# MASS VEGETATIVE PROPAGATION OF LOBLOLLY PINE -- A REEVALUATION OF DIRECTION

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Abstract.--The problems that will be encountered in producing plantable seedlings from vegetative cultures promise to be significant. At best, systems to handle this material from tissue cultures to rooting medium to seedling nursery (or greenhouse) will be expensive.

Rooted cuttings offer an alternative method of vegetative propagation for which *we* envision a system of production that would require little new technology. "Cutting orchards" also compare favorably with seed orchards on a propagule per acre basis, and may be less costly to operate.

The promise of a system for economically producing operational numbers of vegetative propagules justifies a more intensive effort in trying to develop a reliable technique for rooting loblolly pine cuttings.

## INTRODUCTION

The potential for increased genetic gains through the use of vegetative propagation has been demonstrated for many forest tree species. Bypassing sexual recombination allows both general and specific combining ability to be "captured" in production plantings. For loblolly pine (*Pinus taeda* L.), in particular, an additional 5% to 10% gain in growth could probably be made if mass production of superior genotypes via vegetative propagation was possible.

Historically, most emphasis in vegetative propagation has been placed on rooted cuttings. To date, there has been very limited success with rooting southern pine cuttings (Hare 1974, van Buijtenen et *al.* 1975, Greenwood *et al.* 1980). Only juvenile material (usually less than 4-6 years old) has been rooted with a reasonably high degree of success. Research emphasis has shifted to tissue culture because some researchers feel this may avoid problems of decreased rooting and growth potential related to vegetative propagation of more mature tissues in meristems. There is little doubt that tissue culture of loblolly pine will soon be possible. However, providing the large numbers of plants needed for forest regeneration will require more than the ability to do vegetative propagation. Efficient systems for producing and growing vegetative propagules must also be developed. Considerable time and effort will probably be required to research and develop systems that will produce large numbers of tissue culture plantlets economically. It may be possible to develop a system to produce large numbers of rooted loblolly pine cuttings much sooner. If so, it should be implemented to provide vegetative propagules for forest regeneration until economical tissue culture systems can be developed.

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## STATUS OF TISSUE CULTURE RESEARCH

Tissue culture methodology for production of loblolly pine planting stock is still in its infancy. Research to achieve mass propagation of loblolly pine has been in progress for several years, but operational use of tissue culture is still in the future. Three basic methods of multiplication in vitro have been proposed for forest trees and some horticultural crops (Murashige 1977, Minocha 1980):

1. Somatic cell embryogenesis -Somatic embryos are organized structures which are essentially identical to embryos from zygotes. They will germinate in culture and develop into seedling-sized plants.

2. Adventitious shoots and buds -Organogenesis of shoots and buds can develop from excised plant parts or from callus cultures. When shoots form, they can then be rooted.

3. Enhancing axillary and apical buds to produce shoots. Miniature branches are excised from plants in vitro and are later rooted. This is often called micropropagation.

There are certain advantages to each of the three methods. Embryogenesis and organogenesis have been proposed as the most likely methods to use for mass production of trees. Virtually unlimited numbers of trees could he propagated in a very small area in a short period of time. For example, with embryogenesis in cultures of carrots, yields of 500 embryos per gram of callus per month have been achieved (Murashige 1977).

Micropropagation (best described as miniature rooted cuttings) has the broadest applicability among most plant genera. Virtually all commercial applications of tissue culture technology in horticultural crop species employ micropropagation (Boxus and Druart 1980, Murashige 1978). The greatest success has been commercial propagation of orchids through apical and axillary shoot cultures (Murashige 1974). Some researchers (Thompson and Gordon 1977, Minocha 1980, Banga 1974) have proposed microprogation as a technique for large-scale production of forest trees.

The basic problem with micropropagation methods for reforestation is their inefficiency. Micropropagation is very labor intensive, requiring a great deal of handling at each stage of plantlet development. Only species which have a relatively high per plant value such as orchids, ferns, and fruit trees have potential for use in this method. For most forest species, the initial value of an individual seedling is a few cents.

