Comments

Tree Planters' Notes

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Cover: Preparation for lifting at Elkton Nursery Oregon (photograph by the late Steve Omi, USDA Forest Service, Coeur d'Alene, ID).

The Value of Seedling Quality Testing

Ever since people began planting trees, it has been recognized that seedlings need to be in good condition if they are to survive transplanting and thrive afterwards. Deciding what constitutes "good condition" — and tailoring nursery practices to produce it— has been very much an art. When plantations failed and there were disputes between nursery managers and their customers over whose fault it was, it was one person's word against another's, with no objective way to settle differences.

In the 1920's, morphological grading standards were developed based on height, caliper, shoot-to-root (S/R) ratio, and lack of obvious deformity or mechanical damage. This was a major step forward, but it was still decades before the system was vindicated by sound field experiments. Questions like "How tall is tall enough?" or "How much survival do you lose if the S/R ratio is above a certain number?" had to be answered for many different species on many different sites in many different climatic zones.

Even then, there were unexplained failures of stock that had met all of the grading criteria. In the late 1950's, Ed Stone was the first to propose physiological testing as a means to measure the condition of seedlings to determine their fitness for lifting, storage, and outplanting. Since then, his "root-regenerating capacity" has evolved from something measured in a 28day controlled environment pot test to something measured in a 7- to 14day test in a mist chamber. Now known as "root growth potential" (RGP), it is regularly measured operationally, and the results can be in hand in time to make management decisions about the stock.

There is still much debate about the value of the RGP test, but one must keep in mind that every test is based on certain assumptions, and no single test will tell everything you need to know about the condition of a seedling. In this case, the big assumption is that when outplanted, a seedling has a limited time to make root contact with the surrounding soil, otherwise it will desiccate and die. In much of western North America, where most of the seedlings are spring-planted and summers are normally dry, that is a good assumption. In other areas where summer rain is reliable, it may not be.

In the last 15 years, other tests have been developed that measure different aspects of seedling physiology and rest on different assumptions about what seedlings must do to survive. One example is the chlorophyll fluorescence test. This measures the functioning of photosystem II, and it has been shown that a wide variety of agents can impair its function, including heat, cold, drought, and herbicides. The test can distinguish active, dormant, and dead leaves, and it can be run in a matter of minutes using portable equipment that in recent years has declined in price dramatically.

Another characteristic that is tested for is cold hardiness, which can be measured in a variety of ways. The one I prefer is by electrolyte leakage. This test can be adapted to any tissue in the seedling, can provide results within 3 days, and is precise and amenable to rigorous statistical analysis. The assumptions underlying this test are that seedlings must become hardy enough in the fall in a timely manner to tolerate the lowest temperatures they will experience, either outdoors or in cold storage, and not lose their hardiness prematurely in the spring. In addition, experience has shown that cold hardiness is related to, and is a good proxy for, other important attributes such as RGP, bud dormancy, and general ability to tolerate environmental and mechanical stress.

Cold hardiness testing is coming into use operationally to determine in real time when seedlings are ready to lift and pack into cold, especially frozen, storage. The ability to measure the condition of the seedlings quickly will make it possible for nursery management to respond appropriately to year-to-year variations in weather and be able to identify damage if it occurs. Cold hardiness tests can also tell how well seedlings are maintaining their dormancy during storage, shipping, and outplanting.

With the ability to measure quickly— and not have to guess at— the condition of seedlings and their fitness to tolerate nursery operations, shipping, and outplanting, nurseries and reforestation programs are rapidly moving toward a firmer scientific foundation and better accountability in all phases, and away from being practiced as an art.

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Somatic Embryogenesis Tissue Culture for the Propagation of Conifer Seedlings: A Technology Comes of Age

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Somatic embryogenesis is a tissue culture method of asexual propagation used in horticulture, agriculture, and to some extent in forestry as a means of rapidly multiplying elite varieties or clones. This paper reviews the development of somatic embryogenesis tissue culture procedures for interior spruce— Picea glauca *(Moench) Voss* × Picea engelmannii *Parry ex Engelm. Various components of tissue culture protocols, scale-up technology, nursery production, and field performance arc discussed, as is how this vegetative propagation technology is being integrated within an existing tree improvement program. The somatic embryogenesis tissue culture technology is also compared with the other propagation technologies currently used within forest regeneration programs.* Tree Planters' Notes 47(2):48-57; 1996.

There have been major advances over the past 25 years in the development of operational vegetative propagation systems for conifer species used in plantation forestry programs. These propagation systems provide a means of bringing new genetic material into forestry programs through the capture of a greater proportion of the gain from additive and non-additive genetic components inherent within a selected tree species (Libby and Rauter 1984). Vegetative propagation systems also provide a method for multiplying superior families identified in tree improvement programs (Gupta and Grob 1995; Zobel and Talbert 1984). Vegetative propagation systems being developed for use in forestry utilize the following approaches:

1. Rooted cuttings
2. Micropropagation through organogenesis tissue culture
3. Somatic embryogenesis

Currently, rooted cuttings are the most effective propagation technique that is operationally available to multiply specific individuals that have desirable traits. 1n a recent survey, over 65 million conifer cuttings are produced annually around the world, with this number growing rapidly (Ritchie 1991; Talbert and others 1993). The primary use of rooted cutting technology is for bulk production of genetically improved materials.

Production of rooted cuttings is essentially a 2-step process: the production of cutting-donor plants and the production of rooted cuttings. Donor plants can range from selected trees of wild populations to trees grown from seeds of genetically improved families under an intensive nursery cultural regime. Rooted cuttings of forest tree species are most successfully produced from juvenile portions of donor plants because these juvenile portions of a plant will provide cuttings with the potential for good initiation of root primordia (reviewed by Hackett 1988). Cuttings are placed in a rooting environment (that is, high humidity and soil moisture, warm soils, and moderate light levels), allowed to develop roots, then treated as rising 1-year-old seedlings.

Organogenesis is a tissue culture system that relies on the multiplication of shoots or the *de novo* formation of organs originating from either unorganized callus or preformed shoots or induced buds. Propagules produced through this system are essentially treated as microcuttings. Thus, shoot propagules are placed in an optimal rooting environment and treated in a similar manner as cuttings.

Somatic embryogenesis (SE) is a tissue culture approach where proliferative embryo suspensor masses are established from non-meristematic cells and subsequently cultured to produce organized bipolar structures possessing shoot and root meristems (that is, somatic embryos). The term somatic refers to embryos developing asexually from vegetative (or somatic) tissue. This method has been used-in horticulture, agriculture, and to some extent, in forestry-as a means of rapidly multiplying elite varieties or clones. Through the application of bulk-handling techniques, SE is cost-effective as a propagation approach for conifers (Roberts and others 1995). Distinct from conventional cuttings, SE offers the advantage of long-term storage of germplasm through cryopreservation (Cyr and others 1994). Although considerable research focus and efforts have demonstrated the potential for SE propagation of a large variety of plant species, success has been achieved only at the research scale (Aitken-Christie and others 1995).

Research and development in the area of SE of commercial conifers has been driven by the following 2 factors:

- The multiplication of superior families
- The selection of elite clones from among such families to capture a greater proportion of the genetic gain (Mullin and Park 1992; Park and others 1993)

In conifers, embryogenic cultures can be induced from developing and/or mature zygotic embryos or young germinants, but not from older explants (Attree and Fowke 1991; Tautorus and others 1991; Roberts and others 1993). Thus, SE fulfills a role similar to cuttings with respect to the multiplication of families. The value-added traits that can be captured and propagated through SE are those that can be identified through any tree-breeding program and include yield, wood quality, plus stress, pest and disease resistance. Spruce species appear to be most amenable to SE propagation technology (Hakman and Von Arnold 1988). Interior spruce— a complex of white and Engelmann species *Picea glauca* (Moench) Voss × *Picea engelmannii* Parry ex Engelm.— is a major commercial species in British Columbia, Canada. Within British Columbia since the 1980's, approximately 100 million container interior spruce seedlings have been planted annually in forest regeneration programs. To supply improved genetic material for these programs, a tree improvement program has been developed for large areas of the commercial land base (Kiss 1968). This breeding program affords the opportunity to match significant potential genetic gains with the technical opportunity to deliver value-added material to operational reforestation programs through SE technology. In this paper, we review steps that have been taken in developing SE tissue culture procedures for interior spruce. Various components of tissue culture protocols, scale-up technology and nursery production will be discussed. In addition, SE technology will be compared with other propagation technologies currently used in forest regeneration programs.

Basic Laboratory Protocols for Somatic Embryogenesis

In general, the SE process is divided into several phases (Gupta and Grob 1995):

- Culture **initiation** —induction
- **Proliferation** — maintenance, multiplication, suspension culture
- **Cryopreservation** — germplasm storage
- **Maturation** — somatic embryo production
- **Embryo drying**
- *In vitro* **germination and early growth**

All parts of the laboratory process are performed under-sterile conditions to prevent microbial contamination.

Because technology is most advanced for *Picea* spp., the methodologies discussed in this section are those developed for interior spruce (Cyr and others 1995; Cyr 1996). Apart from the stage of commercial development, the most significant protocol difference among the various conifer species is at the initiation phase. For spruce, stored seed can be used for initiation of SE, whereas for many other conifers, most notably *Pinus* spp. and Douglas-fir, fresh immature cones are required, with initiation success restricted to a 1- to 2-week developmental window (Cyr unpublished data).

Initiation. For spruce, mature zygotic embryos are dissected from the seed and placed onto semi-solid medium containing plant growth regulators (Webster and others 1990). The dissected embryo is referred to as the **primary explant**. The induction of SE is first evidenced by the growth of new tissue, usually at the hypocotyl-cotyledon junction of the explant (figure 1). This process takes approximately 8 to 12 weeks and the new tissue is referred to as "putative" embryogenic tissue or embryonal suspensor mass.

Proliferation. The embryonal suspensor mass is characterized by the presence of early-stage somatic embryo structures that are analogous to those occurring during normal seed development. This tissue is isolated

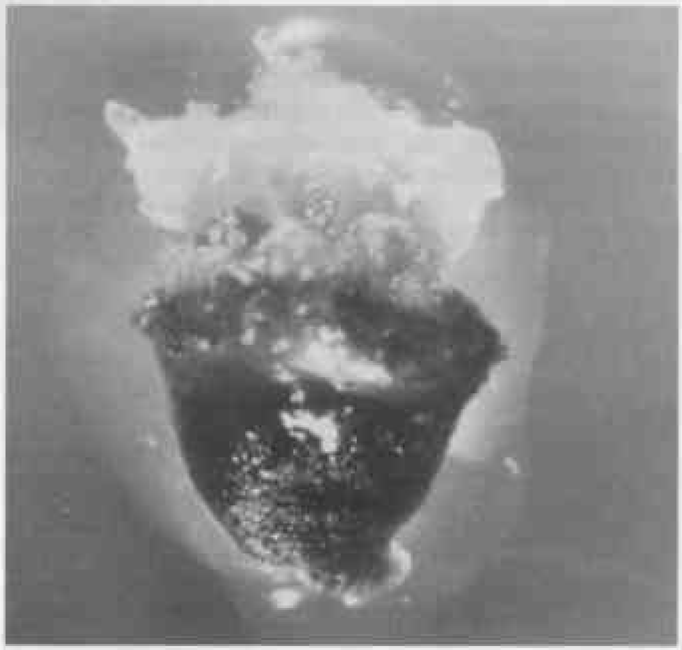

Figure 1—*Initiation of embryogenic tissue from the excised zygotic embryo explant.*

from the primary explant and transferred to fresh medium. Under these conditions, the tissue multiplies and develops as early-stage somatic embryos. Tissue is subdivided (subcultured) and transferred to fresh medium every 7 to 10 days. This material consists of multiple embryos and is often referred to as **embryogenic callus** (figure 2). Sufficient material can be produced for cryopreservation within 4 to 6 weeks.

Cryopreservation. Cryopreservation facilitates long-term storage of the valuable germplasm produced by SE (Cyr and others 1994; Kartha and others 1988). The embryogenic tissue is treated with cryoprotectants, frozen to -35 /C under controlled conditions, and then subsequently stored in liquid nitrogen (-196 /C). Cryopreserved tissue can be regenerated within 1 to 2 weeks after a simple thawing process.

Maturation/embryo drying. To advance the development of somatic embryos, tissue is transferred to a medium containing the phytohormone abscisic acid. Within a period of 4 to 7 weeks, this results in the production of mature somatic embryos that are analogous to their zygotic counterparts (figure 3) (Roberts and others 1993). To complete the process and effect a transition to germination upon exposure to suitable conditions, somatic embryos are harvested and subjected to a drying process (Roberts and others 1993). This step enhances the vigor of the somatic embryo. At this stage, the embryos are often referred to as synthetic seed, or "synseed."

Germination/early growth. Spruce synthetic seed does not require stratification. The embryos are placed on germination media containing no phytohormones and supplemented with a carbohydrate (Roberts and others 1995). *In vitro* germination occurs within 5 to 7 days and proceeds to the development of true needles at 4 to 6 weeks. At this point the plantlets can be transferred to *ex vitro* conditions (figure 4).

Figure 2—*Proliferation of the embryogenic tissue (embryonal suspensor mass). Finger-like projections are early stage somatic embryos.*

Figure 3—*Production of cotyledonary somatic embryos from embryogenic cultures.*

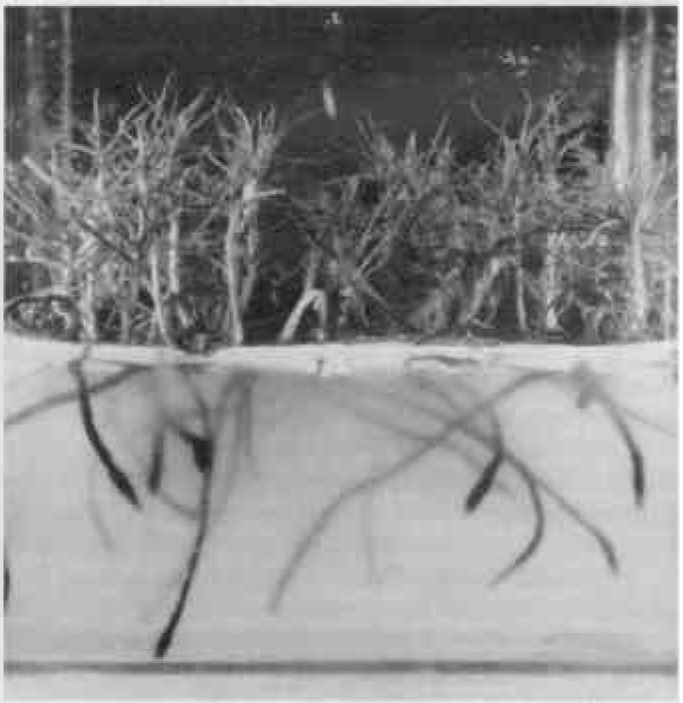

Figure 4—In vitro *germination and early growth of interior spruce somatic seedlings.*

Nursery and Field Performance.

