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THE CAPACITY FOR VEGETATIVE PROPAGATION IN TREES

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I. INTRODUCTION

The roots and shoots of plants are capable of growth throughout their lives, owing to the presence of relatively undifferentiated cells with unrestricted developmental potential (Wareing & Graham 1976). The classic work of Steward (1970), in which whole carrot plants were grow from single cells, clearly demonstrated the totipotency of parenchyma cells, which in many species can dedifferentiate and develop into new plants (Street 1976). For centuries, horticulturists, botanists and foresters have exploited this capacity of plant cells to multiply and differentiate in a variety of propagation techniques.

The capacity of trees to be propagated vegetatively ought in theory to be similar to that of herbaceous plants; however, their greater size and structural complexity at maturity result in a loss in rooting ability which has to be avoided or overcome by using young plants, coppice or 'rejuvenated' shoots (Zimmermann 1976). Also, the capacity for vegetative propagation in trees varies greatly between species and genotypes, and is affected by both their environment and physiological state. Some examples of these influences will be described in this chapter (see also Komissarov 1969; Bonga & Durzan 1982; Hartmann & Kester 1983).

II. VEGETATIVE REGENERATION IN NATURAL STANDS

Many plants, notably weed species, have evolved the ability to regenerate vegetatively from intact or detached plant parts, some of which are specialized

organs (Leakey 1981). In trees, this form of vegetative propagation is relatively uncommon, but a few examples are given below.

Sucker shoots grow from intact root systems in a number of genera, including *Ulmus*, *Robinia*, *Prunus*, *Malus*, *Populus* and *Liquidambar*. Suckers normally develop either from newly initiated meristems on young roots with a developed bark layer and some secondary thickening (Eliasson 1971a), or from preformed shoot primordia developing as protuberances in the phellogen of older roots up to about 2·5 cm in diameter (Schier 1973a). Suckers are apparently prevented from developing, at least in *Populus tremula*, by the accumulation of auxins in the roots, and are stimulated by treatments or events interrupting their translocation from the shoots (Eliasson 1971b, c). Apart from the regeneration of cut aspen and other poplar stands in the USA, little practical use is made of suckering on intact root systems, although sucker shoots could be collected and planted. In a few instances, techniques have been developed to increase the incidence of suckering, as in potted seedlings of *Agathis robusta* (Whitmore 1977) and in natural stands of *Santalum album* (Mahmood Husain & Ponnuswamy 1982).

In the annually burned zone of tropical Australia, rhizomes and lignotubers provide a survival mechanism for a range of *Eucalyptus* species, which maintain a subterranean 'bank' of viable, dormant buds for root and shoot production (Lacey *et al.* 1982). In a somewhat similar way, old and isolated trees of *Doryphora sassafras* and *Eucryphia moorei*, in the cool temperate rainforests of Australia, are adapted to regenerate by producing new shoots from their swollen stem bases (Johnson & Lacey 1983). In North America, *Quercus gambelii* and *Prunus virginiana* produce a shallow network of rhizomes, bearing some large roots which extend vertically downwards to a deep-feeding root system (Schier 1983).

Some intact prostrate branches or buried stems are able to produce roots when in contact with damp ground. This natural 'layering' ability is particularly well seen in some tropical species (Hall & Swaine 1981) and it is exploited in the propagation of various fruit and ornamental tree species (see IIIB, below).

Adventive polyembryony (the asexual multiplication of seeds by agamospermy or apomixis) occurs in *Shorea agami* and *Shorea ovalis* – emergent trees of the Malaysian tropical forests – and it may be common in the Dipterocarpaceae (Kaur *et al.* 1978; Jong 1980). It also occurs in apple, and is well known in cultivated fruit trees like *Citrus*, *Eugenia*, *Garcinia* and *Lansium*, originating from the tropical forest understorey.

III. VEGETATIVE PROPAGATION IN ARTIFICIAL SYSTEMS

A. Graft formation

Scion/rootstock grafting is an age-old practice, and numerous techniques have been developed by horticulturists and foresters (Garner 1979; Hartmann & Kester 1983). They all exploit the ability of cambial cells, placed and held

firmly in close contact, to produce callus, uniting the graft, and subsequently differentiating new vascular tissues. The ability to produce a graft union, which will not disintegrate or break, is a composite function of many genetic, environmental, anatomical and physiological factors.

1. *Genetic factors*

Plants with continuous cambial layers which can readily be placed in direct contact are easiest to graft; they include all true woody species. Grafting success is greatest between closely related plants (Hartmann & Kester 1983); *heteroplastic* grafts between plants of different families and genera are rare, and different species within a genus can be difficult to intergraft, although over 800 combinations are known (Sziklai 1967). Among the spruces, *Picea abies* and *P. glauca* are used as rootstocks for at least six other species (Holst *et al.* 1956). Compatibility in interspecific grafts is sometimes successful only when using specific clonal combinations, as with peach and almond scions on plum rootstocks, in which even reciprocal clonal combinations can fail (Hartmann & Kester 1983). Very easy and compatible unions often result from *homeoplastic* graftings between clones of the same species, as they do from *autoplastic* grafts within a clone. In a few instances, as in peaches, certain species will graft better on to other species than on to themselves.

2. *Environmental factors*

Probably the most common environmental causes of grafting failure are losses of cell turgidity and desiccation, sub- or supra-optimal temperatures for rapid cell growth, the incidence of disease (particularly virus infections) and movement of the scion on the stock. Great care has to be taken to protect the thin-walled, tender parenchyma cells from water stress, which might arise internally through excessive transpiration from the scion and/or externally by inadequate protection of the graft itself.

The need for rapid cell division in the cambium means that grafting should be done at a time of year when temperatures are favourable and the tissues are active or breaking dormancy. In temperate zones, these conditions often occur in spring when, for example, grafting success in *Pseudotsuga menziesii* can exceed 90% (Copes 1970). In the tropics, the equivalent situation occurs prior to the transition from dry to rainy seasons (Okoro 1976). In hot climates, it is often necessary to shade the grafts – this provides a cool, moist environment and can considerably extend the grafting period (Hearne 1971).

3. *Anatomical factors*

The capacity to develop a compatible union is greatly dependent on the close juxtaposition of the cambium across the graft, giving direct and functional connections within the xylem and phloem. Cell recognition, callus formation and differentiation are critical steps in graft formation (Hartmann & Kester 1983). In *Pinus sylvestris* and *Picea abies*, cell division first occurs most vigorously in those regions of the stock that act as storage places for nutrients, like the parenchyma cells of the rays (Dormling 1963), but, in well-matched grafts, tissues external to the cambium produce the most callus.

4. *Physiological factors*

Normally, it is essential for vigorous growth that the stock and scion are correctly orientated. Thus, in stem grafts, the proximal end of scions are inserted into the distal ends of stocks, and, when shoots are grafted on to roots, their proximal ends are brought together (Hartmann & Kester 1983).

Despite a considerable body of practical evidence suggesting that the condition of the stocks and scions is important for the successful development of a graft union, there are few physiological data indicating which aspects are important. Seasonal variation has been demonstrated in apple, by comparing field-grown micro-grafted scions *in vitro* with those grown *in vitro*: consistent year-round success was achieved in the latter but not the former (Huang & Millikan 1980). Greatest success with field-grown material is usually achieved when the scion is dormant, but has been chilled, and when the stock is beginning to make active growth (Holst *et al.* 1956). However, there are few data to explain why this is so. Furthermore, little is known about the role of plant hormones in the development of a graft union.

The compatibility of graft unions is ultimately a function of biochemical events. Two types of incompatibility have been observed: those that are translocated and those that are localized; only the latter can be overcome by the insertion of a compatible interstock or incompatibility bridge. Translocated incompatibilities involve phloem degeneration and necrosis, while localized incompatibilities often result from translocation difficulties, such as the abnormally early termination of xylem growth (Copes 1975).

Biochemically, the compatibility of a graft union may depend on recognition events between the division products of cells within the vascular tissue and cortex, but little work has been done using tree species. In autoplastic grafts of tomato, a pectinaceous common wall complex is produced between stock and scion, which subsequently becomes thin in places to allow the development of plasmodesmatal connections between cells that are in contact. Jeffree and Yeoman (1983) suggested that the cell walls become thin in response to an exchange of diffusible messenger molecules, providing a direct structural linkage between membranes of opposing cells and a pathway for molecules with a recognition function. This may be a critical step in a hierarchy of recognition events, determining the capacity of a graft to form a compatible union. However, incompatibility between pear (*Pyrus communis*) and quince (*Cydonia oblonga*), in warm climates, is attributed to the catabolism of a cyanogenic glycoside (prunasin) ascending into the pear scion from the quince stock (Gur *et al.* 1968). Other evidence on the causes of incompatibility implicates peroxidase activity in the phloem. Thus, grafts between *Prunus* cultivars produce quantitatively more, and qualitatively different, peroxidases above and below the union (Schmid & Feucht 1982), and peroxidase activity is negatively correlated with *in vitro* micro-grafting success in peach (*Prunus persica*) (Pöessel *et al.* 1980).

B. Layering

The stimulation of rooting on intact stems (layering) has traditionally been used in several forms. Mound layering or stooling has been used to propagate

apple and pear rootstocks, where success can depend on the size of the plants established in the stoolbed (Howard 1977). Simple layering is used to propagate filberts (*Corylus maxima*) and air layering, or marcottage, is used on litchi (*Nephelium litchi*) and mature pines (Hartmann & Kester 1983). In all these circumstances, the part of the shoot to be rooted is kept in the dark under the soil or enclosed in a polythene-covered bundle of moss. The bark may be removed or cut, to promote the accumulation of carbohydrates and endogenous hormones, and auxins may be applied to the wound. The greatest success is achieved in spring, using vigorously leafy shoots. In the tropical hardwood *Triplochiton scleroxylon*, root formation was greatest on leading shoot internodes and declined with increasing order of branching. Auxins were beneficial and disbudding was detrimental to rooting (Okoro & Omokaro 1975). Marcotts were more successful on large trees than on saplings, and the capacity to form roots was greatest in August, at the end of the growing season. By contrast, air layering of *Morus alba*, *Ficus carica*, *Grewia optiva* and *Acacia catechu* is most successful before the Indian monsoon (Khosla *et al.* 1982).

