ROOTING OF CONIFER PROPAGULES

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ABSTRACT.--An outline of the general problems involved with the propagation of elite conifer clones by rooted cuttings is drawn from published reports. New approaches for resolving these problems can come from studies of clone production through tissue culture methods. Probable extension of tissue culture techniques will permit the establishment of clones from adult, proven trees and may also provide a tool for *in vitro* propagule evaluation of disease resistance.

Reforestation by vegetative propagation of select clones offers the possibility of rapid improvement of forest yield (Shelbourne and Thulin 1974, Kleinschmit 1974). Clonal reforestation will require a fresh consideration of forest management and the implied biological risks, but the potential for utilization of non-additive genetic variance in trees and the benefit where seed production is slow or inadequate are both strong motives for operational vegetative propagation. Two methods of vegetative propagation offer promise: (1) rooted cuttings or needle fascicles, and (2) rooted propagules produced through tissue culture. The potentials and problems for these two approaches are compared in this paper.

Rooted Cuttings

A comprehensive review of the various reforestation programs using clonal rooted cuttings is presented by Brix and van den Driessche (1977). Clone production by rooted needle fascicles of pine was reviewed by Girouard (1971). General review of rooting practice for horticulturally important species is treated in the book by Hartmann and Kester (1975). Clonal propagation of *Cryptomeria japonica* has been used for centuries in the forests in Japan and provides the only data concerning long-term benefits and problems. Major programs for propagation of Norway spruce with rooted cuttings for operational planting are underway in West Germany (Kleinschmit 1974) and Finland

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(Lepistö 1974). A major research effort with radiata pine is continuing in New Zealand and Australia (Sweet and Wells 1974, Shelbourne and Thulin 1974) but operational plantings are not yet a reality. Research programs for Douglas-fir (Ross 1975), loblolly pine (van Buijtenen et al. 1975) and western hemlock (Boyd 1976) are being conducted in the United States and for white spruce and black spruce in Canada (Rauter 1974).

The above programs are new in terms of forest tree harvest cycles and most of the information deals with rooting behavior and the early field performance of the propagules. Long-term field behavior will be forthcoming. Production of rooted cuttings is difficult or impossible for many species, but where problems can be overcome rapid production of select clones (1/2 million plants from one seedling in 5 years) seems possible. Fully operational cloned planting stock production could be implemented in 10 years given 4-year field evaluation. At that stage in program development the cost of propagules has been estimated at 2 to 3 times higher than for seedlings (Brix and van den Driessche 1977). Time and cost values must be weighed against tree improvement gains through cloning. Such gains can be great.

The problems associated with the production of rooted cuttings vary greatly with species and even individual tree within species. Generally, cuttings taken from young trees in the juvenile stage root more efficiently than cuttings from older trees in the adult stage, e.g., Douglas-fir (Ross 1975), radiata pine (Libby et al. 1972), and white spruce (Rauter 1974). For some species this is not so pronounced (western hemlock,Brix and Barker 1975) and for others even the juvenile stage shows low rooting efficiency (lodgepole pine, Longman et al. 1972) or poor root form (white pine, Thomas and Riker 1950).

The rooted cuttings may grow in the field at rates comparable to seedlings (Norway spruce, Lepistö 1974), or they may achieve these rates after a lag period (western hemlock, Brix and Barker 1975), or continue to grow more slowly (radiata pine, Sweet and Wells 1974). In addition to drastic differences in growth rate, rooted cuttings may assume an irregular growth form (plagiotropism) with age of parent tree or age and position of the shoot on the parent tree, e.g., Douglas-fir (Ross 1975, Copes 1976). On the other hand, rooted cuttings from adult trees may possess some desirable adult tree characteristics such as disease resistance (Thuja plicata, Søegaard 1956).