Throughout the 1990's, germinants from SE technology have shown continued improvement in their development into high-quality somatic seedlings in the nursery. In addition, somatic seedling propagation technology has been successfully integrated into the container seedling production system. This containerized delivery system accounts for upwards of 95% of the conifer seedlings grown for reforestation programs in British Columbia, Canada. Through this time period, the performance of somatic seedlings has been compared to zygotic seedlings.

Production of conifer seedlings in container nursery systems is primarily dictated by 2 operational criteria:

- Maximize greenhouse production through the greatest number of plantable seedlings per unit area
- Minimize the number of seedlings that do not meet defined morphological standards at the end of the production cycle

The goal of the interior spruce somatic seedling program is to produce seedlings that meet these normal operational criteria and have a high level of seedling quality.

For every nursery production cycle over the past 5 years, survival of somatic seedlings after 1 growing season has consistently averaged 95%. This indicates that germinants are of a high quality coming out of the laboratory. This high survival count is important because it demonstrates that a single sowing of these germinants into container cavities is an effective use of both somatic seedlings and greenhouse production space.

How the germinants initially respond to the nursery environment will have a profound influence on subsequent morphological development. During the first stages of development in the nursery, past studies have found variable success in obtaining normal shoot growth of somatic, compared to zygotic, seedlings (Webster and others 1990, Grossnickle and others 1994). As a result of this variable performance, initial production runs of somatic seedlings resulted in a higher than desired culling rate (up to 20%) based on British Columbia Ministry of Forests shoot-height culling standards (Grossnickle and Major 1994a).

Recent nursery performance studies of somatic seedlings have shown that a proper nursery cultural environment (that is, nutrients, temperature, and moisture) during the initial establishment stage will result in normal morphological development of seedlings. Shoot growth of zygotic and somatic seedlings was similar during first-year development for seedlings grown during the 1995 nursery production cycle (figure 5). Due to this improved morphological development, culling rates for 1996 have decreased to 5 to 8% (based on British Columbia Ministry of Forests shoot-height culling standards) (Polonenko unpublished data). Thus, currently produced somatic seedlings consistently meet morphological standards required for production of operational containerized interior spruce seedlings (figure 6).

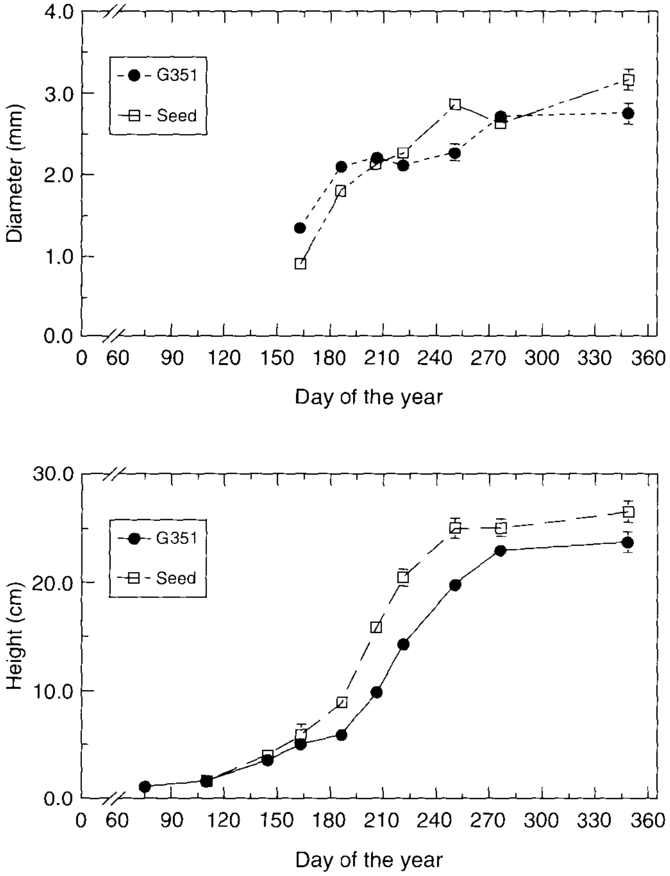

Figure 5 -- *Somatic (G351) and zygotic interior spruce seedling height and diameter growth (mean ±SE) of 1 +0 container stock (for 1 representative clone) from one family during the nursery production cycle.*

Under normal nursery production procedures for interior spruce, seedlings need to develop the proper level of winter hardiness before they are lifted for storage of the 1+0 frozen-stored stocktype or placed outside of the greenhouse into outdoor holding compounds for the second year of a container production cycle for the 2+0 summer-plant stocktype (Simpson 1990). During fall acclimatization, somatic and zygotic seedlings have similar dormancy, freezing tolerance, and root growth patterns (Grossnickle and others 1994). In addition, somatic and zygotic seedlings have similar performance throughout a normal 5-month period of frozen (-2 /C)

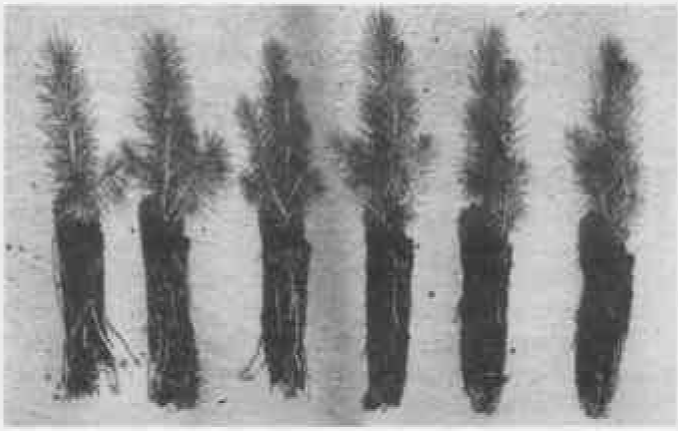

Figure 6—*A comparison of planting stock for conventionally produced interior spruce zygotic (3 on right) and somatic (3 on left) seedlings.*

storage used for holding spring-planted conifer seedlings (Grossnickle and others 1994). This allows somatic seedlings to be treated like normal zygotic interior spruce seedlings during fall and winter nursery cultural practices.

Performance of seedlings on a reforestation site depends upon their inherent growth potential and the degree to which environmental conditions of the field site allow this growth potential to be expressed. To determine a seedling's field performance potential, a stock-quality assessment program needs to use an array of tests that simulate anticipated field environmental conditions (Grossnickle and Folk 1993). This will help forecast seedlings' physiological performance and potential for growth on a reforestation site. Field performance potential testing of somatic and zygotic interior spruce seedlings have found comparable performance capability under both cold (that is, frost and low soil temperature), and drought conditions (Grossnickle and Major 1994a). A similar stock quality assessment program has recently been conducted on the 1996 somatic seedling production run and results are comparable to previous findings (Grossnickle unpublished data). This indicates a consistency in the quality of somatic seedlings to be planted on reforestation sites.

Reforestation site trials have tested the field performance of interior spruce somatic seedlings in comparison to zygotic seedlings (Grossnickle and Major 1994b). These trials have found that somatic and zygotic seedlings have comparable summer seasonal water relation patterns and gas exchange response patterns. In addition, somatic seedlings have comparable or better performance than zygotic seedlings in response to damaging winter conditions. Somatic and zygotic seedlings have corresponding incremental height and diameter growth over at least 2 seasons in the field, resulting in similar new root and shoot development. The survival rates of somatic and zygotic seedlings after 2 growing seasons are comparable, 83 and 77%, respectively. Thus, performance of somatic seedling performance over 2 growing seasons on a reforestation site indicates that they have all of the traits that are desired in container stock for use in forest regeneration programs.

Integration Into a Tree Improvement Program

There is an opportunity with SE technology to not only capture additional gain in height but also take advantage of family differences (Kiss and Yanchuck 1991) and potential clonal differences (Ying 1991) in the resistance to white pine weevil— *Pissodes strobi* Peck— inherent in interior spruce. This clonal strategy involves an "add-on" testing phase to the main breeding program (Sutton and others 1993). The breeding, testing, and selecting of parents for additive gene effects is the main emphasis of the tree improvement program for interior spruce being conducted by the British Columbia Ministry of Forests (Kiss 1968). First-generation clonal seed orchards have been rogued, and genetic gains for improved shoot growth from this orchard were about 11 % based on 10-year height results (Kiss and Yeh 1988). Clonal testing of progeny from first-generation selected parents will capture additional gain for improved shoot growth over and above gains from rogued first-generation seed orchards.

About 1,300 clones have been produced for field trials from 31 full-sib and 14 open-pollinated weevilresistant families of interior spruce using SE technology (Cyr and others 1995; Cyr 1996). Embryogenic callus from all of these clones have been cryopreserved for long-term storage until field selections are made. Parents of the full-sib families were selected, as judged by their progeny, based on 15-year height and 10-year weevil resistance (Kiss and Yanchuck 1991; Sutton and others 1993). The open-pollinated families were selected on the basis of provenance trials (Alfaro 1996). Ramets derived from SE clones are being field-planted to assess for accelerated growth and increased levels of resistance to weevil attacks. Ultimately, a total of 30 to 50 clones will be selected based on mean height growth and weevil damage starting 5 to 6 years after establishment of the trials. The selected clones will be removed from cryostorage and produced as somatic seedlings that will then be deployed operationally to reforestation sites as diverse genetic mixtures. Gains expected from the clonal program will be 22% in height, as well as the deployment of clones with minimal susceptibility to weevil attack.

In parallel with this program, morphological development and physiological performance from a subset of selected clones is ongoing to define parameters that can be used to profile selected clones. Studies have been conducted to assess the clonal variation in morphological development in the nursery and over the first 2 field growing seasons. In the nursery, there is marked variability in the morphological development that occurs between clones within the same family in response to identical nursery culture conditions (figure 7). This variability in nursery performance will have to be addressed in relation to the current concept of producing morphologically uniform stock for reforestation programs in British Columbia (Scagel and others 1993).

Studies have also examined clonal differences in physiological performance at selected times, and within selected environments, throughout the yearly seasonal cycle. These studies have also found that there is a wide range in physiological performance between clones of interior spruce in relation to potential field site conditions (Grossnickle unpublished data). This information will provide a means of defining clones having desired physiological traits that can tolerate limiting field site conditions. The practical application of this program is to develop a series of measurement parameters that can be used to assist in the profiling of clonal material that has been selected through standard genetic selection field trials and will be deployed within operational reforestation programs.

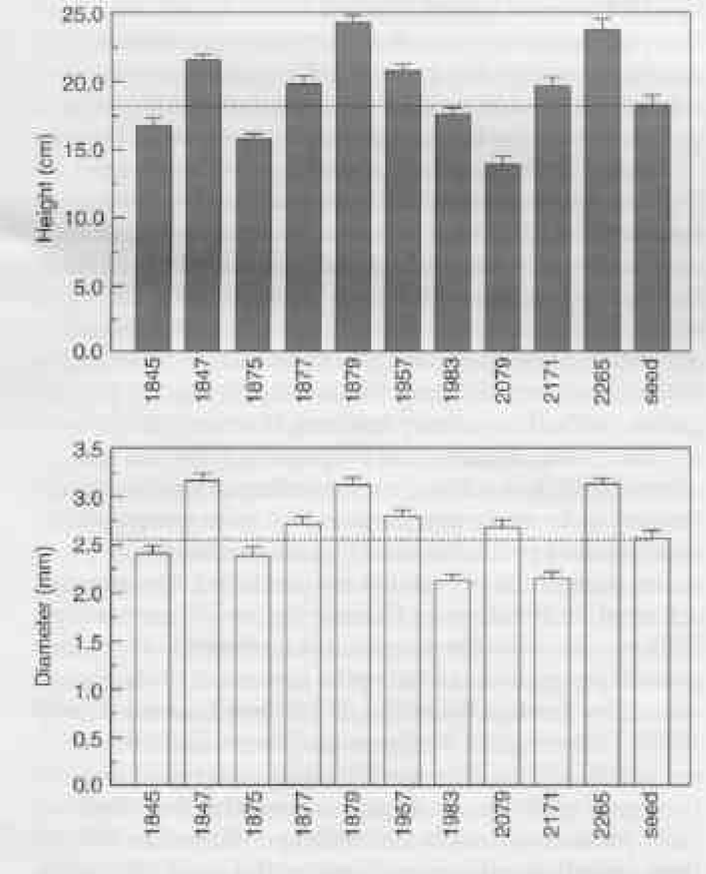

Figure 7—*Somatic and zygotic interior spruce seedling shoot development (height and diameter; mean ± SE) of 1+0 container stock (of 10 representative clones) from 1 family at the end of the nursery production cycle.*

Scale-Up to an Operational SE Production System

The ultimate test for commercial acceptance of a novel technology such as somatic embryogenesis is the ability to develop and implement a successful operational use for the technology. The key components that must be addressed during this stage are

- Development of a cost-effective manufacturing process
- Delivery of high-quality products that provide predictable and reproducible performance
- Technology validation and promotion in the market place

Issues associated with the scale-up of SE production and delivery of SE products will be discussed in this section.

Plant tissue culture processes tend to require significant hands-on manipulation to optimize the quality of plant propagules produced, but the labor costs involved render the products excessively expensive and therefore unacceptable in the marketplace. Attempts to minimize labor costs and improve product quality resulted in the focusing of research priorities during the past decade on automating some or all of the stages required for synseed production and subsequent handling of synseeds and germinated somatic seedlings (Cervelli and Senaratna 1995; Gray and others 1995; Heyerdahl and others 1995; Onishi and others 1994; Walker 1995). However, Cervelli and Senaratna (1995) noted that in spite of all these efforts, there were no successful commercial applications of SE to that date for either angiosperms or gymnosperms.

During the past 18 months, significant progress has been made toward a reliable, high-volume cost-effective SE production system for spruce. The details of the spruce SE process used at the laboratory-scale have been described (Cervelli and Senaratna 1995; Roberts and others 1993, 1995). Rather than trying to develop and optimize automated systems for spruce SE, the major emphasis during this production scale-up period was to overlay the established fundamental principles of manufacturing production planning and control (Wight 1984) on the laboratory-scale SE process. The initial steps required extensive and precise identification and characterization of the inputs, the action steps and related resources and time requirements, and the outputs of each step in the spruce SE process. In addition, computerized inventory tracking programs were developed and incorporated. The information generated provided a clear identification and understanding of the constraints within the process, and enabled numerous modifications and improvements in SE production planning, scheduling and processes. Furthermore, "standard operating procedures" for each stage of spruce SE production were developed and then strictly followed during every production run. The net effect was that SE productivity increased by more than 300% without any additional labor inputs.

Our recent focus of product development has been assessing the compatibility of various commercial horticulture practices with SE, and then use this information to modify SE production practices to improve quality of somatic embryos at the germination stage and somatic seedlings at the nursery production stage. This market-driven approach— that is, awareness and adoption of existing proven technologies-has also resulted in the elimination of problems in the nursery development (establishment and growth vigor) of somatic seedlings (see "Nursery and Field Performance" section, page 51).

