

VEGETATIVE PROPAGATION OF LOBLOLLY PINE

J. P. van Buijtenen and D.V. Shaw¹

Abstract.-- Loblolly pine is currently propagated vegetatively by grafting techniques as well as rooted cuttings. Tissue culture techniques are also becoming feasible as a means of producing vegetative propagules.

Grafting has been done operationally for over 30 years and can be done routinely with very high success rates. However, it is limited in application because of its high cost.

Propagation of loblolly pine by rooting is much more difficult, but the available techniques are being improved and have potential to be cheaper than grafting. A few factors such as donor plant manipulation, maintenance of juvenility, and the correct amount of mist are of critical importance. A large number of additional factors each make a small contribution to rooting success. A combination of all these factors is needed to obtain a high enough success rate for a commercial operation.

Tissue culture has improved immensely in the last decade, but further refinements are needed before commercial application to coniferous forest trees could be considered.

Additional keywords: Pinus taeda, rooting, grafting, tissue culture, girdling, understock.

INTRODUCTION

The purpose of this paper is to outline the methods and opportunities associated with vegetative propagation of loblolly pine. Most of what will be said for loblolly pine holds equally well for several other southern pines, particularly slash, shortleaf and Virginia pines. In most cases the vegetative propagation of these southern pines is actually easier than that of loblolly pine.

Loblolly pine is already being propagated on a fairly large scale by grafting for the purpose of establishing seed orchards. It is estimated that there are currently about 4500 hectares of grafted loblolly pine seed orchard in the southern United States. If vegetative propagation by rooted cuttings becomes practical, however, the potential is far greater and hundred thousands or millions of hectares of forest could be established by vegetative propagules. The same, of course, is true for vegetative propagation with tissue

¹Professor, Texas Agricultural Experiment Station, and Head, Reforestation Department, Texas Forest Service, College Station, TX.; and Vice-President, Breeding and Research, International Forest Seed Co., Odenville, AL. The authors gratefully acknowledge the many helpful comments and suggestions of Mr. T. M. Marino, International Paper Co., Natchez, MS.

culture propagules. The incentive to master vegetative propagation methods is great: by vegetative propagation it is possible to capture 100% of the potential genetic gain. While the potential gains for loblolly pine are not well demonstrated with empirical data, theoretical considerations imply that improvements over conventional seed orchard technology can be substantial. If experience with hardwoods can be used as an example, gains of approximately 50% can be expected. Gains of 10-20% with Norway spruce in Germany have been realized (Kleinschmidt and Schmidt, 1977).

Vegetative propagation is not without problems. The principal difficulty is one of economics. At the moment it costs approximately \$5 to \$10 to produce a healthy, well established graft. Rooted cuttings cost between 25 cents and \$1 each. Compared to the cost of about 2 to 3 cents for a bare-root seedling and 7 to 25 cents for a containerized seedling, this cost is rather prohibitive. Large scale production of rooted cuttings is expected to decrease costs further, due to economics of scale. If the potential genetic gains acquired through clonal technology are attained, the higher cost of cloning forest trees may be justifiable.

Other problems include: the need to use juvenile material at least for propagation by rooted cuttings and tissue culture (or the need for reliable rejuvenation methods); the slower initial growth rate of rooted cuttings as compared to seedlings; and the experimental difficulties of comparing seedlings to propagules produced by vegetative propagation. We will return to some of these problems later.

VEGETATIVE PROPAGATION BY GRAFTING

Grafting techniques for loblolly pine were developed a little over 30 years ago. The methods have been refined continuously since that time and grafting can now be done routinely with a very high success rate: better than 90%.

The methods of choice are the side graft, the side veneer graft and the cleft graft. Best grafting success is obtained, when the understock is just initiating physiological activity and the scion material is still dormant. However, equally high graft success can be achieved with succulent tissue, but more care must be taken because of the tender tissue used. Often the grafts are covered with a plastic bag to conserve moisture and a kraft paper bag to provide shade.

A technique that is recently gaining favor (White et al., 1983), consists of cutting the needles off the scion and covering the graft union and scion completely with melted paraffin wax. This eliminates the need for the plastic and kraft bags and reduces the amount of after-care. The grafts are somewhat slower to develop, since the scions have to grow through the paraffin, but grafting success has been very high and growth by the end of the growing season does not seem to be very different from that of other methods of grafting.

Grafting can also be categorized according to the way in which the understock is handled. One can distinguish pot grafting, nursery bed grafting and field grafting. Pot grafting is convenient, since one can plant the understock six months ahead of time at one location in at least a partially heated facility. Also water and fertility levels are easily controlled. The

drawback is that a certain amount of pot binding occurs and that there is some delay in the development of the grafted tree once it has been transplanted into the field.

In nursery bed grafting the understock is planted in a nursery bed usually at about 30 x 30 cm spacing. The grafts are made in the nursery bed and then successfully grafted plants are transplanted with a ball into their location in the seed orchard. This method has some of the advantages of the central location of the pot grafting method and gets away from the problems of pot binding. On the other hand the logistics of lifting and moving the grafts can be a real problem if the beds are distant from the seed orchard.

The method of choice is the field grafting method. The most common procedure is to plant three understock at each location in the orchard and graft on the two best understock. After the grafts have been made there is still a certain amount of moving to be done, as invariably in some locations both grafts will die and in other locations both will live. This method is more time consuming because of the larger distances between the grafts, but results in grafts that are far more vigorous and root-firm than the other two methods.

VEGETATIVE PROPAGATION BY ROOTED CUTTINGS

Loblolly pine is notoriously difficult to propagate by rooted cuttings, but enough progress has been made in the last few of years that at least one large industry is committed to develop it into a commercial process. A large number of factors influence the success of the rooting process (van Buijtenen et al., 1975) and these will be considered individually in the remainder of this section.

Condition of the Donor Plant

The maturation state of the donor plant is one of the most important factors influencing the rooting of its cuttings. Cuttings from untreated loblolly pine over six years old are extremely difficult to root, while cuttings of one-year-old seedlings can be rooted rather readily (Greenwood and Nussbaum, 1981). Maintaining the donor plant in a juvenile condition or rejuvenating the donor plant is therefore of paramount importance. Hedging is the principal method of maintaining a donor plant in a juvenile condition (Libby et al., 1972). It is important to keep hedges as low as possible. In practice hedges have been anywhere from 25 cm to 1 meter high. The Texas Forest Service has a number of hedges that are now twelve years old, which still produce cuttings that root with a reasonable success rate.

Care of the cutting donor plant (ortet) is of major importance. For example, Bower and van Buijtenen (1977) demonstrated that greenhouse grown donor plants produce cuttings that are easier to root than field grown donors. Conversely, Shaw (pers. comm.) routinely achieves similar results for greenhouse and field grown donors. Such observations probably result from similarities or differences among the environmental factors applied to the donor plants. Fertilization, irrigation, day length and severity of previous donor shearings will all affect the ability of a donor plant to produce rootable cuttings.

Pre-Treatment of the Cuttings

Hare (1971) developed a method of pre-treating the cutting which was refined by Cunningham and van Buijtenen (1983). By girdling the cuttings before removing them from the donor plant and treating them with a hormone mixture rooting success can be enhanced tremendously. There is a parallel here, as pointed out by Mott (personal communication) between tissue culture and rooted cuttings. The conditions needed for root initiation are not the same as the conditions for optimal growth and development of the roots. Pre-treatment of the girdles on the tree produces conditions optimal for root initiation. Once they are transferred to the rooting bench one can concentrate on obtaining optimal conditions for root growth and development.

Unfortunately, such intensive methods result in high costs of production. Except for very high value products (e.g. specialized understock for grafting) such methods will be too costly. Less expensive methods must be developed for rooted cuttings that will be used for operational reforestation.

The rooting environment consists of several independently (but simultaneously) applied treatments aimed at root initiation and development. Improvements in rooting response over the last few years have resulted largely from manipulation of the treatments listed below. Each of the following makes a small contribution to rooting success, and all must be near optimal to achieve an outstanding rooting response.

Conditions in the Rooting Bench or Rooting Chamber

Root-zone heat increases rooting success somewhat and is being used by many growers. Time-to-root, percent rooting and root growth have been shown to increase in the presence of bottom heat (Hansen et al., 1979; Wetherington, 1983).

Mist quantity and quality is very critical for successful rooting (Greenwood et al., 1980), and optimal values are extremely difficult to attain. If the amount of moisture is too low the cuttings will die of dessication. If it is too high the cuttings will rot and algae growth will be a problem. Between .05 and .1 millimeter of moisture per hour seems to be about optimal for a range of temperature and humidity conditions.

Carbon dioxide enrichment of the atmosphere in the rooting chamber is beneficial for most plant species (Molner and Cummings, 1968) and contributes a small amount of additional rooting. The optimum level appears to be around 1000 parts per million of carbon dioxide. This level cannot be maintained continuously if ventilation is needed for cooling.

Nutrient replacement and/or supplementation is beneficial to rooting because frequent misting leaches many of the nutrients out of the foliage and additional nutrients are necessary (Sorenson and Coorts, 1968). A modified Hoagland solution that has been used is described in table 1. The cuttings are sprayed lightly every evening with this solution. Another option is to replace the depleted nutrients with one of several commercial fertilizers injected directly through the misting system.

Table 1.--Composition of nutrient solution used to spray cuttings

Element	Concentration, ppm
N	50
K	50
P	150
Ca	50
Mg	20
Cu	0.016
B	0.01
Mn	0.25
Zn	0.1
Mo	0.01
Fe	5.5
S	44

Hormone treatment is a virtual necessity for rooting of southern pine species. The hormone treatment that has given the best results so far is the rooting powder developed by Hare (1971), the composition of which is given in table 2. All of the components in this mixture make a demonstrable contribution to rooting success.

