Chapter 5

Vegetative Propagation of Southern Pines

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Abstract

Large potential genetic gains have prompted aggressive pursuit of cost-effective cloning methods for southern pines by both micropropagation (tissue culture) and macropropagation (rooted cuttings) during the last 20 years. Although progress has been impressive, methods which can compete with highly efficient nursery production of 1 + 0 seedlings continue to be plagued by high unit costs, maturation, and other unforeseen developmental problems. Research on both types of methods is continuing, and protocols for producing rooted cuttings from seedling material and micropropagation from older tissue (6- to 12-year-old plants) are currently being tested. However, no reliable methods for rejuvenation of mature material are yet available. Nonetheless, there is strong interest in selecting superior trees at an early age and entering them into a cutting orchard and in mass propagating superior full-sibling family material by rooting cuttings from hedged seedlings. With these approaches, gains from improved families may be realized many years earlier than would be possible from seed production (even with supplemental mass pollination) from conventional orchard systems.

5.1 Introduction

The large gains possible from cloning selected phenotypes of forest trees — that is, from vegetatively propagating exact genetic copies of donor trees — combined with the need to increase the productivity of forestland have spurred development of efficient, cost-effective cloning methods. Furthermore, cloning superior half-sibling or full-sibling families where seed supply is limiting would greatly expand genetic gain. In fact, the long time period required for a conventional seed orchard to reach full production could be substantially shortened by using cutting (cloning) orchards.

Over the last 20 years, a number of prototype programs based on rooted cuttings (macropropagation) of Norway spruce *[Picea abies* (L.) Karst.], black spruce *[Picea mariana* (Mill.) BSP], radiata pine *(Pinus radiata* D. Don), and loblolly pine *(Pinus taeda* L.) have been established [6, 9, 62, 68]. Some of these can generate millions of plantable cuttings per year. The development of such programs for southern pines is behind that of programs for the spruces and radiata (Monterey) pine not because of lack of effort, but because southern pine cuttings are more difficult to root, requiring rigorous control of environmental conditions. However, recent advances in rooting methodology, and the use of easily rootable cuttings obtained from seedlings originally hedged (dominant shoots selectively pruned to force outgrowth of fascicular shoots) before 1 year of age, may permit cost-effective production of rooted cuttings from juvenile southern pine [22].

In addition, a number of promising tissue-culture procedures (collectively called micropropagation), which start with tissue fragments (explants) from embryos or seedlings [4] and more recently older trees [5], are being developed. Although these methods are labor intensive at present, they could become cost-effective means of cloning if some steps were automated and might prove useful in rejuvenating mature tissues from select trees [31]. Moreover, the introduction of new genetic material (DNA) directly into cells (genetic engineering) can best be done on single cells or small groups of cells, which can only be regenerated into whole plants through micropropagation [56].

This chapter describes the current status of both micropropagation and macropropagation techniques, recognizing that the latter has the higher short-term potential for large-scale production of plantable material. Macropropagation in conifers has also recently been reviewed by Rauter [71].

5.2 Genetic Advantages of Vegetative Propagation

The major advantage of using vegetative propagules (clones), instead of seedlings, for reforestation arises from genetic principles. A driving force behind any cloning technique is the ability to propagate exact genetic copies of selected donor trees (ortets). In addition to these classical factors, which are true for all organisms, considerable time

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could be saved through cloning, relative to current forestry practices, and gains like faster growth or better disease resistance could more rapidly be realized.

Differences among plants within a species can be divided into an environmental and a genetic component. The environmental component includes, for example, the effects of temperature, nutrient, and moisture status. The effects of the genetic component are further subdivided into two major types: additive and nonadditive. Additive genetic effects for a trait are controlled by many genes, each with a small, but cumulative influence. For example, genetically determined height-growth potential in trees covers a continuous range, and accounts for minor differences among individual trees. If the additive genetic value for height of parent trees is known (e.g., from field test results), then the expected additive genetic values of their offspring can be accurately predicted. Nonadditive genetic gains represent a synergistic phenomenon whereby an individual, or an entire full-sibling family, performs better than predicted by the breeding value of the parents. While additive gains can be predicted based on parental breeding values, nonadditive gains cannot and when detected, can only be exploited by cloning individuals or mass producing families of the two parents whose progeny exceed expected performance. Current tree-improvement programs effectively manipulate only the additive portion of the total genetic variation in the population. In contrast, capturing potential nonadditive genetic value has proven to be difficult, and management of seed orchards, which emphasize gain from nonadditive genes, has been uneconomical. Therefore, the remaining nonadditive genetic variation among individual trees within a population remains untapped. However, in a clonal tree-improvement program, knowing the specific cause of genetic superiority (e.g., whether from additive or nonadditive effects) is unnecessary because the entire genetic constitution of the tree is duplicated.

The relative proportion of nonadditive to additive genetic variation is difficult to demonstrate but may be substantial. In studies with loblolly pine, McKeand et al. [65] reported 0.0 to 2.8 times as much nonadditive as additive variation for a number of traits. If a significant amount of nonadditive genetic variation is present in certain individual trees, then gain from clonal selection may more than double gain from selection for additive genetic value only.

