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Abstract

These proceedings are a compilation of 25 papers that were presented at the regional meetings of the forest and conservation nursery associations and the Intertribal Nursery Council meeting in the United States in 2010. The Joint Meeting of the Southern Forest Nursery Association and Northeastern Forest and Conservation Nursery Association was held at the Peabody Hotel in Little Rock, Arkansas on July 26 to 29, 2010. Subject matter for the technical sessions included marketing strategies, tree improvement programs, nursery certification, fumigation updates, and insect and disease management. Field trips included afternoon tours of the ArborGen Nursery in Bluff City, AR, Baucum Nursery in North Little Rock, AR, and the University of Arkansas at Pine Bluff Small Farm Outreach Lonoke Site. The Joint Meeting of the Western Forest and Conservation Nursery Association and Forest Nursery Association of British Columbia: Target Seedling Symposium-2010 was held at the Sheraton Portland Airport Hotel in Portland, OR, on August 24 to 26, 2010. Subject matter for the technical sessions included the target seedling, seed handling, seedling nutrition, seedling culturing, pest management, nursery research and new technology, and general nursery topics. Afternoon field trips included tours of Blooming Nursery in Cornelius, OR, PRT container nursery in Hubbard, OR, and IFA bareroot nursery in Canby, OR. The Intertribal Nursery Council Meeting was held at the Medallion Hotel in Arlington, WA, on September 21 to 23, 2010. The meeting was hosted by the BankSavers Nursery, owned by the Stillaguarnish Tribe, in Arlington. Subject matter for the technical sessions included native plant production for fisheries restoration, the use of small native plant nurseries in cultural and conservation education, energy conservation and alternative energy sources in nurseries, and the effects of climate change on nursery production. A short workshop on native plant nursery fertilization was also given. Afternoon field trips included tours of the BankSavers Nursery and Buffalo farm, a tour of Pilchuck Park Wetland Mitigation project, and a tour of the Stillaguamish Tribal Fish Hatchery.

Keywords: bareroot nursery, container nursery, nursery practices, fertilization, pesticides, seeds, reforestation, restoration, tree physiology, hardwood species, native species

Papers were edited to a uniform style; however, authors are responsible for content and accuracy.

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National Proceedings:

Forest and Conservation Nursery Associations—2010

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Searchable Internet Database—www.rngr.net

The National Nursery Proceedings database includes papers published in the regional nursery proceedings (Western, Intermountain, Northeastern, and Southern) since 1949. The database can be searched by date, author, or keyword and papers are available in portable document format (PDF).

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**No papers received; for more information, contact authors at the addresses provided in the list of attendees.

Joint Meeting of the Southern Forest Nursery Association and Northeastern Forest and Conservation Nursery Association

Little Rock, Arkansas July 26 to 29, 2010



Illustration courtesy of College of Natural Resources, University of Idaho.

Forests and Forestry in Arkansas During the Last Two Centuries

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Abstract: Arkansas has had a long and storied history related to its forests and forestry. Ever since its acquisition in the Louisiana Purchase, timber has played a large role in the socioeconomic development of this state. In the 1880s, it was estimated that Arkansas had about 13 million ha (32 million ac) of forests and several hundred billion board feet of timber, numbers that fell dramatically as commercial lumbering spread across the state. After reaching historic lows in forest coverage and volume around the end of World War II, better conservation measures and the widespread implementation of sustainable forestry and fire suppression has allowed for some recovery of forested cover (now stabilized at about 7.3 million ha [18 million ac]) and a steady increase in timber volume (currently estimated at over 0.8 billion m³ [27 billion ft³]). Over one-third of the timber volume in Arkansas is pine (*Pinus* spp.), a number that is expected to increase as pine plantations continue to replace natural-origin pine and pine-hardwood stands. Recent changes in ownership, increased management intensity, and threats to the health of Arkansas timberlands will continue to challenge foresters well into the future.

Keywords: Crossett Experimental Forest, hardwoods, history, lumbering, pines, USDA Forest Service

Introduction _

Arkansas, the self-proclaimed "Natural State," has a long tradition of wood utilization that continues to this day. The state has been blessed with abundant rainfall, good soils, and a temperate climate, all of which encourage luxuriant forests when not constrained by local site conditions or disturbance patterns. These forests have long driven the socioeconomic well-being of the state; at one time, the forest products industry provided 60% to 70% of all manufacturing jobs in Arkansas (Bruner 1930). A more recent study on the economic impacts of forest-related industry noted that over 33,000 Arkansans were employed in this field, with over US\$ 1.6 billion in labor income and an estimated economic impact of US\$ 2.83 billion (University of Arkansas Division of Agriculture 2009). The addition of other benefits from contributions to tourism, hunting and fishing, water and air quality, and similar goods and services makes Arkansas forestlands a vital resource to the state.

Geographers often subdivide Arkansas into seven physiographic regions (Figure 1). These include the low rolling hills of the timber-covered West Gulf Coastal Plain, where most of the lobloly pine (*Pinus taeda*) is produced; the Mississippi River Alluvial Plain, a broad, flat, agricultural region now largely cleared of its bottomland hardwood and baldcypress (*Taxodium distichum*) forests; Crowley's Ridge, a prominent (low elevation) outlier in northeastern Arkansas covered in hardwood-dominated forests more typical of the Piedmont Plateau further to the east; the Ouachita Mountains, heavily forested with shortleaf pine (*Pinus echinata*) and mixed upland hardwoods; the Arkansas River Valley, a combination of agricultural and forested lands along the Arkansas River; the Boston Mountains' steeply incised slopes covered in oak-hickory forests; and the Ozark Plateau, also dominated by oak-hickory forests, with scattered shortleaf pine. One hundred tree species were encountered in the most recently completed Forest Inventory and Analysis (FIA) survey of



Figure 1. The topography of Arkansas, overlain by the physiographic provinces of the state.