Table 2.--Preparation of rooting powder

The rooting powder is prepared as follows: dissolve 1.0 gm IBA (indolebutyric acid), 1.0 gm PMPZ (1-phenyl-3-methyl-5-pyrazalone), and 1.1 gm Alar-90 (90% B-Nine, Naugatuck Chemical Co., Naugatuck, Conn.) in 70 ml room temperature anhydrous acetone. Alar will not dissolve completely, so suspend it in the acetone and mix well with the talc. Transfer quantitatively to 67 gm talc (Baker USP) in a bowl. Stir the slurry constantly in a hood over gentle air stream until completely dry. Sift through a stack of sieves, to 50 mesh. Mix well with 20 gm sifted Captan 50W (Orthocide 50) and 10 gm sifted 10X confectionery sugar. Grind the final mixture in a large mortar and sift again.

Photoperiod extension to 16 hours per day can increase rooting percentage by a relatively small amount. Increasing the day length during the winter can also help prevent abnormal dormancy, that is difficult to break when cuttings are removed from the rooting chamber in late spring.

Supplemental light applied during the night with light of photosynthetic intensity can enhance rooting percent. The cost however is very high for the small increase in rooting obtained.

Time of Year

The literature suggests that rooting of cuttings in the dormant season is greatest for a wide range of species. This is not the case when using the hormone combination of Hare; the highest rooting success is obtained in the

spring of the year. Cuttings rooted at different times of the year require different conditions. For example, when using Hare's girdling technique in the spring, 2% indole butyric acid for 2 weeks works best. During the dormant season, from September through the end of January 4% of indole butyric acid for 6 weeks seems to work best. During August rooting success has been so poor that one should not even attempt it.

Rejuvenation

Rejuvenation is the technique whereby an old plant is treated in such a way that certain characteristics known to occur in the juvenile phase of growth of that species are restored. Several techniques have promise but whether these work is still open to debate. Increased rooting and field vigor are the two most important juvenile traits which must be restored to loblolly pine rooted cuttings from old selections.

There are only two techniques that have been reported to rejuvenate plants in terms of restored rooting potential. Rooting has been increased by grafting mature scions to juvenile understocks (Franclet, 1979; Chaperon, 1979; Muzik and Cruzado, 1958). Rejuvenation has occurred as a result of in vitro pruning in tissue culture in the presence of cytokinins (Franclet, 1979). Cytokinin itself seems to inhibit rooting to some extent and one needs to wait for a period of time to let its effect dissipate.

Hedging definitely will increase rooting over non-hedged plants but, this technique appears to be more applicable to maintaining juvenility rather than restoring juvenility. Serial propagation (rerooting) has also been shown to at least slow maturation (Pawsey, 1971); thus, as with hedging this technique may be more applicable in maintaining juvenility.

Selection for Trees That Root Well

Although some people do not like to admit it, one often practices selection for rooting ability, either by design or as a by-product of the procedures followed. Clones that do not root well are not expanded to large numbers and are dropped from the program. Culling for rooting ability is of no advantage when selection is for growth and vigor traits, and propagation is by non-vegetative means.

Table 3.--Comparison of family rooting performance in three trials

Family	Trial 1	Trial 2	Trial 3
	----- <u>Percent Rooting</u> -----		
1	87	100	74
2	44	84	79
3	30	65	33
4	12	67	25
5	29	100	58

In fact, the reduction in population size resulting from such culling is undesirable. However, high rooting percentage is essential for economical large scale production of rooted cuttings, and planned reductions

in population size must be incorporated into the breeding plan. The variation in rooting response for five full-sib loblolly families in 3 trials is given in table 3. Families 1 and 2 are always good or outstanding, while families 3 and 4 always perform poorly in a relative sense.

VEGETATIVE PROPAGATION BY TISSUE CULTURE

In the last few years the technique of propagating loblolly pine by means of tissue culture has been greatly improved. It still might be more appropriate, however, to refer to it as organ culture. In most cases sprouts are obtained first from either embryos or young plants and these are subsequently rooted. Thus far no one has been able to develop complete plants from either single cells or calluses. One organization has been able to re-propagate 6-year-old loblolly pine rooted cutting in tissue culture. The process begins first by producing axillary shoots that appear juvenile by cytokinin sprays, culturing the axillary shoots for elongation in tissue culture, then rooting these in tissue culture (Abo El-Nil, 1982). Others have experienced difficulty in reproducing these results.

Great care is needed in conditioning the tissue culture plantlets, but this has been done successfully and a number of field plantations of tissue culture plantlets have been established and are growing very well (Leach, 1979). There have been reports of genetic instability of tissue culture plantlets but evidence of this in loblolly pine has not been observed (Renfroe and Berlyn, 1984).

APPLICATIONS

The most important current application of vegetative propagation of loblolly pine in the South is no doubt seed orchard establishment. Little attention has been paid to the understock being used, although the vegetative propagation of understock by rooted cuttings is one very useful means for maintaining control of understock.

Experience from apple growers indicates that the understock influences growth rate, fruitfulness and resistance to diseases of the grafted plant; there is every reason to believe that the understock for loblolly pine grafts might be equally important (Rogers and Beakbane, 1957; McKinley, 1975). Research is currently underway at the Texas Forest Service and the Western Gulf Tree Improvement Program to screen for understock for grafting.

The biggest potential of all however for the application of vegetative propagation is in the reforestation of large acreages of production forests. There are many different goals one could pursue. Foremost in most peoples mind is the possibility of capturing all the genetic gain possible each generation, thus achieving extremely rapid genetic gains. There are other applications as well; e.g. one could use rooted cuttings for specialty products to be grown on more limited acreages. A prime example, for instance is the development of highly rust resistant loblolly or shortleaf x loblolly hybrid clones for planting on high rust-hazard sites. Similarly, there might be potential for developing clones that are highly resistant to the southern pine bark beetle. This however would be a much more difficult problem to solve in view of the biology of the insect. It would also be feasible to develop clones with specialized wood properties which would be useful for a narrow range of products. Clones

with either exceptionally high or exceptionally low wood specific gravity or with very high extractives content could be produced fairly readily.

Technology is available to utilize rooted cuttings from seedlings to increase planting stock from improved sources in low supply (Armson et al., 1980). Furthermore, rooted cutting can reduce the time to fully utilize planting stock from seed orchards. This would make the genetic gain at each new generation available sooner than by waiting for the orchard to reach full maturity.

The use of rooted cuttings would allow the adaptation to a changing market much more readily than the seed orchard approach. To establish a new orchard and harvest the first commercial quantities of seed can take approximately 8-10 years with proper site selection and orchard management. Using rooted cuttings one could conceivably reach commercial production in less than 5 years and obtain a greater change in properties at the same time.

An excellent review of 18 advantages associated with clonal forestry has been published by Libby and Rauter (1984).

FUTURE RESEARCH NEEDS

Perhaps the most vexing problem at the moment is the evaluation of the growth potential of rooted cuttings. The experience to date indicates that rooted cuttings may grow somewhat slower (at least initially) than seedlings of approximately the same size (Bailey, 1984). This could very well be an artifact and could be caused by the fact that a rooted cutting has gone through a tremendous shock and is not in the same physiological state as a seedling of comparable size. As horticultural methods for rooting and conditioning cuttings are refined this initial lag may disappear. It is extremely difficult to develop an unbiased method of comparing seedlings with rooted cuttings because of the differences in physiological condition and size of the rooted cuttings. New research is needed in developing an appropriate technology to do so.

Work on rejuvenation is also urgently needed. There are some promising leads, but the surface has hardly been scratched. The methodology for evaluating juvenility itself is not very well developed. Juvenility is not always clearly defined and methods of measuring juvenility have not been fully developed. Increased rooting and field vigor are two measurable juvenile traits being presently evaluated but many others still exist. Until efficient methods are developed one cannot really determine whether rejuvenation has indeed occurred. Despite all these questions an enormous amount of progress has been made and it is a matter of time now before all the available information is combined together in one system and rooted cuttings of loblolly pine are produced economically on a large scale.

LITERATURE CITED

- Abo, El-Nil, M. M. 1982. Methods for asexual propagation of Coniferous trees. U.S. Patent 4,353,184. Weyerhaeuser Company. October 12, 1982.
- Armson, K.A., Fung, M. and Bunting, W.R. 1980. Operational rooting of Black spruce cuttings. J. For. 78(6): 341-343.