Future of this Technology in Reforestation Programs

The use of tree improvement practices to enhance the genetic characteristics of planted seedlings is a forestry practice that consistently shows a high return on investment by increasing yields obtained from planted forests. The use of improved seed is an effective way of bringing genetic improvement to forest regeneration programs. Seed orchards are currently used to produce seeds in large commercial quantities from trees having desired genetic traits. However, improved seed does not provide a method to multiply specific individuals that have desirable traits. Vegetative propagation techniques provide the best means for multiplying the improved genetic resource developed from tree improvement programs.

Two criteria are considered important for the successful implementation of vegetative propagation systems within an operational forestry program (Carson 1986). First, the propagation system must have the ability to preserve superior candidate clones, without genetic change or further maturation, while genetic selection programs are taking place. The ability to propagate elite clones will require a capacity to maintain individuals in a form capable of regenerating after a minimum period of 5 to 10 years that is required in order to test and select clones in the field. Second, the propagation system has to be able to multiply selected clones in large enough numbers at a reasonable cost. If these 2 criteria can be reasonably met, the selected vegetative propagation systems can be implemented within an operational forestry program.

To have a successful rooted cutting production program, donor material must be maintained in a juvenile state to ensure optimum rooted cutting performance. The phenomenon of maturation in conifers is a natural developmental process; this maturation process is a major impediment in maintaining hedge orchards of elite genetic material for continual production of genetically improved rooted cuttings (Zobel and Talbert 1984). Attempts to propagate mature individuals of northern conifer species by this propagation method has been unsuccessful or severely limited by plagiotrophism or other effects of maturation (Hackett 1988).

An alternative practice for rooted-cutting production currently in use to produce juvenile cuttings is seedling-origin hedges. This practice is currently in operational use by the Weyerhaeuser forest company to bulk-up material from elite Douglas-fir— *Pseudotsuga menziesii* (Mirb.) Franco— seed produced in their tree improvement program (Ritchie 1994). With this program, Weyerhaeuser currently produces 3 million rooted cuttings/year. This same cultural approach has been developed for interior spruce (Russell 1990). A limitation of this propagation approach is that it only provides a means of multiplying specific individuals in 1 production cycle, thus eliminating the long-term capture of a genetic source for future regeneration programs.

Vegetative propagation of conifer seedlings from organogenesis on an operational scale has also been conducted on a limited basis. Large scale production and field establishment trials have been conducted on a number of conifer species since the mid 1980's, for example, loblolly pine – *Pinus taeda* L.— in Amerson and others (1984) and Douglas-fir in Ritchie and Long (1986). The major limiting factor in applying this propagation method in forestry has been the cost relative to the low value of individual propagules (Hasnain and others 1986). In addition, most coniferous species have proven fairly recalcitrant to standard micropropagation methods and problems, usually resulting from early maturation of the plants that are produced. The success achieved with radiata or Monterey pine— *Piftus radiata* D. Don— is a notable exception. An operational organogenesis program on radiata pine is currently being conducted by Tasman Forestry Ltd. in New Zealand (Gleed 1995). This program produces just over 1 million propagules, using elite genetic material selected from their seed orchards, on an annual basis for their high-yield plantation forests. Currently, no more than 20% of their annual plantings are clonal, with genetic diversity broadened by selecting only 2 to 3 clones/non-related family within the mixed family groups of 15 to 20 non-related families. If there are currently operational limitations to this system, they are the labor-intensive procedures required in handling cultures to produce quality propagules and the limited ability to store (that is, cold-storage for up to 6 years) while field selections are being conducted (Cheliak and Rogers 1990). The same problem of maturation that occurs in rooted cutting programs can also occur in organogenesis tissue culture programs (Gupta and others 1991). Thus, the higher per-unit propagule cost limits the use of this technology to propagate only the very elite genetic material derived from a tree improvement program, whereas limited storability of cultures reduces the time available to make proper genetic selections.

Somatic embryogenesis is the only vegetative propagation technology that provides long-term preservation of the selected genetic component of a conifer species. Embryogenic cultures can be proliferated in a juvenile form for long periods of time to produce unlimited numbers of propagules from the same clone. These cultures can also be frozen-stored indefinitely while genetic selection is being conducted. Somatic embryogenesis protocols have shown no evidence of somaclonal variation (Eastman and others 1991; Cyr and others 1994), thus ensuring the genetic stability of spruce embryogenic clones. This propagation technology also provides a means of incorporating, through genetic engineering, selected traits that will enhance seedling performance, for example, insect resistance (Ellis and others 1993). Thus, SE is a vegetative propagation technology that will allow for the preservation of superior genetic material that can be used within an operational regeneration program.

Once selections of clones having desired genetic traits are made, these clones need to be brought forward to produce propagules that will then be grown under standard nursery cultural practices for the production of somatic seedlings. Somatic embryogenesis as a viable operational propagation technology for conifer species is just coming of age. Forestry companies and government organization around the world are currently working at bringing this technology to a point where it can produce conifer somatic seedlings, on a cost effective basis, with desired genetic characteristics-for example, Douglas-fir (Gupta and others 1994), loblolly pine (Handley and others 1994), and radiata pine (Smith and others 1994). Currently the program at the Forest Biotechnology Centre and Silvagen Inc., BCRI, is the furthest along in the scale-up process of this propagation technology. The production of somatic interior spruce seedlings has grown steadily from an initial production run of 12,000 somatic seedlings in 1993 to 350,000 somatic seedlings in 1996. It is anticipated that 600,000 somatic seedlings will be produced in 1997 with the target of 1,000,000 somatic seedlings for 1998.

Somatic embryogenesis of interior spruce has developed to the point where an operational production program has been initiated. The emphasis of this program in the coming years will be to scale up this propagation technology while reducing the cost per production unit. At the same time, the overall mandate will be to improve the quality of somatic seedlings from a range of genetic sources having desired field performance characteristics. This will allow for the successful implementation of SE technology as a viable vegetative propagation system within operational forestry programs.

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Container Seedling Planting With Manual Microsite Preparation for Species Restoration

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Difficulties in achieving good soil-root contact when planting container seedlings in undisturbed forest soils containing living roots resulted in the adaption of a manual microsite preparation technique using heavy tree-planting spades. Microsites that were an admixture of mineral and organic soils, were produced on boot-screefed spots by repeated vertical insertion of the spade at various angles. Shortrooted container seedlings planted in these microsites on hardwood strip cuts resulted in good survival and growth. Tree Planters' Notes 47(2):58-61; 1996.

Human activities combined with natural disturbances have altered the species composition of large areas of eastern Canada's forest; for example,

- Harvesting practices have resulted in the conversion of red spruce *(Picea rubens* Sarg.)– balsam fir *(Abies balsamea* (L.) Mill.)– eastern hemlock *(Tsuga canadensis* (L.) Carr.) forests into balsam fir forests that are subject to massive destruction by spruce budworm (Blais 1983; Weetman 1994).

- Fires in the stem-exclusion phase of post-harvest succession often result in hardwoods such as white birch *(Betula papyrifera* Marsh.) and red maple *(Acer rubrurn* L.) dominating the site for over a century (Seymour 1992).

- Forest plantation programs on well-drained, upland, fertile, Acadian mixedwood sites have emphasized pioneer conifer species such as black spruce *(Picca mariana* (Mill.) B.S.P.) and jack pine *(Pinus banksiana* Lamb.), which cannot regenerate themselves on these sites after harvest because of intense competition from various early successional species; the resulting forest often has lowered species diversity and decreased management options.

Restoration programs have been implemented to rebuild original species assemblages and increase future management options. Restoration programs in forestry have recently included ecosystems in which only some species are missing; the goal is the reestablishment of biodiversity and the level of heterogeneity inherent in undisturbed systems (CCFM 1995; Harrington 1995). Restoration strategies may include planting to reintroduce missing species and should provide the conditions for heterogeneity to develop without predetermining which species will predominate (Noss 1994; Trombulak 1996).

Fill planting, underplanting, and gap planting have been dealt with from the standpoint of light availability for planted seedlings (Helgerson 1990) and size of planting stock (Needham and Clements 1991; St-Amour 1995); however, very few published studies deal with the mechanics of planting seedlings in these situations, where soil preparation by tillage equipment is not possible.

There has been some experimental work with rakes mounted on tracked excavators and mixing attachments for small tractors to prepare plantable microsites in forest stands that have been partially cut or treated as shelterwoods (FERIC 1996).

Planting in the absence of mechanical site preparation can be accomplished by **dughole methods** (Gordon and others 1995), however, these are laborious and very time-consuming. More common are **compression methods** (Smith 1986) by which planting slits are produced by pushing soil aside using sharp tools such as dibbles, shovels, and planting guns and **extraction methods** (Cormier 1994) by which a soil core, equivalent in size and shape to the container seedling root plug (StAmour 1996), is removed; both these methods run the risk of compacting or glazing and smearing soil on the walls of the planting hole and deforming the roots' growth and form (Balisky and others 1995).

Operational fill planting in New Brunswick cutovers, stocked at less than 60% with natural softwood regeneration, increased from 10% of the total reforestation effort in 1991 to 30% in 1993. This technique is believed to protect natural regeneration, avoid site disturbance, and increase commercial species stocking at reduced cost. However, planting has been done exclusively by compression methods in unprepared soils to produce planting holes and survival, growth, root form, and tree stability have not been assessed for this fill-planting methodology.

Evolution of a Technique

During our studies involving restoration plantings of shade-tolerant and intermediate species in areas where they had been eliminated by unsustainable harvesting practices, fire, or intensive plantation culture, most of the candidate planting sites were either too small to accommodate heavy tillage equipment or were in areas such as municipal watersheds where the use of such machinery was not allowed.

Our main experience has been in planting container seedlings with dibbles, spades, and planting guns in friable, freshly disturbed, mineral soil resulting from the use of various plows and scarifiers. We tried using these tools on undisturbed soils after boot-screefing off most of the organic horizon, but the presence of living roots of brushy vegetation and adjacent trees, as well as undecomposed roots attached to freshly cut stumps, made for problems in achieving good contact between soil compressed against the sides of the hole and the rooted plugs.

Our solution has been the use of heavy plug spades. Light boot-screefing is done so that the level of mineral soil at the base of the humus layer can be identified. The long (135 cm) wooden-handled spade is plunged through the remaining humus into mineral soil 5 to 8 times to a depth of approximately 15 cm. The blade (measuring 19 x 11.5 cm) is heavy enough (1.46 kg) that this operation is not overly tiring. The blade is rotated about 45/ at each insertion so that organic and mineral soils are mixed together to produce a patch of friable material about 15 cm in diameter. Most of the living roots in the patch are severed by this "cultivation." On the final insertion, the blade is left embedded in the mixed soil and moved back and forth in a 30/ arc to open a hole for inserting the rooted plug. Short-rooted plugs (6 to 8 cm long) from PANTH and Jiffy container systems are easier to insert than longer rooted plugs from various multipot container systems. Loose soil is pulled around the rooted plug by hand and pressed in place by the palm of the hand or a boot. Finally (and this is recommended with any container seedling planting method), loose organic material (leaves, needles or organic horizon materials) is swept over and around the base of the planted seedling to diminish the wicking of moisture from the exposed organic-mineral soil mixture.

Disadvantages

The planting rates achieved by people using a variety of tools on mechanically prepared soil microsites are not possible when producing spade-mixed microsites. A minimum of 30 seconds is required to find (probing with the planting spade) an area free enough from large subsurface rocks and to "cultivate" a 15-cm-diameter patch and then plant the seedling. This is in contrast to approximately 10 seconds of actual planting time when a variety of tools are used on mechanically prepared sites. Mechanical site preparation usually provides slash-free travel lanes for planters as they move from one spot to the next; this ease of movement is not possible when moving across debris-strewn cutovers.

Advantages

Manual microsite preparation can be used when planting steep slopes and partial harvest situations such as shelterwoods where tillage equipment is difficult or impossible to use. The costs of capital and energy-intensive tillage are avoided. There is little destruction of natural regenerants as manual microsite preparation produces soil disturbance on less than 1% of the ground surface at the highest planting densities. Discretionary planting is facilitated in the absence of regular planting lines produced by tillage equipment; planting spots can be chosen far enough to the south of major light competitors, such as sprouting stumps, to enhance the chances of survival and adequate growth rates. Proximity of nutrient-rich humic material and uninhibited root extension (Balisky and others 1995) is facilitated by a mixed planting spot many times the diameter of the rooted plug. Frost heaving of the seedlings, which is a problem on the large planting spots of bare mineral soil produced by mechanical tilling, can be diminished by the production of small microsites that are an admixture of mineral and organic soils (BCMF 1994).

Trial Plantation

We planted winter greenhouse-reared Jiffy '96' seedlings in July 1994 and summer greenhouse-reared seedlings, which were hardened-off and freezer-stored over winter, in May 1995. The Jiffy system uses dry mesh-enclosed peat pellets that are expanded by water soaking before seeding. The round peat plugs when fully expanded are capable of supporting free-standing seedlings that are completely air-pruned because the plugs are held together by mesh. Expanded size is 38 mm diameter and 68 mm high, with a rooting volume of about 80 cm^3; growing density is 590/m^2. Seedling characteristics at the time of field planting are shown in table 1.

Planting was done in strip cuts of several widths in white birch-red maple stands on a municipal watershed forest. The planting intervention was extensive on a very wide grid (5 by 5 m) and was designed to facilitate the study of competitor-influenced light regimes on the

Table 1— *Container seedling characteristics at planting on manually prepared microsites and field measures after several growth seasons*

Species	Strip width	Planting date	Planting height. (cm)	Planting volume (cm^3)	% Survival 8/96	Volume 8/96 (cm^3)
Red spruce	10	7/94	20.4	0.66	97	11.8
	20				83	10.1
	30				96	11.5
	10	5/95	21.4	0.86	94	5.9
	20				75	6.8
	30				83	7.0
White spruce	10	7/94	18.1	0.72	100	9.4
	20				100	10.3
	30				89	22.1
	10	5/95	22.3	0.89	100	11.5
	20				100	11.6
	30				94	13.1
White pine	10	7/94	9.5	0.50	100	14.2
	20				79	19.2
	30				100	13.0
	20	5/95	7.15	0.14	79	4.2
	30				100	9.0

growth of shade-tolerant and intermediate species. This trial will complement similar work with planted pioneer species in large clear-cuts (Salonius and others 1991). Planting in the north to south-oriented strips included positions 5 m within the uncut leave strip, the edge of the cut strip, and at 5-m intervals across the strip. The long-term goal was to reintroduce species to the Acadian mixedwood site that would ultimately produce regenerants capable of being managed by some form of partial harvest silviculture.