Genetic gains through reforestation with superior clones can be realized sooner than through a recurrent selection and seedling propagation program. The advantage arises chiefly from the shorter time needed between initial selection and production of plantable reforestation stock [63]. For comparison purposes, Table 5.1 illustrates the basic time intervals in a traditional seed orchard and a clonal cutting orchard. Superior trees are selected in progeny tests at approximately age 6 years in both cases. For the traditional case, the selected trees are then grafted into a seed orchard. Loblolly pine seed orchards require approximately 15 years from establishment to reach full seed production, although significant production starts at about age 10. Therefore, 21 years (6 + 15 years) would elapse before the seed orchard had reached full production. Alternatively, for the clonal case, a cutting orchard could be in full production after 9 years (6 + 3 years), reducing the time of forest-stand establishment by 12 years. Nonetheless, genetic improvement will continue steadily, if somewhat slowly, in a traditional program. In fact, this type of program forms a necessary basis for any clonal reforestation program, providing new genotypes for selection each generation [17, 63].

Table 5.1. Time to full production for a traditional loblolly pine seed orchard and a new clonal loblolly cutting orchard (adapted from Greenwood [29]).[1]

Activity	Seed orchard	Cutting orchard
Selection age, years (A)	6	6
Time to full production, years (B)	15	3
Total (A + B)	21	9

[1] 2.4×10^6 plants produced/ha, assuming 85 trees/ha, 1.27 kg seed/tree, 22,000 seedlings/kg for the seed orchard; and 17,000 hedges/ha, 200 cuttings/hedge, 70% rooting success for the cutting orchard.

5.3 Micropropagation

5.3.1 Terms and Types

This section outlines micropropagation techniques used for the southern pines, including loblolly, slash *(Pious elliottii* Engelm.), longleaf *(P. palustris* Mill.), shortleaf *(P. echinata* Mill.), and Virginia *(P. virginiana* Mill.). The term micropropagation as used here encompasses all manner of *in vitro* (in glass under sterile conditions) or tissue-culture vegetative propagation. Tissue culture studies which deal only with the growth or metabolism of undifferentiated cells (callus) will not be addressed here.

If propagation studies with all pine species are taken collectively, four main types of micropropagation can be recognized (Fig. 5.1):

Type A: organogenesis from organized (typically embryonic or very juvenile) explants.

Type B: axillary or fascicular shoot micropropagation. (Axillary shoots develop in from leaf axis; fascicular shoots develop from needle fascicles.)

Type C: somatic embryogenesis.

Type D: organogenesis from callus.

Organogenesis refers to a general pattern of propagation in which specific plant organs are initiated in sequence, i.e., shoots followed by roots to yield a complete plantlet (plant produced by tissue culture). Organogenesis may occur from organized tissue (explants) as in type A micropropagation or from unorganized tissue (callus) as in type D micropropagation (see Fig. 5.1). The important points in

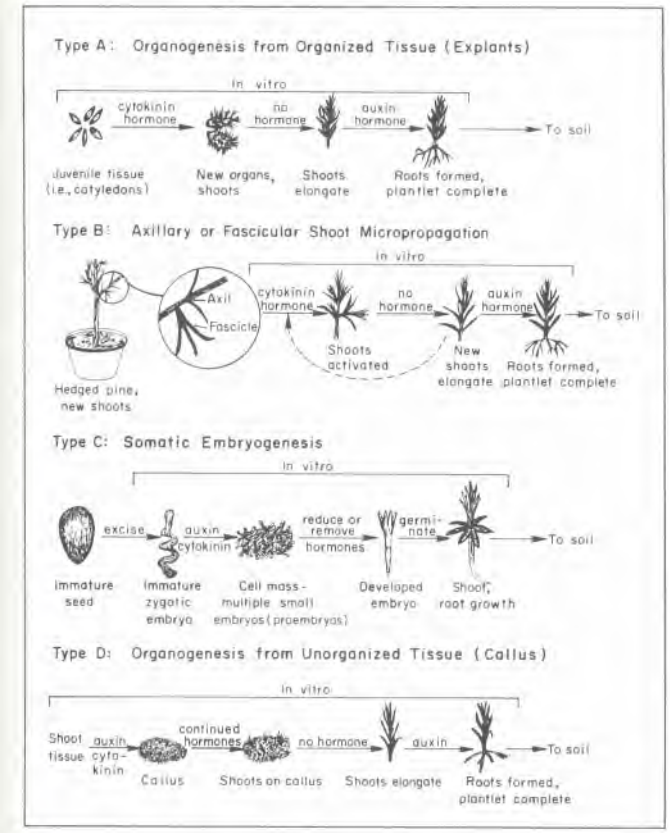

Figure 5.1. Schematic representations of the four main types of pine micropropagation techniques in which selected pieces of tissue are cultured *in vitro.* See text for details.