- Bailey, James K. 1984. Juvenile cutting production techniques and early field performance of cutting versus seedlings of slash pine. PhD Dissertation, Texas A&M University, College Station, Texas. 93 p.
- Bower, R.C. and van Buijtenen, J. P. 1977. A comparison of rooting success of greenhouse-grown and field-grown slash pine cuttings. *Can. J. For. Res.* 7(1):183-185.
- Chaperon, H. 1979. Maturation and propagation by cuttings of forest trees. *AFOCEL Etudes et Recherches* 12:19-31.
- Cunningham, M.W. and van Buijtenen, J.P. 1983. Effect of shortened auxin treatments on the rooting of girdled slash pine shoots. *Can. J. For. Res.* 13(5):917-920.
- Francllet, A. 1979. The Rejuvenation of adult trees with a view to their vegetative propagation. *AFOCEL Etudes et Recherches. Micropropagation d'arbres Forestiers* 12:3-28.
- Greenwood, M.S., Marino, T.M., Meier, R.D. and Shahan, K.W. 1980. The role of mist and chemical treatments in rooting loblolly and shortleaf pine cuttings. *For. Sci.* 26(4):651-655.
- Greenwood, M.S. and Nussbaum, E.S. 1981. Rooting ability and growth performance of stem cuttings from one and five year old ortets of loblolly pine. *Proc. 16th South. For. Tree Imp. Conf.* p 176-183.
- Hansen, E.A., Phipps, H.M. and Tolsted, D.N. 1979. Rooting greenwood top cuttings of a difficult-to-root populus clone. *Tree Planter's Notes*, Spring, 1979. 9-11.
- Hare, R.C. 1971. Factors promoting rooting of tree cuttings. Paper presented at Sixth Southern Forest Physiology Workshop - Gainesville, Florida. Sept. 9-10, 1971.
- Kleinschmidt, J. and Schmidt, J. 1977. Experiences with Picea abies cutting propagation in Germany and problems connected with large scale application. In: Vegetative Propagation of Forest Trees - Physiology and Practice. p 65-86.
- Leach, G.N. 1979. Growth in soil of plantlets produced by tissue culture. Loblolly pine. *Tappi* 62(4):59-61.
- Libby, W.J., Brown, A.G. and Fielding, J.M. 1972. Effects of hedging Radiata pine on production, rooting, and early growth of cuttings. *N.Z. J. For. Sci.* 2(2):263-283.
- Libby, W.J. and Rauter, R.M. 1984. Advantages of Clonal Forestry. *For. Chron.* 60(3): 145-149.
- McKinley, C.R. 1975. Growth of loblolly scion material on rootstock of unknown genetic origins. *Proc. 13th South. For. Tree Imp. Conf.* p 230-233.
- Molnar, J.M. and Cummings, W.A. 1968. Effect of carbon dioxide on propagation of Softwood, Conifer, and herbaceous cuttings. *Can. J. Plant Sci.* 48:595-599.

- Muzik, T.J. and Cruzado, H.J. 1958. Transmission of juvenile rooting ability from seedlings to adults of Hevea brasiliensis. Nature 181:1288.
- Pawsey, C.K. 1971. Development of grafts of Pinus radiata in relation to age of scion source. Aust. For. Res. 5:15-18.
- Renfro, M.H. and Berlyn, G.P. 1984. Stability of nuclear DNA content during adventitious shoot formation in Pinus taeda L. tissue culture. Am. J. Bot. 71:268-272.
- Rogers, W.S. and Beakbane, A.B. 1957. Stock and scion relations. Ann. Rev. Plant Phys. 8:217-236.
- Sorenson, D.C. and Coorts, G.D. 1968. The effect of nutrient mist on propagation of selected woody ornamental plants. Am. Soc. Hort. Sci. Proc. 92:696-703.
- van Buijtenen, J.P., Toliver, J., Bower, R., and Wendel, M. 1975. Operational rooting of loblolly and slash pine cuttings. Texas Forest Service Publication No. 111. 9 p.
- Wetherington, J.L. 1983. Propagation of Juniperus chinensis 'Torulosa' using bottom heat. Inter. Plant Prop. Soc. Proc. 33:589-594.
- White, G., Lowe, W.J., and Wright, J. 1983. Paraffin grafting techniques for loblolly pine. South. J. Appl. For. 7(3):116-118.

VEGETATIVE PROPAGATION OF RADIATA PINE IN NEW ZEALAND

M.I. Menzies, T. Faulds, M. Dibley, and J. Aitken-Christie^{1/}

Abstract. For many years mature clones of radiata pine plus trees have been vegetatively propagated by grafts and cuttings for the establishment of archives and seed orchards. There is now considerable interest in vegetative propagation of juvenile trees to produce planting stock directly for afforestation.

Seed of superior genetic quality from controlled crosses is becoming available; vegetative propagation has been developed to bulk up the seedlings. Options include field collection of stem cuttings from 3 to 5-year-old trees, propagation of cuttings in the nursery from 1-year-old seedlings, micropropagation, or combinations of these.

Field collection of stem cuttings is time consuming and expensive because of travel and the small number of cuttings that can be collected from each tree. Cuttings from older trees have advantages in that ortets can be selected for desired characteristics, and malformation is greatly reduced, but they do have reduced initial diameter growth and are not always easily rooted.

Propagation from seedling ortets in the nursery is cheaper than from field cuttings; a range of methods is available, depending on multiplication rate desired and time available. Methods involve manipulation of stool beds by topping and pinning down of seedlings to produce cutting material that can be set in the open nursery bed or in containers. A higher multiplication rate can be obtained by using small fascicle cuttings set in containers. The percent rooting normally exceeds 90% and cuttings are ready for field planting the winter following setting in open beds, or earlier if set in containers. Effective selection is not possible with seedling ortets so that all improvements come from the seed itself.

Extensive multiplication of individual seeds is a possibility for clonal forestry using micropropagation techniques. A trial to clonally test and micropropagate 200 clones from the best 20 controlled-cross families has been started. Clones are being held in a juvenile state by cold storage of the micropropagated shoots, while the clonal test is being evaluated in the field over a 5 to 8-year period.

^{1/} Scientist, Technical Officer, Technician, and Scientist, respectively, New Zealand Forest Service, Forest Research Institute, Rotorua, New Zealand. The helpful criticism of Dr M.D. Wilcox, Dr D.R. Smith, and Dr M.J. Carson is gratefully acknowledged.

The comparative cost of different types of planting stock is \$50/1000 for seedlings, \$160/1000 for field cuttings, \$70-115/1000 for cuttings from nursery stool beds, and \$450/1000 for micropropagated plantlets.

Additional keywords: Mature cuttings, juvenile cuttings, micropropagation.

INTRODUCTION

Radiata pine plantations traditionally have been established using seedlings. The current New Zealand annual planting programme, including replanting of cutover areas, is about 40 000 hectares. This programme uses about 5000 kg of seed each year, with over three quarters of it being improved seed from open-pollinated seed orchards. Seed orchards are progressively being improved as a result of ongoing selection, progeny testing, and breeding research, with the aim of supplying all New Zealand's requirements from seed orchards with the highest possible quality. This programme involves vegetative multiplication of mature material from superior trees, using both grafts and cuttings, for the establishment of seed orchards and clonal archives. Cuttings are preferred, because of the problem grafts often present with delayed incompatibility. Techniques have been developed to reliably produce planting material using both methods (N.Z. Forest Service, 1984a).

Cuttings have often been advocated for establishing radiata pine plantations, particularly when combined with selection and clonal testing (Fielding 1964, 1970; Thulin and Faulds 1968; Libby *et al.* 1972; Wilcox *et al.* 1976; Burdon 1982; Libby 1983). In general, these authors envisaged selecting superior trees in the forest at age 5-10 years, propagating them by cuttings and holding the clones in cutting archives while a clonal test was concurrently established and evaluated. The archives would be kept hedged to minimise any ageing effects. Once results from the clonal tests were available, the desired clones could be propagated from the hedges. Advantages of the scheme would be an ability to establish clonal forests of uniform, superior, tested clones. As it has turned out, there have been insurmountable difficulties, including cost in time and manpower in establishment and maintenance of hedges, a low multiplication rate, difficulty of rooting cuttings from hedges with an effective ortet age older than 7 years, a lower growth rate of cuttings from older ortets, and the need for a long lead time of about 15 years before cuttings could be produced, reducing flexibility for introducing new selection criteria.

However, it is not necessary to use individual clones to provide planting stock superior to that of conventional open-pollinated seed orchards. In one study (NZ Forest Service 1974), seedlings from controlled pollinations gave a 43% gain in volume and a 36% gain in straightness over an unselected bulk seedlot, while seedlings from an open-pollinated orchard gave only a 24% increase in volume and a 17% increase in straightness over the unselected bulk seedlot. In a conventional open-pollinated orchard, clones vary in their reproductive

capability and timing of anthesis, and so pollination is not random, neither can it be controlled (Sweet and Krugman 1977). Also, there is contamination from pollen sources outside the orchard which prevents clones of optimum genetic quality from contributing to the quality of orchard seed to the extent desired. Wilcox et al. (1975) demonstrated that there were significant additional gains to be made from controlled pollinations between the best general combining parents, or of crosses showing high specific combining ability. Controlled pollinations would also allow selection for special-purpose traits not available from seed from an open-pollinated orchard. One commercial-scale method entails maintaining hedges of the desired clones in an orchard, and using controlled pollination to produce seed (Sweet and Krugman 1977; Smith et al. 1981). It would be feasible, but logistically difficult, to produce all New Zealand's seed requirement in this way. An alternative method is to combine a programme of controlled pollination with some form of vegetative amplification (Smith et al. 1981; Burdon 1982).

Control-pollinated seed is now being commercially produced in one seed orchard, with 40 kg becoming available in 1986 and over 100 kg/year expected within a few years. Also, a new 20 ha controlled-pollination seed orchard is now being developed on a coastal Bay of Plenty site to provide a reliable supply of seed of progeny-tested superior families (NZ Forest Service 1985).