Many people who are not familiar with tissue culture techniques suggest using horticultural tissue culture methods as models for plantlet production systems of forest trees. Unfortunately, forest regeneration requires that millions of trees, not just a few thousand, be produced each year. Micropropagation currently appears to be feasible only on a relatively small scale to mass produce vegetative propagules of forest trees.

Callus and cell suspension cultures which utilize embryogenesis or organogenesis as a propagation method are the most feasible tissue culture methods for forest trees. Growing trees from single cells or small groups of cells allows for very large-scale production. The critical limitation of this technology will be the development of radically different engineering systems for producing propagules so that producing plantlets in very large numbers will not be extremely labor-intensive. Innovative laboratory, greenhouse, and nursery procedures must be developed before tissue culture propagation of large numbers of trees becomes a reality.

## STATUS OF ROOTED CUTTING RESEARCH

Experimental techniques for rooting southern pine cuttings with rigorous environmental control (Hare 1974) have been adapted to root relatively large numbers of loblolly pine stem cuttings with considerable consistency. Cuttings from 1-year-old loblolly and slash pine (*Pinus elliotti* var. elliotti) have consistently been rooted with about 50% success (van Buijtenen *et al.* 1975). Furthermore, rigorous control of the rooting environment promises to improve the success of rooting larger numbers of cuttings. The amount of mist applied was a critical factor in rooting cuttings from 4-year-old loblolly pine in one experiment where the best treatment had 64% rooted cuttings (Greenwood *et* al. 1980).

Unfortunately, the rooting success and subsequent growth rate of loblolly pines declines with increasing age of ortet (McAlpine and Jackson 1959, and Greenwood 1981). This is an important problem since the optimum genetic selection age for loblolly pine seems to be greater than ages at which loblolly pine cuttings can be rooted with consistency. For example, the optimum genetic selection ages for rotation ages of 30 and 40 years was 6 and 8 years, respectively (Lambeth 1980). If selection is at an age when rooting success and growth have declined, the benefits from vegetative propagation may be lost. Fortunately, work with other species of pines has shown that the decline in rootability and growth of propagules can be arrested, i.e. juvenility can be maintained. Hedging *Pinus radiata* D. Don. slows the maturation effects on rootability (Libby *et* al. 1972). Micropropagation of brachyblasts (short shoots of needle fascicles) that are just beginning to elongate "rejuvenates" *Pinus pinaster* Ait. (Franclet 1979). The most important priority for rooted cutting research with loblolly pine is to test these techniques to determine whether they will arrest maturation or "rejuvenate" mature genotypes.

Genetic variation in rooting ability of loblolly pine must be considered in developing a vegetative propagation system. Rooting percentages of clones from 4-year-old loblolly pines ranged from 0 to 100 percent (Foster 1978). Selection for rooting ability could be combined with selection for growth to choose clones which are superior for both traits.

Rooting loblolly pine is possible now. If systems are developed to "rejuvenate" or slow the maturation of older material, and if efficient systems for producing large numbers of propagules are developed, the use of rooted cuttings may be a practical method of operational vegetative propagation.

Producing rooted cuttings for field planting can be broken down into two phases: 1) producing cuttings, and 2) rooting and growing cuttings to a plantable size. We conceived a model for a cutting orchard to see if large numbers of cuttings could be produced on a reasonable land area and compared our model to a mature seed orchard to get an idea of comparative efficiency.

Operational procedures for rooting and growing Norway Spruce (Picea abies (L.) Karst.) cuttings were used as a model for the second phase.