both cases are the production of *new organs* in sequence. In contrast, embryogenesis (type C) is a propagative process in which both shoot and root apices are *simultaneously* (or nearly so) initiated to yield a plant in the same manner that a zygote (fertilized egg) develops into an embryo in a seed. The natural development of zygotes into embryos is termed zygotic embryogenesis, whereas tissue-culture production of embryos from vegetative (somatic) cells is termed somatic embryogenesis. Axillary or fascicular shoot micropropagation (type B) differs from both organogenesis and embryogenesis in that a new apex or bud is not initiated. Instead, this process relies initially upon outgrowth or elongation of *already existing organs,* such as shoot apices (meristems) in leaf axils or the apex of a short shoot (needle fascicle). Outgrowth of the existing shoot is followed by rooting (an organogenic step) to yield complete plantlets. All of these propagative procedures are regulated by plant hormones, generally auxin(s), cytokinin(s), or both. In most cases, auxin(s) is (are) associated with root formation and cytokinin(s) with shoot formation, but both may be required for a particular step and their normal associations can be reversed in some species.

Micropropagation types A, B, and C, all reported to be successful with at least some of the southern pines, are described in subsequent paragraphs. Type D is not yet available for southern pines, but a reliable system for shoot production, with one instance of rooting to yield a complete plantlet (plant produced by tissue culture), has been reported for *Pinus eldarica* [28]. Recently, shoots derived from *P. eldarica* callus, which were then multiplied via axillary micropropagation, have been rooted with 85% success, and plantlets are now established in greenhouse soil [unpubl. data, 27]. Both organogenesis and embryogenesis from callus, if perfected for southern pines, would be extremely valuable propagation methods. Both have the potential for automated production of the large numbers of plantlets needed by the forest industry, and they are well suited for application of direct insertion of DNA [56].

5.3.2 Micropropagation Methods for Southern Pines

As for rooted cuttings, methods for tissue culture will have their greatest value starting with explants from mature trees. All four micropropagation types could in theory begin with such explants. But in practice, type A, which has been studied extensively in conifers for more than 10 years, almost uniformly requires starting with embryonic or seedling explants. As trees mature, they undergo developmental changes which include reduced growth rate and increased reproductive ability, but these changes are also accompanied by a progressive decline in the regenerative capacity of tissues removed from the plant, whether as cuttings or explants placed on a tissue-culture medium [31]. Given the lack of success with mature explants despite 10 years of effort, the likelihood of routinely extending type A to mature trees appears small. Propagation types B, C, and D are still in the developmental phase or are wholly absent from use with southern pines; consequently, the probability of applying these methods to mature trees is difficult to predict. However, limited propagation via type B has been reported with older (6.5- to 11-year-old) loblolly pine trees [1, 5].

The first of the southern pines to be micropropagated was longleaf, with type A methods [76]. Shortly thereafter, all southern pine species were successfully cultured to produce shoots or complete plantlets [12], also by type A methods. Micropropagation studies with pine hybrids where southern pines serve as one or both parents have yielded adventitious shoots (shoots formed in abnormal places) and, in some cases, complete plantlets. Longleaf x slash hybrid embryos have produced adventitious shoots *in vitro* via type A propagation [77], and pitch *(P. •igida* Mill.) x loblolly hybrids have been micropropagated to complete plantlets via type A [53]. In that same study, Kim et al. [53] also reported lateral (axillary) shoot micropropagation (type B) for the pitch x loblolly hybrid.

The most frequently planted southern pines are loblolly and slash. These two species are also the ones most studied for tissue culturing. Several reports on type A procedures for slash pine have recently been published. Studies

focusing on *in vitro* adventitious shoot initiation and growth starting with embryonic explants [70, 77] have provided shoot culture procedures and some understanding of regulatory features in those procedures. Lesney et al. [57] also produced adventitious shoots from embryonic cotyledons and reported that 90% of them rooted. Lastly, ITT Rayonier scientists and North Carolina State University (NCSU) researchers, following methods similar to those published by Mott and Amerson [69], have jointly produced and carried more than 250 slash pine tissue-cultured plantlets to the field in a single test planting [unpubl. data, 3].

Although tissue-culture research with slash pine has increased recently, the number of studies on loblolly pine far exceeds that on any of the other southern pines. Indeed, NCSU researchers established the first field planting of tissue-cultured loblolly pine trees in 1978. Since that time, some forest-industry companies have followed suit. NCSU researchers now have at least 17 field locations [5]. Micropropagation methods developed or developing for loblolly pine currently include type A [4, 5, 67, 69], type B [1, 5], and type C [8, 39]. Type D is as yet completely undeveloped for loblolly pine.

5.3.3 General Protocols for Loblolly Pine Micropropagation and Field Performance of Plantlets

In the remainder of this section, we summarize general procedures for micropropagation of loblolly pine and comment on field performance data of resultant plantlets. For detailed information on propagation techniques, times, environments, media, and hormones, the reader should refer to [1, 4, 5, 8, 39, 67, 69]. For detailed information on field performance, the reader should refer to [5, 24, 64].

5.3.3.1 Type A

Type A micropropagation requires adventitious shoot production, which has been reported from hypocotyls, needle fascicles, and cotyledons. Of these explants, cotyledons work best and are the ones most commonly used.