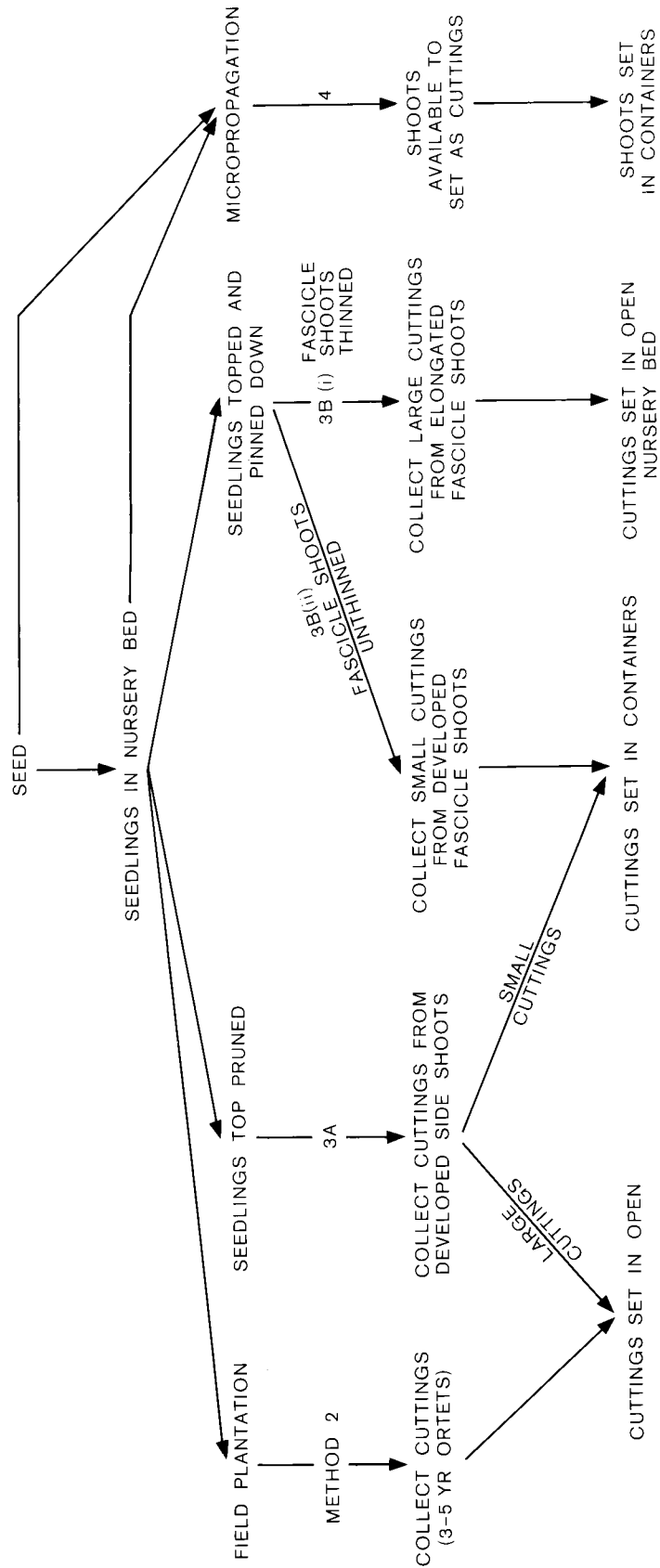
Rapid advances are also being made in methods of vegetative propagation of juvenile planting stock. These methods include collection of cuttings from plantation trees of improved seed origin, manipulation of young seedlings in the nursery bed as seedling stools, and micropropagation (Diagram 1).

To help evaluate propagation options, it is desirable to have production costs of the various methods, but it is difficult to get accurate comparable costs for all methods at one time. The base cost is the cost of producing 1/0 seedlings, and there is a wide range of prices in New Zealand, from about \$35-\$90/1000. This cost varies depending on nursery size and the number of problems, such as soil type, bird or animal predation, fungal or insect infections, amount of conditioning done, need for irrigation, and number of seedlings finally produced per kg seed sown. An average cost would be about \$50/1000, and this will be used as a base figure. Labour for all propagation methods will be costed at NZ\$7/hour.

1. Vegetative propagation of old material for seed orchards and archives

Grafts and cuttings are used for cloning physiologically old (10 years or older) radiata pine to establish seed orchards and clonal archives. Cuttings are preferred because of the problem grafts often present with delayed incompatibility. However, cuttings taken directly from old parent ortets rarely root satisfactorily, necessitating grafting as a first step in propagation procedures for newly-selected trees.

DIAGRAM 1 - METHODS OF VEGETATIVE PROPAGATION OF GENETICALLY IMPROVED SEED



Bud scions are taken in winter from parent ortets for grafting, and the grafts used for establishing temporary clonal orchards (usually hedged), or for initiating new seed orchards. Once the grafts are large enough for repropagation (3 years after planting), they are used as a source of cuttings for setting up permanent archives and seed orchards. Archives, generally hedged, provide a continued source of cutting material for seed orchards and may also be used for controlled pollination.

Important factors in the successful propagation of old clones by cuttings are health of foliage, topping and ring-barking treatment, and the rooting environment (NZ Forest Service 1984a). In mid-February the current season's shoots are cut back to where foliage is dense and fully developed (topping). In March, 3 to 4 weeks after topping when the cuttings have developed small needle fascicle buds, a strip of bark 10-mm wide is taken from around the stem of each cutting (ring-barking) 50 to 150 mm below the terminal topping. Petrolatum grease is applied to the ring-barked area, which is then covered with aluminium foil. Then in April, 4 to 6 weeks after ring-barking, the cuttings are ready for collection. At this stage, each cutting should have callus formed at the ring-bark, and a basal swelling. Cuttings are set in polythene tunnels which maintain a temperature of 15-28°C during daylight hours and a humidity of 70-100%. When rooting of cuttings has started in about October, the polythene tunnel is removed and replaced with shade cloth until December when it is removed. From December to April an intensive root-pruning regime, both horizontal and lateral, is imposed on the cuttings.

If the prescribed propagation system is adhered to, 70% usable rooting can be achieved. Rooting, both in quality and quantity, is greatly reduced if any treatment is omitted.

In the future micropropagation of mature radiata pine may also provide a fast reliable method of obtaining large numbers of plants from selected mature trees for seed orchard establishment (NZ Forest Service 1984a).

2. Vegetative propagation using field cuttings

Cuttings can be propagated readily from young radiata pine trees in the forest. Plantations suitable for cutting collection are being established with seedlings from special seedlots from the best seed orchard clones (Arnold and Gleed, 1985) or from control-pollinated crosses. Collection from field trees also allows some selection for vigour and form of the parent ortet. Tips of lateral branches growing in full sunlight are used. It is essential that only healthy disease-free material is used. Cutting material should have dense, fully elongated healthy needles, with a cutting length between 100 and 150 mm, and a minimum diameter of 6 mm.

The number of cuttings available for collection from each tree varies depending on age of the ortets and site. While up to 10 cuttings can

be collected from a large 3-year-old tree with lower branches unsuppressed by weed competition, the average from all 3-year-old trees is five. These numbers treble for 5-year-old ortets.

The cuttings are collected in late April-late June during the period of slowest growth. Setting is done as soon as possible after collection, to avoid fungal infection, although cuttings can be stored for up to a month in a cool store. Cuttings are set outside at a depth of 5 cm in raised nursery beds. Overhead irrigation is necessary during warm or dry windy weather, particularly during the first few weeks after setting.

Rooting of cuttings occurs in the spring following setting, with cuttings from younger ortets rooting in October/November and cuttings from older ortets rooting later in November/December or even later. The percentage of cuttings forming an acceptable root system declines with increasing age of ortets; while 80% of cuttings from 3-year-old ortets might be expected to form acceptable root systems, about 65% of cuttings from 6-year-old ortets do so at the FRI Nursery.

Cuttings are conditioned by undercutting at a depth of 10-15 cm in early January, with lateral root pruning 2-3 weeks later, and wrenching at approximately 3-weekly intervals. If nitrogen deficiency symptoms occur during this conditioning, a 2% urea solution can be applied at 400-500 l/ha until the symptoms disappear.

Cuttings are ready for lifting one year after setting, and are planted during June to mid-July. After mid-July, they start to flush and successful establishment is more difficult.

Thirty thousand cuttings from 3-year-old ortets set in the FRI nursery last year produced 75% plantable cuttings; 8% died without rooting, 10% died after rooting from root rot or other causes, and 7% had unacceptable root systems. More than half a million cuttings are being produced annually by this system by one large private forestry company (Arnold and Gleed 1985).

The cost of field cuttings varies depending on distance to the collection site, and ease of collection (e.g., slope, weed competition, tree size). Last year 79,000 cuttings were collected. Based on labour at \$7/hour, collecting 1235 per man-day, and setting at 6000 per man-day, with 75% rooting, the collection and setting cost was \$110/1000. After setting, they accrue the normal 1/0 seedling costs of \$50/1000 for weed control, fungal and insect sprays, fertiliser, conditioning, and lifting and packing, for an overall cost of \$160/1000 cuttings. Cuttings are set at a wider spacing than seedlings are sown at, but there is a better percentage of plants at the nursery gate. With present returns an extra 12% of nursery bed space would be required for cuttings; this is not included in the cost estimates.

There are many effects of ortet age which may affect the use of field cuttings for plantation forestry. The major ones are:

a) Rooting Ability

Historically it has been considered that percent rooting, and number of roots forming on a cutting, decline with increasing ortet age. While at least 90% of cuttings from one-year-old seedlings could be expected to form roots when set in an open nursery bed at the Forest Research Institute (FRI), Rotorua, this percentage declines to about 80% by ortet age 5, and 40% by ortet age 15, at full sexual maturity (Thulin and Faulds 1968). Plantable cuttings from young ortets have a relatively large number of roots arising from the base of the cutting, and root wrenching produces a compact fibrous root system (Thulin and Faulds 1968). However, there can be problems getting cuttings from older ortets to form enough roots, and this has often contributed to their poor survival (Jacobs 1939; Thulin and Faulds 1968; Fielding 1969; Menzies and Chavasse 1982).

b) Growth Rate

There have been a number of studies comparing the growth rate of seedlings and cuttings. One of the earliest experiments in New Zealand showed that cuttings set from 1.5-year-old nursery trees had similar growth rates to seedlings up to four years after planting (Field 1934). Similar results have been reported in Australia (Fielding 1970), although there was a lower growth rate of cuttings from 15-year-old ortets, particularly in the first few years. Brown (1974) compared the growth of cuttings from 1 to 7-year-old ortets with seedlings and found no significant difference, although there was a trend to a lower diameter growth of cuttings with increasing age of ortet after 3 years. Trials in New Zealand have also indicated that cuttings tend to have lower relative growth rates, particularly with cuttings from older ortets (Sweet and Wells 1974; Shelbourne and Thulin 1974; N.Z. Forest Service 1984b). West (1984) found that cuttings from 4-year-old ortets had similar height growth but significantly smaller diameter than seedlings after 5 years at four sites.

c) Tree Form

It has been observed that cuttings, even from unselected trees, tend to be straighter than seedlings (Thulin and Faulds 1968), and that juvenile seedlings and cuttings are not as straight as more mature cuttings and grafts (Sweet and Wells 1974). However, there is also a wide range of straightness noted within an age group of cuttings (Shelbourne and Thulin 1974).