A Model for a Loblolly Pine Cutting Orchard

Dimensions of hedges and numbers of cuttings per hedge were determined from six ornamental hedges of loblolly pine growing in a yard in Hot Springs, Arkansas (Foster and Bridgwater 1978). The trees were 11-years-old and had been pruned annually in June for the previous nine years. This treatment produced hedges that averaged 1.60 meters (m) tall with a somewhat circular and flat top, averaging 1.64 $m^2$ in area (Table 1). In May, 1977, the total number of stem tips was counted on each hedge. Since the optimum size for a loblolly cutting had not been determined, every branch tip at least 2.5 cm long was counted. An average of 653 stem tips were growing on the top surface of each hedge or 413 stem tips per square meter of hedge top (Table 1). The numbers of potential cuttings growing from the sides of the hedges were neglible and therefore, not counted.

Table 1.--Tree dimensions and numbers of stem tips on 6 ornamental hedges of loblolly pine

| Variable | Average of 6 hedges | Minimum | Maximum |
|---|---|---|---|
| Total Height | 1.63 (m) | 1.50 | 1.75 |
| Diameter of Top | 1.42 (m) | 0.93 | 1.73 |
| Surface Area of Top | 1.64 ($m^2$) | 0.68 | 2.35 |
| Number of Stem Tips $\geq$ 2.5 cm on the Top | 653 | 309 | 1253 |
| Number of Stem Tips per $M^2$ | 413 | 259 | 623 |

(After Foster and Bridgwater 1978)

One acre (0.41 ha) section of cutting orchard, illustrated in Figure 1, can be extended to any number of hectares or clones. Our illustration has 18 rows of continuous hedges, each with 63 plants whose tops are about 1 m wide. Thus, there are 1144.8 $m^2$ hedges per acre (0.41 ha) of cutting orchard in our model. The tops of adjacent plants will join to give one continuous surface for each row; and would be maintained at a height to faciliate management. This configuration would seem to lend itself to the development of mechanical and harvesting systems. A distance of 2.5 m (just over 8 ft.) was left between rows of hedges for the passage of equipment.

We assumed that 300 stem tips (75% of the 400 average) would be suitable for rooting. Even this reduced number may be optimistic since labially pine cuttings 10 to 15 cm (4 to 6 in) long are used by van Buijtenen et al. (1975). Only 129 acceptable cuttings were produced per square meter of hedge after hedging P. radiata for 3 years (Libby et al. 1972). However, the number of cuttings suitable for rooting may be increased by cultural treatments such as fertilization and irrigation and if more than one crop of cuttings can be harvested per year.