C. Propagation from root and rhizome fragments

As mentioned above, shoot bud primordia can form on intact roots (in *Populus*, *Salix*, *Prunus*, etc). The initiation and development of these buds can be stimulated in summer by artificial fragmentation, which releases them from the effects of high levels of endogenous auxin and β inhibitor (Eliasson 1971c). In *Populus tremula*, the levels of β inhibitor were ten times greater in root fragments in the light than in the dark, but shoot growth was unaffected. The absence of β inhibitor in dark shoots may enable suckers to emerge on roots at great depths in the soil (Eliasson 1971b).

In *Populus tremuloides*, sucker production was increased by treating root fragments with (a) ethylene-releasing 'Ethepon' at 100 mg l^{-1} (Schier & Campbell 1978), (b) the anti-auxin α-(p-chlorophenoxy)isobutyric acid (CPIBA) in June, when their auxin content was greatest (Schier 1975), and (c) gibberellic acid (GA_3) in July, applied to visible buds (Schier 1973b). Clones differed considerably, with an average of 6 to 24 sucker shoots forming on root fragments of different genotypes (Schier 1974). The use of long root fragments (up to 1 m) did not increase the total number of shoots formed or the numbers overwintering (Perala 1978).

In apple cultivars, adventitious shoots form only after root fragmentation. The greatest number of shoots formed on root cuttings collected in early winter, when they were rich in stored polysaccharides (Robinson & Schwabe 1977b). Shoot formation was enhanced by cold storage and cytokinin application, and shoot cuttings from them were subsequently rooted easily with applied auxins (Robinson & Schwabe 1977a).

In contrast to the development of sucker shoots from *Populus* root fragments, shoot production by oak (*Quercus gambelii*) and chokecherry (*Prunus virginiana*) rhizomes was greater in the light than in darkness (Schier 1983). Rhizome fragments of both species produced similar numbers of shoots, perhaps owing to the development of dominance (Leakey 1981), although chilling dormant

rhizomes of *Q. gambelii* enhanced sprouting. It appears that these shoots, from underground stems, have topophytic variation similar to that in aerial shoots. This variation is not found in sucker shoots from roots (Schier 1983).

D. Bud formation *in vitro*

Few plant tissues are completely unsuitable as the starting point for *in vitro* culture. The capacity for propagation by *in vitro* culture is limited by the establishment and maintenance of the tissue in an appropriate condition to induce the rapid division and subsequent differentiation of cells. The explant must be kept in sterile conditions, and provided with (a) macro- and micro-nutrients, (b) a source of energy, usually sucrose, (c) vitamins, amino-acids, etc, and (d) the correct balance and sequence of plant growth regulators, co-factors, etc, to regulate the subcellular and cellular processes of cell division and differentiation of shoot, root, or embryo. Success will also depend on the osmotic pressure and pH of the medium, which can be a solid or liquid, and the physical environment. The details of these requirements are presented in many books on this subject and will not be considered further here.

Three *in vitro* propagation systems have evolved: organogenesis, embryogenesis and meristem proliferation or micro-propagation. A problem common to these methods is the exudation of toxic phenolic compounds into the medium. Various techniques have been used to reduce this problem, including the use of only a short period of sterilization in sodium hypochlorite rather than alcohol (Staritsky & van Hasselt 1980), soaking the explants in sterile water for 3 h prior to culturing them (Chevre *et al.* 1983; Vieitez *et al.* 1983), culturing in the dark, and using activated charcoal (Monaco *et al.* 1977).

1. *Organogenesis*

Although a number of tree species have been successfully propagated by organogenesis from callus culture (see David 1982; Brown & Sommer 1982), this approach has been relatively unsuccessful in trees (Jones 1983). It remains important, however, because the rates of multiplication from individual cells are very great. On the other hand, there is a risk that genetic changes will occur.

In forest trees, successful organogenesis has usually occurred in callus cultures derived from embryos, or from hypocotyl and cotyledon explants, although tissues from large trees have also been found to be capable of developing adventitious primordia (Biondi & Thorpe 1982). Seed or seedling-derived material is generally recommended, and attention should be paid to their physiological state, including the conditions of germination and seed stratification (Sommer & Caldas 1981).

Differences in media composition cannot be considered here, except to say that, with various modifications, the Murashige and Skoog basal medium (M & S) is the most commonly used. The performance of species and cultivars differs on media with different mineral composition, with high levels of organogenesis in *Picea abies* occurring on media with slow callus formation (Bornman 1983). Generally, the differentiation of shoot primordia from callus

requires higher concentrations of cytokinins than auxins, but the relative levels of these have to be determined for each species, and possibly modified for different clones. The different auxins (Durzan 1982) and cytokinins (von Arnold 1982) used can have varying optimal concentrations, depending on the production system. Organogenesis in *Pinus radiata* is promoted by withdrawing cytokinins after a 21-day bud initiation phase (Biondi & Thorpe 1982). In *Picea abies*, the cytokinin requirement for organogenesis can best be met by a short-duration (3 h), high-concentration (125 μM) pulse, or by lower concentrations (5 μM) with vacuum infusion (Bornman 1983). Other advances in the generally poor performance of *P. abies* have resulted from attention to the details of basal media concentration, to the optimal balance of different cytokinins, and to photoperiod (von Arnold 1982).

The culture of protoplasts – the living parts of plant cells removed from their cell walls by enzymatic digestion – is a necessary prerequisite to the production of somatic hybrids by the fusion of cells from different plant species, and their subsequent regeneration by callus culture. In trees, protoplasts have been isolated and cultured for a number of species (reviewed by Ahuja 1982), but their capacity for propagation remains virtually unknown, as this technique is still in its infancy. The main exception to this generalization is the *Citrus sinensis* cultivar Shamouti, which has a high optimal plating density (4×10^{-5} cells ml^{-1}) for cell division with a lower optimum (10^5 cells ml^{-1}) for colony formation, and active callus colonies free from protoplasts subjected to X-ray treatments have subsequently formed embryoids (Vardi *et al.* 1975).

In common with the previously mentioned *in vitro* techniques, the physiological condition of the starting material for protoplast isolation is critical, with seedling material, particularly that already cultured with auxins and cytokinins *in vitro*, being the most amenable. In *Betula*, the yield of protoplasts from 3- to 4-week-old seedling shoot cultures exceeded, by 30 times, that from 12- to 16-week-old cultures (Smith & McCown 1983). The reformation of cell walls in culture is prevented by the inclusion of cellulase and pectinase in the liquid media, together with osmotic stabilizing agents like 0·4–0·8 M mannitol (see Kirby 1982). *Pinus taeda* protoplasts can form cell walls in 48 h, divide every four to nine days, and produce numerous callus colonies in three weeks (Teasdale & Rugini 1983). The future prospects for long-term culture, and probably protoplast fusion, are reasonably good. Furthermore, the occurrence of genetic variability in rapidly dividing single-cell cultures may be a source of somaclonal variation of benefit to future tree improvement programmes (Larkin & Scowcroft 1981).

2. *Embryogenesis*

Cells can form somatic embryos, under basically similar conditions to the above, as in internode and leaf explants of coffee (*Coffea arabica*) (Monaco *et al.* 1977), nucellus tissues of citrus fruits (Button & Kochba 1977) and immature embryos of cacao (*Theobroma cacao*) (Pence *et al.* 1979). In coffee, different combinations of auxins and cytokinins seem to determine both the speed with which embryo initiation occurs and the final extent of multiplication (Staritsky & van Hasselt 1980). In four *Citrus* cultivars, the carbohydrate

source is thought to be important for embryo formation, with galactose in particular enhancing embryogenesis (Kochba *et al.* 1982). Mango (*Mangifera indica*), like *Citrus*, is a naturally polyembryonic species (Rangaswamy 1982), and seems to be highly amenable to embryogenesis *in vitro* (Litz *et al.* 1984). In mango, success followed a series of media transfers, in which auxin (2, 4–D) replaced coconut milk, and was then omitted, leaving the basal medium free from plant growth regulators. Similarly, auxins, cytokinins and gibberellins inhibited embryogenesis of *Citrus* on a galactose-containing medium (Kochba *et al.* 1982), although benzyladenine was later needed for embryo germination. Contrary to these experiences, the capacity for embryogenesis in forest trees has been stimulated by the addition of gibberellic acid to the medium, both with and without cytokinins, in *Santalum album* and *Eucalyptus citriodira* (Sita *et al.* 1980; Sita 1982). We now know that a number of tropical tree species are naturally polyembryonic, so the prospects look encouraging for further exploitation of embryogenesis.

3. *Meristem proliferation of shoot cultures*

This technique starts with an organized meristematic explant – normally a shoot tip or axillary bud (preferably with little callus) containing a terminal bud and many lateral buds. The objective is to stimulate the continued growth of all these meristems by enhancing the capacity for sylleptic branching (as defined by Tomlinson & Gill 1973) and preventing the establishment of apical dominance. Large multiplication rates (4- to 10-fold every four to six weeks) have been achieved by regularly subculturing and cropping the shoots for subsequent rooting. Over the last eight years, this approach has been applied successfully to an increasing range of tree species, notably apple and some other horticultural and plantation crops (Jones 1983).

As in material for all forms of propagation, the state of the explant is important, but a year-round supply of young apple shoots has been successfully achieved by removing leafless winter shoots from cold storage at regular intervals. Although successful proliferation occurs most frequently from vigorous young shoots, explants from mature trees of *Tectona grandis*, *Tamarindus indica*, *Punica granatum* and *Eucalyptus citriodora* have been cultured and rooted (Mascarenhas *et al.* 1982). Similarly, successful propagation has occurred using explants from 4-year-old apple scion cultivars (Jones *et al.* 1979).