Cuttings from older ortets also tend to have fewer and smaller branches than seedlings (Fielding 1970; Pawsey 1971; Tufuor and Libby 1973), with more horizontal branching (Pawsey 1971).

Because of these improved form traits of less stem sweep and better branching habit, stands of older ortet cuttings have significantly more acceptable final crop trees than seedling stands. In a 6 ha trial at Kaingaroa planted with seedlings and cuttings from 5-year-old ortets, when the stand was thinned to 500 stems per hectare, 50% of the remaining seedlings were malformed, compared with only 14% of cuttings (N.Z. Forest Service 1984b). This would allow stands to be established at wider than normal spacings (Gleed 1983; N.Z. Forest Service 1984b). However, recent results of trials of seedlings from new generation seed orchards (now coming into production) indicate that they should have 75-80% acceptable stems on most sites, leaving less margin of gain from the use of older ortet cuttings.

d) Other Morphological Characteristics

(i) Bark thickness

Cuttings from ortets older than 6 years tend to have markedly thinner bark, particularly in the first 4.6 m above ground (Fielding 1970; Pawsey 1971).

(ii) Taper

Cuttings generally have less taper than seedlings (Fielding 1970; Pawsey 1971; Sweet and Wells 1974), particularly in the lower bole (Fielding 1970; Pawsey 1971) and with older ortets (Sweet and Wells 1974). These differences in bark thickness and taper may help compensate for the smaller d.b.h.o.b. of older ortet cuttings (Pawsey, 1971).

(iii) Stem cones

As would be expected with their greater maturity, cuttings bear male and female strobili earlier than seedlings. With cuttings from ortets older than 10 years, male catkins and female strobili were present on some trees a year after planting, and were more prevalent two years after planting (Sweet 1973). Female strobili were common on cuttings from 5 to 6-year-old ortets 5.5 years after planting, with stem cones prevalent on lower stems, while these were much less common on seedlings (Fielding 1970). Stem cones on mature cuttings would need to be pruned off to avoid stem degrade.

e) Wood properties

(i) Wood density

There is little difference in the average wood density between seedlings and cuttings from juvenile ortets, but cuttings or grafts from mature ortets have a lower average wood density than the ortets from which they were taken (Sweet and Harris, 1976; Nicholls et al., 1977). Maximum and minimum within-ring

wood density decreased with increasing ortet age for grafts (Sweet and Harris, 1976). Similar non-significant trends were found by Nicholls et al. (1977).

(ii) Tracheid length

Sweet and Harris (1976) found that tracheid length in second and fifth growth rings was significantly greater in grafts than in seedlings, and increased with increasing age of the original ortet. This result was confirmed for cuttings by Nicholls et al. (1977). These differences diminished with increasing age of propagules and may not exist at all beyond the ring tenth from the pith.

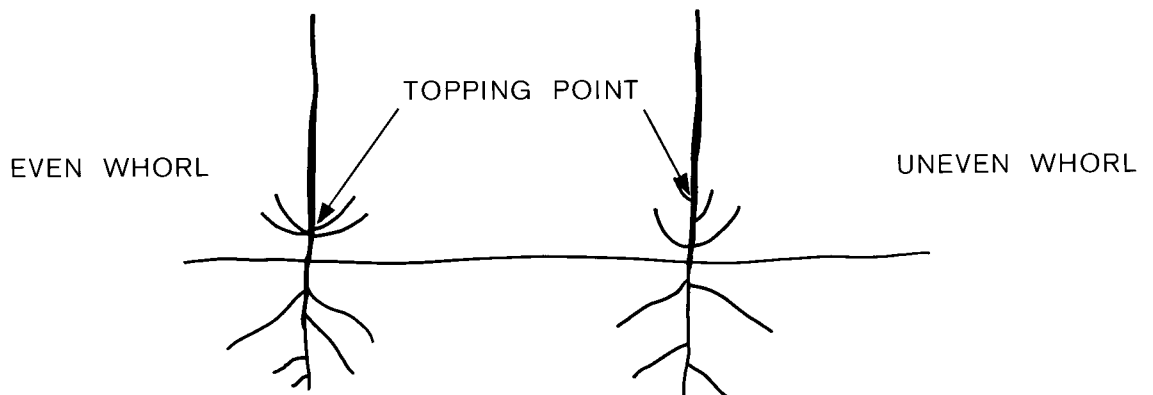
Trees are at least three years old from sowing before they are large enough to collect cuttings from; as the trees become older and larger, more cuttings can be collected. Ortet age 5 appears to be the oldest practicable age before the advantage of improved stem form is outweighed by the disadvantages of decreased rooting ability and reduced diameter growth. Field trials have been established to determine the optimum ortet age.

3. Vegetative propagation of juvenile material using nursery stool beds

(a) Seedling pruning

Seed is sown in spring (September/early October). In February, when the seedlings are 100-180 mm tall, they are topped with secateurs at a point 5 mm above the highest side shoot (Diagram 2). The side shoots develop into stem cuttings, with 2-6 cuttings per plant (Plate 1).

Diagram 2. Topping position for development of side shoots.



Top growth and callusing occur in October in both open-bed and containerised cuttings, with rooting in October.

Open bed cuttings are conditioned as for field collected cuttings and are ready for planting the following winter (Plate 2).

Containerised cuttings are grown on a low nutrient regime to produce woody-stemmed plants 100-200 mm tall.

Rooting percentages for these types of stem cuttings are given below (Table 1).

Plate 2. Open bed cuttings ready for field planting.

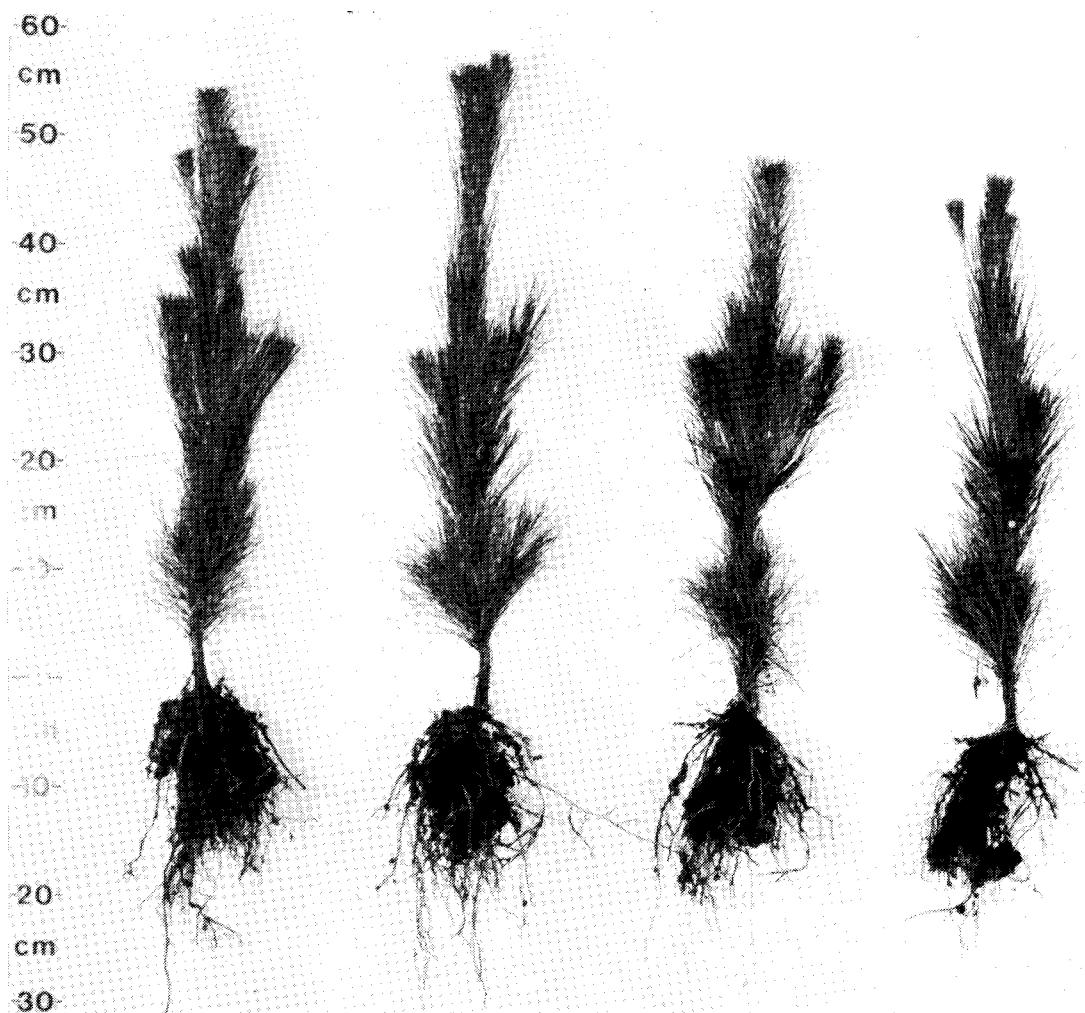


TABLE 1 - Percentage rooting for stem cuttings set in 1984 at the Forest Research Institute Nursery, Rotorua

Cutting type	No. cuttings set	% died without rooting	% died rootrot	% died other causes	% alive	% plantable*
Open bed						
1st order	23000	0.4	5	1.5	93	88
2nd order	11000	0.3	5	0	95	80
Containers						
open environment	8000	0.6	4	0	95	90
Open bed						
3-year-old	30000	8.0	6	4	82	75

* Less than those alive owing to cuttings rejected for poor root form or no single dominant leader.