Arkansas, but only a relative handful (Table 1) contributed most of the volume (Rosson and Rose 2010). This paper will summarize the forest conditions of Arkansas over a two-century period, from its initial acquisition by the United States to the present-day, and describe the major events that shaped the development of these timberlands. In addition, some anticipated trends of Arkansas forests will be provided to help suggest the future.

Forest Conditions Prior to 1880

The first persons to enter Arkansas over 12,000 years ago, the Paleoindians, encountered considerably different landscapes than we see today. These lands were still strongly influenced by glacial activity much further north, and supported vegetation assemblages notably different than those that appeared following a climatic stabilization

Tree species	Volume (millions of ft ³)*	Percentage of total	Cumulative total
Loblolly pine (<i>Pinus taeda</i>)	6,040.1	22.29	22.29
Shortleaf pine (Pinus echinata)	3,467.5	12.80	35.08
White oak (Quercus alba)	2,555.4	9.43	44.51
Sweetgum (Liquidambar styraciflua) 1,922.2	7.09	51.61
Post oak (Quercus stellata)	1,441.5	5.32	56.93
Northern red oak (Quercus rubra)	974.3	3.60	60.52
Black oak (Quercus velutina)	876.2	3.23	63.75
Southern red oak (Quercus falcata)	850.9	3.14	66.89
Black hickory (Carya texana)	639.7	2.36	69.25
Water oak (Quercus phellos)	612.9	2.26	71.52
All other 90+ species	7,719.3	28.48	100.00
Totals:	27,100	100.00	

 Table 1. Live tree volume of stems at least 12.7 cm (5-in) dbh reported in the 2005 FIA survey of Arkansas forests (Rosson and Rose 2010).

*1 ft³ = 0.03 m³

approximately 4000 to 5000 years ago (Delcourt and Delcourt 1981; Royall and others 1991). However, dendroecological records suggest that the mild contemporary climate Arkansas enjoys today was punctuated by periodic "megadroughts," with intense, if brief, impacts on forest conditions (Stahle and others 1985; Cleaveland 2000; Stahle and others 2007).

Written records of Arkansas forests at first contact are limited. The dense populations of Native Americans described in the chronicles of Spaniard Hernando de Soto in the early 1540s had drastically declined by the time French missionaries and traders returned to the region 150 years later. Few Europeans remained during the next century; with the notable exception of an occasional hamlet or hunter/trapper. there were almost no white settlers living in Arkansas prior to 1800. Reports of forest conditions during this period are even scarcer, and largely limited to passing mentions in early explorer accounts. American control following the 1803 Louisiana Purchase brought increasing numbers of settlers, especially after the General Land Office public land surveyors began subdividing the territory starting in 1815 (Gill 2004). These early surveyors viewed the lands they worked on as wilderness, with few traces of civilization; the wide expanses of agricultural fields cleared by the prehistoric native peoples encountered by de Soto had long since been replaced by mature forests. The scattered groups of Indians removed from Arkansas Territory by the early 1830s had limited impact on the forests of the region. They (as would the EuroAmerican settlers that followed them) periodically set fires to clear the forest undergrowth, especially in the uplands, and would carve out small patches of timber to build their homes and plant a few crops, but nothing on the scale of the prehistoric cultures.

For most of the 19th century, the infrastructure to exploit the virgin forests of Arkansas was too limited to support much in the way of commercial lumbering. Limited quantities of timber were felled for local consumption and a relatively small amount of cutting occurred along the major rivers of the state, either to provide fuel wood for steamships or pine or cypress logs to raft to mills in Louisiana. This was soon to change, however; a rapidly growing and wood-hungry nation would soon drive land speculation and commercial lumbering on a massive scale across the entire southern US.

Arkansas Forests During the Exploitive Lumbering Period _

Environmental historians consider 1880 a benchmark year for forests in the Arkansas region. By then, railroads had penetrated the region, and lumber companies that had cut out their timberlands in the Lake States and New England were scouring the South for new opportunities (Heyward 1958). Early reports on the forest conditions of the US showed only superficial exploitation of the timber resources of Arkansas in the immediate proximity of the major railroads (for example, Sargent 1884; Mohr 1897). Even though shortleaf and loblolly pine were considered the major commercial species of the period, the majority of wood volume of the virgin forests of Arkansas was mixed hardwood, with large quantities of baldcypress and eastern redcedar (*Juniperus virginiana*) in certain habitats (Record 1910).

By the late 1800s, the initial quantifications of the forests of Arkansas were made. Professor FL Harvey (of what would become the University of Arkansas-Fayetteville) reported that Arkansas had at least 51,800 km² (20,000 mi²) of "pine land" thought to have 0.19 to 0.24 billion m³ (40 and 50 billion board feet) of lumber (Harvey 1883). Others estimated that the "original" forests of Arkansas had a total of 0.94 to 1.42 billion m³ (200 to 300 billion board feet) of timber at this time (for example, Bruner 1930). There is no way to confirm these numbers, nor which trees (either by size or species) were included in these assessments; documenting standing timber volume was a challenge prior to the mid-1930s. By this time, the USDA Forest Service (USFS) had begun formal inventories of Arkansas forest conditions, culminating in a series of reports that included the southwestern portion of the state (Cruikshank 1937), the northern Arkansas Delta (Winters 1938), the Ouachita Mountains (Cruikshank 1938), the southern Arkansas Delta (Winters 1939), and finally, the Ozark Mountains (Duerr 1948).