Not surprisingly, clones vary in their media requirements, and in their capacity to proliferate, perhaps reflecting differing degrees of apical dominance. For instance, whereas shoot proliferation of apple rootstocks M7 and M26 and several scion cultivars was greatly enhanced by phloridzin and phloroglucinol (PG) in the presence of 0·5 mg l^{-1} 6-benzlaminopurine (BAP) (Jones 1976; Jones *et al.* 1979), cytokinin-induced proliferation in M9 was not enhanced by PG, although it did improve subsequent rooting (James & Thurbon 1981). Cytokinins alone (0·5–1·0 mg l^{-1}) were similarly found to be sufficient for proliferation of M27, M26 and the scion cultivar Macspur (Lane & McDougald 1982).

Beneficial effects of PG have been reported in plum and cherry (Jones & Hopgood 1979). In plum, it enhanced shoot numbers three-fold in BAP-treated cultures of the cultivar Pixy, but it had no additive effect in the

cherry rootstock F12/1, while in the Myrobalan plum rootstock cytokinin was effective on its own (Hammerschlag 1982). In cacao, on PG-free media, zeatin and zeatin-riboside (10^{-5} M) were equally as effective as BAP (10^{-6} M), but the cultures died after 12 to 14 months (Passey & Jones 1983). In *Pistacia vera*, on the other hand, kinetin was not as effective as BAP, and gibberellins improved neither proliferation nor shoot growth (Barghchi & Alderson 1983).

In chestnut (*Castanea* spp.), the number of shoots formed per culture, and the elongation of the longest shoots were affected by the nutrient content of the media (Vieitez *et al.* 1983), and in common with some forest trees the M & S medium was not found to be the best. Chevre *et al.* (1983) overcame this difficulty by lowering the pH to 4, doubling the Ca and Mg concentrations and adding ascorbic acid. However, cultures derived from mature trees had to be subcultured to a BAP-free, auxin-containing medium for elongation to occur prior to rooting.

Among the forest tree species, shoots of *Eucalyptus* spp. have proliferated on various media. *E. citriodora* was successfully propagated on media similar to those used on horticultural crops, except that phloroglucinol was not tried (Sita & Vaidyanathan 1979; Mascarenhas *et al.* 1982). In *E. ficifolia*, on the other hand, proliferation of explants occurred on media in which the auxin concentration exceeded the cytokinin concentration, although only seedling origin cultures produced rooted plantlets (de Fossard *et al.* 1977).

Clonal variation in growth and proliferation in culture have been observed in *Populus* spp. (Ahuja 1983) and in a preliminary study of African mahogany (*Khaya ivorensis*). In the former species, 26 out of 48 clones failed to grow in culture, while, in the latter, shoots of different clones proliferated on an NAA-containing medium with BAP ranging from 0·9 to 1·5 mg l^{-1} (England & Leakey, unpublished).

Similar success has been achieved in coniferous trees, for example in *Sequoia sempervirens* where activated charcoal enhanced shoot elongation (Boulay 1977). Explant origin affected the level of success in *Pinus radiata*, a species now reliably propagated *in vitro*, with cultures of embryo origin rapidly producing an average of nine shoots each, while those from seedling shoot tips took longer to produce 25 shoots each (Horgan & Aitken 1981).

E. Rooting stem cuttings and *in vitro* shootlets

The capacity of stem cuttings to form roots can be assessed by (a) the percentage of cuttings rooted, (b) the number of roots per rooted cutting, and (c) the speed with which roots emerge and grow. These three criteria are not necessarily related, although generally the longer a cutting takes to root the fewer roots develop. It is also important commercially that there are at least three or more well-branched roots dispersed on all sides of the cuttings. In African mahogany (*Khaya ivorensis*), the form of the root system is affected by the slope of the cutting base (Leakey, unpublished), and the number of roots formed on apple winter cuttings can be increased by splitting the base of the cutting (Howard *et al.* 1984).

The rooting process can be divided into four stages: (a) dedifferentiation, which, in woody plants, usually occurs in cells close to the central core of

vascular tissue, often in parenchyma cells near immature or secondary xylem and phloem (see Haissig 1974a), (b) the formation of root initial cells in these newly meristematic areas, (c) the organization of these cells into root primordia, and (d) their subsequent growth and emergence. It should be remembered that the requirements for root initiation and root elongation often differ, the former being particularly influenced by the genetic and physiological state of the plant, while the latter is more sensitive to environmental factors.

Rooting ability varies between tree species, between clones within species, and among plants within clones. The genetic component of this variability may sometimes be attributed to (a) a lack of endogenous auxins, phenolic or other rooting co-factors, (b) a lack of enzymes or their activators for synthesis of auxin-phenol complexes, (c) the presence of inhibitors, or (d) the presence of enzymes that oxidize or degrade auxins or their co-factors. Variation among plants within clones is attributable mainly to the physiological condition of the stockplant. This condition can be affected by (a) the environment and season, (b) the position of the harvested shoots on the plants, (c) the age and size of the tree, and (d) the incidence of pathogens, virus particles and mycorrhizal organisms (Howard 1972; Hartmann & Kester 1983; Leakey 1983). Additionally, and very importantly, the capacity to develop roots is strongly influenced by the propagator's treatment of the cuttings, (a) chemically, by the application of auxins, other growth regulators, rooting co-factors, minerals and fungicides, (b) physically, either by influencing the size of the cutting, its leaf area, or by wounding or splitting the base, and (c) environmentally, by manipulation of moisture/humidity, light, temperature and the type of rooting medium used.

Clearly, the capacity of cuttings to root is influenced by many factors, so it is perhaps not surprising that tree breeders have sometimes been disappointed by the slow progress made towards large-scale mass propagation (see Bridgwater and Franklin, this volume). This viewpoint is especially strong in forestry, because of the importance attached to conifers, which until recently have been relatively difficult to root, particularly *in vitro* (Jones 1983).

Among broadleaved species, there are differences in ability to root, but experience suggests that most species can be rooted easily (Leakey *et al.* 1982b) although detailed studies are necessary to overcome specific problems in a few difficult-to-root species.

The choice of propagation technique has been extended in recent years by the development of *in vitro* systems. Although technologically more difficult than traditional systems of propagation, an increasing number of species are being successfully cultured *in vitro*; however, few species have yet been propagated commercially *in vitro*. Explants collected from large mature trees are particularly difficult to propagate. Space does not allow a full discussion of the many factors influencing rooting of stem cuttings, but a brief synopsis follows, using examples from recent work (see also Haissig 1985).

1. *The role of auxins*

Auxins are basipetally translocated in plant stems, and are largely responsible for the polarity of shoots. Since the discovery fifty years ago that auxins greatly

increased the capacity of cuttings to produce roots in most plant species, auxins have become universally used alone, or in combination with other chemicals, as an aid to propagation in horticulture and forestry.

Synthetic auxins are now usually preferred to endogenous indoleacetic acid (IAA). Indole-3-butyric acid (IBA) is the most commonly used, often combined with α-napthalene acetic acid (NAA) or one of the phenoxyacetic acids. Recently, equimolar concentrations of aryl- and phenyl-esters of IAA and IBA have been reported to outperform the unmodified acids in *Pinus banksiana* (Haissig 1979, 1983).

The effects of auxins on rooting capacity may depend on the method of application (Howard 1973). Common forms of treatment include (a) a quick dip in relatively concentrated solution, in which a proportion of the solvent, if not all, is an alcohol, and (b) a soak in relatively weak aqueous solutions. In both cases, the amount of auxin taken up is unknown and a more precise approach would be to apply known weights of auxin to cuttings of known sizes and sensitivities (Bowen *et al.* 1975; Leakey *et al.* 1982a; Amerson & Mott 1982; James 1983a).

Differing responses to auxins between species are well known (Nanda *et al.* 1970), but there can also be within-species variation in auxin preference, as in *Triplochiton scleroxylon* (Leakey *et al.* 1982a). In apples, scion cultivars are generally more difficult to root than the clonal rootstocks, although there is considerable variation between rootstocks (Delargy & Wright 1979; James 1983b).

In addition to species and clone, auxin applications also commonly interact with season of treatment (eg *Pinus sylvestris*, Eliasson *et al.* 1977) and age and size of the stockplant (eg *Olea europaea*, Portlingis & Therios 1976).

2. *The role of co-factors*

Although auxins play an important role in the rooting process, there are many occasions when a range of other substances are required to enhance auxin activity. A major group of these has been called the 'rooting co-factors' (Haissig 1974b) which are a complex of indole and phenolic substances, together with their oxidative enzymes that may directly affect the initiation of root primordia. However, there may be intricate interactions between these co-factors, auxins and other substances. In pear (*Pyrus communis*) there is evidence that indole-phenolic complexes exist (Fadl & Hartmann 1967), while in hardwood cuttings of the apple rootstock M26 no such evidence has been found (Bassuk *et al.* 1981), although polyphenyl oxidase (PPO) activity, and levels of phloridzin (a phenolic glycoside), increased prior to increases of a number of endogenous co-factors, which in turn were related to improvements in rooting ability (Bassuk & Howard 1981). The importance of phenols is emphasized by the enhanced rooting *in vitro* of apples (Jones & Hatfield 1976; James & Thurbon 1981), plums (Jones & Hopgood 1979) and cacao (Passey & Jones 1983), following the addition of phloroglucinol to the culture medium.

Cytokinins are involved in cellular differentiation processes, but there are only a few reports of cytokinin-enhanced rooting. More commonly, applied cytokinins inhibit rooting. Higher levels of endogenous cytokinins were found in difficult-to-root *Populus tremula*, than in easy-to-root *Populus*×*euramericana*

(Okoro & Grace 1978). It appears, however, that the balance of enzymes important to the formation of indole-phenolic complexes may sometimes by complicated by sensitivity to plant growth regulators (*Rhododendron*, Foong & Barnes 1981; *Mangifera indica*, Sadhu *et al.* 1978). As will be seen later, these balances can also be affected by the environment and stockplant condition.