The cost of cuttings, using topped stools, open-bed setting, an 8x multiplication and 85% rooting is \$77/1000 (stool bed cost \$7.45, collecting and setting at 3000 per man-day \$19.20, and \$50 for subsequent raising). The cost of cuttings set in containers outdoors, an 8x multiplication and 90% rooting is \$90/1000 (stool bed cost \$6.30 and \$84 for the cost of raising the cuttings).

A small field trial of 50 cuttings set in roottrainers in June 1983 was planted in the Long Mile area of FRI in February 1984. There was initially 100% survival, although 4 cuttings were browsed by rabbits. Cuttings were between 40 and 70 mm tall when set, 170 mm tall when planted in February, 280 mm tall in August of the first year, and 740 mm tall in April 1985 indicating that there have been no establishment problems. Planting on a production basis started last summer (February 1985).

(b) Seedling pinning down

There is a range of options available to raise elongated fascicle shoots suitable for open bed or container setting. The two most promising options are:

- (i) Open-bed cuttings: Seed is sown in September. Stock plants are grown for 14 months, when they are about 1 metre tall. They are then topped to about 750 mm to remove soft top growth, and pinned down to the ground.

Inter-fascicular buds in the fascicles elongate into fascicle shoots. These are thinned to 30 mm apart along the seedling stem after 4-6 weeks (Plate 3). By May, 20 months after sowing, the seedlings have produced shoots 100-150 mm in length (Diagram 3, Plate 4). These shoots are collected and set in outside nursery beds.

Plate 3. Fascicle shoots after thinning (scale approx. actual size)

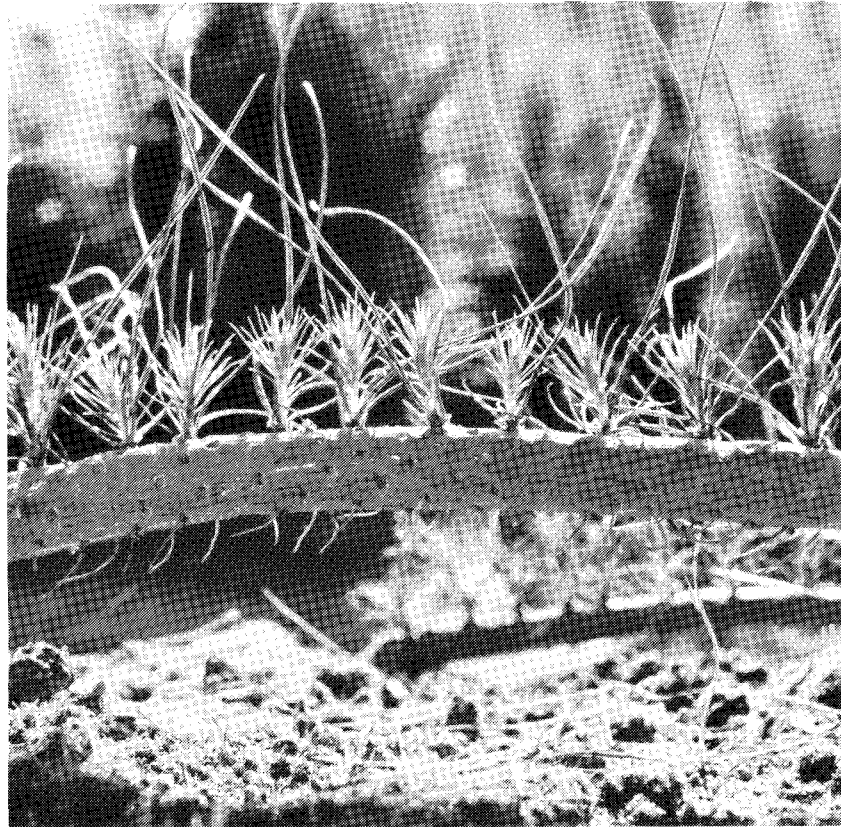


Diagram 3. Production of large cuttings from pinning down method.

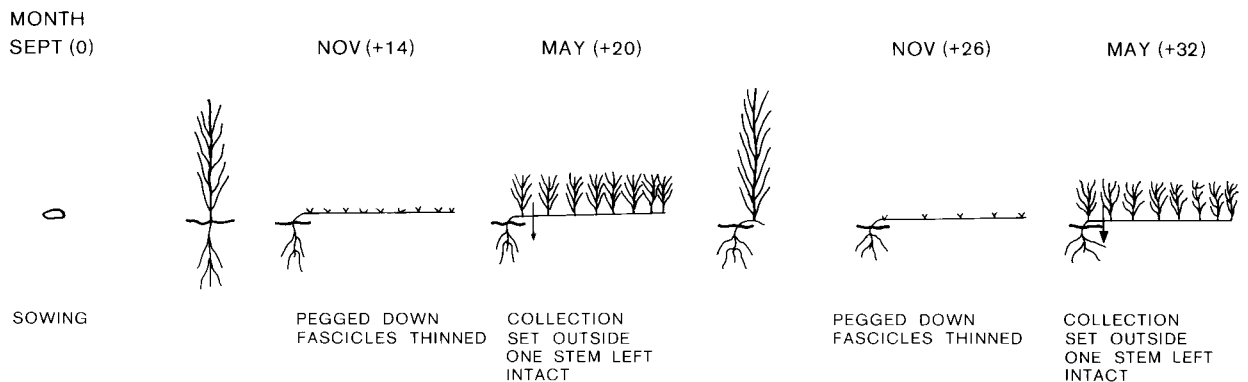
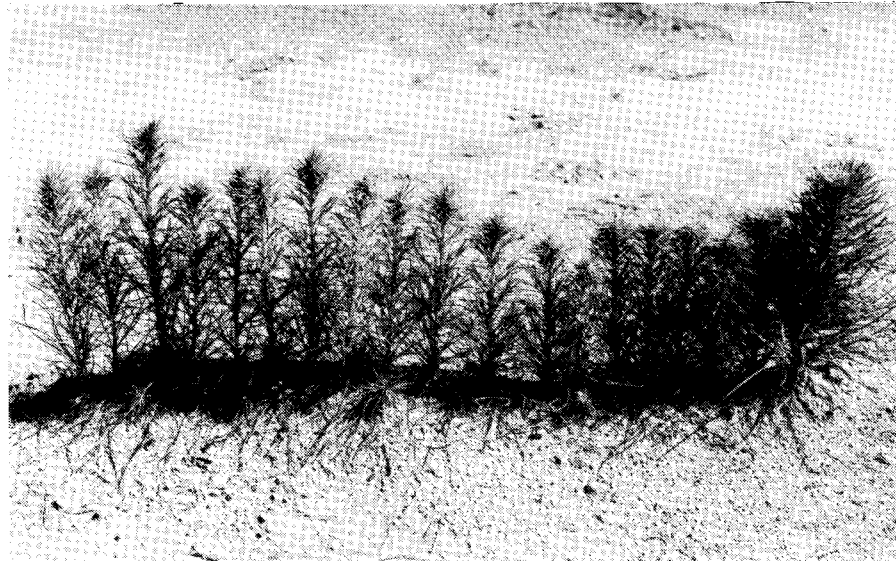


Plate 4. Elongated fascicle shoots on stool plant, ready for setting as large cuttings (scale approx 1cm = 5cm)



The multiplication rate is 30-40x if only first-order cuttings are collected, or up to 80x if second-order cuttings are collected as well. Cuttings from this method were set for the first time on a large scale in June 1985.

The cost of cuttings set in open beds, an 80x multiplication and 85% rooting is \$70/1000 (stool bed cost \$0.75, collecting and setting \$19.20 and \$50 for subsequent raising

One fascicle shoot can be left on the root system (Diagram 3) and the rest of the stem cut off. This shoot then develops into a new stem, ready for pinning down the following November, to repeat the cycle. It is envisaged that stock beds might be maintained indefinitely in this manner, although this has yet to be evaluated. Ageing of cutting material may be a problem, although there is no evidence for this yet in the second season.

- (ii) Container cuttings: Seed is sown in September. In March, six months later, when seedlings are 100-200 mm tall, they are topped and pinned down (Diagram 4). The developing fascicle shoots are left unthinned and are collected in June, 9 months from sowing, and set in rootrainers. The rootrainers can be kept outdoors, producing cuttings ready for planting in February-March (Plate 5), or they can be put in a polythene tunnel, producing cuttings ready for planting earlier, by

November-December. The average multiplication rate has been 17x (1984 setting). Rooting success was 99.3% for outside containers, and 98.6% for containers kept in a polythene tunnel.

Diagram 4. Production of small cuttings from pinning down method.