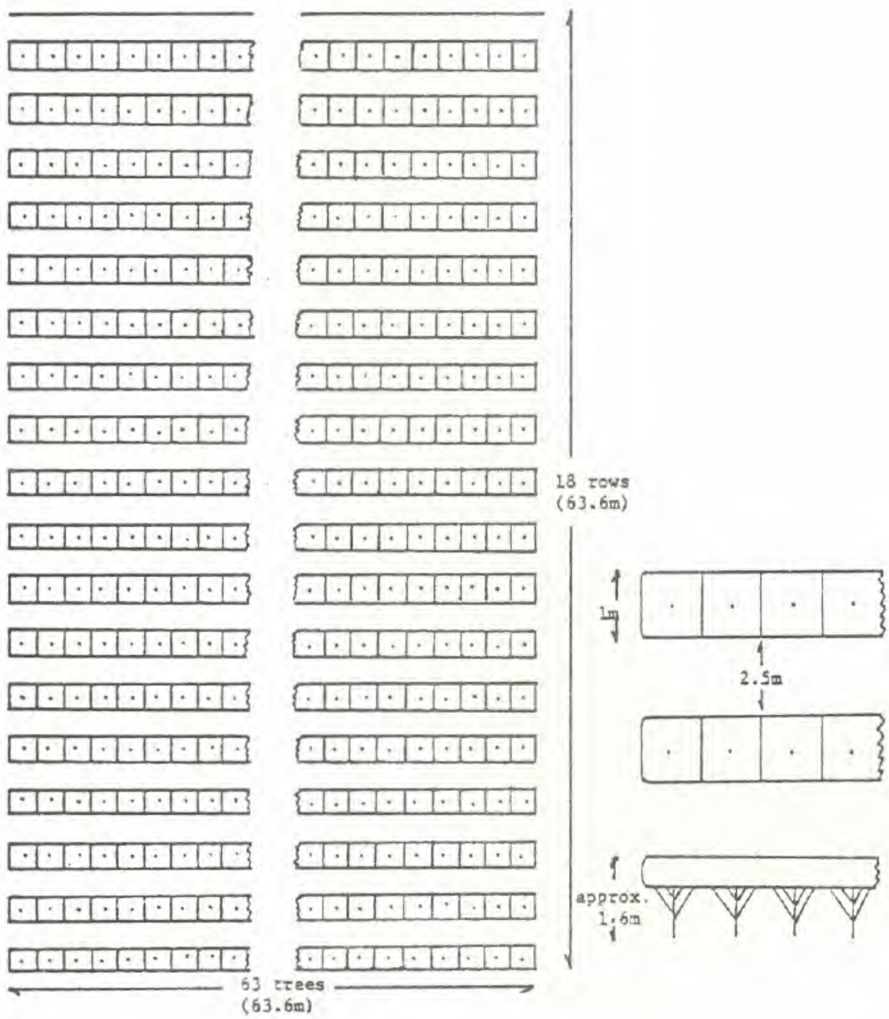


Figure 1.--Diagram of a 1 acre (0.41 ha) section of a cutting orchard to mass produce stem cuttings of labially pine. (after Foster and Bridgwater 1978)

We assumed that at least 64% rooting, the best reported by Greenwood et al. (1980), can be consistently achieved. Experience from other of our rooting trials with loblolly pine has shown that about 85% of cuttings which rooted will survive through their hardening-off period until they are outplanted.

The number of plantable cuttings per acre (0.41 ha) of orchard is the product of:

1) The area of hedges per acre (1144.8 m2);
2) The number of suitable cuttings per square meter of hedge (300);
3) Rooting success (64%);
4) Survival after a hardening-off period (85%).

Over 186,000 propagules per acre of orchard will be produced if our assumptions are met. This is about the same as a loblolly pine seed orchard producing 30 lbs. (13.5 kg.) of seed per acre (0.41 ha) from which 6,200 plantable seedlings are realized per pound (0.45 kg.) of seed.

Yield estimates for both seed orchards and cutting orchards are conservative. But we believe our examples show that cutting orchards can compete favorably with seed orchards on the basis of the number of outplants produced per hectare. Furthermore, cutting orchards may be more economical to operate than seed orchards since they will not require working in lift-buckets as in mature loblolly pine seed orchards, and will better lend themselves to mechanization, should require a shorter time to reach full production, and should have less annual fluctuation in numbers of propagules.

Rooting and Growing Large Numbers of Cuttings

Developing systems to produce large numbers of rooted cuttings for outplanting will mean "scaling-up" the system for producing a few thousand propagules described by van Buijtenen et al. (1975). This system includes:

1) treating cuttings with hormones (usually IBA) to enhance rooting,
2) extending natural daylengths with artifical lighting,
3) controlling ambient air temperatures within 20°-25° C,
4) bottom heating,
5) carbon dioxide enrichment,
6) humidity control (usually by misting),
7) a "hardening-off" period before field planting to reduce planting shock.

Similar systems employing plastic greenhouses for environmental control have been used to produce large numbers of rooted cuttings of Norway Spruce for operational outplanting in Germany for the last decade (Kleinschmit and Schmidt 1977) and in Finland since the mid-1970's (Lepisto 1974). Trials should be initiated to adapt available techniques to systems for producing large numbers of loblolly pine rooted cuttings.