To begin this propagation sequence, the explants (cotyledons) are exposed to a culture medium rich in cytokinin(s) (a class of plant hormones promoting cell division) to stimulate cell divisions in the surface layers of the explant. After cell division is sufficient to start shoot formation processes, the cotyledons must be removed from the cytokinin(s) to assure continued shoot development (Fig. 5.1). These shoots generally elongate 1 to 2 cm *in vitro* without hormones and are then rooted. The rooting process begins with an auxin pulse (short-term application of auxin(s), a class of hormones promoting rooting) applied at the stem base to stimulate cell divisions in the cambium. In the program at NCSU this auxin treatment is routinely performed *in vitro*, but the root development phase that follows may occur either in hormone-free tissue-culture medium or in soil in a mist bench. Upon leaving *in vitro* culture, auxin-pulsed shoots or rooted plantlets are acclimated to nonsterile growth in a greenhouse mist area for several weeks and then transferred to a nonmisted greenhouse bench for approximately 6 months to produce plantlets suitable for transfer to the field.

As already noted, tissue-cultured loblolly pine plantlets (produced via the cotyledon method) have been established at multiple field locations across the southeastern U.S. for up to 9 years. In general, plantlet survival upon transfer to the field is high (> 90%). The initial height growth of plantlets in the field lags behind that of genetically similar seedlings, but after acclimating, plantlets appear to grow as well as seedlings. Indeed, height measurements have shown the growth increments of plantlets and seedlings to be equal in year 4 despite a total height differential resulting from the early growth lag.

The shoot morphology of type A tissue-cultured plantlets is subtly but nonetheless clearly and recognizably different from that of seedlings. Early field assessments of plantlet shoot characteristics such as bud length and diameter, needle dry weight, branching patterns, and growth cycles have shown these characteristics to more closely resemble the characteristics of mature plants than do those of comparably aged seedlings [64]. However, in later assessments, morphological differences between plantlets and seedlings were slight, and it is uncertain whether any differences will persist.

Besides differences in shoot morphology and early growth, tissue-cultured plantlets produced via the cotyledon method are more resistant to fusiform rust, a feature which is of potential value [5]. Field comparisons of genetically similar seedlings and tissue-cultured plantlets have consistently shown the plantlets to be less infected with the rust fungus, *Cronartium quercuum* f. sp. *fusiforme*. The basis for this increased resistance is unknown, although hypotheses related to physiology and development have been advanced [66]. Whether elevated rust resistance in loblolly pine also results from other vegetative propagation methods, or is limited to the cotyledon method only, remains to be determined. However, if elevated rust resistance is generally available from vegetatively propagated loblolly pine, it will be a big plus for southern forestry (see also chapter 20, this volume).

5.3.3.2 Type B

Type B micropropagation of loblolly pine begins with initially stimulating axillary or fascicular meristems on field-grown or potted trees via hedging and/or cytokinin sprays (Fig. 5.1). The shoots that develop are then placed into culture and restimulated via cytokinin treatments (dips or through the medium) to produce additional axillary or fascicular shoots; these, in turn, through sequential elongation and restimulation can produce many successive generations of shoots to yield large numbers of specimens much like hedging of successive generations of rooted cuttings. The elongated shoots root via an auxin pulse and

can be transferred to the greenhouse, as for the type A cotyledon method.

Type B micropropagation has been applied to yield complete plants from juvenile (< 1-year-old), adolescent (6-1/2-year-old), and mature (11- to 12-year-old) tree explants [unpubl. data, 2]. At present, propagules from mature tree explants have not been transferred from the greenhouse to the field, but in a single field planting established by NCSU researchers, juvenile micropropagules have grown as well as seedlings in the first growing season [unpubl. data, 23]. Type B micropropagules produced by Weyerhaeuser Company researchers have previously displayed high sensitivity to cold weather, and large numbers of these plantlets have now been established in field trials to investigate cold sensitivity [72]. Type B methods are still only experimental and far from operational, but their application to mature tree tissues is promising. Even if type B methods cannot be made economical, they may be useful in efforts to rejuvenate specimens to be included in rooted-cutting programs.

5.3.3.3 Type C

Type C micropropagation is based on *in vitro* production of "somatic embryos" that can "germinate" to form plants like normally occurring (zygotic) seed embryos (Fig. 5.1). In tissue-culture systems, however, we seek to mass produce genetically identical embryos, in contrast to the natural one embryo per seed. Ultimately, somatic embryogenesis in conifers, and in pines in particular, could arise from a suspension culture (friable-cell culture suspended in liquid medium) system which could produce thousands of plants per flask.

In vitro embryogenesis from coniferous tree cultures has been pursued for more than 15 years, but only in 1985 was successful somatic embryogenesis reported from calluslike masses derived from embryonic explants of Norway spruce [43]. Among southern pines there is a single report that loblolly pine [39] has been successfully cultured to yield complete plantlets via embryogenesis. Researchers at the Institute of Paper Chemistry have produced embryogenic masses which form very small proembryos (immature, incomplete embryos) of loblolly pine [8], and NCSU researchers likewise have obtained proembryogenic cultures in both loblolly and slash pine [unpubl. data, 2].