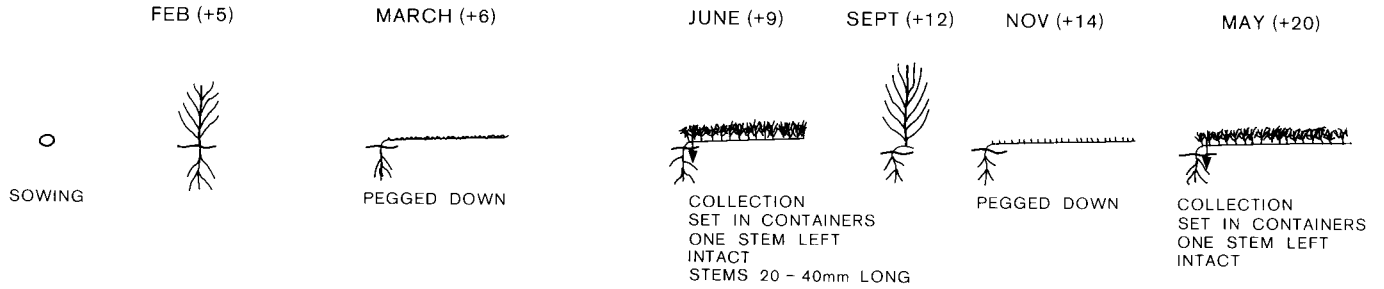


Plate 5. Container cuttings ready for field planting (potting mix washed off)



Similar size cuttings have been planted in a small trial in the Long Mile area at FRI. The cuttings were less than 40 mm long when set in June 1983, and were 140 mm tall when planted in February 1984. By August they were 230 mm tall, and in April 1984 750 mm. They had, therefore, caught up with the initially taller cuttings from the topped method discussed earlier.

As with the stock beds for open-bed setting, the fascicle shoot closest to the root system can be kept to develop into a new stem for pinning down the following November to produce cuttings for open-bed setting. This second year crop should give a multiplication rate of about 100x because of a longer pinned-down stem, although cuttings have not yet been set using this method.

The multiplication rate can be increased by sowing the seed earlier to increase plant size. Seed can be sown in rootainers in July in a glasshouse. When outside conditions warm up in spring (September) seedlings can be lined out in the nursery bed at the desired spacing. An increase from 17x to 25x multiplication in the first year should be possible from this earlier sowing, but at an increased cost.

The cost of cuttings set in containers outdoors, a 17x multiplication and 90% rooting is \$87/1000 (stool bed cost \$3.30 and \$84 for the cost of raising the cuttings). If containers are kept in a polythene tunnel for earlier rooting, then the cost would increase to \$112/1000. If seed was sown early in containers in a glasshouse and lined out, increasing the multiplication rate to 25x, then the cost would be \$89/1000 outdoors, and \$114/1000 in a polythene tunnel.

5. MICROPROPAGATION

Micropropagation methods have been developed for a variety of explants, including embryos, and cotyledon and seedling shoot tips (Reilly and Washer, 1977; Aitken *et al.*, 1981; Horgan and Aitken, 1981; Aitken-Christie and Thorpe 1984; Smith 1985). There are many steps involved, including shoot initiation, shoot elongation, shoot multiplication, and rooting.

Shoot Initiation: Excised embryos, cotyledons from 5- to 7-day-old germinated seeds, or induced fascicle shoots from 9-month-old seedlings can be used as explants. After sterilisation in a 50/50 bleach solution all explants are cultured on a LePoivre (LP) medium containing 5 mg/litre benzylaminopurine (BAP), 3% sucrose, and 0.8% Difco Bacto agar for 3 weeks.

Shoot Elongation: After BAP or cytokinin treatment, explants are cultured on an LP medium exactly the same as the initiation medium but without BAP. Several transfers (2 to 6) are necessary to get fully elongated shoots of about 15 to 20 mm. Usually clones with large numbers of shoots (200 or more) require at least 6 transfers. Each time clumps of shoots are transferred they are cut into smaller pieces and the newly-cut surface placed in contact with the medium. Adventitious shoots, which would not have formed in nature, develop on embryos and cotyledons, while axillary shoots form from already existing meristems on fascicle explants.

Shoot Multiplication: Small shoots can be multiplied in culture to build up numbers per clone by topping shoots and allowing new side shoots to grow out or by placing shoots on to the LP medium with 5 mg/litre BAP. The latter method is used when shoots older than 18 months from seed produce secondary needles.

Cold Storage of Shoots: Shoots can also be cold-stored in culture at any stage of elongation, thereby arresting growth. Shoots resume growth when placed back in the controlled environment chamber under normal culture conditions. So far, shoots have been kept successfully in cold storage for up to 5.5 years. Plantlets have been produced from shoots cold-stored for 17 months.

Rooting: For root initiation, shoots are given a 5-day auxin treatment in water agar, plus 1.0 mg/litre IBA, and 0.5 mg/litre NAA. After this they are washed and planted in a non-sterile peat/perlite/pumice (50%/25%/25%) mix in trays. Trays of shoots are kept in a high humidity chamber which is misted and aerated daily until roots are formed in 2 to 8 weeks. Some clones root much better than others. The plantlets are transferred to a plastic tent in the glasshouse, hardened off, and then lined out in nursery beds under shade cloth. The shade cloth is later removed and plantlets then receive the standard forest nursery treatments given to seedlings. From auxin treatment of shoots to planting out of micropropagated stock takes 12 months.

Environmental Conditions for Plantlet Formation: A controlled environment cabinet is necessary for the sterile shoot formation stages. A 16-hour light/8-hour dark photoperiod is maintained throughout all stages of culture development; the "day" temperature varies between 20 and 28°C, with higher temperatures favoured for shoot initiation and cooler ones for shoot elongation. Night temperatures are 5°C lower. Cultures are grown under "Cool-white" fluorescent lights at an average light intensity of 80 $\mu\text{E m}^{-2}\text{s}^{-1}$. Root formation is best under the same photoperiod and light intensity as that for shoot formation but at 25/20°C (day/night).

Cost of Micropropagated Shoots: The cost of producing micropropagated planting stock ranged from 30 cents to \$1 per plant, depending on process efficiency (Smith, 1985). Rooting success is an important factor in the final cost. The cost of planting stock rises sharply if shoots fail to form roots. For example, if shoots are produced in vitro at a rate of

50/hr and only 50% of them form roots then the cost is \$770/1000. Over 80% of the cost of micropropagated planting stock can be attributed to labour costs and 25% is labour during the in vitro shoot production stages. Approximately \$450/1000 was seen to be a reasonable cost for a commercial operation.

The multiplication rate from micropropagation is dependent on the time in vitro, and 1000 x multiplication rate would be feasible for 22 months from seed to plantlets at the nursery gate. Higher multiplication rates would be possible with a longer time with in vitro stages of remultiplication.

The ability to hold micropropagated shoots in cold storage (Aitken-Christie 1984) may allow a programme of field clonal testing while ramets are kept juvenile as micropropagated shoots in the cold store, rather than in hedged archives, as previously described (Thulin and Faulds 1968; Libby et al. 1972; Libby 1983).

In an experiment started in 1983, seed of two hundred clones of the best 19 FRI genetically improved families plus one control seedlot were sown in October. After undercutting and wrenching seedlings were potted up into 4 litre containers in May and kept in a glasshouse. In June, seedlings were topped to stimulate fascicle shoot development and after eight to twelve weeks, fascicle cuttings were collected for use as explants for micropropagation and as small cuttings for rooting. Some ramets will be planted in a clonal field trial, while the rest are cold-stored. After five years, the best clones can be identified from the field trial, and the clones vegetatively multiplied by micropropagation from the juvenile shoots stored in the cold store.

DISCUSSION

There are two main reasons for the upsurge in interest in vegetative propagation of radiata pine in New Zealand. Firstly, the tree breeding programme at the Forest Research Institute is continually producing control-pollinated seed in limited quantities from the best available parents. Progeny from these seedlots are measurably superior to those from open-pollinated seed orchards. Vegetative propagation would extend the use of this scarce seed, thereby permitting greater areas of forest to be planted with the best genetic stock. Secondly, the improved form of cuttings from older ortets has become apparent from field trials (Wilcox et al. 1976; Gleed 1983; N.Z. Forest Service 1984b; West 1984) and there is a demand for cuttings from older ortets (Arnold and Gleed 1985). As a consequence of the improved genetic quality of the seed stock, and the improved form associated with age, cuttings can be planted confidently at comparatively wide spacing. Although the cost per plant is higher than for seedlings, the cost per hectare appears very competitive.

There is a wide variation in multiplication rate, time taken to produce cuttings, and cost of the various forms of vegetative propagation (Table 2). The method used will depend on the relative importance of these three factors.

One of the easiest methods for multiplication of seedlings is the top pruning method using nursery stool beds. Cuttings can be set bare-root or in containers, and the time in the nursery is less than two years, although the multiplication rate is low. A higher multiplication rate can be obtained from stool beds using the pinning-down method, although the cuttings must be set in containers unless the time in the nursery is extended to three growing seasons. However, the multiplication rate is more than doubled with an extra year in the nursery and the stool beds can be used for more than one crop of cuttings.

Containerised planting stock has not been widely used in New Zealand with radiata pine because of the successful development of bare-root technology and also because of root distortion problems with containerised seedlings. However, root development of cuttings in Spencer Lemaire roottrainers has been promising (Plate 5); field trials have been established to verify if there are any long-term root development problems with containerised cuttings. Containerised stock needs less nursery space and has a shorter nursery production time than bare-root stock. The ability to plant containerised stock in late summer and autumn may also be an advantage.