Preliminary Results

Field measures of seedlings, after several growing seasons, are shown in table 1. Growth of container seedlings planted on manually prepared microsites has been encouraging. Survival is similar to that achieved by the largest planting program in Canada (Johnson 1994). Growth has generally been more vigorous in the wider strips, which offer both competing vegetation and planted seedlings access to greater amounts of direct sunlight during the growing season. We are especially interested in the growth of red spruce in the wider strips. Red spruce tends to suffer from exposure and winter drying when planted in the open and we surmize that the orientation of the cut strips, perpendicular to the most damaging winter winds, has produced this result.

Conclusions

Manual mixed microsite preparation, coupled with the planting of short-rooted container seedlings, has been successful. Controlled experiments are necessary to compare the growth and survival of seedlings planted in slits produced by compression methods with growth and survival of seedlings planted using the method developed in this study.

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Cold Hardiness Testing to Time Lifting and Packing of Container Stock: A Case History

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A model of cold hardening and dehardening was developed for ponderosa pine (Pinus ponderosa *var.* scopulorum *Engelm.) and Douglas-fir* (Pseudotsuga menziesii *var.* glauca *(Beissn.) Franco), and was used in conjunction with cold hardiness testing to decide when to begin lifting and packing container stock into cold storage, and when packing should be completed.* Tree Planters' Notes 47(2):62-67; 1996.

Over the last 15 years, physiological tests have been developed and are coming into widespread use in reforestation programs to measure and track the quality of planting stock (Tanaka and others 1994). One of the most useful characteristics to test for is cold hardiness— not only because trees usually need some degree of hardiness to tolerate cold storage and the conditions they will encounter on the planting site but also because it is a useful indicator of root growth potential (RGP) and bud dormancy (Burr and others 1989; Tinus and others 1986). Furthermore, cold hardiness is quicker to measure than RGP or bud dormancy, and given enough data, lends itself to creation of predictive models. This paper describes the use of cold hardiness testing to

- Aid in deciding whether to accept a contract-grown container seedling crop.
- Produce a predictive model of cold hardiness.
- Determine when lifting and packing into cold storage should begin, and when it should be completed.

In February 1995, there was concern about container seedlings received at the USDA Forest Service Payson Ranger District in Arizona that (1) they may not have been dormant, (2) buds were not present or not well developed, (3) there was too much root growth, (4) they got too warm in transit, and/or (5) the caliper was too small. Although a morphological examination yielded tentative answers to all of the concerns, I tested them to provide additional information in support of a management decision on whether to accept and plant the seedlings.

Samples of ponderosa pine— *Pinus ponderosa* var. *scopulorum* Engelm.— seedlings were tested for cold hardiness by freezeinduced electrolyte leakage (FIEL) and RGP. The test for FIEL can give precise answers and be completed in 3 days. Determination of RGP takes 7 days (Burr and others 1987). Both tests have proven useful for management decisons (Burr and others 1986, 1989). The 50% index of injury from FIEL was -18 /C, which is about half of maximum cold hardiness, and the shape of the cold injury versus temperature curve suggested that although the seedlings were losing hardiness, the RGP was high. On this basis, I recommended that the trees be accepted and planted. However, the cold hardiness tests indicated that packing should have been completed some time earlier.

Because the Forest Service had contracted with the same nursery to produce a crop for 1996, I offered to model cold hardiness as a function of temperature and photoperiod for ponderosa pine and Douglas-fir— *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco— so that, in the fall and winter, cold hardening could be tracked in real time using local weather records. The model could be used in conjunction with FIEL and RGP tests to tell the nursery when to begin lifting and packing into cold storage and when it should be finished.

The model was developed using data from a growth-room study in which ponderosa pine and Douglas-fir were exposed to a simulated fall, winter, and spring over 25 weeks (Tinus and others 1995). Cold hardiness was measured weekly as the trees hardened and dehardened under 5 different combinations of photoperiod lengths and day and night temperatures (figure 1). Rates of hardening and dehardening were calculated by linear regression on segments of the hardening curve (figure 2), and the regressions assembled to yield daily rates of hardening as a function of temperature (figure 3). Other rules governing whether the trees would harden or deharden in response to photoperiod, temperature, and degree of hardiness were factored in based on previous work (Burr and Tinus 1988; Leinonen and others 1995). The complete model is presented on page 67.

Daily maximum, minimum, and average temperatures from the reporting weather station at the New Mexico State University Horticulture Farm for the last 4 years (figure 4A-D), were used to model the cold hardiness of ponderosa pine and Douglas-fir for the months of October through February (figure 5A-D). The lifting and packing window was considered to open when the 50% index of injury fell below -22 /C. Previous work has shown that at this point RGP has doubled or more

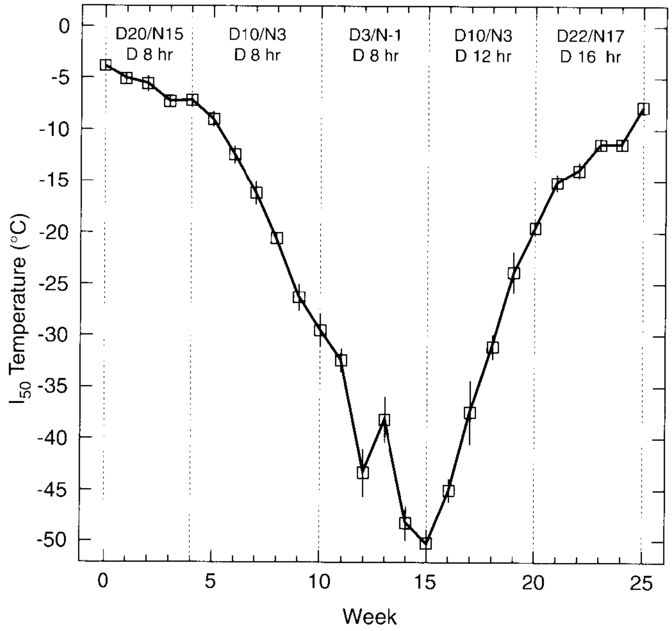

Figure 1—*Douglas-fir cold hardiness measured by freeze-induced electrolyte leakage during a 25- week growth-room experiment. Day/night temperatures and photoperiod are indicated for each segment at the top. Error bars are the 95% confidence intervals.*

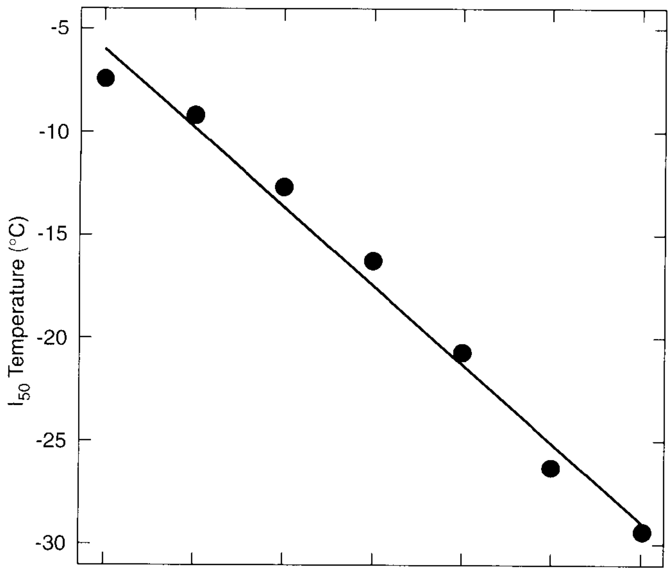

Figure 2—*One of several regression lines calculated from segments of figure 1. The slope of the line is the rate of cold hardening.*

from its late summer low, the chilling requirements for budbreak have been met, and the seedlings will continue to gain cold hardiness when placed in cold storage (Burr and others 1989; Tinus and others 1986). The

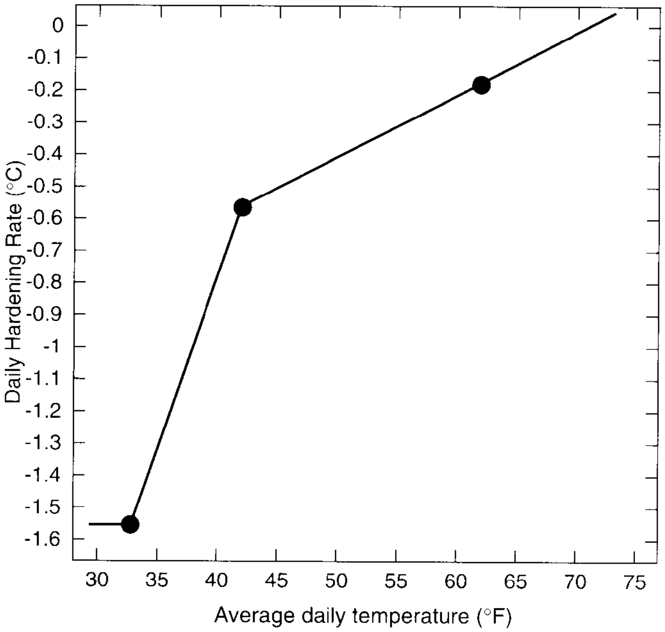

Figure 3—*Cold hardening rates of Douglas-fir as a function of average daily temperature (°F were used to avoid having to convert all the weather records).*

packing window was considered to close when a significant amount of cold hardiness had been lost, in this case about 5 /C from the maximum.

Examination of figure 4A-D shows that year-to-year temperature patterns are different, causing differences in timing and rate of hardening and dehardening, and the degree of hardiness achieved (figure 5A-D). The 1 measurement taken in February 1995 (figure 50 showed that the model underestimated cold hardiness of ponderosa pine by about 3 /C. A possible reason for this is that the New Mexico State University Horticulture Farm weather station is about 100 to 150 m lower in elevation and on the edge of the heat island created by the city of Las Cruces but the nursery is 16 km away. This means that the seedlings at the nursery probably had been exposed to temperatures a few degrees colder than the temperatures used to drive the model. The model also indicated that packing should have been completed in midJanuary.

I began modelling cold hardiness in early November 1995 so that the Forest Service contracting officer could be notified when the nursery should start lifting and when packing into cold storage should be finished. The NMSU Horticulture Farm's weather data are updated daily and are available on the Internet (http: / /weather.nmsu.edu/sum96/nmsue96.sum gets you the 1996 data, for instance), which allowed weekly updating of the model (figure 5D). This was important, because each year is different, and one cannot predict the weather accurately more than a week in advance.

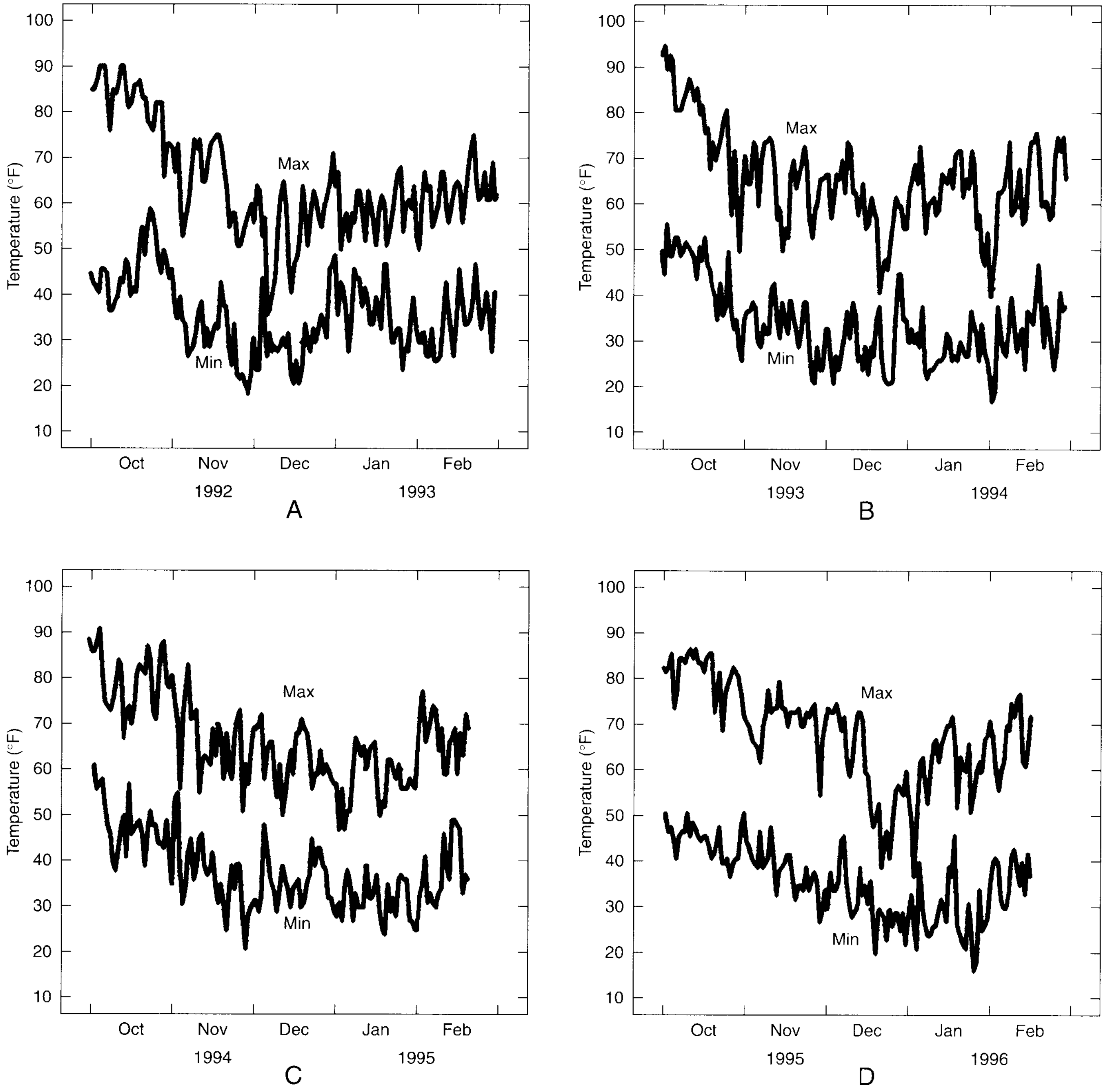

Figure 4—*Daily maximum and minimum temperatures at the New Mexico State University Horticulture Farm, Las Cruces, NM.*