Overall, propagation by rooted cuttings varies greatly in the rooting efficiency and growth of cuttings from species to species and from tree to tree. Furthermore, the performance of cuttings may be affected profoundly by tree age, position of the cutting on the tree, tree vigor, and the season cuttings were taken. Many of these problems can be overcome in many species by refining methodology, but the solution of the general problems summarized in figure 1 remain.

The major dilemma with forest improvement through vegetative propagation by rooted cuttings is that cuttings propagate better from juvenile trees, but selection of elite mother trees must come after they have reached the adult stage; by then propagation by cuttings becomes a severe problem (fig 1). A practice of repeated shearing of juvenile trees, "hedging", has aided in retaining the juvenile character of cuttings over a longer period (Libby and Hood 1976). Repeated cycles using rooted cuttings as the source of new cuttings, "re-rooting", has also helped to maintain juvenility (Fielding 1969). These practices may be adaptable to a variety of species but, failing that, there remains the possibility of devising more reliable selection procedures for juvenile trees or of using methods that can generate cuttings of juvenile character from adult trees (fig. 1).

Tissue Culture Propagules

The recently emerging application of tissue culture techniques to conifers provides an alternative to rooted cuttings for clonal forest propagation. Present tissue culture techniques can provide rooted propagules of forest species from multiple adventitious buds formed on embryos or on embryo and seedling parts cultured *in vitro* (Mott *et al.* 1977 and references there cited). The present tissue culture capabilities are similar to those of the rooted cutting methods (fig. 1) in that rooted propagules may be produced in a short time from juvenile seedling material. Reliable and routine rooting of the shoots produced in culture, on the scale needed in operational planting, is difficult to achieve. Cost per tissue culture propagule is high and the propagules are just beginning to be field tested and are not ready for operational planting.

The only published data on propagule production from improved tree genotypes (Mott *et al.* 1977) shows that multiple adventitious shoots can be obtained from nearly all seedling progeny of select loblolly pine trees from piedmont or coastal plain sites. An average of 20 shoots per clone can be expected on the first cycle, but methods to root a high percentage of these shoots reliably and on a production scale are lacking. As much as 70 percent rooting of these shoots has been achieved in some experiments, but production of 3-10-rooted individuals per clone is more common. Work is continuing in the laboratory (Mehra-Palta *et al.* 1977) and elsewhere to improve the rooting efficiency.

The early growth and form in soil of such propagules compare favorably with those of seedlings, and clones of few individuals can be tested simultaneously on several sites, thus multiplying the information to be gained on genotype/environment interactions during the field tests. In this way the clones can aid efforts to develop reliable methods for selecting elite trees while they are still juveniles.

Figure 1.--Generalized outline of problem areas in the propagation of elite clones of conifers by means of rooted cuttings.

Possible improvements	Source of cuttings	Rooting	Propagule behavior in field
Increase selection efficiency for young trees	Juvenile Trees (poor clone selection at juvenile stage)	+ good efficiency - poor root form - variable with tree - season dependent	+ favorable comparison with seedling
Prolong juvenile phase (hedging; re-rooting)	↓		
Reversion from adult to juvenile phase	↓		
Devise better means of rooting adult tree cuttings	Adult Trees (better clone selection at adult stage)	- poor efficiency: age/size of tree, position on tree - poor root form - inconsistent rooting - season dependent	+ adult wood qualities + disease resistance - loss of juvenile growth - plagiotropism - abnormal growth

Obviously, the tissue culture methods are at a much earlier stage of development than rooted cuttings. But all indications are that the problems in rooting the adventitious buds from tissue culture will be resolved. Furthermore, there are similarities between the two technologies that might help to resolve some of the problems encountered with cuttings. Cuttings must be studied intact because they rely on photosynthesis and shoot growth to support root initiation. Consequently, subtle influence of the mother tree on the shoot growth behavior of cuttings is inherent in the rooting process. Tissue culture procedures offer a more tractable system for the study of the controlling factors in rooting. Carbon source, nutrients, and growth regulators are supplied in the culture medium and thus buds, shoots, stems, etc. can be excised and cultured separately to discover the influence of each on the rooting process. For example, growth regulators normally produced within the plant can be supplied exogenously in an effort to mimic the contribution made by the young shoot tip that has been removed. Finally, the more extensive information on growth behavior of rooted cuttings may point the way for tissue culture studies.