3. *Role of the leaf*

It is common experience that leafless summer cuttings rarely root, while leafless winter cuttings root well, especially at the end of the winter. This difference exists because winter cuttings have greater amounts of stored reserves and endogenous co-factors than summer cuttings, and winter cuttings can have preformed root initials (Cheffins & Howard 1982a, b). Winter cuttings are, however, dependent on the rapid emergence of new shoots to replenish dwindling carbohydrate reserves (*Populus* spp., Okoro & Grace 1976). Summer cuttings, by contrast, are entirely dependent on the leaf for photosynthates. Hence, the carbohydrate content of leafy cuttings of *Triplochiton scleroxylon* almost doubled in nine days, whereas carbohydrate reserves in leafless cuttings were virtually depleted over the same period (Leakey *et al.* 1982a). However, not all the effects of leaves are beneficial (Reuveni & Raviv 1980), and there is evidence for optimal leaf areas per cutting. For instance, leaf areas greater than 50 cm² per cutting were detrimental in *T. scleroxylon* and *Cleistopholis glauca*, but not in *Terminalia ivorensis*

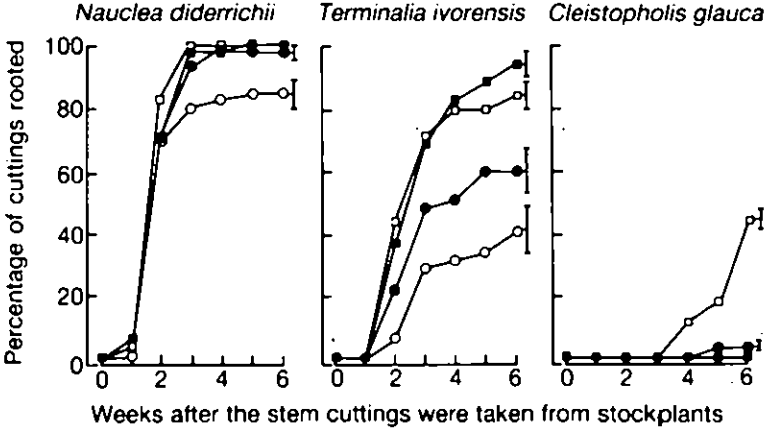

FIGURE 1. Differences in the rooting ability of leafy stem cuttings of three tropical hardwood species, and the effects of different leaf areas per cutting. ○=5 cm², ●=10 cm², □=50 cm² and ■=100 cm². The cuttings were treated with 20 μg indole-3-butyric acid, and were rooted under intermittent mist. The vertical bars denote ± one standard error.

and *Nauclea diderrichii* (Fig. 1; Leakey *et al.* 1982a). The deleterious effects of sub- or supra-optimal leaf areas seem therefore to be greater in difficult-to-root species. In *T. scleroxylon* the optimum leaf area was shown to represent a balance between photosynthetic gains and transpirational losses (Fig. 2).

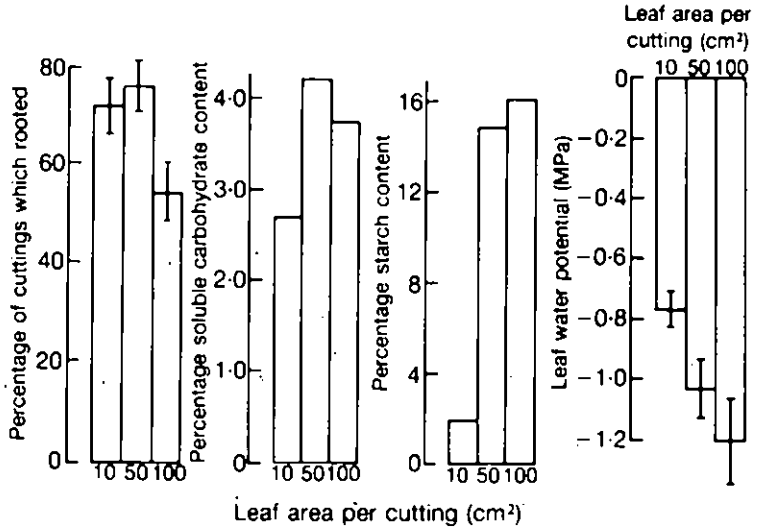

FIGURE 2. Effects of leaf area on the percentage rooting, stem-soluble carbohydrate and starch contents, and leaf water potential of *Triplochiton scleroxylon* leafy stem cuttings after 28 days under intermittent mist. Note that cuttings with 100 cm² of leaf had the largest total (non-structural) carbohydrate content, but suffered the greatest water stress and so rooted least well. Vertical bars denote ± one standard error (MPa = megapascals).

4. *Carbohydrate metabolism*

The supply and redistribution of carbohydrates within cuttings can sometimes limit their capacity to root, and to some extent this limitation may be associated with the absence of root respiration (Haissig 1984). In other instances, the carbohydrate content of cutting can be supra-optimal. Auxin enhances starch hydrolysis (Haissig 1974c) and several enzymes have been identified which increase in activity during primordium development, suggesting the involvement of both the Embden-Meyerhof-Parnas pathway of glycolysis and the pentose phosphate pathway (Haissig 1982).

5. *Nitrogen metabolism*

Nutrient deficiencies are detrimental to rooting, presumably because threshold levels are necessary for such processes as protein and nucleic acid synthesis (Hartmann & Kester 1983). Responses to nutrients are not always predictable; for example, when given to *Triplochiton scleroxylon* stockplants, complete fertilizers enhanced the rooting ability of only suppressed basal shoots (Leakey 1983), while halving the macro-nutrient content of the culture medium enhanced rooting *in vitro* of *Pistacia vera* (Barghchi & Alderson 1983).

A considerable body of evidence suggests that high carbohydrate/nitrogen ratios in cuttings favour root initiation, but this evidence can be misleading because the C/N ratio at the base of cuttings can become very different from that in the cuttings as a whole (Haissig 1974c).

6. *Water relations*

The rooting capacity of cuttings is frequently related to the water balance. Experimentally, rooting has been shown to suffer when leaf water potentials fall to about −0·8 to −1·0 MPa (Loach 1977; Loach & Gay 1979), below which there is a linear relationship between declining leaf water potential and decreased rooting.

The water balance of cuttings is governed by the rate of uptake, principally through the cut basal ends, but sometimes also through the leaf, and the rate of transpirational losses. With increased duration in the propagation bed a resistance to water uptake develops, which is apparently unrelated to callus formation or embolism in the vascular tissues. Water losses are affected by the vapour pressure deficit of the air, radiation levels and leaf resistances to water loss. Stomatal conductance typically drops rapidly in fresh cuttings and rises again as roots develop (Gay & Loach 1977). Rooting can normally be enhanced by shading and by maintaining a water film over the leaves, often associated with a lowering in leaf temperature (Loach & Gay 1979). The effects of different propagation systems and media on the water balance of leafy cuttings have been discussed by Grange and Loach (1983).

In leafless winter cuttings, one might expect that the water balance might not be too critical. However, root formation on hardwood plum and apple cuttings can be greatly influenced by applying antidesiccants to the cut ends, increasing the humidity of the rooting environment, and by preventing water stress (Howard 1980; Howard *et al.* 1983).

7. *Light*

Cuttings from conifers and deciduous trees of temperate and tropical origin root more readily after the stockplants have been kept in light levels well below the photosynthesis saturation point (*Pinus sylvestris*, Hansen *et al.* 1978; *Populus* and *Salix*, Eliasson & Brunes 1980; apple, Christensen *et al.* 1980). However, in *P. sylvestris*, the beneficial effects of applied auxins were enhanced by keeping the stockplants under high irradiances, suggesting that light changed either the auxin or co-factor content of the shoots (Strömquist & Hansen 1980).

In *Triplochiton scleroxylon*, low stockplant irradiances enhanced rooting and changed the dominance relationships between the shoots of 2-shoot stockplants (Fig. 3). This result supported earlier findings that competition between shoots decreased the rooting ability of cuttings taken from dominant shoots (Leakey 1983). Additionally, four weeks' growth of *T. scleroxylon* at 155 $W m^{-2} s^{-1}$ for 19·5 h day^{-1} resulted in lower rates of net photosynthesis than at 75 $W m^{-2} s^{-1}$, perhaps owing to end product inhibition; in this instance, rooting was greatest in cuttings taken from stockplants grown at the low irradiances and seemed to be related to the fact that they had low starch contents. In contrast, increased illumination has enhanced rooting in apple explants *in vitro* (25 to 100 $\mu E\ m^{-2} s^{-1}$, Sriskandarajah *et al.* 1982). It is possible, however, that the importance of light environments *in vitro* may change at different stages in the growth and development of explants. The effects of different levels of irradiance on the cuttings themselves during the rooting phase have been found to be relatively minor, provided that water

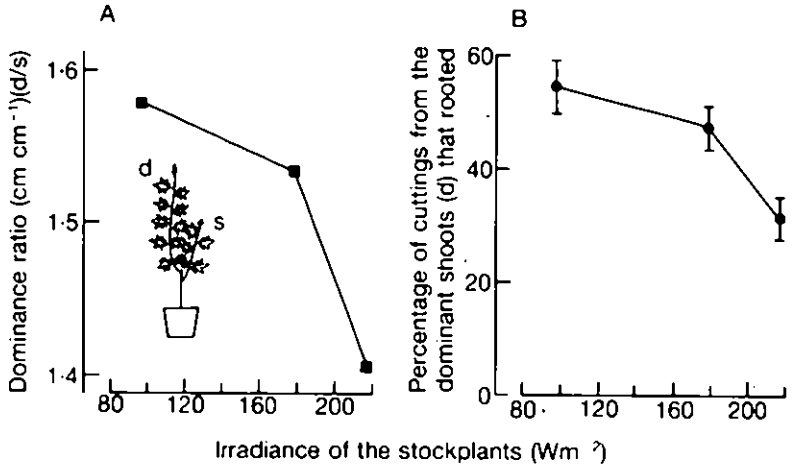

FIGURE 3.