Figure 2 provides the best available estimates of both forest area and volume for Arkansas from about 1880 until 2005. The volumes prior to the forest inventories of the late 1930s represent cubic foot yield estimates based on board foot totals (assuming that a cubic foot of wood, adjusted for kerf and log shape, yields 6 board feet), while those after this point were reported in cubic feet. There was an estimated 1.4 billion m³ (50 billion ft³) of live standing sawtimber in Arkansas in 1880 (Bruner 1930). Industrial exploitation peaked in Arkansas in 1909, with over 9.3 million m³ (2 billion board feet) of lumber cut in this year, most of which was then shipped to markets out of state (Harris and Maxwell 1912). In addition, almost 12 million m³ (2.6 billion board feet) of timber were cut for firewood and hundreds of millions of board feet were turned into cooperage, lath, shingles, veneer, crossties, and other forest products (Harris and Maxwell 1912).



Figure 2. Long-term trends (1880 to 2005) in forest coverage and wood volume for Arkansas. Data before 1935 are based on poorly documented estimates, while data after this date are from the Forest Inventory and Analysis (FIA) program of the USDA Forest Service.

This rate of consumption substantially exceeded the growth of Arkansas forests. By the late 1920s, sawtimber volumes had dropped to about 0.2 billion m³ (7 billion ft³) (Bruner 1930), a number that would continue to decline well into the mid-20th century. The apparent spike in volume noted in the late 1930s (Figure 2) probably arises from better inventories rather than a rapid jump in tree growth or stocking. At the lowest reliable estimates, Arkansas probably had less than 0.3 billion m^3 (10 billion ft^3) of live timber by the end of World War II. This decline was then followed by decades of continuous increase until the present-day quantity of 0.8 billion m³ (27.1 billion ft³) was reached (Figure 2; Rosson and Rose 2010). Interestingly, during this same period, total forest cover in Arkansas remained largely unchanged. After reaching a historic high of about 13 million ha (32 million ac) prior to 1880, forested land decreased steadily over the next half-century before stabilizing between 7.3 to 8 million ha (18 to 20 million ac) (Figure 2). Fire control, better silvicultural techniques, pine plantations, conservation programs, and the reforestation of former farmlands have all helped to maintain forest cover.

The federal government reserved large parcels of public domain in western and northern Arkansas to establish the Arkansas (now Ouachita) and Ozark National Forests in 1907 and 1908, respectively. While this provided some additional protections to these timberlands, one of the primary management policies of the USFS at this time was to harvest timber and other resources from these lands when possible (Strausberg and Hough 1997). Cutover lands dominated the state by 1930, with most lands sold to farmers or simply abandoned after the valuable timber was removed. These cleared lands, often covered in logging debris, frequently burned and many communities became increasingly destitute as the lumber mills ran out of timber and closed their operations. Public outcry, the promotional efforts of private citizens, and pressure from industry eventually prodded the legislature to establish the Arkansas State Forestry Commission in 1931, but adequate funding for the agency was lacking until well into the 1930s (Lang 1965). Arkansas A&M College (now the University of Arkansas-Monticello) opened the first formal course of study in forestry, offering a 2-year degree starting in 1945 and a 4-year degree in 1950.

Forestry Brings Recovery_

By the 1920s, it was obvious that the once extensive virgin forests of Arkansas had been all but exhausted by decades of lumbering, land clearing, and catastrophic events such as fire and tornadoes. A few of the large family-owned timber companies (for example, the Crossett Lumber Company, Dierks Lumber and Coal Company, Long-Bell Lumber Company, and the Fordyce Lumber Company) began to experiment with sustainable forestry practices by the mid-1920s with the notion of engaging in "permanent operations" (Hall 1925a,b; Williams 1925; Woods 1925; Gray 1954). However, very little was known about proper silvicultural techniques during this period; additional technical support was thus needed to ensure the success of these operations. The USFS Southern Forest Experiment Station, headquartered in New Orleans, began providing direct technical assistance to a number of cooperating lumber companies, eventually culminating with the establishment of the Crossett Experimental Forest (CEF) in southeastern Arkansas by late 1933 (Reynolds 1980). The first scientist stationed at the CEF, Russell R Reynolds, helped firms such as the Ozark-Badger Lumber Company and the Crossett Lumber Company evaluate different options in the harvest and delivery of wood and the management of standing timber (including regeneration techniques), laying the groundwork for decades of close cooperation (Reynolds 1980).

Once silvicultural techniques for the most productive forest types were developed, the timber industry quickly returned to southern states (Heyward 1958). The favorable growing conditions and valued timber species, coupled with relatively inexpensive land, existing infrastructure, and a capable workforce, helped encourage corporations such as International Paper Company, Georgia-Pacific, Weyerhaeuser, and Potlatch to acquire large tracts of Arkansas timberland during the middle decades of the 20th century, especially in the West Gulf Coastal Plain and Ouachita Mountains. Georgia-Pacific, for example, entered the picture by purchasing the lands of the Fordyce Lumber Company and Crossett Lumber Company. A number of the original family-owned lumber firms, such as Anthony Timberlands and Deltic Farm and Timber also transitioned into sustainable forestry operations.

These large companies sought to increase the productivity of their lands, typically favoring even-aged approaches over the uneven-aged silviculture that had initially dominated second-growth forests. Seed tree- and shelterwood-based systems soon rose to prominence, with prescribed fire a common technique for controlling competing vegetation. Growing international competition, changes to tax and investment laws, and continuing improvement in both genetics and stand density management, however, increasingly prompted timber companies to use even more productive loblolly pine plantations, especially after 1980.