In vitro embryogenesis in conifers generally starts from very juvenile explants. In loblolly pine, the starting material is immature zygotic embryos, including the suspensor cells at the base of the embryo. Gupta and Durzan [39] stimulated the suspensor regions of immature zygotic embryos in a dark environment to produce embryogenic callus-like masses on a culture medium containing very high auxin and cytokinin levels. For embryo development, these masses with proembryos were transferred to a culture medium with reduced hormone levels and maintained in darkness. Complete embryos and plantlets were eventually produced on hormone-free medium in the light. At least

Figure 5.2(A). Cutting orchard (hedge) of loblolly pine cultured for 4 years; trees are 0.5 m tall.

Figure 5.2(B). Collecting cuttings from a 2-year-old hedged seedling of loblolly pine.

Figure 5.2(C). Loblolly pine cutting to which rooting powder has been applied on the basal 2 cm.

Figure 5.2(D). Greenhouse at International Forest Seed Company specifically designed for rooting up to 145,000 cuttings of loblolly pine in containers over a 4-month period.

Figure 5.3(A). Tray of container-grown loblolly pine rooted cuttings, 1 year after setting for rooting.

some of these plantlets have been transferred to soil, but none are yet established in field trials.

5.4 Macropropagation

5.4.1 Status of Current Programs

Vegetative propagation of conifers by rooted cuttings dates back to the 15th century, with reports of plantation establishment of Japanese cedar *(Cryptomeria)* in Japan [79]. Contemporary programs for the mass propagation of conifers by cuttings all share a number of common features. Most programs begin with cuttings from juvenile trees which are hedged to multiply cuttings and prevent further maturation by the ortet (see Fig. 5.2A). Rooting procedures are also similar, involving mist and treatment of the cutting base with auxin and a variety of other chemicals which promote rooting (e.g., [34, 44, 80]; see Fig. 5.2C). Once rooted, cuttings can then be handled like 1 + 0 seedlings and transplanted into the nursery or directly into the field (see Fig. 5.3). A brief discussion of several contemporary rooted-cutting programs for conifers provides a background for considering similar programs for southern pines.

5.4.1.1 Radiata pine

The discovery that stem cuttings of radiata pine root easily, and can even be rooted directly in nursery beds, prompted extensive studies on the vegetative propagation of that species in New Zealand and Australia [e.g., 14, 59]. Cuttings from both juvenile and mature trees root well, but cuttings from older ortets show progressively declining growth potential and increased flowering [78]. Although cuttings from older trees grow slower, they produce straighter stems with better form and smaller branches [10, 49]. In Australia, an operational rooted-cutting program that starts with tissue-culture material has recently been

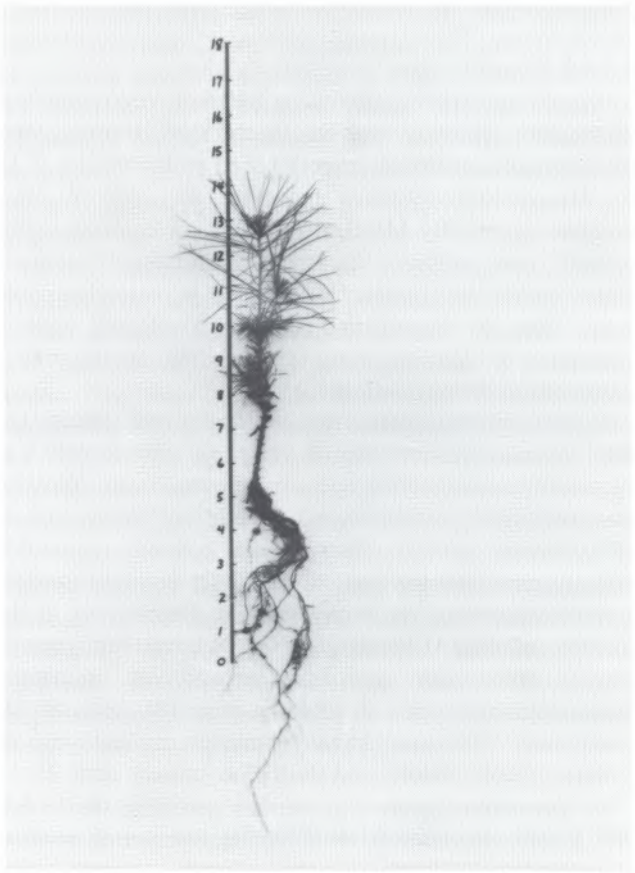

Figure 5.3(B). Similar loblolly cutting with potting medium removed.

proposed by a private timber company. In this effort, plantlets obtained from seed of superior full-sibling families would be multiplied by tissue culture. These plants would be allowed to grow 5 years, and then the best ortets selected and hedged (see Fig. 5.2A, B) for rooted-cutting