A. Relationship between the light intensity, given for 19·5 h to stock-plants of *Triplochiton scleroxylon* at 30 °C and 80% relative humidity, and the ratio of the length of the dominant/suppressed shoots (d/s, on potted plants with two shoots). Note that dominance was greatest at the low light intensities.

B. The percentage of cuttings from the dominant shoots that rooted. Note that the poorly illuminated, most dominant, shoots rooted best.

stress was prevented, presumably because of the limited photosynthetic activity of detached cuttings (Hansen *et al.* 1978; Strömquist & Hansen 1980). However, irradiation of the basal ends of *Populus* and *Salix* cuttings can inhibit rooting (Eliasson & Brunes 1980). Thus, it is important to keep cutting bases in the dark.

Etiolation and/or blanching the stockplants in complete darkness can enhance rooting, for instance in difficult-to-root apple cultivars (Delargy & Wright 1979). Similarly, the *in vitro* rooting ability of M9 apple rootstocks can be enhanced by a period of darkness prior to severance (James 1983a), and in the variety 'Jonagold' this was associated with an increased phenol content and decreased peroxidase activity (Druart *et al.* 1982). Similar benefits of darkness in plum were offset by the application of the phenolic compound, chlorogenic acid (Hammerschlag 1982). Some recent evidence suggests that the etiolation effect is due to a mobile factor from the shoot apex, and that the enhanced rooting is closely related to the irradiance effects mentioned above (Harrison-Murray 1984).

8. *Temperature*

Temperature influences cambial activity and could therefore be expected to be important in propagation, especially in winter, as demonstrated in leafless apple cuttings (Cheffins & Howard 1982a), where warm temperatures enhanced rooting, but increased respiration losses, and so hampered successful establishment. In *Larix*, early rooting was best at 27 °C, but maximum rooting

occurred at 15 °C (John 1977) emphasizing the importance of minimizing respiratory losses.

9. *Season*

The date at which both leafy softwood cuttings, and leafless hardwood cuttings are collected can markedly affect their rooting ability. Softwood cuttings often decrease in rooting ability during the summer (Klahr & Still 1979), while hardwood cuttings increase in rooting ability during the winter (Lux 1982). There are, however, exceptions to this generalization (eg *Olea europaea* can root well throughout the year, Portlingis & Therios 1976). *Pseudotsuga menziesii* cuttings were unrootable in September, and became progressively more rootable, without auxins, as winter dormancy declined, principally in response to winter chilling (Roberts *et al.* 1974). Applications of auxin to *P. menziesii* in winter, and artificial chilling of *Larix*, have enhanced the rooting of dormant shoots (John 1979).

Seasonal variations in rooting of leafy cuttings are less well understood, but high summer levels of irradiance, water stress, and the incidence of flowering may all contribute to decreased rooting ability. In non-dormant plants, it is difficult to obtain truly comparable cuttings at different times throughout the growing season.

10. *Gravity*

Cuttings taken from vertical *Populus* plants produced more roots than cuttings taken from horizontal plants, although the number of newly initiated wound roots were the same in both treatments (Smith & Wareing 1971). In *Triplochiton scleroxylon*, the overall percentage of cuttings rooted per stockplant was unaffected by different orientation treatments, but basal shoots rooted better than apical shoots when taken from vertical plants (Leakey 1983).

11. *Mycorrhizal fungi*

In some woody plants, the addition of mycorrhizal inoculum to the propagation medium enhances rooting (Linderman & Call 1977; Navratil & Rochon 1981), possibly because growth regulators are exuded into the medium prior to the development of mycorrhizal associations.

12. *Stockplant factors*

Some important differences in the capacity to root can be traced to differences in the physiological condition of different parts of the stockplants, and to their interactions with the environment.

(a) *Within-shoot variables*. In *Triplochiton scleroxylon*, the rooting ability of single-node leafy cuttings decreased markedly down the stockplant shoots (Leakey 1983), which could be due to any of the many differences between them, such as leaf age, internode length, extent of lignification and secondary thickening, gradients in carbohydrate, nitrogen and auxin contents, etc. Currrent experiments indicate that, like carbohydrate contents, the gradient in leaf water potential down the shoot is reversed in the cuttings after two

weeks' propagation, and so may not be too important (Leakey & Coutts, unpublished). Perhaps more relevantly, cutting size, as determined by the normal pattern of internode lengths, was closely correlated with percentage cuttings rooted – the longer cuttings at the apical end rooting best (Leakey 1983). Interestingly, when all cuttings were cut to the same length, basal cuttings rooted best, and hence the normal gradient in rooting ability was reversed (Leakey & Mohammed 1985). The benefits of using large cuttings have also been reported for multi-node cuttings of leafless and winter cuttings (Richardson *et al.* 1979; John 1977). In apple, and other fruit trees, the swollen shoot base with many short internodes is a favoured site for root development (Howard 1981).

(b) *Between-shoot variables.* Cuttings from main stems and branches have different rooting abilities, the latter being best in plum, pine, spruce (Hartmann & Kester 1983) and fir (Miller *et al.* 1982). A comparison, using different *T. scleroxylon* plants of identical size, form and growth rate, has confirmed this intrinsically higher rooting ability of lateral cuttings, although many factors affect their rooting ability (Leakey 1983). For example, there was a negative correlation between the numbers of shoots per plant and the rooting ability of the uppermost shoot, with two shoots per potted stockplant being optimal. The rooting ability of cuttings from these shoots was strongly affected by the application of nutrients and the extent of mutual shading; heavy shading was detrimental, but some shading was beneficial. Thus, much of the between-shoot variation in rooting ability appeared to be attributable to competition between shoots and to their different light environments.

Bearing the above in mind, it is not surprising that there are topophytic effects of cutting origin on the rooting ability of cuttings collected from different parts of tree crowns. For example, in 6- and 21-year-old *Picea abies*, rooting decreased by 2·5% with each successive branch whorl up the trees (Roulund 1973). Commonly, throughout the propagation literature, the very low rooting ability of crown shoots from large trees is attributed to the attainment of the reproductive or mature phase ('phase-change'). While this may be so, many of the comparative studies between juvenile and mature shoots make no attempt to distinguish between 'phase-change' *per se*, and the numerous other differences between cuttings collected from seedlings or coppice shoots and those from the structurally complex crowns of large trees (eg Morgan & McWilliams 1976). In particular, attention should be paid to: internode length, rates and periodicity of growth, leaf size, frequency of branching, and differences in light environments, leaf water potential, carbohydrate and nutrient contents. Some studies do partially offset this criticism by using cuttings from mature shoots grafted on to juvenile rootstocks; significantly, the rooting ability of the cuttings from these more vigorous and comparable shoots is often considerably improved, and such material has been said to be 'rejuvenated' (eg *Eucalyptus grandis*, Paton *et al.* 1981), although 'reinvigorated' may be a more appropriate term. In a similar way, mature, second-generation cuttings (cuttings from cuttings) of *Triplochiton scleroxylon* have rooted well, even following flowering, and despite persistent plagiotropism (Leakey, unpublished). Reinvigoration has also enhanced rooting of shoots on large, but heavily pruned trees of *Artocarpus heterophyllus* (Mukher-

jee & Chatterjee 1979). Similar changes may also account for the reported 'rejuvenation' by successive subculturing of apple shoots *in vitro* (Sriskandarajah *et al.* 1982).

Accepting the above-mentioned difficulties, it is clear that cuttings from crown shoots do differ from seedling or coppice shoots in their content of inhibitors (Vieitez & Vieitez 1976), various growth regulators, nucleic acids and rooting co-factors (Heuser 1976; Paton *et al.* 1981). Additionally, as in chestnut (*Castanea sativa*) and avocado (*Persea americana*), there can be differences in anatomy or leaf retention which may limit rooting (Vieitez & Vieitez 1976; Reuveni & Raviv 1980).

To minimize topophytic variation in rooting ability, horticulturists and foresters have developed methods of stockplant management which encourage the continued formation of young vigorous shoots, Traditionally, some fruit rootstocks are propagated by stooling, and stone fruits by layering (Howard 1981). However, recent improvements in the techniques for rooting winter cuttings of apple, plum and cherry have led to the increased use of more productive 'hedged' stockplants. These stockplants have also been developed with some success for *Pinus radiata*, yielding more than 100 shoots per square metre (Libby *et al.* 1972).

Recently, there has been evidence that rooting can be enhanced by preconditioning *in vitro* explants, either by changing the medium (Horgan & Aitken 1981) or the light environment (Druart *et al.* 1982).

IV. CONCLUSIONS

In recent years, rapid progress has been made in developing and refining a range of vegetative propagation techniques. These techniques exploit numerous facets of tree physiology that centre around the inherent totipotency of many plant cells. Provided with appropriate environmental and hormonal stimuli to prevent physiological stresses, promote division, and activate differentiation, the cells of most tree species will multiply and organize new tissues to form grafts, develop root initials, proliferate shoot apices, or even produce whole new plantlets from single cells. While the techniques used on trees are basically similar to those developed for herbaceous plants, horticulturists and foresters have had to overcome the within-plant difficulties associated with both the greater age, size and complexity of trees, and the effects of season on their patterns of growth.

Despite the large number of variables presented in this review, many tree species are easily propagated, provided that physiological stresses are avoided and appropriate material is used. For example, using the techniques and regimes developed for rooting *Triplochiton scleroxylon* stem cuttings, over 90% of 50 other tree species have been successfully rooted (Leakey *et al.* 1982b). Experience suggests, moreover, that even species considered difficult to propagate may become relatively easy following detailed study, as has been demonstrated for hardwood cuttings of apple (*Malus* spp.) by Howard *et al.* (1983), softwood cuttings of *Triplochiton scleroxylon* by Leakey *et al.* (1982a), and *Pinus sylvestris* by Whitehill and Schwabe (1975).