By the late 1990s, most of the vertically integrated timber companies began to divest themselves of their forests, choosing instead to focus on their core business of manufacturing and purchasing their raw materials on the open market. During the last 20 years, most of the industrial timberlands in Arkansas were transferred to a variety of real estate investment trusts (REITs) or timberland investment management organizations (TIMOs). Major firms such as Georgia-Pacific and International Paper Company left the land management business entirely, and were replaced by operations such as Plum Creek Timber Company, Hancock Timber Resources Group, Resource Management Service, and The Campbell Group. A few of these timber companies reorganized their timberlands, converting them into separate investment operations (examples of this include Potlatch and Weyerhaeuser).

During this period of rapid ownership change, a number of large parcels were also acquired for conservation purposes by government agencies and non-governmental organizations, such as The Nature Conservancy. This occurred while public land management in Arkansas became less intensive, with federal lands shifting away from clearcutting and planting and more towards ecosystem restoration (Guldin and Lowenstein 1999). In particular, large-scale commercial harvesting on the Ouachita National Forest in the latter decades of the 20th century triggered considerable public pressure to modify how national forest lands were managed. By the early 1990s, ecosystem management research and demonstration programs were installed by the Ouachita National Forest and the USFS Southern Forest Experiment Station (now the Southern Research Station). As a part of this program, a 62,725-ha (155,000-ac) block of the Ouachita National Forest has been dedicated towards restoration of an open. mature shortleaf pine-bluestem community (Bukenhofer and Hedrick nd). Extensive controlled burning, in conjunction with the targeted removal of midstory hardwoods and other habitat manipulations, have been installed to aid in the recovery of a number of sensitive or endangered species, including the red-cockaded woodpecker (*Picoides borealis*) and the pale purple coneflower (Echinacea pallida) (USFS 1999; Bukenhofer and Hedrick nd). Similar pine-bluestem restoration efforts are being implemented on the Ozark National Forest, which is also interested with the return of naturally regenerating shortleaf pine back to its historical distribution on the forest.

Private non-industrial ownerships have remained the least consistently managed forestlands within Arkansas, with large tracts harvested with little concern for future stand conditions. It is not unusual, for example, for a logger to contact small private landowners and cut their timber without specific plans to regenerate the forest. Estate-related issues are also a major concern for private landowners; many feel forced into cutting the timbered properties they inherited in order to pay the taxes arising from their acquisition. Forestry consultations are available to most private landowners, often at little to no expense, via the Arkansas Forestry Commission or major timber companies. For-profit forestry consultants often steer private landowners towards intensively managed pine plantations, although many such landowners place wood fiber production as relatively low on their list of ownership objectives (Rosson and Rose 2010).

Current Silvicultural Trends

The abundance of naturally regenerated pine and bottomland hardwood forests in Arkansas has declined steadily since the early 1960s, although they still comprise 84% of current forests (Rosson and Rose 2010). During this same period, upland hardwoods coverage has remained relatively constant, and both oak-pine forests and pine plantations have increased significantly (Conner and Hartsell 2002). Pine plantations (primarily loblolly pine) have increased most dramatically (Figure 3), from approximately 22,260 ha (55,000 ac) in 1952 to just over 1.19 million ha (2.94 million ac) in 2005 (Conner and Hartsell 2002; Rosson and Rose 2010). Most of the increase has occurred since the early 1980s; the 2005 total represents 675% more land in pine plantations than the 1982 FIA estimate of 176,500 ha (436,000 ac). To meet the demand for plantations, Arkansas currently has three major tree nurseries that supply the majority of the planting stock: ArborGen Fred C Gragg SuperTree Nursery (near Bluff City), the Weyerhaeuser nursery near Magnolia, and the State of Arkansas Baucum Nursery in North Little Rock. These facilities are capable of producing over 100 million pine seedlings and 10 million hardwood seedlings every year.



Figure 3. Change in plantation acreage in Arkansas from FIA data from 1952 until 2005 (1 ac = 0.4 ha).

Silvicultural practices have intensified over the last 20 years. During this period, many landscapes once dominated by naturally regenerated, even-aged stands have become short rotation (< 30 year) pine plantations, often with intensive site preparation, mid-rotation thinnings, and competition control. Many stands in southern Arkansas receive significant site preparation treatments immediately following harvest, including ripping and bedding. Genetic improvements and density management have been identified as particularly important in maximizing fiber yield while shortening rotation length (Stanturf and others 2003). For these reasons, foresters often plant improved pine seedlings at lower densities and conduct precommercial thinnings in more heavily stocked pine plantations (often to remove naturally seeded "volunteer" pines). A variety of herbicide treatments have been developed to control undesired vegetation, both prior to and after planting, and landowners often employ mid-rotation herbicides to further reduce competition. Arkansas forest owners generally do not use large quantities of fertilizer on their properties, as is commonly done in other parts of the southeastern US. Most plantations, however, receive one or two commercial thinnings before the stand is cleared and a new one established, often on a rotation length of 25 to 35 years.