TABLE 2 - Summary of approximate costs for various propagation options

Method	Multipli- cation rate	Bare-root or containers	Set outside or polythene tunnel	Minimum time in nursery (months)		Cost (NZ\$/ 1000)	
				Stool bed	Rooting	Total	
Seedlings	1	B	O	-	-	10	50
Field	3-5	B	O	-	12	12	160
Topped Stools	8	B	O	9	12	21	77
		C	O	9	8	17	90
Pinned down stools +	17	C	O	10	8	18	87
			P	10	5	15	112
Sown early	25	C	O	12	8	20	89
			P	12	5	17	114
Pinned down stools	80	B	O	20	12	32	70
Micro- propagation	1000	B	-	-	11	22	450

Field cuttings are more expensive than stool bed cuttings (Table 2) because of travel involved in their collection, but this has not prohibited their use. They have been widely established in recent years, particularly on agroforestry sites where they have been planted at lower than normal stockings because of their expected better form (Arnold and Gleed 1985). This has resulted in less interference with animal grazing. These advantages appear to outweigh potentially lower diameter growth rates, although the long-term significance of this has yet to be determined. Further trials have been established with cuttings from 1- to 5-year-old ortets to determine what the optimum ortet age is to gain worthwhile improvement in form without sacrificing growth rate.

Early trials using cuttings were established from trees of unimproved seed origin. Second-generation seed orchard seedlings have shown superior growth and form compared with unimproved controls and first generation seed orchard seedlings. Trials with control-pollinated seedlings have shown significant improvement in growth rate and form as well as other characteristics, compared with controls or first generation seed orchard seedlings (New Zealand Forest Service 1984c). Therefore, there may be no extra benefit from the ageing effect of older ortet cuttings.

There may be an added benefit in combining both the effects of control-pollinated seed of progeny tested families and the effect of ageing by multiplying cuttings from seedling ortets of differing maturation stage. This option is being evaluated in field trials established in 1984 on 10 sites. If the results from these field trials show that an increased ortet age produces desirable growth and form characteristics, this could be done by lining out cuttings rather than seedlings for the stool beds. Serial propagation could be used to manipulate the age required, and the method would be cheaper than field collection, as well as increasing the multiplication rate with each cycle. However, this would increase time in the nursery.

Experiments with field-collected cuttings and nursery stool cuttings discussed so far would make use of control-pollinated seed of genetically improved families. The original idea of using rooted cuttings for plantation forestry was to use clones that had been field tested (Fielding, 1964, 1970; Thulin and Faulds, 1968; Libby *et al.*, 1972; Burdon 1982; Libby 1983), but there were found to be several problems including that of maintaining hedges in a juvenile state. The cold storage option with micropropagation could solve this problem. Micropropagation is very expensive compared with other propagation methods for multiplication of control-pollinated families (Table 2). Either the labour cost of micropropagation would need to be significantly reduced or alternative techniques (e.g., somatic embryogenesis) become available for micropropagation to become competitive. Besides the present high cost of micropropagated plantlets there can be problems developing a balanced root system on plantlets. A method of overcoming both these problems would be to line plantlets out as nursery stools after initial micropropagation from seed and, after pinning down stools, collecting stem cuttings for open-bed or container setting (Smith,

1984). If micropropagated plantlets cost \$450/1000 to produce, this would add only \$5.60/1000 to the resulting cuttings if there was an 80x multiplication rate from the stools.

CONCLUSIONS

With seed of improved genetic quality becoming available from controlled crosses between progeny-tested parents, vegetative propagation is seen as a useful way of extending this scarce seed. Several methods of propagation can be used, but the most promising involve use of nursery stool beds, by topping and pinning down seedlings to produce cutting material that can be set in the open nursery bed or in containers.

If clonal forestry is judged desirable for gaining benefits of further genetic improvement, then use of micropropagation techniques may allow clonal testing in the field while maintaining clones in a juvenile state in cold storage as micropropagated shoots.

A combination of several of these techniques would allow manipulation of high multiplication rates and effective ortet age at a reasonable cost.

Cuttings will be used more widely for planting radiata pine in the future as these new propagation methods are developed and adopted by forest nurseries.

REFERENCES

- Aitken, J., Horgan, K.J., and Thorpe, T.A. 1981. Influence of explant selection on the shoot-forming capacity of juvenile tissue of Pinus radiata. Can. J. For. Res. 11: 112-117.
- Aitken-Christie, J. 1984. Micropropagation of Pinus radiata. The Plant Propagator 30(3): 9-11.
- _____ and Thorpe T.A. 1984. Clonal propagation of Gymnosperms. In Cell culture and Somatic Cell Genetics of Plants. Vol. 1. Ed. by I.K. Vasil. Academic Press, Inc. pp. 82-95.
- Arnold, R., and Gleed, J.A. 1985. Raising and managing radiata pine vegetative cuttings for production forests. In Proc. Joint Conference of Inst. Foresters of Australia and N.Z. Edited by D.J. Mead and R.C. Ellis, Univ. of Canterbury, N.Z.
- Brown, A.G. 1974. Comparison of early growth in radiata pine raised by cuttings from parents of different ages with that of seedling trees. Aust. For. Res. 6(3): 43-47.
- Burdon, R.D. 1982. The roles and optimal place of vegetative propagation in tree breeding strategies. Proc. IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies Including Multiclinal Varieties: 66-83.

- Field, J.F. 1934. Experimental growing of insignis pine from slips. N.Z.J. For. 3(4): 185-186.
- Fielding, J.M. 1964. The possibility of using cuttings for the establishment of commercial plantations of Monterey Pine. Proc. World Consultation For. Gen. and Tree Impt., Stockholm (FAO), Vol II: 5/10, 7pp.
- _____ 1969. Factors affecting the rooting and growth of Pinus radiata cuttings in the open nursery. Aust. For. and Timber Bur. Bull. 45. 38pp.
- _____ 1970. Trees grown from cuttings compared with trees grown from seed (Pinus radiata D. Don). Silvae Genetica 19: 54-63.
- Gleed, J.A. 1983. Tree improvement - first results from a radiata pine production forest. Appita 36(5): 386-390.
- Horgan, K., and Aitken, J. 1981. Reliable plantlet formation from embryos and seedling shoot tips of radiata pine. Physiol. Plant. 53: 170-175.
- Jacobs, M.R. 1939. The vegetative reproduction of forest trees. 1. Experiments with cuttings of P. radiata Don. Aust. Comm. For. Bur. Bulletin 25. 30pp.
- Libby, W.J. 1983. The clonal option. Norsk Institutt for Skogforskning. 32pp.
- _____ Brown, A.G., and Fielding, J.M. 1972. Effects of hedging radiata pine on production, rooting, and early growth of cuttings. N.Z.J. For. Sci. 2(2): 263-283.
- Menzies, M.I., and Chavasse, C.G.R. 1982. Establishment trials on frost-prone sites. N.Z.J. For. 27(1): 33-49.
- New Zealand Forest Service 1974. Report of the Forest Research Institute for 1973. p.22.
- _____ 1984a. Report of the Forest Research Institute for 1983. p.21-22.
- _____ 1984b. Report of the Forest Research Institute for 1983. p.23-25.
- _____ 1984c. Report of the Forest Research Institute for 1983. p.11-14.
- _____ 1985. Report of the Forest Research Institute for 1984. (In press).
- Nicholls, J.W.P., Pederick, L.A., and Brown, A.G. 1977. A summary of the ortet-ramet relationship in wood characteristics of Pinus radiata. Appita 30(6): 496-502.

- Pawsey, C.K. 1971. Comparisons of vegetatively propagated and seedling trees of Pinus radiata. Aust. For. Res. 5(3): 47-57.
- Reilly, K.J., and Washer, J. 1977. Vegetative propagation of radiata pine by tissue culture: Plantlet formation from embryonic tissue. N.Z. J. For. Sci. 7, 199-206.
- Shelbourne, C.J.A., and Thulin, I.J. 1974. Early results from a clonal selection and testing programme with radiata pine. N.Z.J. For. Sci. 4(2): 387-398.
- Smith, D.R. 1984. Tissue culture for clonal forestry: present and future technologies. Proc. 16th New Zealand Biotechnology Conf: 9-20.
- _____ 1985. Micropropagation of forest trees: Pinus radiata in New Zealand as a model system. In Micropropagation of Fruit and Forest Trees. Ed. by Y.P.S. Bajaj. Springer Verlag (in press).
- _____ Aitken, J., and Sweet, G.B. 1981. Vegetative amplification - an aid to optimising the attainment of genetic gains from Pinus radiata? Proceedings of the Symposium on Flowering Physiology XVII IUFRO World Congress, Kyoto, Japan 1981. Working Party S2.01.05 "Reproductive Processes": 117-123.
- Sweet, G.B. 1973. The effects of maturation on the growth and form of vegetative propagules of radiata pine. N.Z.J. For. Sci. 3(2): 191-210.
- _____ and Harris, J.M. 1976. Wood properties of Pinus radiata: Seed grown trees compared with grafts from different-aged ortets. N.Z. J. For. Sci. 6(1): 114-121.
- _____ and Krugman, S.L. 1977. Flowering and seed production problems - and a new concept of seed orchard. Invited special Paper, Third World Consultation on Forest Tree Breeding, Canberra, Australia.
- _____ and Wells, L.G. 1974. Comparison of the growth of vegetative propagules and seedlings of Pinus radiata. N.Z.J. For. Sci. 4(2): 399-409.
- Thulin, I.J., and Faulds, T. 1968. The use of cuttings in the breeding and afforestation of Pinus radiata. N.Z.J. For. 13(1): 66-77.
- Tufuor, K., and Libby, W.J. 1973. First-lift pruning times of radiata pine seedlings and rooted cuttings in a small California experiment. N.Z.J. For. 18(1): 124-132.
- West, G.G. 1984. Establishment requirements of Pinus radiata cuttings and seedlings compared. N.Z.J. For. Sci. 14(1): 41-52.

Wilcox, M.D., Shelbourne, C.J.A., and Firth, A. 1975. General and specific combining ability in eight selected clones of radiata pine. N.Z.J. For. Sci. 5(2): 219-225.

_____ Thulin, I.J., and Vincent, T.G. 1976. Selection of Pinus radiata clones in New Zealand for planting from cuttings. N.Z.J. For 21(2): 239-247.