REFERENCES

Ahuja, M. R. (1982). Isolation culture and fusion of protoplasts: problems and prospects. *Silvae Genet.*, **31**, 66–77.

Ahuja, M. R. (1983). Somatic cell differentiation and rapid clonal propagation of aspen. *Silvae Genet.*, **32**, 131–135.

Amerson, H. V. & Mott, R. L. (1982). Improved rooting of western white pine shoots from tissue culture. *Forest Sci.*, **28**, 822–825.

Barghchi, M. & Alderson, P. G. (1983). *In vitro* propagation of *Pistacia vera* L. from seedling tissues. *J. hort. Sci.*, **58**, 435–445.

Bassuk, N. L., Huster, L. D. & Howard, B. H. (1981). The apparent involvement of polyphenol oxidase and phloridzin in the production of apple rooting co-factors. *J. hort. Sci.*, **56**, 313–322.

Bassuk, N. L. & Howard, B. H. (1981). A positive correlation between endogenous root-inducing co-factor activity in vacuum extracted sap and seasonal changes in M26 winter apple cuttings. *J. hort. Sci.*, **56**, 301–312.

Biondi, S. & Thorpe, T. A. (1982). Clonal propagation of forest tree species. In: *Tissue culture of economically important plants*, edited by A. N. Rao, 197–204. Singapore: Committee on Science and Technology in Developing Countries, and Asian Network for Biological Sciences.

Bonga, J. M. & Durzan, D. J., eds. (1982). *Tissue culture in forestry*. The Hague; London: Martinus Nijhoff/Junk.

Bornman, C. H. (1983). Possibilities and constraints in the regeneration of trees from cotyledonary needles of *Picea abies in vitro*. *Physiologia Pl.*, **57**, 5–16.

Boulay, M. (1977). Multiplication rapide du *Sequoia sempervirens* en culture *in vitro*. *Ann. Rech. sylvic.*, 37–67.

Bowen, M. R., Howarth, J. & Longman, K. A. (1975). Effects of auxins and other factors on the rooting of *Pinus contorta* Dougl. cuttings. *Ann. Bot.*, **39**, 647–656.

Brown, C. L. & Sommer, H. E. (1982). Vegetative propagation of dicotyledonous trees. In: *Tissue culture in forestry*, edited by J. M. Bonga and D. J. Durzan, 109–149. The Hague; London: Martinus Nijhoff/Junk.

Button, J. & Kochba, J. (1977). Tissue culture in the citrus industry. In: *Applied and fundamental aspects of plant cell, tissue and organ culture*, edited by J. Reinert and Y. B. S. Bajaj, 70–92. Berlin; New York: Springer.

Cheffins, N. J. & Howard, B. H. (1982a). Carbohydrate changes in leafless winter apple cuttings. I. The influence of level and duration of bottom leaf. *J. hort. Sci.*, **57**, 1–8.

Cheffins, N. J. & Howard, B. H. (1982b). Carbohydrate changes in leafless winter apple cuttings. II. Effects of ambient air temperature during rooting. *J. hort. Sci.*, **57**, 9–15.

Chevre, A. M., Gill, S. S., Mouras, A. & Salesses, G. (1983). *In vitro* vegetative multiplication of chestnut. *J. hort. Sci.*, **58**, 23–29.

Christensen, M. V., Eriksen, E. N. & Andersen, A. S. (1980). Interactions of stockplant irradiance and auxin in the propagation of apple rootstocks by cuttings. *Sci. Hortic. (Neth.)*, **12**, 11–17.

Copes, D. L. (1970). Effect of date of grafting on survival in Douglas-fir. *Res. Note Pacif. NW Forest Range Exp. Stn*, PNW-135.

Copes, D. L. (1975). Graft incompatibility in *Pinus contorta*. *Res. Note Pacif. NW Forest Range Exp. Stn*, PNW-260.

David, A. (1982). *In vitro* propagation of gymnosperms. In: *Tissue culture in forestry*, edited by J. M. Bonga and D. J. Durzan, 72–108. The Hague; London: Martinus Nijhoff/Junk.

Delargy, J. A. & Wright, C. E. (1979). Root formation in cuttings of apple in relation to auxin application and to etiolation. *New Phytol.*, **82**, 341–347.

De Fossard, R. A., Barker, P. K. & Bourne, R. A. (1977). The organ culture of nodes of four species of *Eucalyptus*. *Acta Hortic.*, **78**, 157–165.

Dormling, I. (1963). Anatomical and histological examinations of the union of scion and stock in grafts of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.). *Stud. for. suec.*, **13**, 1–136.

Druart, P., Kevers, C., Boxus, P. & Gaspar, T. (1982). *In vitro* promotion of root formation by apple shoots through darkness effect on endogenous phenols and peroxidase. *Z. Pflanzenphysiol.*, **108**, 429–436.

Durzan, D. J. (1982). Cell and tissue culture in forest industry. In: *Tissue culture in forestry*, edited by J. M. Bonga and D. J. Durzan, 36–71. The Hague; London: Martinus Nijhoff/Junk.

Eliasson, L. (1971a). Growth regulators in *Populus tremula*. IV. Apical dominance and suckering in young plants. *Physiologia Pl.*, **25**, 263–267.

Eliasson, L. (1971b). Growth regulators in *Populus tremula*. II. Effect of light on inhibitor content of root suckers. *Physiologia Pl.*, **24**, 205–208.

Eliasson, L. (1971c). Growth regulators in *Populus tremula*. III. Variation of auxin and inhibitor level in roots in relation to root sucker formation. *Physiologia Pl.*, **25**, 118-121.

Eliasson, L. & Brunes, L. (1980). Light effects on root formation in aspen and willow cuttings. *Physiologia Pl.*, **48**, 261–265.

Eliasson, L., Strömquist, L.-H. & Brunes, L. (1977). Influence of light, nutrients and plant hormones on root formation in woody cuttings. In: *Vegetative propagation of forest trees – physiology and practice*, 43–54. Garpenburg: Swedish Institute for Forest Improvement, College of Forestry and University of Agricultural Sciences.

Fadl, M. S. & Hartmann, T. H. (1967). Isolation, purification and characterization of an endogenous root-promotion factor obtained from basal sections of pear hardwood cuttings. *Pl. Physiol., Lancaster*, **42**, 541–549.

Foong, T. W. & Barnes, M. F. (1981). Rooting 'co-factors' in *Rhododendron*: the fractionation and activity of components from an easy-to-root and a difficult-to-root variety. *Biochem. Physiol. Pflanz.*, **176**, 507–523.

Garner, R. J. (1979). *Grafter's handbook*. 4th ed. London: Faber.

Gay, A. P. & Loach, K. (1977). Leaf conductance changes on leafy cuttings of *Cornus* and *Rhododendron* during propagation. *J. hort. Sci.*, **52**, 509–516.

Grange, R. I. & Loach, K. (1983). Environmental factors affecting water loss from leafy cuttings in different propagation systems. *J. hort. Sci.*, **58**, 1–7.

Gur, A., Samish, R. M. & Lifshitz, E. (1968). The role of cyanogenic glycoside of the quince in the incompatibility between pear cultivars and quince rootstocks. *Hort. Res.*, **8**, 113–134.

Haissig, B. E. (1974a). Origins of adventitious roots. *N.Z. J. For. Sci.*, **4**, 299–310.

Haissig, B. E. (1974b). Influences of auxins and auxin synergists on adventitious root primordium initiation and development. *N.Z. J. For. Sci.*, **4**, 311–323.

Haissig, B. E. (1974c). Metabolism during adventitious root primordium initiation and development. *N.Z. J. For. Sci.*, **4**, 324–337.

Haissig, B. E. (1979). Influence of aryl-esters of indole-3-acetic and indole-3-butyric acids on adventitious root primordium initiation and development. *Physiologia Pl.*, **47**, 29–33.

Haissig, B. E. (1982). Activity of some glycolytic and pentose phosphate pathway enzymes during the development of adventitious roots. *Physiologia Pl.*, **55**, 261–272.

Haissig, B. E. (1983). N-phenyl indolyl-3-butyramide and phenyl indole-3-thiolobutyrate enhance adventitious root primordium development. *Physiologia Pl.*, **57**, 435-440.

Haissig, B. E. (1984). Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. *Physiologia Pl.*, **61**, 13–19.

Haissig, B. E. (1985). Metabolic processes in adventitious rooting. In: *New root formation in plants and cuttings*, edited by M. B. Jackson. The Hague: Martinus Nijhoff/Junk. In press.

Hall, J. B. & Swaine, M. D. (1981). *Distribution and ecology of vascular plants in a tropical rain forest – forest vegetation in Ghana. Geobotany 1*. The Hague: Junk.

Hammerschlag, F. (1982). Factors influencing *in vitro* multiplication and rooting of the plum rootstock 'Myrobalan' (*Prunus cerasifera* Ehrh.). *J. Am. Soc. hort. Sci.*, **107**, 44–47.

Hansen, J., Strömquist, L. H. & Ericsson, A. (1978). Influence of the irradiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). *Pl. Physiol., Lancaster*, **61**, 975–979.

Harrison-Murray, R. S. (1984). Light intensity and site of sensitivity. *Rep. E. Malling Res. Stn 1983*, 77–79.

Hartmann, H. T. & Kester, D. E. (1983). *Plant propagation: principles and practices*. 4th ed. New Jersey: Prentice Hall.

Hearne, D. A. (1971). The development of grafting techniques for *Pinus caribaea* in the northern territory of Australia. *Proc. IUFRO Meeting, Gainesville, Florida, USA, March 1971*, 220–225. Oxford: Commonwealth Forestry Institute.

Heuser, C. W. (1976). Juvenility and rooting co-factors. *Acta Hortic.*, **56**, 251–261.

Holst, J. J., Santon, J. A. & Yeatman, C. W. (1956). Greenhouse grafting of spruce and hard pine at the Petawawa Forest Experiment Station, Chalk River, Ontario. *Tech. Notes Forest Res. Div. Can.*, no. 33.

