting origin. *Forest Ecology and Management* **92**, 45-54
- Mesén, F, Leakey, RRB and Newton, AC (2001). The influence of stockplant environment on morphology, physiology and rooting of leafy stem cuttings of *Albizia guachapele*. *New Forests* **22**, 213-227.
- Minghe, L and Ritchie, GA (1999a). Eight hundred years of clonal forestry in China: I. Traditional afforestation with Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.). *New Forests* **18**, 131-142.
- Minghe, L and Ritchie, GA (1999b). Eight hundred years of clonal forestry in China: II. Mass production of rooted cuttings of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.). *New Forests* **18**, 143-159.
- Monteuuis, O (1994). Effect of technique and darkness on the success of meristem micrografting of *Picea abies*. *Silvae Genetica* **43**, 91-95.
- Monteuuis, O, Vallauri, D, Poupard, C and Chauviere, M (1995). Rooting Acacia mangium cuttings of different physiological age with reference to leaf morphology as a phase change marker. *Silvae Genetica* **44**, 150-154.
- Mudge, KW and Brennan, EB (1999). Clonal propagation of multipurpose and fruit trees used in agroforestry. In: Buck LE, Lassoie JP and Fernandes ECM (eds.), *Agroforestry in Sustainable Agricultural Systems*, pp 157 – 190, CRC and Lewis Publishers, New York, USA.
- Newton, AC and Jones, AC (1993a). Characterization of microclimate in mist and non-mist propagation systems. *Journal of Horticultural Science* **68**, 421-430. ???
- Newton, AC and Jones, AC (1993b). The water status of leafy cuttings of four tropical tree species in mist and non-mist propagation systems. *Journal of Horticultural Science* **68**, 653-663.
- Newton, AC, Muthoka, PN and Dick, JMCP (1992). The influence of leaf area on the rooting physiology of leafy stem cuttings of *Terminalia spinosa* Engl. *Trees* **6**, 210-215.
- Newton, AC, Dick, JMCP, McBeath, C and Leakey, RRB (1996). The influence of R:FR ratio on the growth, photosynthesis and rooting ability of *Terminalia spinosa* Engl. and *Triplochiton scleroxylon* K.Schum. *Annals of Applied Biology* **128**, 541-556.
- Ofori D, Newton, AC, Leakey, RRB and Grace, J (1997). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: Effects of auxin concentration, leaf area and rooting medium. *Forest Ecology and Management* **84**, 39-48.
- Ofori, D, Newton, AC, Leakey, RRB and Grace, J (1997). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: Effects of maturation, coppicing, cutting length and position on rooting ability. *Journal of Tropical Forest Science* **10**, 115-129.

- Palanisamy, K and Kumar, P (1997). Effect of position, size of cuttings and environmental factors on adventitious rooting in neem (*Azadirachta indica* A. Juss). *Forest Ecology and Management* **98**, 277-280.
- Pellicier, V, Cazet, M, Verger M and Riviere, LM (1998). Effect of stockplant lighting on bulk vegetative propagation of hybrid larch (*Larix x eurolepis* Henry). *Forest Ecology and Management* **102** 323-332.
- Pellicier, V, Guehl, J-M, Daudet, F-A, Cazet, M, Riviere, LM and Maillard, P (2000). Carbon and nitrogen mobilization in *Larix x eurolepis* leafy stem cuttings assessed by dual ¹³C and ¹⁵N labelling: Relationships with rooting. *Tree Physiology* **20**, 807-814.
- Pinker, I, Zoglauer, K and Goering, H (1989). Influence of light on adventitious root formation in birch shoot cultures *in vitro*. *Biologia Plantarum* **31**, 254-260.
- Puri, S and Swamy, SL (1999). Geographical variation in rooting ability of stem cuttings of *Azadirachta indica* and *Dalbergia sissoo*. *Genetic Resources and Crop Evolution* **46**, 29-36.
- Sanchez, MC, Smith, AG and Hackett, WP (1995). Localized expression of a proline-rich protein gene in juvenile and mature ivy petioles in relation to rooting competence. *Physiologia Plantarum* **93**, 207-216.
- Schneck, V (1997). Studies on the influence of clone on rooting ability and quality in the propagation of cuttings from 40 to 350-year old yews (*Taxus baccata* L.). *Silvae Genetica* **45**, 246-249.
- Shiembo, PN, Newton, AC and Leakey, RRB (1996). Vegetative propagation of *Gnetum africanum* Welw., a leafy vegetable from West Africa. *Journal of Horticultural Science* **71**, 149-155.
- Subramani, KK, Vijayakumar, N, Farooqi, AA, and Ramu BS (1996). Influence of season in the biochemical composition and rooting in pre-conditioned stem cuttings of pomegranate (*Punica granatum* L.) cultivars. *Crop Research (Hisar)* **11**, 347-352.
- Swamy, SL, Puri, S and Singh, AK (2002). Effect of auxins (IBA and NAA) and season on rooting of juvenile and mature hardwood cuttings of *Robinia pseudoacacia* and *Grewia optiva*. *New Forests* **23**, 143-157.
- Tchoundjeu, Z and Leakey, (1996). Vegetative propagation of African Mahogany: effects of auxin node position, leaf area and cutting length. *New Forests* **11**, 125-136.
- Tchoundjeu, Z and Leakey, (2000). Vegetative propagation of *Khaya ivorensis* (African Mahogany): effects of stockplant flushing cycle, auxin and leaf area on carbohydrate and nutrient dynamics of cuttings. *Journal of Tropical Forest Science* **12**, 77-91.
- Teklehaimanot, Z, Tomlinson, H, Lemma, T and Reeves, K (1996). Vegetative propagation of *Parkia biglobosa* (Jacq.) Benth., an undomesticated fruit tree from West Africa. *Journal of Horticultural Science* **71**, 205-215.
- Tousignant, D, Villeneuve, M, Rioux, M and Mercier, S (1995). Effect of tree flowering and crown position on rooting success of cuttings from 9-year-old black spruce of seedling origin, *Canadian Journal of Forest Science* **25**, 1058-1063.
- Wareing, PF and Frydman, VM (1976). General aspects of phase change, with special reference to *Hedera helix* L. *Acta Horticulturae* **56**, 57-69.