Some preliminary data from studies of basic rooting factors are summarized in table 1. Rooting efficiency in response to the growth regulators NAA (α-napthaleneacetic acid) and BAP (6-benzylaminopurine) is compared for seedlings lacking shoot and root -- "hypocotyl sections"; seedlings lacking roots -- "derooted seedlings"; the terminal growing bud of seedlings -- "excised seedling shoots", and for similar "adventitious buds" produced in culture. The rooting efficiency is affected by other factors including the age of the seedling and the culture environment, etc. These effects are reflected in the range of percentage rooting given for each growth regulator concentration in table 1.

Table 1.--Summary of *in vitro* rooting efficiency to be expected for different seedling parts and tissue culture buds in response to growth regulator stimuli

Growth regulator concentration (mg/l) supplied in agar medium		Percent rooting			
NAA	BAP	Excised hypocotyl sections	Derooted seedlings	Excised seedling shoots	Adventitious tissue culture buds
0	0	0-6	0-37	0	0
0.1	0	0-27	0-12	--	20-30
0.1	0.1	0-27	80-90	70-80	40-70
0	0.1	0	0-12	0	0

Experiments of this type suggest that different exogenous growth regulator supplies are needed depending on which parts have been excised. Further experiments should define corrective measures to be taken when certain parts of seedlings or shoots are absent or functioning improperly. This information should lead to more efficient methods for the rooting of cuttings from trees. For example, the poor rooting of cuttings taken from adult trees might be corrected by suitable treatments to improve the vigor of the shoot bud on such cuttings.

Buds of loblolly pine produced by tissue culture are adventitious and possess juvenile character. The adventitious buds may be obtained from seedling hypocotyl and stem sections and the adventitious buds themselves are capable of successive rounds of new adventitious bud formation. The juvenile character of adventitious buds so produced holds great promise for vegetative propagation of adult trees. Procedures are now being tested for adventitious bud production from cultured parts of older trees. These buds develop on parts cultured after removal from influence of the adult mother tree, so they can be expected to revert to juvenile character. Once established, the adventitious buds could be used in successive rounds of propagule generations in the short time and small space characteristic of tissue culture propagation of herbaceous plants (Murashige 1974). This would, of course, permit vegetative propagation of elite trees selected in the adult stage (fig.1).

Adventitious buds can be obtained from continuously subcultured, disorganized callus cultures of aspen but there is evidence that clones produced in this way may not be uniform (Lester and Burbee 1977) and this might argue for bud production from excised tree parts rather than from subcultured callus.

Propagule Evaluation in vitro

It seems certain that the methods for rapid vegetative propagation of conifers on a production scale will soon be at hand. The capacity of rapid propagation brings with it the need for early and efficient testing for such things as disease and pest resistance. An elite adult tree may have merely escaped certain diseases in its present site; the propagules may present a substantial hazard for other sites. Early selection for resistance in the laboratory on cultured tissues would permit tests for disease resistance to keep pace with potential propagule production. Tissue culture may also provide a more tractable system for study of mechanisms of disease resistance, similar to the approach outlined earlier for rooting.

We have succeeded in rearing southern pine beetle from eggs on a loblolly pine callus substrate (Mott and Thomas 1977). This system can now be used to study pest/host interactions and host resistance. Fusiform rust resistance of young loblolly and slash pine seedlings and

excised seedling parts can be identified in culture and the rust may also be cultured axenically (Amerson and Mott, in press). This opens the way for pathogen/host interactions and disease resistance mechanisms to be studied on this and other important diseases under controlled in vitro conditions.

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