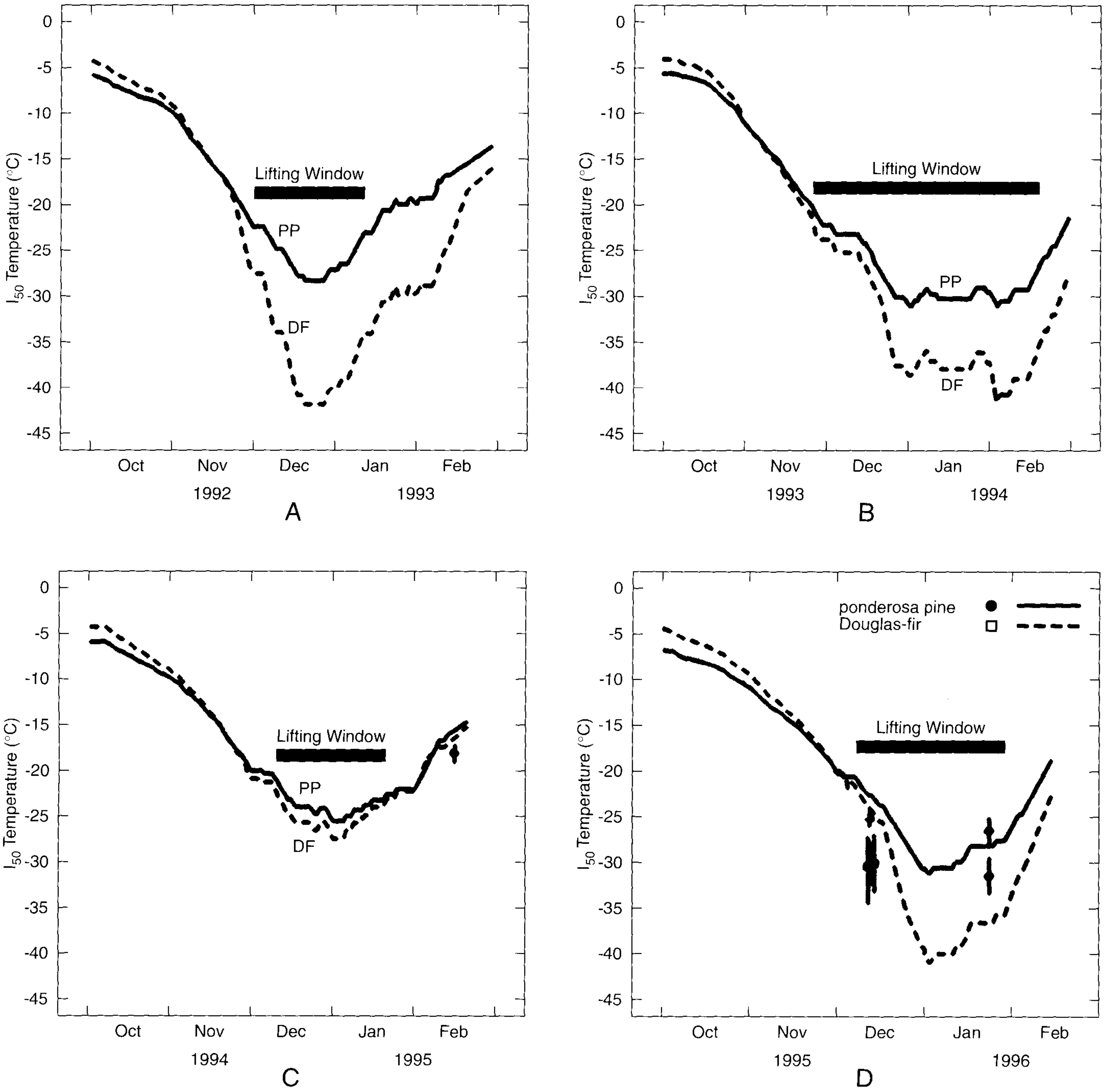

Figure 5—*Cold hardiness of ponderosa pine and Douglas-fir as estimated by the model (solid and dashed lines) and measured by FIEL (data points; error bars are the 95% confidence intervals). The lifting window opens when the 50% index of injury reaches −22 °C and closes when 5 °C of cold hardiness have been lost from the maximum.*

The model indicated that the lifting window opened December 8, because the 50% a index of injury for both ponderosa pine and Douglas-fir had reached -22 /C. The nursery sent samples from 2 lots of each species by overnight mail for FIEL and RGP testing. Once again, the model underestimated cold hardiness, and by December 13, it was clearly time to begin lifting and packing. This was 3 weeks earlier than originally planned (figure 5D). The RGP was very satisfactory, ranging from 23 to 33 roots/seedling after 7 days in a mist chamber at 19 /C.

The model indicated that maximum cold hardiness was reached on January 2 and was being lost thereafter due to rising maximum day temperatures (figure 4D). However, the loss was slow thoughout January because of continued subfreezing night temperatures. By January 18, it looked as though the end of the packing window was about a week away, so another set of samples was tested. Two lots of ponderosa pine and no Douglas-fir were sent, representing only trees that had not yet been packed and from different seedlots than were tested in December. The trees were still quite hardy and close to what the model predicted, but the 2 lots were significantly different (figure 5D). The lot with the greater hardiness had been stored outside, while the less hardy one had been kept in a greenhouse. The RGP was 30 and 39 roots/seedling for the 2 lots. The measurements confirmed that as of January 24, it was still OK to pack, but that packing must end soon, as local temperatures were rising.

Packing was completed January 30, and it is expected that the seedlings in cold storage were fully dormant and in good physiological condition. Had the model not been run in real time and its results made available to the contracting officer weekly and confirmed by 2 sets of FIEL and RGP tests, lifting and packing would have been started after January 1 on a schedule planned months earlier based on guesswork and managerial convenience, and probably would have continued into late February once again, well after the packing window ended.

This case history shows how the use of a cold hardiness model in conjunction with testing can be a valuable and practical strategy for determining lifting and packing windows. However, the information will not be complete until field performance of seedlings lifted and packed within and outside of the modelled windows are compared.

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Cold Hardiness Model of Rocky Mountain Ponderosa Pine and Douglas-fir

A model was produced to estimate the cold hardiness of ponderosa pine and Douglas-fir being grown at Las Cruces, NM, based on daily maximum, average, and minimum temperatures and the dynamics of cold hardiness that have been learned over the last 10 years, primarily at Fort Valley, AZ, in 1988 and the experiments run in New Zealand in 1993–1994. The model is not very smooth but gives answers that look very reasonable.

General Rules for Both Species

1. No hardening occurs before October 1. Photoperiod is too long and temperatures too warm.
2. Minimum hardiness is assumed on October 1 and hardening proceeds according to the temperature-dependent first stage.
3. Warm temperatures do not deharden until LT_{50} reaches − 22 °C. Then warm temperatures do reverse hardening. This could happen in November.
4. In December below-freezing nights permit hardening with maximum day temperature up to 15.5 °C. No hardening occurs if maximum day temperature is warmer. If minimum night temperature is warmer than +1.5 °C, then no hardening occurs, if day temperature is greater than 10 °C. If maximum day temperature is greater than 15.5 °C and minimum night temperature is greater than 4.5 °C, then dehardening occurs.
5. In January and February, dehardening occurs if maximum day temperature >10 °C, and mimimum night temperature >3 °C. Dehardening occurs if maximum day temperature >15.5 °C and minimum night temperature >0 °C. Hardening occurs when maximum day temperature <5.5 °C and minimum night temperature <0 °C. If none of these conditions prevail, hardiness does not change.

Species-Specific Rules

PONDEROSA PINE:

Minimum cold hardiness: $LT_{50} = -6.5$ °C

Hardening rates:
When average daily temperature (T) >5.5 °C, then daily change (CH) in LT_{50} is: $CH = -0.675 + 0.0318*T$
When average daily temp < 5.5 °C, then daily change in LT_{50} is 0.50 °C and does not change with temperature.

Dehardening rates:
When LT_{50} is lower than −16 °C, daily loss of hardiness = 0.59 °C.
When LT_{50} is warmer than −16 °C, daily hardiness loss = 0.19 °C.

DOUGLAS-FIR:

Minimum cold hardiness: $LT_{50} = -3.9$ °C

Hardening rates:
When average daily temperature (T) >5.5 °C, daily change in LT_{50} is: $CH = -0.75 + 0.0345*T$
When average daily temperature is 5.5 °C, >T<0.5 °C, daily change in LT_{50} is: $CH = -1.649 + 0.198*T$
When average daily temp T< 0.5 °C, daily change in LT_{50} is −1.55 °C and does not change with temperature.

Dehardening rates:
When LT_{50} is lower than −18.3 °C, daily loss of hardiness is 0.87 °C.
When LT50 is warmer than −18.3 °C, daily loss of hardiness is 0.27 °C.

Influence of Seed Position on First-Year Survival and Growth of Directly Seeded Northern Red Oak

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A study was conducted in Quebec to select the best technique for sowing northern red oak (Quereus rubra *L.*) *in the field. This study examined the effect of planting position on seedling growth and the direct seeding methods to achieve the best early survival and growth. The seed positions tested were sideways and with tips down or up. Sowing methods compared were hand throwing and deposition of seeds through a tube held at 3 different angles (vertical, 45%, and 30%). The recommended sowing method takes into account the survival and growth for three planting positions, the proportion of seeds landing in the prepared slits, and the sowing time. Acorns planted with tips pointing down resulted in the lowest root biomass, crooked taproots, and the highest shoot-to-root dry weight ratios after the first growing season. Planting position had no statistically significant effect on seedling early survival and growth. Weed competition reduced the straightness of the taproot. Two sowing techniques were tested. Sowing was more efficient when seeds were hand-thrown into perpendicular slits cut with a lawn edger.* Tree Planters' Notes 47(2):68-75; 1996.

Direct seeding can be an appropriate means of regenerating northern red oak *(Quercus rubra* L.) on sites where the natural seed supply is deficient, provided that sowing techniques are appropriate (Kolb and others 1989). Various factors, notably site quality, depth of sowing (Auchmoody and others 1994; Schopmeyer 1974; Wilkinson and others 1992), and predation (Johnson 1994; Thorn and Tzilkowski 1991) are thought to affect survival and early growth of oaks. The influence of seed weight has also been extensively studied (Auchmoody and others 1994). The position of seeds as a factor in the survival and early growth of oaks has not been considered. Theoretically, some seed positions could initiate morphological deformation, mainly of the root system. In this article, we seek answers to the following questions:

- What morphological differences in seedlings result from the position of the seed in the ground?
- In which positions do seeds tend to fall?
- Can the proportion of seeds in the best position for

Survival, height growth, dry weights of organs, and number of roots are among the most frequently used variables to assess the success of regeneration (Beck 1970; Gordon 1988; Kolb and Steiner 1989; Stroempl 1985; Trencia 1995). Differences in architectural characteristics of root systems also occur with changes in growth conditions (Fitter and Strickland 1992; Trencia 1995). Topological variables are useful for succinctly describing the architecture of the root system independently of its dimensions. The topological diameter of a root system is the highest number of ramifications from the root collar to the apex of a root tip; it is correlated to the energy cost of building the root system and to the efficiency for water transportation (Fitter 1985). Acorns will germinate on the surface of the ground. However, most unburied acorns are subsequently displaced or destroyed by rodents (Auchmoody and others 1994). Natural burial of red oak acorns is mostly performed by grey squirrels in single--acorn caches in the ground about 3 cm deep (Thorn and Tzilkowski 1991). Recommended planting depth varies from 0.5 to 3 cm (Schopmeyer 1974; Williams and Hanks 1976). Direct seeding of oak usually involves dropping seeds into spots in the soil. The incision can be made with a light instrument. Lawn edgers are well suited for this task (figure 1); they cut to the appropriate depth and are relatively light, inexpensive, and readily available. In this study

- We compare first-year growth of seedlings developing from seeds placed in the soil in 3 positions.
- We also evaluate how sowing techniques may increase the ratio of seeds falling in what is determined as the ideal position.
- We choose a sowing technique that combines efficiency of delivery with optimal positioning of seed for growth.

Materials and Methods

Northern red oak seeds were collected in October 1991 from a single stand located at Sainte-Croix, Quebec. All the seeds were immersed in fresh water for

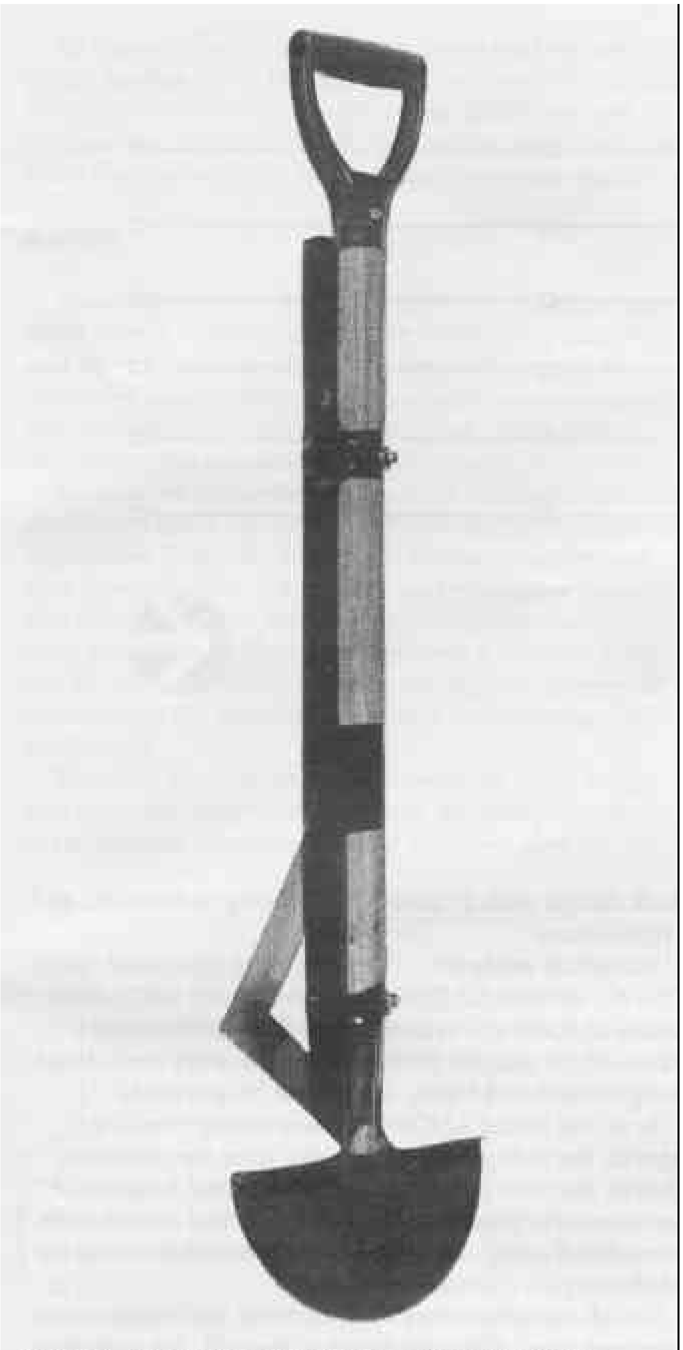


Figure 1—*Lawn edger with tube and triangle attachments*

2 days; floating seeds were then discarded. The healthy seeds were sun-dried and inspected for insect larvae, mainly *Curculio* sp. The seeds were then stored at 5 /C until mid-April 1992 and stratified afterwards (Struve and others 1991).

Sowing was carried out in May 1992. The trials were conducted at Cap-Tourmente (latitude 47/ 04' north, longitude 70/ 48' west), approximately 50 km east of Quebec city. The soil was a loamy clay of the Maheux series (Marcoux 1981), with no clearly defined horizons. The site had been cultivated until 1963; a poplar plantation was subsequently established on it.