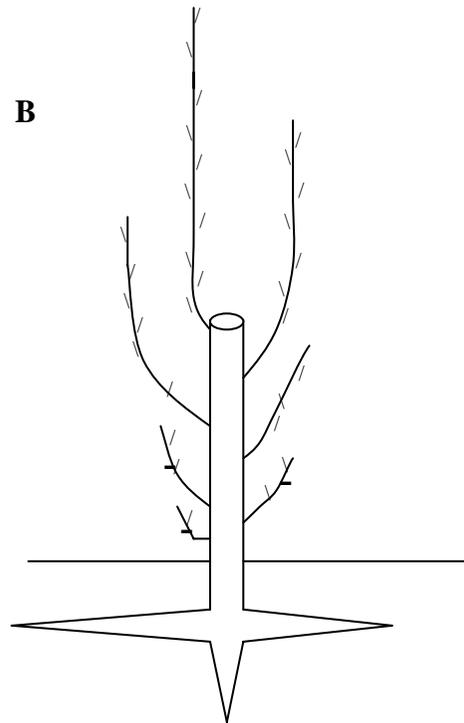
Figure 1. Diagram showing how % rooting varies in optimally treated cuttings, depending on which cuttings are used and on how the stockplant is managed (leaves omitted for clarity).

Standard stockplant:
No knowledge about
rooting behaviour



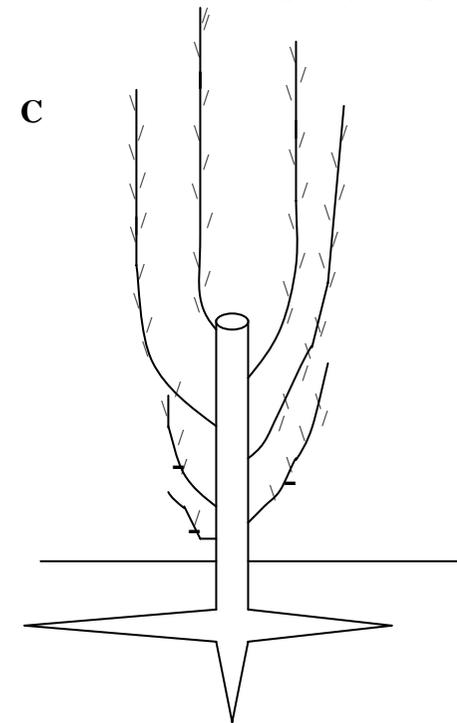
Harvest all 31 cuttings:
16 rooted
= 52% = 16 plants

Standard stockplant: with
knowledge that rooting is
best from upper shoots



Harvest 16 cuttings from
top 2 shoots: 16 rooted
= 100% = 16 plants

Stockplant under far-red light
with nutrients: Shoot co-
dominance enhanced with
increased rooting capacity



Harvest 45 cuttings from
top 3 shoots: 40 rooted
= 89% = 40 plants