Public landowners in Arkansas vary considerably in their silviculture practices. Federally owned timberlands (primarily national forests and national wildlife refuges) have reduced most of their fiber production efforts and now focus more on ecosystem restoration, especially to help endangered species. Some state agencies still manage their lands primarily for timber or natural gas production, while private landowners engage in a range of activities. Extensive forest conversions to non-timber activities (for example, farming) have largely ceased in recent years, helping stabilize Arkansas forest cover at about 7.3 million ha (18 million ac) over the last decade (Figure 2). Residential development in parts of the state, especially the northwestern corner between Fayetteville and Bentonville and central Arkansas just west of Little Rock, have consumed large tracts of forests during this period, but this loss has largely been offset by the afforestation of former agricultural lands (Wear and Greis 2002).

Forest Health

Forest health issues represent an increasing concern for Arkansas landowners. Many invasive species are present in the state, although few are at crisis levels. Kudzu (Pueraria montana var. lobata), for example, is locally abundant but is generally not considered a major forest management concern in Arkansas. A number of other invasive plant species, however, are poised to increasingly challenge the state's forests. Japanese climbing fern (Lygodium japonicum) and Chinese tallowtree (Triadica sebifera) have just started to invade forests in extreme southern Arkansas, and cogongrass (Imperata cylindrica) is found in the adjoining states of Louisiana, Mississippi, and Texas, and is expected to eventually reach Arkansas (Miller 2004). Numerous exotic insects and diseases also threaten the state's forests, including emerald ash borer (Agrilus planipennis) and laurel wilt disease (Raffaelea lauricola).

Native pests, such as the southern pine beetle (*Dendroctonus frontalis*), have been a widespread problem in the past, but are largely of local concern today. A major exception to this trend is a recent outbreak of the red oak borer (*Enaphalodes rufulus*) in parts of the Interior Highlands of Arkansas, Missouri, and Oklahoma. A combination of drought, poor quality hardwood sites, and an aging forest produced very favorable conditions for the borer, which reached unprecedented levels and contributed to the widespread decline and death of various red oaks (*Quercus* spp.) over the last decade (Stephen and others 2003; Fierke and others 2007).

The Future of Arkansas Forests____

The future of Arkansas forests depends heavily upon commodity demands and land use practices, both of which can be simulated. Models generally predict increased demand for forest products well into the 21st century (for example, Prestemon and Abt 2002). The Midsouth region of the US, which includes Arkansas and most of its adjoining states, is also predicted to increase in forest cover and overall timber volume, largely because of slower population growth and the continued reforestation of former agricultural lands (Wear 2002). It also seems likely that long-term declines in the coverage of naturally regenerated pine and hardwood forests (Conner and Hartsell 2002) should continue, supplanted in most cases by loblolly pine plantations and housing/commercial developments. Given recent trends, eastern redcedardominated forests are also likely to increase significantly into the future (for example, Rosson and Rose 2010).

Much uncertainty remains regarding the impact of climate change upon the forests of Arkansas. The region is predicted under most scenarios to be getting warmer and somewhat wetter, although the magnitude and nature of these trends is still far from certain. Some projections have a number of more southerly tree species moving into the state, while other species are greatly reduced or vanish completely (for example, Iverson and others 2008). For instance, slash pine (*Pinus elliottii*), not currently native to the state, is predicted to arrive under most climate scenarios, while sugar maple (*Acer saccharum*), an uncommon hardwood found primarily in sheltered coves in the Ozark Plateau, is forecast to all but disappear from Arkansas (Iverson and others 2008).

Arkansas forestlands have always been in a state of change, whether responding to species biogeography, large-scale climatic patterns, human influences, or any of a number of other factors. Many of these changes are predictable, others are not; some of these drivers have yet to even appear in the region. We know, for example, that our forests will continue to be altered by invasive species. In fact, the only seemingly certain future for Arkansas forests is one where demands will continue to be placed upon this resource for timber, water, recreation, wildlife, and air quality at the same time a series of challenges threaten its ability to meet these needs.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

Weyerhaeuser in Arkansas

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Abstract: Weyerhaeuser Company has undergone many transitions since it was founded in 1900. Changes have occurred in the timberlands and wood products divisions as well as other divisions, based on both the US and global economies. This paper will present a synopsis of the current status of Weyerhaeuser generally, and in Arkansas in particular.

Keywords: wood products, fiber, plywood, nursery

Weyerhaeuser Company_

Weyerhaeuser Company was incorporated in 1900 in Washington by Frederick Weyerhaeuser, with profits realized from timber resources in the Lake States of the US. He bought approximately 364,000 ha (900,000 ac) in Washington at a cost of US\$14.60/ha (US\$ 6/ac) in what was considered to be a huge risk at the time.

The corporate headquarters of Weyerhaeuser Company is in Federal Way, Washington, located between Seattle and Tacoma. Company operations are found in ten countries, and include timberlands, wood products, cellulose fibers, real estate, and transportation. Annual sales total US\$ 5.5 billion, with 14,900 employees currently involved in various aspects of the company. At present, Weyerhaeuser controls approximately 9 million ha (22 million ac) of timberland.

Current figures are a big change from very recent history, reflecting a drop in annual sales from as high as US\$ 20 billion and a workforce of over 50,000 employees in expanded operations. The company has sold the paper, container-board, and recycling businesses, and developed a strategy to return to core businesses.

Timberlands

Weyerhaeuser Timberlands operate in nine states, owning 2.3 million ha (5.8 million ac) and leasing 307,500 ha (760,000 ac). A large amount of the leased land is located in the southern US. In addition to US operations, 6.2 million ha (15.2 million ac) are licensed in Canada; 127,500 ha (315,000 ac) are under management in Uruguay; and 18,200 ha (45,000 ac) are managed with a partnership in China.