Horgan, K. & Aitken, J. (1981). Reliable plantlet formation from embryos and seedling shoot tips of radiata pine. *Physiologia Pl.*, **53**, 170–175.

Howard, B. H. (1971). Propagation techniques. *Scient. Hort.*, **23**, 116–126.
Howard, B. H. (1972). Depressing effects of virus infection on adventitious root production in apple hardwood cuttings. *J. hort. Sci.*, **47**, 255–258.
Howard, B. H. (1973). Factors affecting the rooting response of plants to growth regulator application. *Acta Hortic.*, **34**, 93–105.
Howard, B. H. (1977). Effects of initial establishment practice on the subsequent productivity of apple stoolbeds. *J. hort. Sci.*, **52**, 437–446.
Howard, B. H. (1980). Moisture change as a component of disbudding responses in studies of supposed relationships between bud activity and rooting in leafless cuttings. *J. hort. Sci.*, **55**, 171–180.
Howard, B. H. (1981). Propagation of fruit and other broadleaved trees. *Jl R. agric. Soc.*, **142**, 110–128.
Howard, B. H., Harrison-Murray, R. S. & Fenlon, C. A. (1983). Effective auxin treatment of leafless winter cuttings. In: *Growth regulators in root development*, edited by M. B. Jackson and A. D. Stead, 73–85. (Monograph 10). Wantage: British Plant Growth Regulator Group, Letcombe Laboratory.
Howard, B. H., Harrison-Murray, R. S. & Mackenzie, K. A. D. (1984). Rooting responses to wounding winter cuttings of M26 apple rootstock. *J. hort. Sci.*, **59**, 131–139.
Huang, S.-C. & Millikan, D. F. (1980). *In vitro* micrografting of apple shoot tips. *HortScience*, **15**, 741–743.
James, D. J. (1983a). Adventitious root formation *in vitro* in apple rootstocks (*Malus pumila*). I. Factors affecting the length of the auxin-sensitive phase in M9. *Physiologia Pl.*, **57**, 149–153.
James, D. J. (1983b). Adventitious root formation *in vitro* in apple rootstocks (*Malus pumila*). II. Uptake and distribution of indol-3yl-acetic acid during the auxin-sensitive phase in M9 and M26. *Physiologia Pl.*, **57**, 154–158.
James, D. J. & Thurbon, I. J. (1981). Shoot and root initiation *in vitro* in the apple rootstock M9 and the promotive effects of phloroglucinol. *J. hort. Sci.*, **56**, 15–20.
Jeffree, C. E. & Yeoman, M. M. (1983). Development of intercellular connections between opposing cells in a graft union. *New Phytol.*, **93**, 481–509.
John, A. (1977). Vegetative propagation in hybrid larch (*Larix×eurolepsis* Henry) in Scotland. In: *Vegetative propagation of forest trees – physiology and practice*, 129–136. Garpenburg: Swedish Institute for Forest Improvement, College of Forestry and University of Agricultural Sciences.
John, A. (1979). Propagation of hybrid larch by summer and winter cuttings. *Silvae Genet.*, **28**, 220–225.
Johnson, R. D. & Lacey, C. J. (1983). Multi-stemmed trees in rainforest. *Aust. J. Bot.*, **31**, 189-195.
Jones, O. P. (1976). Effect of phloridzin and phloroglucinol on apple shoots. *Nature, Lond.*, **262**, 392-395, with Erratum. *Nature, Lond.*, **262**, 724.
Jones, O. P. (1983). *In vitro* propagation of tree crops. In: *Plant biotechnology*, edited by S. H. Mantell and H. Smith, 139–159. (S.E.B. seminar series 18). Cambridge: Cambridge University Press.
Jones, O. P. & Hatfield, S. G. S. (1976). Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds. *J. hort. Sci.*, **51**, 495-499.
Jones, O. P. & Hopgood, M. E. (1979). The successful propagation *in vitro* of two rootstocks of *Prunus*, the plum rootstock Pixy (*P. insititia*) and the cherry rootstock F12/1 (*P. avium*). *J. hort. Sci.*, **54**, 63–66.
Jones, O. P., Pontikis, C. A. & Hopgood, M. E. (1979). Propagation *in vitro* of five apple scion cultivars. *J. hort. Sci.*, **54**, 155–158.
Jong, K. (1980). A cytoembryological approach to the study of variation and evolution in rainforest tree species. In: *Tropical ecology and development*, edited by J. I. Furtado, 213–218. (*Proc. int. Symp. on Tropical Ecology, Kuala Lumpur, Malaysia, 16–21 April, 1979*).
Kaur, A., Ha, C. O., Jong, K., Sands, V. E., Chan, H. T., Soepadmo, E. & Ashton, P. S. (1978). Apomixis may be widespread among trees of the climax rain forest. *Nature, Lond.*, **271**, 440–442.
Khosla, P. K., Nagpal, R. & Puri, S. (1982). Propagation of some forestry species by air-layering. *Indian J. For.*, **5**, 171–174.
Kirby, E. G. (1982). The use of *in vitro* techniques for genetic modification of forest trees. In: *Tissue culture in forestry*, edited by J. M. Bonga and D. J. Durzan, 369-386. The Hague; London: Martinus Nijhoff/Junk.

Klahr, M. & Still, S. M. (1979). Effect of indole-butyric acid and sampling-dates on the rooting of four *Tilia* taxa. *Sci. Hortic. (Neth.)*, **11**, 391–397.

Kochba, J., Spiegel-Roy, P., Neumann, H. & Saad, S. (1982). Effect of carbohydrates on somatic embryogenesis in subcultured nucellar callus of *Citrus* cultivars. *Z. Pflanzenphysiol.*, **105**, 359–368.

Komissarov, D. A. (1969). *Biological basis for the propagation of woody plants by cutting*. Jerusalem: Israel Programme for Scientific Translations.

Lacey, C. J., Gillson, A. N. & Whitecross, M. I. (1982). Root formation by stems of *Eucalyptus botryoides* Sm. in natural stands. *Aust. J. Bot.*, **30**, 147–159.

Lane, W. D. & McDougald, J. M. (1982). Shoot tissue culture of apple: comparative response of five cultivars to cytokinin and auxin. *Can. J. Pl. Sci.*, **62**, 689–694.

Larkin, P. J. & Scowcroft, W. R. (1981). Somaclonal variation – a novel source of variability from cell cultures for plant improvement. *Theor. appl. Genet.*, **60**, 197-214.

Leakey, R. R. B. (1981). Adaptive biology of vegetatively regenerating weeds. *Adv. appl. Biol.*, **6**, 57–90.

Leakey, R. R. B. (1983). Stockplant factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum., an indigenous hardwood of West Africa. *J. hort. Sci.*, **58**, 277–290.

Leakey, R. R. B. & Mohammed, H. R. S. (1985). Effects of stem length on root initiation in sequential single-node cuttings of *Triplochiton scleroxylon* K. Schum. *J. hort. Sci.*, **60**. In press.

Leakey, R. R. B., Chapman, V. R. & Longman, K. A. (1982a). Physiological studies for tropical tree improvement and conservation. Factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum. *For. Ecol. Manage.*, **4**, 53–66.

Leakey, R. R. B., Last, F. T. & Longman, K. A. (1982b). Domestication of tropical trees: an approach in securing future productivity and diversity in managed ecosystems. *Commonw. Forest. Rev.*, **61**, 33–42.

Libby, W. J., Brown A. G. & Fielding, J. M. (1972). Effects of hedging radiata pine on production, rooting and early growth of cuttings. *N.Z. J. For. Sci.*, **2**, 263–283.

Lindermann, R. G. & Call, C. A. (1977). Enhanced rooting of woody plant cuttings by mycorrhizal fungi. *J. Am. Soc. hort. Sci.*, **102**, 629–632.

Litz, R. E., Knight, R. J. & Gazit, S. (1984). *In vitro* somatic embryogenesis from *Mangifera indica* L. callus. *Sci. Hortic.*, **22**, 233–240.

Loach, K. (1977). Leaf water potential and the rooting of cuttings under mist and polythene. *Physiologia Pl.*, **40**, 191–197.

Loach, K. & Gay, A. P. (1979). The light requirements for propagating hardy ornamental species from leafy cuttings. *Sci. Hortic. (Neth.)*, **10**, 217–230.

Lux, A. (1982). The annual cycle of rhizogenesis of poplar stem cuttings. *Biologia (Bratisl.)*, **37**, 31–41.

Mahmood Husain, A. M. & Ponnuswamy, P. K. (1982). An innovation in the vegetative propagation of Sandal (*Santalum album* Linn.). *Indian J. For.*, **5**, 1–7.

Mascarenhas, A. F., Gupta, P. K., Kulkarni, V. M., Mehta, U., Iyer, R. S., Khuspe, S. S. & Jagannathan, V. (1982). Propagation of trees by tissue culture. In: *Tissue culture of economically important plants*, edited by A. N. Rao, 175–179. Singapore: Committee on Science and Technology in Developing Countries, and Asian Network for Biological Sciences.

Miller, N. F., Hinesley, L. E. & Blazich, F. A. (1982). Propagation of Fraser fir by stem cuttings: effects of type of cutting, length of cutting and genotype. *HortScience*, **17**, 827-829.

Monaco, L. C., Söndahl, M. R., Carvalho, A., Crocomo, O. J. & Sharp, W. R. (1977). Applications of tissue culture in the improvement of coffee. In: *Applied and fundamental aspects of plant cell, tissue and organ culture*, edited by J. Reinert and Y. R. S. Bajaj, 109–129. Berlin; New York: Springer.

Morgan, D. L. & McWilliams, E. L. (1976). Juvenility as a factor in propagating *Quercus virginiana* Mill. *Acta Hortic.*, **56**, 263–268.

Mukherjee, S. K. & Chatterjee, B. K. (1979). Effects of forcing etiolation and indolebutyric acid on rooting of cuttings of *Artocarpus heterophyllus* Lam. *Sci. Hortic. (Neth.)*, **10**, 295–300.