## COST CONSIDERATIONS

Loblolly pine seed orchard and nursery management has been refined to the extent that high-quality seedlings can be reliably produced at a low cost per plant. Systems for vegetative propagation are not well developed and undoubtedly will be more costly than for seedlings. These costs must be minimized so they do not negate the returns from increased genetic gains from vegetatively produced stands of selected genotypes. Estimating the costs for producing large numbers of vegetative propagules will require more specific information about systems than is available at present. Therefore, we estimated the amount that might be spent on vegetative propagation under different sets of assumptions (Table 2)0

Table 2. Present value of genetic gains from vegetative propagation under different set of assumptions.[1/]

| Stumpage Price and Rotation | Planting Density[2/] in trees/acre | 10% Discount Rate | | | 5% Discount Rate | | |
|---|---|---|---|---|---|---|---|
| | | Improved Growth Rate | | | Improved Growth Rate | | |
| | | 10% | 15% | 25% | 10% | 15% | 25% |
| $10/cord @ 25 years[3/] | 681 | 0.5¢ | 0.8¢ | 1.3¢ | 1.6¢ | 2.4¢ | 4.1¢ |
| | 538 | 0.6 | 1.0 | 1.6 | 2.1 | 3.1 | 5.1 |
| | 436 | 0.8 | 1.2 | 2.0 | 2.5 | 3.8 | 6.4 |
| $35/cord @ 30 years[3/] | 681 | 1.3¢ | 2.0¢ | 3.3¢ | 5.4¢ | 8.0¢ | 13.4¢ |
| | 538 | 1.7 | 2.5 | 4.2 | 6.8 | 10.2 | 16.9 |
| | 436 | 2.1 | 3.1 | 5.2 | 8.4 | 12.5 | 20.9 |
| $50/cord @ 30 years[4/] for S.I. 90+ land | 681 | 2.5¢ | 3.8¢ | 6.3¢ | 10.2¢ | 15.3¢ | 25.5¢ |
| | 538 | 3.2 | 4.8 | 8.0 | 12.9 | 19.4 | 32.3 |
| | 436 | 3.9 | 5.9 | 10.0 | 15.9 | 23.9 | 39.8 |

[1/]Value indicates how much additional can be spent for a single plantable propagule to realize the indicated genetic gain and realize a given rate of return on the investment over and above inflation.

[2/]Spacing levels are: 8' x 8' = 681 trees per acre
9' x 9' = 538 trees per acre
10' x 10' = 436 trees per acre

[3/]Assumes unimproved growth rate of 1.5 cords/acre/year

[4/]Assumes unimproved growth rate of 2.0 cords/acre/year (only for best sites of Site Index 90+). (After McKeand 1981).

The per plant present value of genetic gains realized at harvest varied from 0.5¢ to almost 40i per tree with different assumptions. These values apply to vegetative propagules derived from tissue culture or rooting cuttings. Loblolly pine seedlings can be purchased in the southeastern U. S. for about 1.5 ¢ each ($15/M). If costs for mass vegetative propagation are 3 times that for seedlings as for Norway spruce (Kleinschmit and Schmidt 1977) then the present value in Table 2 must exceed 3.0¢ per plant to recoup this additional cost of vegetative propagation. Present values exceeding 3.0¢ require higher stumpage prices to make vegetative propagation profitable, particularly for lower levels of genetic gain and a 10% discount rate. Unless the cost differential between seedlings and vegetative propagules is less than 3.0¢ per tree then vegetative propagules will probably be used only to regenerate high site index lands and for rotation lengths that will produce larger, more valuable trees.

## SUMMARY

Techniques for vegetative propagation of loblolly pine by tissue culture are not known. Once known, innovative techniques for producing the very large numbers required for operational regeneration will take additional time to develop. Techniques for rooting loblolly pine stem cuttings are known, but need refinement and "scaling-up" to produce the numbers required. A model for a loblolly pine cutting orchard was proposed which compared favorably to a mature seed orchard with regard to the number of outplants produced per hectare of orchard.

The development of systems to produce large numbers of rooted cuttings of loblolly pine should receive more emphasis. Rooted cuttings offer a way to realize the benefits from vegetative propagation until tissue culture systems are perfected.

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