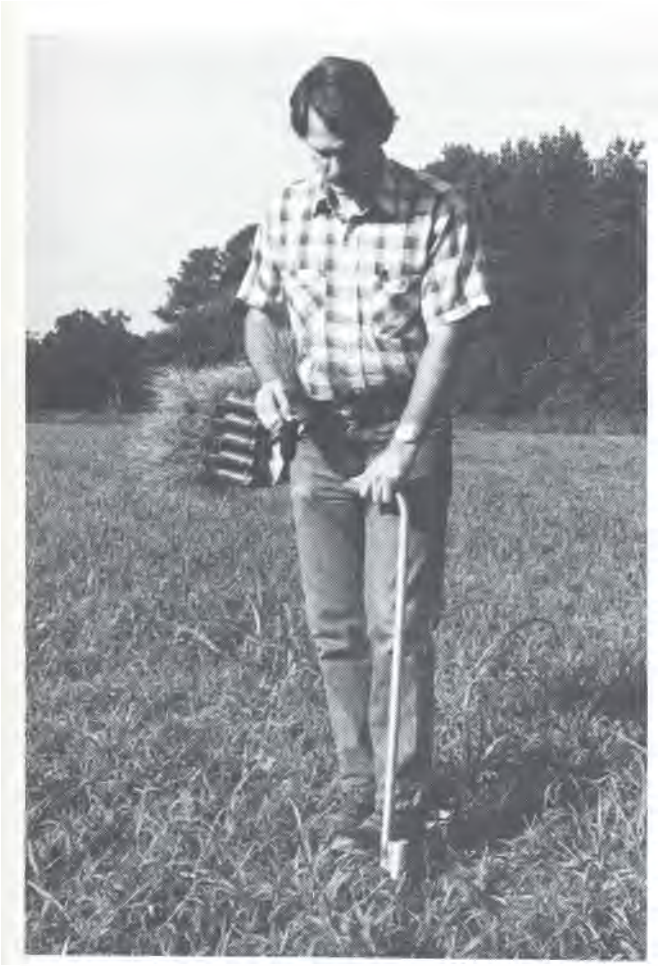

Figure 5.3(C). Field planting container-grown loblolly pine rooted cuttings.

Figure 5.3(D). Loblolly cuttings after four growing seasons in the field.

production. Although some maturation will have occurred, further maturation could be prevented or slowed by hedging. A mill study has shown that timber produced by cuttings from ortets about 5 years old will yield double the face grade veneer, as well as provide 43% more veneer volume and 8% more sawtimber than timber produced by cuttings from hedged seedlings [55; pers. commun., 58]. The trade off of higher product quality and value at the expense of more total volume appears justified.

5.4.1.2 Norway spruce

Vegetative propagation of this species by rooted cuttings is well established in Europe. Kleinschmit [54] reports that production costs for a large-scale (1 million plants/year) rooted-cutting operation may be only 20% greater than those for 2 + 2 transplants. After rooting, the cuttings are handled like seedlings, and a plant comparable to a seedling can be produced in 3 years. However, a 1-year-old rooted cutting of Norway spruce costs about 3 times as much as a 1+0 seedling, which is similar for southern pines [19]. In Denmark, spruce cuttings are produced by hedging, with an annual yield of about 5 million cuttings/ha [pers. commun., 73]. Over a 3- to 4-year period, about 1,000 to 1,500 cuttings/seedling can be produced, so a limited amount of seed from selected full-sibling families can be used to produce many offspring. Rooted cuttings of Norway spruce appear to grow as well as seedlings, even when cuttings are taken from 9-year-old trees, although the cuttings are more plagiotropic (tend to grow horizontally) and do not look like seedlings [74]. Rooted cuttings from clones selected for good height and volume growth can substantially outperform seedlings [54, 74].

5.4.1.3 Black spruce

In Canada, the Ontario Ministry of Natural Resources is currently producing about 1 million rooted cuttings/year. The cuttings, taken from seedlings of selected full-sibling families, at present are screened only for lack of plagiotropism and early rapid growth [pers. commun., 48]. Long-term evaluation of the selected clones has only recently begun.

5.4.2 Developmental Physiology of Rooting Cuttings of Southern Pines

Rooting cuttings of conifers involves the regeneration of adventitious root meristems directly from tissues associated with the vascular tissue or from callus (or wound) tissue which has formed at the base of the cutting [37]. This chapter discusses only root primordia induced on the stem after the cutting is taken, since preformed primordia (which form during the normal course of branch or stem development) are absent on conifer stem cuttings [41]. Induction of root regeneration on cuttings is a function of species, genotype, level of maturation (phase change) of the ortet, and developmental state of the donor branch (which varies with season). During root regeneration, auxins, both naturally occurring (endogenous) and externally applied

(exogenous), and environmental factors such as mist, light, and temperature are of particular importance [e.g. 34, 80].

5.4.2.1 Auxins

Although a number of exogenously applied substances promote rooting by cuttings [45], auxins, either synthetic or natural, are consistently the most important. Whether auxin works alone to promote rooting [13, 33] or in concert with other substances remains to be resolved [42].