Wood Products

The wood products division of Weyerhaeuser Company includes 23 lumber mills, 12 engineered products mills, seven oriented strand board (OSB) mills, two plywood plants, five veneer plants, and seven hardwood mills in the US. A plywood facility in Uruguay and a hardwood lumber mill in Brazil are also part of the wood products division.

Net sales in wood products in 2009 totaled approximately US\$ 2.2 billion (Figure 1). Sales of softwood lumber comprised 40% of the total, but those profits have been heavily impacted by recent declines in the housing market. The next largest sales were in engineered solid section products (18%), followed by OSB (10%), hardwood lumber (9%), and plywood (4%). The remaining 19% were comprised of sales of other products.

Cellulose Fibers

Weyerhaeuser operates five mills that manufacture absorbent fluff pulp, one use of which is in diapers. These mills are located in Georgia, Mississippi, North Carolina, and Alberta, Canada. In cooperation with Procter & Gamble Company, an additional mill is scheduled to open in Poland in 2012.



Figure 1. Net sales for the wood products division of Weyerhaeuser Company in 2009.



Figure 2. State of Arkansas map.

Real Estate

The real estate division of Weyerhaeuser runs six different home-building companies in the real estate business. These businesses are located in the states where housing demand has been the greatest, including California, Arizona, Nevada, Texas, Virginia, Maryland, and the District of Columbia. Each of these companies has an independent strategy to manage for the different needs in those communities.

Transportation

Westwood Shipping, a transportation division of Weyerhaeuser, runs seven large ships that haul logs and containers off the west coast of the US. These ships run on set schedules across the Pacific Ocean to the Pacific Rim countries. There were also four short-line railroads located in Mississippi, Arkansas, and Oregon, but Weyerhaeuser has recently sold these to a third party.

Weyerhaeuser in Arkansas_

The company first came to Arkansas in 1956. In 1969, the company bought Dierks Forest in the southwestern part of the state (Figure 2). The Arkansas branch of the company currently has 691 employees and oversees 254,500 ha (628,800 ac).

Arkansas operations include a plywood mill in Emerson, a town located almost on the Louisiana border; a large sawmill in Dierks that is a carry-over from the original Dierks Forest; timberlands that are located predominantly in the southwestern part of the state; tree improvement operations and a research and development facility in Hot Springs, that also houses various sales offices for the lumber and plywood businesses, as well as administrative and support offices; and the nursery in Magnolia.

Magnolia Nursery

The Magnolia Nursery (Figure 3) was founded in 1972 as the company was gearing up to reforest Arkansas operations. Since its inception, the nursery has grown 1.9 billion seedlings of various species and has developed a seed extraction and processing lab on site. They are currently growing approximately 50 million pine species, as well as 2.5 million hardwood species. The hardwood species are grown for outplanting on company lands as well as for sale to other parties for both the Wetlands Reserve Program and the Conservation Reserve Program.



Figure 3. Weyerhaeuser Magnolia Nursery hardwood seedling crop.

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Panel Discussion: Marketing Hardwoods at the George O White State Forest Nursery

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Keywords: seedling ordering, native species, large stocktypes

George O White State Forest Nursery_

The George O White State Forest Nursery is a hardwood nursery located in a state dominated by hardwood species. Marketing and selling our hardwoods is what we do. Depending on the year and seed availability, we grow about 65 species of hardwood trees and shrubs. During the last 5 years, we have grown about 60 hardwood species per year. We also grow about six species of pine. During the last 10 years, about 75% of the 47 million trees we distributed were hardwood trees and shrubs.

Recent Additions to Operations

Due to the variety of species available, we offer *a lot* of choices for our customers. The following are some of the things we are doing to sell more trees.

Improved Order Form

Several years ago, we improved our order form by adding color and photographs. This of course, results in greater cost, but customers tell us they love the improvements. We try to key in on fall color, fruit, and nut production rather than flowering in the photos we have of each species. We also have an online order form with links to photos and information on each species we offer.

Species Changes

We are constantly adding, and occasionally dropping, species. Several years ago our agency published a guide called *Tried and True Native Plants*. Within the guide, we have sections that list shrubs and trees. However, because our list of species changes so frequently, we generally do not add all of the trees and shrubs available. Instead, our focus for the last few years was to have the same trees available that we advise landowners to plant. Species that are new to our order form are highlighted with a special acorn symbol that says "NEW!" in the center of the acorn. Customers can now contact us during the summer or fall to find what is new this year so they can plan their planting schedule in advance.

Extra Large Trees

In the southern US, we believe you can grow big hardwoods in one season. For us, it is often hit or miss for size in our 1+0 hardwoods, but it depends on the specifications that define big. Our extra large seedlings are specified by a minimum height (not caliper). For the oaks (Quercus spp.), blackgum (Nyssa sylvatica), walnut (Juglans spp.), tulip poplar (Liriodendron tulipifera), and a few other species, 76 cm (30 in) is the minimum height; for bald cypress (Taxodium distichum), 91 cm (36 in) is the minimum height; and for pecan (Carya *illinoensis*), 61 cm (24 in) is the minimum height. A bundle of 25 extra-large seedlings is double the price of the same bundle of 25 regular-size seedlings of that same species. In general, most of our extra large seedlings are 1+0 trees. With the exception of pecan and hickory (Carya spp.), we do not carry any of our hardwoods for 2 years. Extra-large seedlings are a huge hit with our customers; we nearly always sell out. In 2008, we sold as many as 119,000. When you are selling and advertising large trees, make sure your inventory is up-to-date. If you promised a customer large trees and do not have them, it may be impossible to find them at another nursery.