The experiment consisted of 1 "biological" trial to assess the growth of seedlings and 3 "direct seeding techniques" trials to evaluate sowing techniques. We used a randomized complete block design, located in a small clearing of a poplar plantation, for the seedling survival and growth trial. The 3 trials of direct seeding techniques comparing the sowing methods were done under planted rows of hybrid poplar, approximately 50 m from the growth trial.

Seedling survival and growth trial. Seedling growth for 3 positions of acorns in the seed spot were replicated 6 times, for a total of 18 cells, in a randomized complete block design (figure 2). The cells consisted of 1 row of 50 seeds, each spaced 15 cm apart within rows and 1 m between rows. The seed positions compared were sideways (position 1) and with tips down (position 2) or up (position 3).

One-year-old seedlings and 4 samples of herbaceous vegetation were collected from each cell of the block design. To extract the seedlings, the surrounding soil was lifted and seedlings were soaked in water to remove the soil from the roots. We analyzed 222 seedlings that did not suffer root breakages at extraction and collected aboveground portions of herbaceous vegetation from 4 small plots of 400 cm^2, 15 cm from each row of seedlings.

The topological diameter of each seedling was calculated as the maximum number of root ramifications from any root tip to the root collar. The angle between the sections above and below the root collar was measured with a protractor. The maximum angle between taproot sections above and below the point of attachment of the acorn was 180/, corresponding to a straight taproot.

All dry weights were measured after oven drying at 110 /C for 24 hours. Variables calculated were total height, stem and root dry weight, topological diameter, angle of deviation of the taproot, and aboveground dry mass of herbaceous vegetation.

Direct seeding techniques. The acorns were spaced about 60 cm apart, the planters sowing a seed with each step. A T-shaped seed spot was created by cutting 2 perpendicular slits in the ground with a lawn edger. Seeds were then directed into the prepared slits either by hand or through a guiding tube attached to the lawn edger (figure 1). The objective of the using a guiding tube is similar to that of the Pottiputki planter, which deposits container seedlings vertically (shoot up) in the intended spot. We assumed that a seed rolling within the tubing would turn on its side and fall in that position into the slit. Seeds were also expected to roll better if the tube was held at an angle instead of vertically.

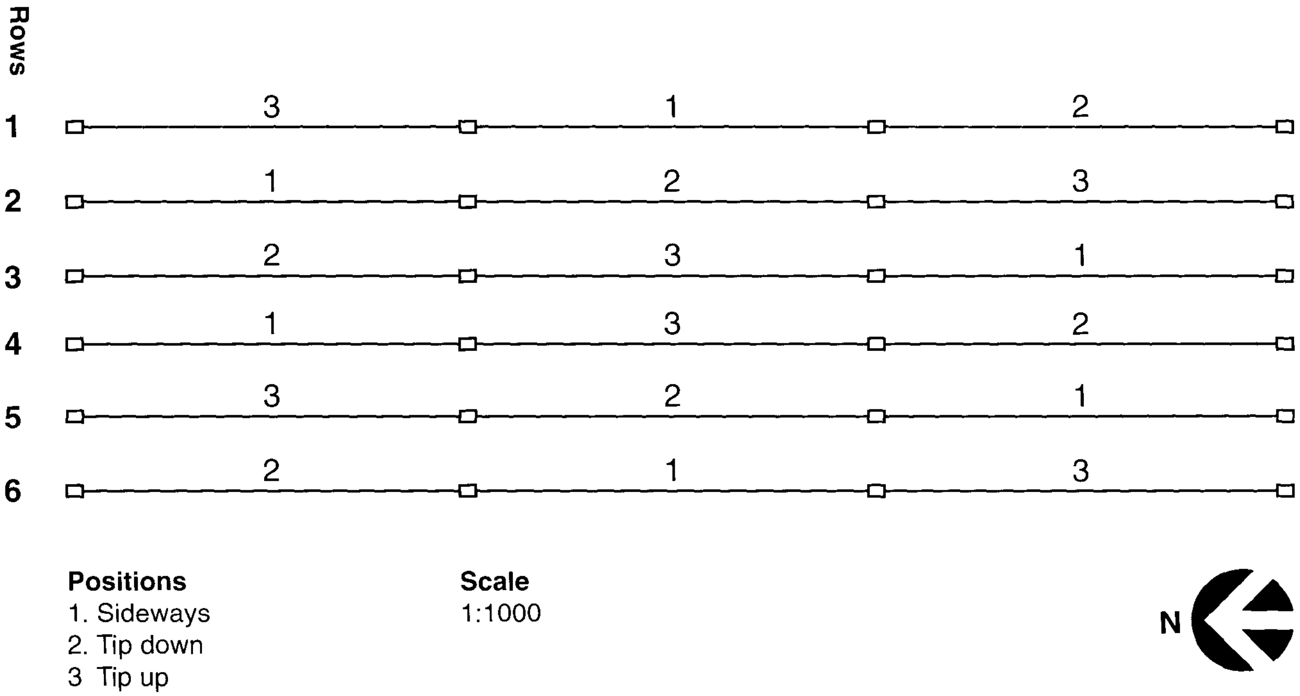


Figure 2—Experimental layout of the seedling survival and growth trial.

Thus, attaching a tube to the side of the lawn edger seemed to be a low-cost practical way of controlling the location and positioning of acorns. The seed positions were the same as in the growth trial: acorns on their sides (position 1) or with tip down (position 2) or tip up (position 3).

In the first trial of direct-seeding techniques, we looked at how the angle of the guiding tube relative to the soil affected the positioning of the seeds in the slit. The design compared the effect of 3 angles of the seeding device (vertical, 45/, or at 30/) and of the 2 operators. The angle was controlled with an appropriate triangle attached to the handle (figure 1).

In the second trial of direct-seeding techniques, we compared the 2 different sowing methods: hand-throwing the seeds into the slits or depositing them through the tube attached to the lawn edger's handle. Because we believed that the operators would improve with practice, we sowed 2 replications of 50 seeds. We recorded the number of seeds falling in the intended location (that is, in or out of the slit) and the position of seeds in the slits. Thus, the 2 sowing methods, the 2 operators, and 2 replications were tested in this second trial.

In the third trial of direct-seeding techniques, we compared the time it took to sow batches of 100 acorns by hand or through the guiding tube mounted on a lawn edger. The layout was a randomized complete block design with 2 operators, 2 sowing techniques, and 4 replications.

Statistical analysis. Statistics were computed using SYSTAT, version 5.2 (Wilkinson and others 1992). All values of F and chi square higher than the threshold values of 5% and 1% probability levels were considered as significant and highly significant, respectively.

In all the initial ANOVA models testing seedling growth, the independent variables were the treatment (that is, the seed positions), replication and biomass of herbaceous vegetation (VegGM2). Survival results were normalized using the weighted logit transformation for small samples (Fernandez 1992).

For all variables other than survival, cell values were averaged over all the seedlings of the cell. For each significant ANOVA, orthogonal contrasts tested 2 hypotheses. First, growth for positions 1 and 3 did not differ. Second, growth for position 2 differed from that of the 2 other positions.

Significant factors of the first 2 direct-seeding-techniques trials were determined using log linear models. These models compared the predicted and observed number of seeds in each position or location. The models retained showed no significant difference at P values above 0.05, according to Agresti (1990) between the observed and predicted values and

In the last direct-seeding-techniques trial we compared sowing times by analysis of variance, where independent variables were instruments, operators, and replications. In this analysis, interactions were removed from the final analysis because they were not significant.

Results

Seedling survival and growth trial. Median survival after 3 months was 66% for seeds lying sideways and 40 to 44% for seeds placed vertically (figure 3). Weighted logit transformations of survival were not significantly different (F = 0.886, P = 0.44). Herbaceous vegetation did not affect survival (F = 4.3, P = 0.068).

Biomass of competing herbaceous vegetation had no significant effect except in relation to the straightness of the taproot (F = 7.29, P = 0.024). Herbaceous competition, however, was not random and was more important along a line running from centre front (row 3, column 1) to back left of the design (row 6, column 3, figure 4). Topological diameters were slightly different (P = 0.046) when the effect of vegetation biomass was not considered.

The seed position had no influence on mean height and stem dry weight (table 1). For the morphological characteristics considered (table 1), there were no significant differences between seedlings from acorns sown sideways (position 1) and with their tip up (position 3). Sowing the acorns vertically with the tips down (position 2) resulted in a reduction of the root dry weight, the straightness of the taproot (figure 5), and the topological diameter. Lower root dry weight increased shoot to root ratio for acorns sown tips down, compared with acorns in the other 2 positions.

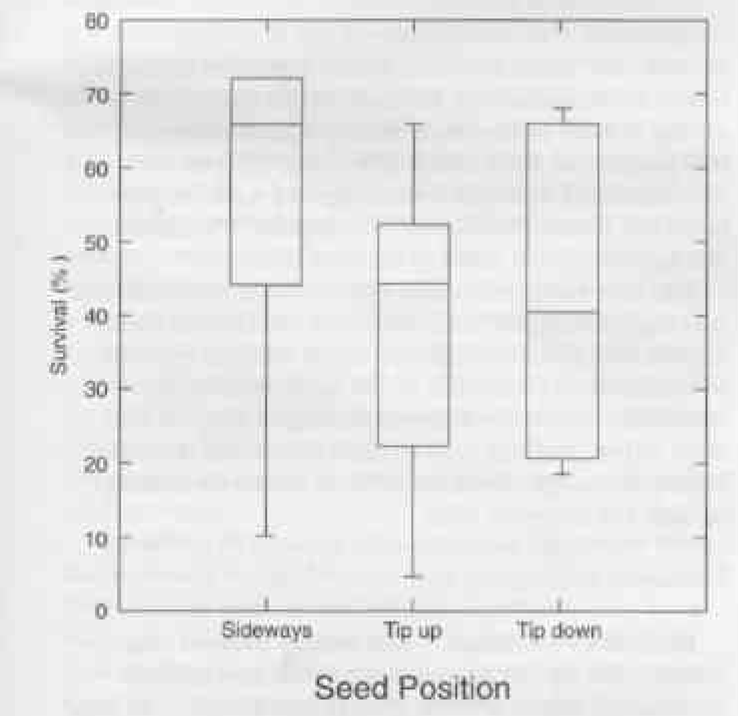


Figure 3—*Box plot of survival ratio per treatment. The horizontal bar in the box shows the median. Upper and lower edges of the boxes represent the 25th and 75th percentiles, respectively, and the bars show the 5th and 95th percentiles.*

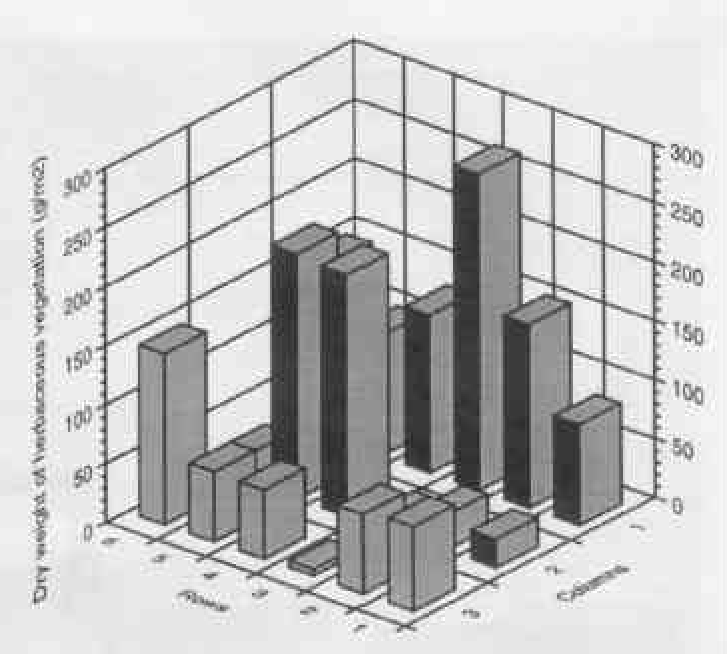


Figure 4—*Dry weight of herbaceous vegetation for the cells of the randomized complete block design. Distances between rows and columns are not to scale. The columns' axis represents the southern edge of the design.*

Table 1—*Summary results of analysis of variance for the main characteristics of first-year northern red oak seedlings*

	Values of F		Mean for acorn position		
	Treatment	Covariant[1]	Sideways	Tip down	Tip up
Survival (%)	0.89		55	39	42
Height (cm)	1.80		149	158	142
SDW (&g)	0.53		485	476	432
RDW (&g)	5.04*		637 a	478 b	599 a
S/R (%)	9.37**		84 a	111 b	79 a
Angle[2] (degrees)	7.04*	7.29*	159 a	110 b	136 a
Diameter[3]	4.24*		101 a	80 b	97 a

SDW = stem dry weight, RDW = root dry weight, S/R = shoot-to-root dry weight ratio. Means for acorn position followed by different letters are statistically different at the 5% probability level. The asterisks (*) and the double asterisks (**) indicate significant (5% probability level) and very significant (1% level) differences respectively.

[1] Dry weight of aerial parts of herbaceous vegetation per square meter (g/m2).

[2] Deviation of taproot from a straight line.

[3] Topological diameter (Fitter 1985).