Auxins are actively transported towards the base of the plant (polar transport), even if the plant is inverted for long periods [75]. This basal polarization of auxin transport appears to be associated with the basal formation of roots, and in fact may be essential to the process [32, 51]. Inhibiting polar transport of auxin inhibits rooting even if auxin is applied to the cutting base; this implies that the internal allocation of natural auxins may be vital to the rooting process [51]. A rooting powder developed for southern pines by Hare [44], which includes auxin in addition to other substances, is sometimes more effective than auxin alone (see Fig. 5.2C). However, its effectiveness varies with species, time of year the cuttings are taken [61], and solution concentration [34].

5.4.2.2 Genotype

Even within a species, genotype plays a highly significant role in the ability of cuttings to form roots. For example, rootability of cuttings from 10 clones of radiata pine showed over a 3-fold (22 to 74%) variation [15]. Bower and van Buijtenen [11] report similar variation in rooting success among six clones of slash pine. While clonal variation in rooting is well documented, variation at the family level is less important. Over 2-fold (22 to 60%) variation in rooting by cuttings from half-sibling families of loblolly pine has also been reported [35], although a more comprehensive study by Foster [16] showed insignificant differences in rooting among 19 half-sibling families. Applying auxins or other root-promoting substances cannot overcome a genetic predisposition of an individual to not root [e.g., 11]. In both the Norway and black spruce rooted-cutting programs described earlier, clones which do not root well are dropped from the program.

5.4.2.3 Maturation

Maturation, or phase change, occurs gradually in woody plants, and is usually accompanied by, among other things, decreased rooting competence (see the review by Hackett [40]). In southern pines, a steady decline in rooting ability with age has not been thoroughly documented. However, pooling the results from several studies leads to the tentative conclusion that rooting ability drops sharply after the first year or so, then plateaus. Greenwood and Nussbaum [35] report no difference in rooting of cuttings from ortets aged 2 and 5 years. However, cuttings from loblolly pine seedlings <1 year old appeared to root faster and more frequently than woody cuttings from older material [unpubl. data, 82]. Marino [62] reported a drop in rooting ability by loblolly pine between I and 3 years old. Grigsby [36] noted that cuttings from 25-year-old ortets of loblolly pine rooted only slightly better than those from 6-year-old trees.

The capacity of rooted cuttings to produce height and diameter growth, as well as branches, decreases markedly with ortet age [21, 25, 30, 35]. In addition, reproductive competence and needle size increase with ortet age. These developmental changes resulting from maturation are persistent and at present appear difficult to reverse. However, there have been some promising attempts to rejuvenate conifers (for example, type B tissue culture), but these results are preliminary and must be verified (see also the review by Greenwood [31]). As mentioned earlier, value loss from decreased growth could be offset by an increase in straightness and branching characteristics [21].

5.4.2.4 C effects

Many environmental considerations, such as cultural factors or position within the crown of the tree, can influence the behavior of cuttings. Geneticists use the term "C effect" to describe the extent to which these considerations alter the clonal mean for a character of interest [20]. In this manner, C effects may mask true genetic effects and usually inflate estimates of genetic variance. Because the true performance of a clone (e.g., height growth or rooting ability) may be increased or decreased by C effects, the clone may be mistakenly chosen or rejected for use in a tree improvement program.

Cultural manipulation of stock ortets can result in beneficial C effects [45]. For example, cuttings from vigorous, greenhouse-grown ortets of slash pine appear to root better than field-grown cuttings [11]. In addition, the time of year the cuttings are taken affects rooting. Marino [62] and Mahalovich [61] present evidence that loblolly pine cuttings root well if taken during the dormant period (September through February), whereas Bower and van Buijtenen [11] report that loblolly pine cuttings also root well if taken in May. Seasonal effects on ortet condition and the rooting chamber environment, both of which affect rooting, are probably confounded in these experiments and need further clarification.

5.4.2.5 The rooting environment

The southern pines generally are difficult to root, so optimizing the rooting-chamber environment is critical. Marino [62] summarizes the state-of-the-art environmental conditions for rooting southern pines: these include carefully controlled mist, supplemental CO_2 (1,500 to 2,000 ppm), bottom heat (26.6°/80°F), and photoperiod extension to 18 to 20 hours. These recommendations are workable, but established levels are based on only a few studies on southern pines. Further refinement is probably possible. For example, high humidity and moist soil, typically provided by mist, are essential for rooting southern pines, but too much mist can greatly inhibit rooting [34]. Therefore, uniformity of mist must be

regulated by devices such as gantry-mounted nozzles or fogging nozzles (ultrasonic or high-pressure atomizers).

Other treatments which affect the health of cuttings during the rooting process are described by Marino [62] and will not be dealt with here.