Special Bundles

Our nursery offers four or five specialty bundles of seedlings in a typical year. Each bundle contains five or six species, with five to ten seedlings per species, depending on the package offered. When we have a limited number of one species available, we typically offer these exclusively within a bundle. This past year, our Conservation Bundle included five seedlings of each of the six species. Four of the six species, paw paw(*Asimina triloba*), arrowwood(*Viburnum* spp.), red-osier dogwood(*Cornus stolonifera*), and American beautyberry (*Callicarpa americana*), were only available in the bundle. If a customer wanted paw paw, they had to purchase this bundle. We allocated trees for 1000 bundles and sold them all.

Our method of marketing is not advertising or writing articles, it is our ability to offer something new, different, large, and of high quality. To sell our hardwoods, we grow lots of them, lots of species, offer big and regular size seedlings, add new species, and do anything else to get landowners to buy more.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

Who Pays for Tree Improvement?

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Abstract: Tree improvement has been one of the most successful collaborative research efforts in history, eliciting participation from a wide variety of players. This effort has included state forestry agencies, research universities, integrated forest industries, and the USDA Forest Service. Tree improvement was organized through cooperatives whose objectives were to distribute responsibilities, rights, and rewards fairly and equally. Mergers, which accelerated in the 1990s, followed by land divestitures from integrated forest industries to institutional investors, and the rise of nursery businesses marketing genetics directly to landowners, have resulted in a much more heterogeneous business environment. With increasing disparity in organizational capabilities, changing economic goals, and the increasing costs along with potential benefits of biological research, it is unclear as to whether collaborative tree improvement efforts will remain viable. Game theory offers an explanation as to why tree improvement collaborations have been successful in the past, points out shortcomings in the current cooperative structure, and offers some insights into how we may choose to manage our future.

Keywords: tree improvement, silvicultural cooperatives, game theory, collaborative research

Introduction

Tree improvement programs, since their inception more than 50 years ago, have been collaborative efforts between state forestry agencies, research universities, and large, integrated forest industries. The USDA Forest Service has led the effort in some regions and has supported basic research in forest genetics in all parts of the country. Tree improvement programs have been responsible for much of the gain experienced in forest productivity, either directly by providing better planting material or indirectly by serving as a model for many other silviculture cooperatives (Todd 1995; McKeand and others 2006; see also Vance and others 2010). Key to this success was a model of distributed ownership where responsibilities for selecting, preserving, and breeding plant material were shared among participants. This made it possible to evaluate the large numbers of individuals needed to make rapid initial gains despite the fact that trees are large, long-lived organisms that require many large plantings to adequately evaluate performance.

One of the drivers behind this success has been the belief that "a rising tide raises all boats." Increasing forest productivity was seen as desirable public policy supported by the state forestry agencies because it benefited the family forest owners directly and contributed to overall economic activity. Integrated forest industries benefited from increased forest productivity through development of a stable and inexpensive source of raw material. Under these conditions, it made sense to keep the cost of seedlings low. This was done primarily by pricing seedlings on a cost-plus basis accounting for the expenses of orchard management and nursery production. The value added by tree improvement was largely ignored and the cost of genetics programs was subsidized from other sources. Financial support was primarily from publicly appropriated funds and corporate research budgets that could be partially written off corporation taxes (Figure 1). In other words, tree improvement was supported by a number of organizations with more or less similar goals, capabilities, and motivations. Historic investments in tree improvement and the current structure of tree improvement cooperatives reflect these equities and also encapsulate the elements that will cause future organizational strains (Byram and others 2005).

What do we currently spend on TI effort in SE? Approximately \$5.4 M spent annually Benefit to Cost ~ 4.5:1



Figure 1. In-kind support for southern pine tree improvement (in millions of US dollars) from a 2007 survey of the members of the North Carolina State University Cooperative Tree Improvement Program, the Cooperative Forest Genetics Research Program, University of Florida and the Western Gulf Forest Tree Improvement Program (McKeand and others 2007).

What Prevents Us from Using the Same Highly Successful Model Going Forward?

The ugly truth is that while making the best genetics widely available may make good public policy, it also makes genetics a low-value commodity from which it is difficult to make a direct profit. This situation is not unique to forest trees but is a problem shared by all minor crops, many that have breeding programs supported primarily by the public (Berland and Lewontin 1986). This constraint has actually been less problematic in forest industry than in other breeding programs for at least two reasons. First, the cost of tree improvement, including in-kind contributions, has been relatively modest given the value of the crop. Second, and more importantly, most participants in forest genetics programs made their profits with their manufacturing facilities, not from the sale of genetic improvement, where most seed companies make their incomes.

Mergers among integrated forest companies, that rapidly accelerated during the 1990s, were followed closely by the divestment of corporate forest land to institutional investors. This resulted in a reduction in the number of players that could logically participate in breeding programs; these programs benefit from the economies of scale. Concurrently, several state agencies adopted the position that economic development should be left to the private sector, and have closed tree improvement programs, abandoned orchards, and shuttered nurseries. Furthermore, the new class of large institutional forestland investor/owners frequently has different investment criteria than those historically held by integrated forest industry. Some have recognized that they have a vested interest in forest productivity to reduce the cost of their own production and to maintain a viable manufacturing customer base. These organizations have been both aggressive and innovative in maintaining their commitment to tree improvement programs. Other institutional owners, representing a sizeable proportion of the landbase previously supporting tree improvement programs, have opted to buy seedlings on the open market and essentially forego the in-kind cost of tree improvement. This is a completely rational decision where fragmented ownership reduces the size of holdings within any one breeding and deployment zone below the level needed to support a stand-alone tree improvement program.