Seeding techniques trials. In the first seeding techniques trial, we determined that the operator and the angle of the tube used to deposit the seeds had no effect

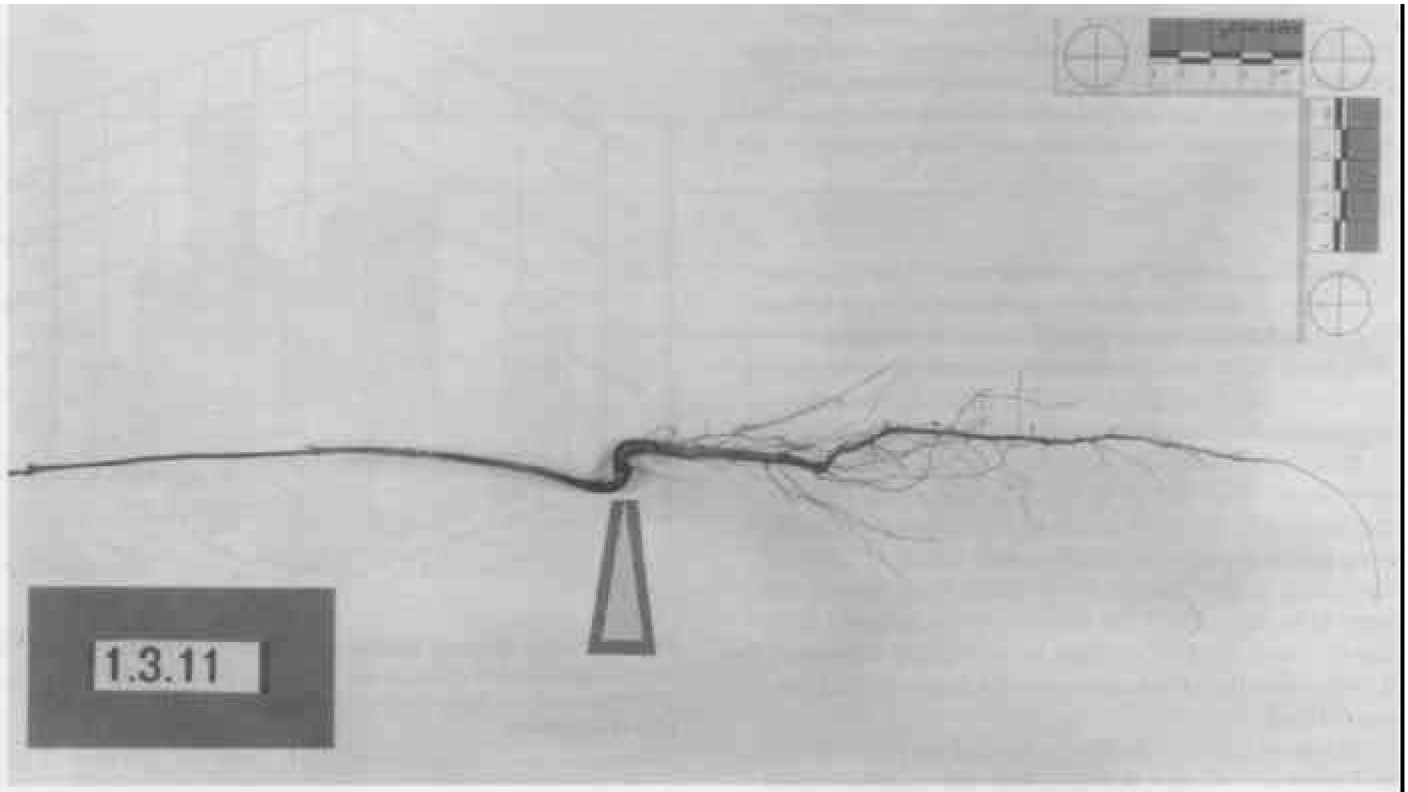
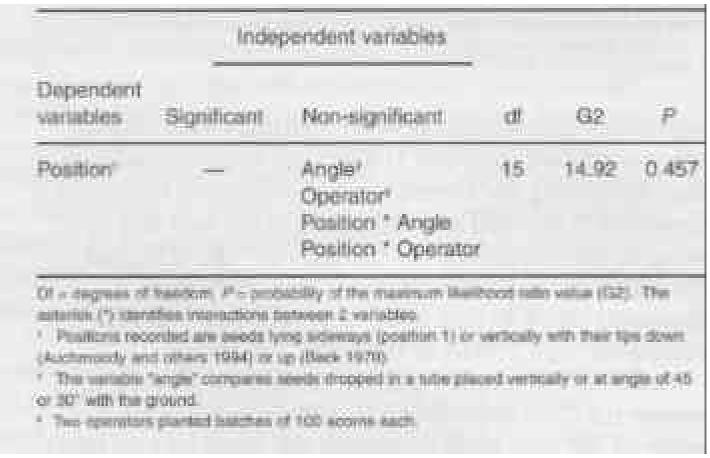


Figure 5—*Angle between the sections of the taproot above and below the junction with the radicle. The seedling represented was planted vertically with the acorn's tip pointing downward.*

on the resulting position of the seeds in the slits (table 2); 80% of the seeds fell sideways, 11% with the tips pointing down, and 9% with the tips pointing up.

In the second trial, the proportion of seeds falling onto the prepared seed spot increased with the use of a guiding tube (table 3); 5.9% of the seeds fell outside the intended location when they were hand thrown, whereas only 1.3% landed outside when deposited through a tube. Therefore, sowing through a tube resulted in a saving of 4.6% of the seeds. Seed position, however, was independent of the method of sowing, of operators, and of replications (table 3). Percentages of seeds in positions 1 to 3 were 80, 12, and 8%, respectively, almost identical results to those of the first trial.

Table 2— *Effect of the angle o f the sowing tube with the ground on position of seeds in the ground; significant and nonsignificant variables are determined by log-linear models*



	Independent variables				
Dependent variables	Significant	Non-significant	df	G2	P
Position[1]	—	Angle[2] Operator[3] Position * Angle Position * Operator	15	14.92	0.457

Df = degrees of freedom, P = probability of the maximum likelihood ratio value (G2). The asterisk (*) identifies interactions between 2 variables.

[1] Positions recorded are seeds lying sideways (position 1) or vertically with their tips down (Auchmoody and others 1994) or up (Beck 1970).

[2] The variable "angle" compares seeds dropped in a tube placed vertically or at angle of 45 or 30° with the ground.

[3] Two operators planted batches of 100 acorns each.

The time required to sow 100 acorns in the third trial was significantly reduced when the seeds were handthrown into the slits: adjusted mean number of seeds sown decreased from 275 to 230 seeds/person-hour when the seeds were directed through a tube on the lawn edger. The 20% gain in time with hand throwing largely outweighs the loss of 5% of the seeds landing outside the intended spot.

Discussion

Growth of seedlings. Our results showed that pointing the tips of acorns down while sowing had detrimental effects on root growth and possibly, on seed germination. However, sowing technique had no influence on this because the seeds tended to fall in the same position whether they were hand thrown in the slits or sown through a tube.

Table 3— *Location and position; significant variables are determined by log-linear models*

Independent variable	Dependent variables				
	Significant	Non-significant	df	G2	*P*
Location [1]	Method Location * method	Operator Replication [2] Location * operator Location * replication Method * operator Method * replication Operator * replication	4[1]	6.02	0.197
Position [3]	—	Method Operator Position * method Position * operator Position * replication Method * operator Method * replication Operator * replication	21	9.32	0.986

The asterisk (*) identifies interactions between 2 variables. df = degrees of freedom, *P* = probability of the maximum likelihood ratio value (G2).

[1] The variable "location" is the frequency of seeds falling in or out of the prepared seed spot.

[2] Two operators planted replicates of 55 acorns.

[3] Positions recorded are seeds lying sideways (position 1) or vertically with their tips down (Auchmoody and others 1994) or up (Beck 1970).

Survival rates were similar to those reported in previous studies (Johnson 1994). Although the survival rate was 20% higher for acorns lying sideways than for the other 2 seed positions, such differences were not significant. Absence of significant differences in survival rate is a consequence of the lowest survival rate values of seeds lying sideways (figure 3). Weed competition can affect survival in different ways. Beck (1970) obtained survival rates of approximately 30% after 2 years when there was no control of competition from low vegetation or overstory In a controlled environment, Kolb and others (1989) reported higher germination rates in the presence of competition. Surveys in forest stands show poor regeneration success when competition from low vegetation increases (Johnson 1994). Our mean values of herbaceous biomass ranged from 60 to 132 g/m^2, compared with 100 to 230 g/m^2 in a study by Kolb and others (1989). Sampling biomass late in the fall when some species (as dandelion) had completely disappeared from our plots may have affected the significance of the effect over survival.

Variations in biomass of herbaceous vegetation were likely caused in part by inter-plot competition. Neighboring trees or herbaceous vegetation may have reduced available light or affected available moisture. This resulted in patches of dense competing vegetation separated by areas with lower biomass values for competing vegetation (figure 4). The influence of competition on survival in our trial can be considered low because the high biomass values in Figure 4 do not match low survival values in figure 6.

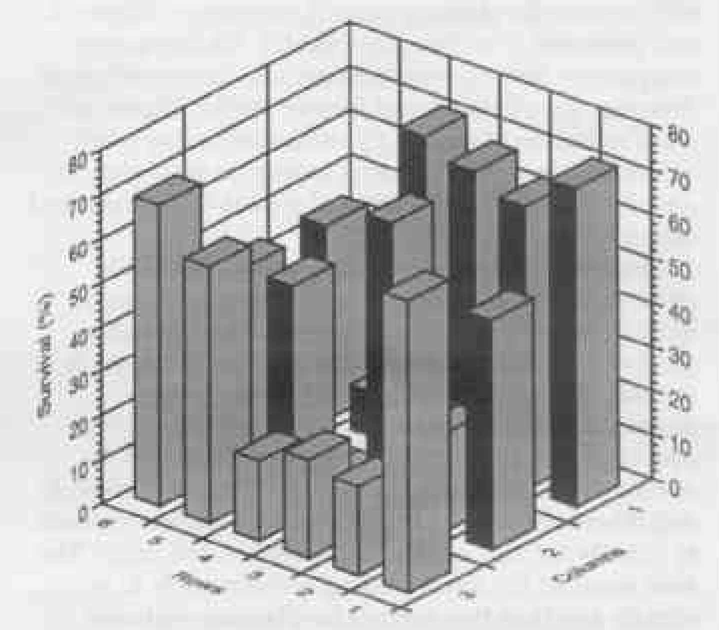


Figure 6—*Survival percentage for the cells of the randomized complete block design. Distances between rows and columns are to scale. The columns' axis represents the southern edge of the layout.*

Our seedlings were relatively small by nursery standards. Seedlings grown in nurseries for 1 season weigh approximately 3 times more than our seedlings (Kolb and Steiner 1989a, Trencia 1995). Heights reported are 2 to 4 times higher than the ones we obtained.

Generally, seed position in the ground does not change aboveground growth of seedlings. Root systems of northern red oak are generally considered as more site-sensitive than the aboveground parts: Kolb and Steiner (1989a) reported that during the first year, red oak has a tendency to modify allocation of its reserves to the production of roots whereas stem growth is controlled genetically. Schultz and Thompson (1990) recommended that northern red oak seedlings not be graded by stem measurements. Sowing seeds with their tips down mainly decreases the straightness of the taproot and the biomass of the root system. One-year-old nursery-grown seedlings generally have shoot-to-root dry biomass values (SRR) ranging from 0.3 (Kolb and Steiner 1989a) to 6.0 (Tworkoski and others 1983). Tworkoski and others (1983) observed 2-fold differences with changes in soil bulk density. Our SRR values diminished mainly with increased root dry weight. Herbaceous vegetation also obstructed the elongation of the taproot vertically and reduced the topological diameter of the root system.

Overall role of weed competition does not appear to warrant efforts to control weeds during the first year. Kolb and others (1989) report that weed competition reduces height growth by 29% for red oak compared to 60% or more for yellow-poplar *(Liriodendron tulipifera* L.) and white ash *(Fraxinus americana* L.). Within-species competition between oak seedling also has no effects on first-year growth (Kolb and Steiner 1989b). Higher levels of reduction of seedling growth warranting weed control are, however, reported mostly on good sites for a study spanning over 3 years of growth (Cogliastro and others 1990).

Sowing techniques. Sowing method had little effect on the positioning of the seeds in the ground: hand throwing the acorns or rolling them through a guiding tube did not modify the position of the seeds in the soil. Sowing through a guiding tube mostly increased the length of time required for the sowing operation. Sowing through a tube also increased the number of acorns buried in the soil by 5%. Auchmoody and others (1994) reported that nearly all seeds exposed to rodents are lost, mostly to chipmunks and mice. The time required for direct seeding is comparable to or slightly less than that needed for planting container seedlings.

Conclusion

The object of this study was to select the best manual method of sowing northern red oak acorns, taking into account biological and technical constraints. The lawn edger proved a useful and appropriate tool for preparing the seed spots in the clay loam soil. Considering that the same number of seeds fell in the best position for seedling growth regardless of sowing method, hand throwing the seeds is recommended because it reduces the sowing time: it took about 20% less time to hand-throw the seeds than to use the planting tube. Our results support seedling grading methods including root systems characteristics. Short-term observations suggest that operational methods should aim at maximizing the proportion of seeds lying sideways. Acorns lying sideways had the best survival although the differences between techniques were not significant. All sowing techniques ensured a good early growth for over 80% of the seeds germinated. In an ideal scenario, only a few seedlings with negative characteristics would originate from seeds with the tips pointing down. We ran the study over one growing season mainly in order to first obtain short-term data. Longer-term studies of 2 or 3 years are needed to evaluate the persistence or appearance of existing or new morphometric differences. In addition, new direct seeding techniques trials could be run in order to find ways of increasing the proportion of seeds that land sideways.

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Importance of Cutting Diameter and Method of Production on Early Growth of Hybrid Poplar

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Rapid early growth of hybrid poplars— Populus spp.– established from hardwood cuttings is critical to the success of plantations. Without rapid growth, weed competition and drought can significantly affect tree survival and productivity. Producing vigorous cuttings, quickly developing roots, and initiating shoot growth are therefore important. Hardwood cutting diameter was not an indicator of tree vigor for 8 of 15 hybrid poplar clones tested and only a weak indicator for 7 clones. The angle at Vehicle cuttings are excised from stems significantly influenced early growth of 1 poplar clone. The assumption that cutting diameter is an important index of subsequent growth is challenged, and the lack of criteria for excising cuttings is addressed. Tree Planters' Notes 47(2):76-80; 1996.

Vegetative propagation from dormant-season hardwood cuttings is an efficient and increasingly used practice for a number of deciduous tree species. The initial success of plantation establishment using cuttings depends upon site quality, the intensity of competing vegetation, and weather. The physiological rooting capability of various hybrid poplar-Populus spp.— clones, the vigor and health of the parent tree, cutting storage environment, care of handling, and treatment during processing and planting are also important to tree establishment and early growth (Hansen and Phipps 1983; Hansen and others 1983; Morin and Demeritt 1984; Stuhlinger and Toliver 1985).

Cutting diameter is often used as an index of potential cutting vigor. Larger diameters are generally considered to be positively correlated with the success of plant establishment and early growth, and nursery practices generally exclude cuttings smaller than 0.6 cm diameter (Dickmann and others 1980; Hansen and others 1983; Morin and Demeritt 1984). In the current study, we report 2 experiments testing the hypothesis that cutting diameter influences tree establishment and growth.

In a third experiment, we assess the impact of the method of cutting production on tree growth. The manner in which cuttings are physically excised from harvested "whips" (stems growing off the poplar stools) can be variable. Root development and productivity may be affected by the angle at excised relative to the stem. The "angle of excision" has not previously been studied, and there are no standard recommendations for nursery practice. Lesser angles increase the bottom surface area and circumference of exposed cambial tissue from which roots might emerge. By better defining the characteristics of vigorous cuttings, more productive cuttings can be produced and wasteful practices curtailed.

Materials and Methods

Relationship of cutting diameter to survival and growth in the field. The soil on the site (University of Wisconsin—Madison's Arlington Experiment Station in Columbia County) was a Plano silt loam, 0 to 2% slope (fine-silty; mixed, mesic Typic Argiudolls). All site preparation was conducted during early fall 1987. The existing alfalfa, *Medicago sativa,* was killed with 1.1 kg (active ingredient)/ha 2,4-D (dimethylamine salt of 2,4dichlorophenoxy acetic acid) and 0.6 kg ai/ha Banvel™ (diethylamine salt of 3,6-dichloro-o-anisic acid), followed by tillage and site treatment with 3.6 kg ai/ha simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) to prevent weed reestablishment.