Nanda, K., Anand, V. K. & Kumar, P. (1970). Some investigations of auxin effects on rooting of stem cuttings of forest plants. *Indian Forester*, **96**, 171–187.

Navratil, S. & Rochon, G. C. (1981). Enhanced root and shoot development of poplar cuttings induced by *Pisolithus* inoculum. *Can. J. For. Res.*, **11**, 844–847.

Okoro, O. O. (1976). Some factors affecting successful 'take' of *Pinus caribaea* grafts. *Niger. J. For.*, **6**, 20–23.

Okoro, O. O. & Grace, J. (1976). The physiology of rooting *Populus* cuttings. I. Carbohydrates and photosynthesis. *Physiologia Pl.*, **36**, 133–138.

Okoro, O. O. & Grace, J. (1978). The physiology of rooting *Populus* cuttings. II. Cytokinin activity in leafless hardwood cuttings. *Physiologia Pl.*, **44**, 167–170.

Okoro, O. O. & Omokaro, D. N. (1975). Marcotting *Triplochiton scleroxylon* K. Schum. *Proc. Symp. Variation and Breeding Systems of* Triplochiton scleroxylon *(K. Schum.), Ibadan, Nigeria, 1975*, 93–98.

Passey, A. J. & Jones, O. P. (1983). Shoot proliferation and rooting *in vitro* of *Theobroma cacao* L. type Amelonado. *J. hort. Sci.*, **58**, 589–592.

Paton, D. M., Willing, R. R. & Pryor, L. D. (1981). Root-shoot gradients in *Eucalyptus* ontogeny. *Ann. Bot.*, **47**, 835–838.

Pence, V. C., Hasegawa, P. M. & Janick, J. (1979). Asexual embryogenesis in *Theobroma cacao* L. *J. Am. Soc. hort. Sci.*, **104**, 145–148.

Perala, D. A. (1978). Aspen sucker production and growth from outplanted root cuttings. *Res. Note N. Cent. For. Exp. Stn*, NC-241

Pôessel, J.-L., Martinez, J., Macheix, J.-J. & Jouard, R. (1980). Variations saisonnières de l'aptitude au greffage *in vitro* d'apex de Pêcher (*Prunus persica* Batsch). Relations avec les teneurs en composés phénoliques endogènes et les activités peroxydasique et polyphenol-oxydasique. *Physiol. Vég.*, **18**, 665–675.

Portlingis, I. C. & Therios, I. (1976). Rooting response of juvenile and adult leafy olive cuttings to various factors. *J. hort. Sci.*, **51**, 31–39.

Rangaswamy, N. S. (1982). Nucellus as an experimental system in basic and applied tissue culture research. In: *Tissue culture of economically important plants*, edited by A. N. Rao, 269–286. Singapore: Committee on Science and Technology in Developing Countries, and Asian Network for Biological Sciences.

Reuveni, O. & Raviv, M. (1980). Importance of leaf retention to rooting of avocado cuttings. *J. Am. Soc. hort. Sci.*, **106**, 127–130.

Richardson, S. G., Barker, J. R., Crofts, K. A. & van Epps, G. A. (1979). Factors affecting rooting of stem cuttings of salt desert shrubs. *J. Range Mgmt*, **32**, 280–283.

Roberts, A. N., Tomasovic, B. J. & Fuchigami, L. H. (1974). Intensity of bud dormancy in Douglas-fir and its relation to scale removal and rooting ability. *Physiologia Pl.*, **31**, 211–216.

Robinson, J. C. & Schwabe, W. W. (1977a). Studies on the regeneration of apple cultivars from root cuttings. I. Propagation aspects. *J. hort. Sci.*, **52**, 205–220.

Robinson, J. C. & Schwabe, W. W. (1977b). Studies on the regeneration of apple cultivars from root cuttings. II. Carbohydrate and auxin relations. *J. hort. Sci.*, **52**, 221–233.

Roulund, H. (1973). The effect of cyclophysis and topophysis on the rooting ability of Norway spruce cuttings. *For. Tree Improv.*, **5**, 21–41.

Sadhu, M. K., Bose, S. & Saha, L. (1978). Auxin synergists in the rooting of mango cuttings. *Sci. Hortic. (Neth.)*, **9**, 381–387.

Schier, G. A. (1973a). Origin and development of Aspen root suckers. *Can. J. For. Res.*, **3**, 45–53.

Schier, G. A. (1973b). Effects of gibberellic acid and an inhibitor of gibberellin action on suckering from aspen root cuttings. *Can. J. For. Res.*, **3**, 39–44.

Schier, G. A. (1974). Vegetative propagation of aspen: clonal variation in suckering from root cuttings and in rooting of sucker cuttings. *Can. J. For. Res.*, **4**, 565–567.

Schier, G. A. (1975). Promotion of sucker development on *Populus tremuloides* root cuttings by an antiauxin. *Can. J. For. Res.*, **5**, 338–340.

Schier, G. A. (1983). Vegetative regeneration of Gambel oak and chokecherry from excised rhizomes. *Forest Sci.*, **29**, 499–502.

Schier, G. A. & Campbell, R. B. (1978). Effect of ethephon on suckering of excised roots and rooting of cuttings in trembling aspen. *Forest Sci.*, **24**, 66–72.

Schmid, P. P. S. & Feucht, W. (1982). Changes in peroxidases in the phloem of *Prunus avium/Prunus cerasus* graftings during the initial stage of the union formation. *Angew. Bot.*, **56**, 93–98.

Sita, G. L. (1982). Tissue culture of *Eucalyptus* species. In: *Tissue culture of economically important plants*, edited by A. N. Rao, 180–184. Singapore: Committee on Science and Technology in Developing Countries, and Asian Network for Biological Sciences.

Sita, G. L. & Vaidyanathan, C. S. (1979). Rapid multiplication of *Eucalyptus* by multiple shoot production. *Curr. Sci.*, **48**, 350–352.

Sita, G. L., Shobha, J. & Vaidyanathan, C. S. (1980). Regeneration of whole plants by embryogenesis from cell suspension cultures of sandalwood. *Curr. Sci.*, **49**, 196–198.

Smith, M. A. L. & McCown, B. H. (1983). A comparison of source tissue for protoplast isolation from three woody plant species. *Plant Sci. Lett.*, **28**, 149–156.

Smith, N. G. & Wareing, P. F. (1971). The effect of gravity on root emergence from cuttings of some tree species. *Forestry*, **44**, 177–187.

Sommer, H. E. & Caldas, L. S. (1981). *In vitro* methods applied to forest trees. In: *Plant tissue culture: methods and applications in agriculture*, edited by T. A. Thorpe, 349–358. New York: Academic Press.

Sriskandarajah, S., Mullins, M. G. & Nair, Y. (1982). Induction of adventitious rooting *in vitro* in difficult-to-propagate cultivars of apple. *Plant Sci. Lett.*, **24**, 1–9.

Staritsky, G. & van Hasselt, G. A. M. (1980). The synchronised mass propagation of *Coffea canephora* in vitro. *Proc. int. Colloq. Coffee, 9th, London*, vol. 2, 597–602.

Steward, F. C. (1970). From cultured cells to whole plants: the induction and control of their growth and differentiation. *Proc. R. Soc.*, **175B**, 1–30.

Street, H. E. (1976). Experimental embryogenesis – the totipotency of cultured plant cells. In: *The developmental biology of plants and animals*, edited by C. F. Graham and P. F. Wareing, 73–89. Oxford: Blackwell Scientific.

Strömquist, L.-H. & Hansen, J. (1980). Effects of auxin and irradiance on the rooting of cuttings of *Pinus sylvestris*. *Physiologia Pl.*, **49**, 346–350.

Sziklai, O. (1967). Grafting techniques in forestry. *Comb. Proc. I.P.P.S.*; **17**, 124–129.

Teasdale, R. D. & Rugini, E. (1983). Preparation of viable protoplasts from suspension-cultured loblolly pine (*Pinus taeda*) cells and subsequent regeneration to callus. *Plant Cell, Tissue and Organ Cult.*, **2**, 253–261.

Tomlinson, P. B. & Gill, A. M. (1973). Growth habits of tropical trees: some guiding principles. In: *Tropical forest ecosystems in Africa and South America. a comparative review*, edited by B. J. Meggers, E. S. Ayensu and W. D. Duckworth, 124–143. Washington: Smithsonian Institute.

Vardi, A., Spiegel-Roy, P. & Galun, E. (1975). Citrus cell culture: isolation of protoplasts, plating densities, effects of mutagens and regeneration of embryos. *Plant Sci. Lett.*, **4**, 231–236.

Vieitez, E. & Vieitez, A. M. (1976). Juvenility factors related to the rootability of chestnut cuttings. *Acta Hortic.*, **56**, 269–273.

Vieitez, A. M., Ballester, A., Vieitez, M. L. & Vieitez, E. (1983). *In vitro* plantlet regeneration of mature chestnut. *J. hort. Sci.*, **58**, 457–463.

Von Arnold, S. (1982). Factors influencing formation, development and rooting of adventitious shoots from embryos of *Picea abies* L. Karst. *Plant Sci. Lett.*, **27**, 275–287.

Wareing, P. F. & Graham, C. F. (1976). Problems of development. In: *The developmental biology of plants and animals*, edited by C. F. Graham and P. F. Wareing, 3–4. Oxford: Blackwell Scientific.

Whitehill, S. J. & Schwabe, W. W. (1975). Vegetative propagation of *Pinus sylvestris*. *Physiologia Pl.*, **35**, 66–71.

Whitmore, T. C. (1977). *A first look at* Agathis. (Tropical forestry papers no. 11). Oxford: Commonwealth Forestry Institute.

Zimmerman, R. H., ed. (1976). Juvenility in woody perennials. *Acta Hortic.*, **56**, 3–317. (Proc. int. Soc. for Hort. Sci. Symp., Maryland and West Berlin, 1975).