5.4.3 Performance of Seedlings Versus Vegetative Propagules

Few field trials have been established to assess growth performance of rooted-cuttings of the southern pines (see Fig. 5.3D). In early results from a rooted cutting trial of slash pine, Franklin [25] found a dramatic decline in height growth of the cutting as age of the ortet advanced. Maturation effects on growth persist for many years, perhaps indefinitely. Foster et al. [21] provided results from two field trials of loblolly pine comparing rooted cuttings and seedlings of the same half-sibling families. Rooted cuttings from 1-year-old ramets (clonally propagated cuttings from superior trees) actually were taller than seedlings through four growing seasons. Rooted cuttings from 5-year-old ortets were taller than seedlings after one season's growth but were the same height after four seasons. In a second study [21], rooted cuttings from 4-year-old ortets grew significantly slower than seedlings. In both studies, initial size or vigor of the rooted cuttings and seedlings strongly influenced performance. Foster et al. [21] concluded that growth of rooted cuttings from 1-year-old ortets should compare favorably with that of seedlings but that ortets of age 5 or older should be avoided for producing cutting stock. In a study comparing up to third-year performance of loblolly pine, Foster [18] found no difference in growth and morphology traits between rooted cuttings from ortets < 1 year old and seedlings from the same five full-sibling families. The only exception was that rooted cuttings displayed slightly less stem taper.

Knowing the relationship between tree age and physiological and morphological changes within a tree allows stock with a known set of maturation-induced, as well as inherited, traits to be selected. Like inherited traits, maturation-induced traits also persist for vegetative propagules, such as grafts [30] or rooted cuttings [21]. In one study, rooted cuttings derived from 4-year-old loblolly pine were significantly straighter, had narrower crowns, and had less tapered stems than seedlings [21]. It appears possible to choose trees which are old enough to avoid particularly negative juvenile traits (e.g., seedling grass stage in longleaf pine) but which are still young enough to root well and subsequently grow well [21, 25].

5.5 Recommended Use of Micropropagation and Macropropagation: Concerns and Limitations

5.5.1 Operational Use of Clones

Once a practical reforestation program using vegetative propagules exists, questions arise concerning clonal deployment. For example, how many clones should be used in a plantation [60]? Should clones be planted in a complete mixture or as a mosaic of small pure clone blocks [46]?

Unfortunately, few empirical results exist in forestry to answer either of these questions. However, both relate to the stability of a population of trees in the face of changing environments — both climatic events (i.e., unusual freezes, droughts, or ice storms) and pest attack (i.e., disease or insect) — especially over time. The risk, then, is whether enough trees can survive and prosper to develop into an adequate stand at rotation age [60].

Deployment strategies are currently guided by both theoretical studies and empirical results from large-scale plantations. Theoreticians have taken different approaches yet have reached similar conclusions, stating generally that 10 to 25 clones per location will result in an acceptably low risk of plantation failure [50, 60]. On the basis of empirical results, eastern cottonwood *(Populus deltoides* Bartr.) plantations have successfully and repeatedly been established with from 1 [52] to 10 clones [pers. commun., 81] with no apparent detrimental effects. The spatial distribution of pure clone blocks and complete mixes also varied. The solutions to these deployment concerns depend upon the findings of well-designed field studies to supplement existing theoretical and empirical results.

5.5.2 Economic Considerations

Unlike the cost of seedling production in a bareroot nursery or even a container nursery [38], production costs, and therefore economic benefits, of a reforestation program using vegetative propagules are uncertain for southern pines [19]. To date, the rooting of southern pine cuttings has required a high-quality greenhouse environment [62], whereas the production of tissue-culture plantlets has required growth rooms [69]. Undoubtedly, these facilities will augment production costs beyond those of a bareroot nursery.

Large-scale production of southern pine vegetative propagules is in its infancy. International Forest Seed Company set 80,000 rooted cuttings of loblolly pine for rooting in 1987 as part of their tree-improvement program using macropropagation [22]. Facilities, cutting hedges, and cutting establishment are shown in Figure 5.2. A greenhouse designed specifically for this purpose costs approximately $\$150/m^2$ ($\$14/ft^2$) (see Fig. 5.2D). The finely controlled environment needed to root cuttings of southern pines [62] exceeds that needed by most horticultural species [45], which require fairly simple rooting facilities that cost as little as $\$43/m^2$ ($\$4/ft^2$) [47]. Therefore, production costs will be greater for macropropagation of southern pines. Tissue culture of southern pine is even farther from operational production than rooted cuttings, and the attendant costs are even more uncertain. However, as both types of vegetative propagation near an operational mode, the production systems will become streamlined and more efficient, as have their closely aligned horticultural counterparts.

Even though the production cost of vegetative propagules may be higher than that of seedlings [7, 26, 54], the ultimate economic benefit of using clones may offset the higher initial cost. Unfortunately, few economic analyses of clonal forestry have been published, and those that have emphasize Norway spruce [54], sitka spruce *[Picea sitchensis* (Bong.) Carr.][26], and radiata pine [7] rather than the southern pines.

As with any type of economic analysis in forestry, that for clonal forestry depends upon the particular species and management practices. With radiata pine, even an additional cost of over 500% for rooted cuttings compared with seedlings is more than offset by the discounted value of savings in cultural treatment of the forest stand and higher value of the final harvest [7].

Forest stands of the southern pines are being established in the United States with rooted cuttings and tissue-cultured plantlets. As foresters become more familiar with the cost of propagules, required cultural regimes, and resultant product values, precise economic analyses can be conducted better to assess system effectiveness.

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