Dormant hardwood cuttings (25 cm long) with viable buds, cut perpendicular to the original stem, were made from 15 hybrid poplar clones during the winter of 1987-88 (table 1). Cuttings were kept frozen and sealed in plastic bags until planting (about 6 months). Frozen cuttings were soaked in water for 12 hours immediately before planting in saturated soil on 23-24 April 1988. Cuttings were planted so that their tops were flush with the soil surface at 1.22 x 1.22-m spacing. Cuttings were carefully inserted into dibble holes to prevent the surface soil containing the herbicide simazine from falling into the rooting zone. Trees were established in 4 tree blocks, in a randomized block design, from which 6 of 12 replicates were used in this study. Long-term results from this plantation are described in Robison and Raffa (1997).

Because of the dry conditions during the summer of 1988 (Trenberth and others 1988), supplementary spot irrigation was applied as 4 to 8 liters of water/tree 8 times between 2 June and 7 July 1988 (40 to 75 days after planting), respectively (Robison and Raffa 1997).

Table 1— *Hybrid poplar clonal designations and parentages*

Designation(s)[*]	Parentage	Taxonomic sections[†]
NC5339, Crandon	*alba* × *grandidentata*	100% L
NC5271, NE19	*nigra* 'Charkowiensis' × *nigra* 'Caudina'	100% A
NC5377, Wisconsin #5	*deltoides* × *nigra*	100% A
NC11004, Siouxland	*deltoides*	100% A
NC5260, Tristis #1	*tristis* × *balsamifera*	100% T
NC11505, NE388, NE88	*maximowiczii* × *trichocarpa*	100% T
NC5262, NE387	*balsamifera* 'subcordata–Candicans' × *berolinensis*[‡]	75% T × A
NC11396, NE49	*maximowiczii* × *berolinensis*[‡]	75% T × A
NE332	*simonii* × *berolinensis*[‡]	75% T × A
NC5331, NE299	*nigra* 'Betulifolia' × *trichocarpa*	50% T × A
NC11432, NE252	*deltoides* 'Angulata' × *trichocarpa*	50% T × A
NC11445, NE280, NE157	*nigra* × *laurifolia*	50% T × A
NM6, 'Max 5'	*nigra* × *maximowiczii*	50% T × A
NC11382, NE27	*nigra* 'Charkowiensis' × *berolinensis*[‡]	25% T × A
DTAC2	*deltoides* 'Angulata' × *berolinensis*[‡]	25% T × A

[*] Designations beginning with NC refer to clones (re)named by the USDA North Central Forest Experiment Station and with NE by the USDA Northeastern Forest Experiment Station; NM and DTAC were clones developed by the Ontario Ministry of Natural Resources, Canada.

[†] Percentage of *Populus* spp. taxonomic section represented in each hybrid: L = Leuce, T = Tacamahaca, A = Aigerios.

[‡] *berolinensis* = *nigra* × *laurifolia*.

The top diameter of each cutting was measured (±0. 1 mm) after planting, and tree heights (from soil surface to top of apical bud) were measured at 56 and 79 days after planting (± 1 cm). The difference between cutting diameter of surviving and dead trees was examined by Student's t-test for each clone. Homogeneity of variance was verified graphically. The relationship between cutting diameter and tree height was examined by correlation analysis.

Relationship of cutting diameter to growth in the glasshouse. Fifteen hybrid poplar clones (table 1) were established as 12-cm-long dormant hardwood cuttings in a glasshouse, following the same initial procedures described previously. However, they were planted flush with the soil surface in 20-cm-diameter plastic pots in saturated Redi-Earth Peat-Lite® potting soil, and each fertilized with 15 g of Osmocote® slow-release fertilizer (17:6:12 plus micronutrients). The environmental conditions were 16 hours light and 8 hours dark cycle, 20 to 27 °C, and 30 to 55% RH. The top diameter of each cutting was measured after planting. Height growth at 43 days post planting was measured and related by correlation analysis to cutting diameter.

Effect of cutting angle on growth. Clone NM6 was planted as 10-cm-long dormant hardwood cuttings in saturated potting soil in plastic pots in a glasshouse, as described previously. These cuttings were planted frozen, without a period of soaking. In addition, both ends of each cutting were freshly clipped immediately before planting so that half were cut perpendicular (90°) to the length of the stem at the top and bottom, and half were cut at a 45° angle to the length of the stem at the top and bottom. One 90° and one 45° cutting were made from each original (frozen) 25-cm-long cutting. All freshly cut end-surfaces exposed green, healthy cambium.

The surface area of the bottom of each cutting was measured before planting; 2 diameter measurements perpendicular to each other (± 0.1 mm) were taken and the area was calculated). Seventy-nine days after planting, the height of each stem was recorded I cm and the basal diameter (± 0.1 mm) of each primary stem per plant measured at 5 cm above the soil. Intact plants were removed from the potting soil by submerging the pots in water and gently washing the soil away from the roots. The number of primary roots originating along the length of the cuttings and those around the circumference of the cut surface were recorded. The basal diameters (± 0.1 mm) of the 3 largest roots/plant and the length of the single longest root (±1 cm) were recorded. Harvested plants were separated into roots, stems, leaves including petioles, and residual cutting, and oven-dried at 65 °C to constant weight (± 10 mg). Differences between these measures of plant productivity and cutting angle were evaluated by analysis of variance (Abacus Concepts 1989). Homogeneity of variance was verified graphically for each parameter.

Results and Discussion

Only clones NC5260 and NC5262 showed significant variation in survival related to cutting diameter at P# 0.10 and P# 0.05, respectively (table 2). For all clones, except NC11505, mean cutting diamteter was larger for surviving trees than for those which had died, although not statistically larger (table 2). Height growth of clones NC5331, NC11396, NC11445, and DTAC2 at 79 days was significantly correlated with cutting diameter in the field, but not in the glasshouse at 43 days (P #0.1) (table 3). Only clone NE332 had height growth significantly correlated with cutting diameter in both environments (P # 0.1).

Standard nursery practice recommendations are for cuttings of at least 0.6 to 1 cm diameter (Hansen and others 1983, Morin and Demeritt 1984). Given the range of cutting diameters in the current study, our results corroborate this criterion, but indicate that sensitivity to cutting diameter is generally weak among the clones tested. Cutting diameter was related to survival of 2 clones (table 2), and height growth of 5 other clones (table 3), but the results for these 7 clones were not consistent across all experiments. Four of the 5 clones for which cutting diameter was related to height growth were also among those with high field survival (NC11396, NC11445, NE332, DTAC2) (tables 2 and 3). Clones with high field survival are likely to have a high physiological rooting capability. These clones appear to be the most sensitive to cutting diameter.

Although clonal differences in survival and growth were observed, the relationships among cutting diameter, physiological rooting capability, survival and growth are unclear. Cutting mass might be a more useful index of potential survival and/or growth than diameter. This possibility requires testing. If it were found true, larger diameter cuttings would require shorter lengths to satisfy survival and growth expectations.

The potential for high survival and growth of cuttings smaller than 0.6 cm diameter is unknown. Therefore it may be appropriate to test each clone to determine a minimum diameter requirement. Field observations suggest that, for many *Populus* spp. and *Salix* spp. clones, cutting diameters less than 0.6 cm are adequate, even in dry years. Expectations that larger diameter cuttings will be more productive, and that cuttings below the established minimum diameter will be relatively unproductive, may be false. For the large-scale production of cuttings, clone-specific minimum diameter recommendations may be appropriate to minimize stool-bed waste.

Cuttings produced at a 45/ angle had significantly greater cut surface areas than those cut at 90/, as expected (2.54 versus 1.54 cm^2; F=80.62, P= 0.0001, df=1.8) (table 4). Both types of cuttings had equivalent residual dry mass (5.09 versus 5.05 mg). The ovendry weight of leaves (F=3.4055, P=0.0756, df=1,28) and roots (F=5.4575, P=0.0269, df=1.8) were significantly greater on plants grown from 90/ cuttings. All other parameters were equivalent between the cutting types at P# 0.10.

Table 2—*Mean cutting top diameters and hybrid poplar clonal survival at 56 and 79 days after planting at the Arlington Experiment Station, Wisconsin*

	Mean cutting diameter (mm)											
	56 days post planting					79 days post planting						
Clone	Surviving trees	(n)		Dead trees	(n)	Surviving trees	(n)		Dead trees	(n)	Cutting diam. range (mm)	Height at 56/79 days (cm)
NC5339	11.4	(11)	ns	11.2	(12)	—	(0)	—	11.3	(23)	8.0–15.0	4 / —
NC5260	10.5	(21)	**	7.7	(3)	11.4	(13)	**	8.7	(11)	7.5–15.5	10 / 37
NC11505	8.0	(15)	ns	8.8	(8)	8.0	(13)	ns	8.6	(10)	6.0–13.5	5 / 23
NC5271	15.5	(22)	—	13.0	(1)	15.5	(22)	—	13.0	(1)	5.5–20.5	32 / 64
NC5377	9.6	(22)	ns	7.5	(2)	9.8	(13)	ns	9.0	(11)	5.5–15.5	6 / 26
NC11004	12.4	(20)	ns	12.3	(3)	12.5	(18)	ns	11.8	(5)	7.5–18.5	22 / 53
NC5262	5.7	(18)	ns	5.5	(6)	5.8	(15)	*	5.3	(9)	4.5– 7.0	12 / 42
NC5331	7.8	(8)	ns	6.8	(16)	7.4	(7)	ns	6.8	(17)	6.0– 9.5	10 / 30
NC11382	9.6	(20)	ns	8.1	(4)	9.5	(17)	ns	8.9	(7)	7.0–16.0	13 / 37
NC11396	10.5	(21)	ns	8.9	(2)	10.6	(18)	ns	9.6	(5)	7.5–14.5	25 / 70
NC11432	10.0	(12)	ns	9.0	(12)	9.8	(7)	ns	9.4	(17)	6.0–12.5	12 / 50
NC11445	11.1	(22)	—	7.5	(1)	11.2	(21)	ns	9.0	(2)	7.0–16.5	22 / 54
NE332	9.2	(22)	—	7.5	(1)	9.2	(22)	—	7.5	(1)	6.0–12.5	26 / 54
NM6	14.5	(24)	—	—	(0)	14.5	(24)	—	—	(0)	12.0–19.0	44 / 83
DTAC2	15.1	(20)	ns	11.8	(2)	14.7	(19)	ns	10.0	(3)	9.5– 1.0	14 / 36

**, * refer to significant differences in cutting diameter between surviving and dead trees at 56 and 79 days, at $P \leq .05$ and .10, respectively.

Note: for some clones, total n is less than 24 due to unrecovered cuttings (that is, dead trees or buried cuttings) at the time of diameter measurement.

Table 3— *Correlation coefficients between cutting diameter (CD) and tree height (Ht) at 56 and 79 days after planting hybrid poplar clones in the field at the Arlington Experiment Station, Wisconsin, and 43 days after planting indoors in a glasshouse*

	Correlation coefficients for CD vs. Ht				
Clone	56 days—field	79 days—field	43 days—indoors	CD range (mm) indoors	n indoors
NC5339	.48	—	.57	7.6– 8.9	4
NC5260	.30	.22	−.12	4.6– 6.5	8
NC11505	.04	.00	.62	8.4– 9.9	6
NC5271	−.04	−.04	−.04	8.9–11.6	8
NC5377	.17	.16	.37	6.7– 8.4	8
NC11004	.29	.18	−.27	7.1–11.0	8
NC5262	.39	.06	−.12	4.0– 9.0	8
NC5331	.01	.69*	.30	6.5– 9.5	8
NC11382	−.22	−.21	.27	6.6– 9.4	8
NC11396	.39*	.42*	.44	7.2– 9.4	8
NC11432	.32	.54	−.10	7.5– 9.0	8
NC11445	.47**	.46**	−.05	7.8–10.8	8
NE332	.49**	.38*	.85*	5.2– 8.4	5
NM6	.11	−.03	.17	11.0–13.5	8
DTAC2	.34	.51**	.21	8.1–10.7	6

**, * refer to significant correlations at P<0.05 and 0.10, respectively; field study cutting diameter and n are in table 2.

The smaller size of the plants from cuttings prepared with 45/ cuts (table 4) may be due to the greater number of cambial cells damaged in a 45/ cut, and the relative distance to healthy cells from the cut surface. Because cambial cells are parallel to the length of the stem, a 90/ cut results in approximately 1 cell damaged for each nearest healthy cell. However, at 45/, the ratio of damaged to near-healthy cells could approach 2:1. Thus, roots from the 45/ cut-end would have to arise from and bridge a greater area of necrotic tissue than those from a 90/ cut. Nearly one-third more roots arose from the cut surface of the 90/ cuttings (table 4).

The greater aboveground surface area of the top of the 45/ cuttings, relative to the 90/ cuttings, may have hastened drying and also contributed to retarded growth. The current data suggest that 45/ cutting is not a beneficial practice. Although other clones need to be tested, these results suggest that cuttings should be made at a strictly 90/ angle.

Management Implications

Cutting diameters above 0.6 cm were not well related to improved survival or growth. Thus there appears to be little reason for favoring larger cuttings. Cutting diameters less than 0.6 cm may be suitable for some clones but would need to be tested explicitly. Such testing may be justified for clones to be mass-produced. Increasing the length of exposed cambium from which roots emerge, by reducing the cutting angle from 90/ to 45/, reduced rooting and growth of clone NM6.

Table 4— *Mean characteristics (± SD) of hybrid poplar clone NM6 79 days after establishment with 2 types of cuttings*

Characteristic	90° Angle cutting (n=14)	*P*	45° Angle cutting (n=16)
Area of the bottom surface of the cutting (cm2)	1.5±0.2	.0001	2.5±0.4
Height of primary stem (cm)	70.1±17.7	.7498	68.5±9.5
Cumulative height of all stems (cm)	85.5±30.2	.3488	77.4±15.0
Main stem diameter at 5 cm (mm)	5.2±1.1	.2822	4.9±0.6
Total number of primary roots	14.4±6.4	.5085	13.2±3.5
Total number of primary roots arising at the cut end	7.7±5.0	.1860	5.8±2.3
Total number of primary roots arising along cutting length	6.7±3.0	.5177	7.4±2.6
Primary roots basal diameter (mm)	1.7±0.5	.8087	1.6±0.3
Length of longest root (cm)	31.3±6.7	.2335	34.4±7.4
Oven-dry weight of stem(s) (mg)	2.9±1.3	.1564	2.3±0.8
Oven-dry weight of leaves (mg)	5.6±2.3	.0756	4.3±1.3
Oven-dry weight of roots (mg)	1.1±0.4	.0269	0.8±0.3
Oven-dry weight of residual cutting (mg)	5.1±0.6	.9031	5.1±0.8

Therefore, as a general practice, a 90/ cutting angle is recommended and with an automated production system is easier to make.

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