Concurrent to the withdrawal of state forestry agencies from seedling production and the rise of a new base of customers, a stand-alone forest tree nursery business has arisen. This was made possible, in part, by the divestiture of land by forest industry. As some organizations no longer had an internal need for seeds, the consequence was that the best genetics from existing orchards have become more widely available. These new ventures market a wider range of genetics than previously available, but must make their profit on the sale of seedlings rather than higher value stumpage. Strategies for a nursery business can include selling a low cost commodity where there is little incentive to develop new products. Alternatively, market differentiation can be developed by selling full-sib families, varietal lines, or seedlings that differ genetically from other sources on the market. These seedlings offer good genetic value to the customer. Unless the market recognizes their economic value, there will be little incentive to invest in the future of tree improvement. Market differentiation can also drive increased competition. As tree improvement is primarily a pre-commercial population development program, this need not limit collaboration and, in fact, sharing the cost of development could be an incentive for more intensive cooperation.

Tree improvement is now poised to make remarkable gains. Swift progress is occurring in vegetative propagation, selection efficiencies, and deployment strategies. Substantial investments are occurring in silviculture research (Vance and others 2010) and basic genetics (Whetten and Kellison 2010) that can make our forest potentially far more productive. The USDA National Institute of Food and Agriculture (NIFA) is making very large investments in basic research and proof-of-concept type experiments (NIFA 2010a, 2010b); the results from this government-sponsored research, however, will have to be translated into operational programs through applied breeding programs. As a consequence, future tree improvement programs will require community resources far beyond what has been considered normal in the past (Table 1). In addition, it is likely that it will be desirable to measure attributes such as BTU content or nanostructure reactivity that will involve investments in new equipment or the development of service laboratories (Briggs 2010; Wegner and others 2010). These factors offer tremendous opportunities to improve productivity and to develop novel products, but they will come at an increased cost and complexity that we have not yet incorporated into our current system.

program.
Germplasm Conservation Scion banks and long-term seed storage
DNA Stock Centers
cDNA libraries
BAC libraries
PCR primer sets
Research Populations
Association and mapping populations beyond standard progeny testing
(crossing, establishing, maintaining, and measurements)
Specialized Skills in Biometrics
Laboratory Facilities for Phenotyping
Wood density
Microfibril angle
BTU content
Fermentation /conversion efficiency
Nanocrystals
Nanostructure reactivity

1. Infrastructure that may be needed to support a modern tree breading

Complexity comes in many forms, but probably the most challenging to the current structure will be the need to manage intellectual property. In theory, this can be done by assigning responsibilities, rights, and rewards according to each organization's contribution. Tree improvement programs have been extremely successful over the past 50 years, working with handshake agreements as these three factors have been more or less equally shared. It seems unlikely that this structure will be successful going forward as organizational capacities and goals diverge.

What Can Game Theory Teach Us about Tree Improvement?_____

These substantial organizational difficulties can be overcome. Game Theory, the study of how individuals interact and organize (see for example Myerson 1991), offers an explanation as to why tree improvement collaborations have been successful in the past, points out shortcomings in the current cooperative structure, and offers some insights into how we may choose to manage our future. Briefly, collaborations develop when the benefits to the participants exceed what they can expect from acting as individuals. In other words, individuals will work together when the whole is greater than the sum of the parts. This has certainly been true in tree improvement programs where population development is primarily a pre-commercial development program that benefits directly from economies of scale. In Game Theory terminology, shortly after collaboration develops, "cheaters and freeloaders" emerge that reap the benefits of the collaboration without contributing to the group. The system conveys a competitive advantage to them, so their decision is completely justified from an individual's perspective. Their emergence within the system is inevitable and unavoidable.

Collaborations can tolerate some level of defection, which again reflects conditions in tree improvement's past. For example, nurseries operated by integrated forest industries could afford to subsidize seedlings for outside sales because this reduced the per-unit cost of seedlings used internally. When the motivation to "cheat or freeload" reaches a critical level, however, one of two things will happen. Collaborations collapse and a competitive environment predominates, or methods are found to promote continued teamwork through incentives and/or punishments applied to limit defectors. An important corollary is that collaboration is not always the best option. When development of a product is anticipated to be costly and economically risky, the prospect of having a competitive advantage is frequently a necessary motivation for making the required investment. Examples from our industry include the development of varietal lines and genetically modified trees. A second corollary is that enforcement of collaboration in the face of increasing pressures to defect comes at a cost to the partnering organizations. This is frequently in the form of increased complexity, lack of individual flexibility, or increased operating expenses.

Therefore, according to Game Theory, three conditions must be met for continued collaborative tree improvement efforts to be warranted: 1) net benefits of collaboration must exceed those of competition; 2) benefits must also exceed the cost of enforcement against defectors; and 3) participants must perceive that responsibilities, rights, and rewards are fairly distributed. Condition 1 will be true as long as population improvement is deemed a priority because very large breeding programs are out of reach of single organizations. In fact, the opportunity cost in allowing collaboration to atrophy would be substantial if the comparison to corn yields is accurate (Figure 2). Conditions 2 and 3 can be met with appropriate organizational structure that fairly distributes costs and manages intellectual property. If done properly, this would encourage investment from landowners and investors with a vested interest in improved forest productivity that currently opt out of directly supporting tree improvement research.



Figure 2. Estimated progress of forest tree breeding from realized gain and number of generations in the breeding program compared to the actual US corn yields reflecting the application of modern breeding programs (corn yields from Ruttan 1999).

Other crops have done this by collecting a mandatory check-off fee at the point of sale that is then used to support research (for example, the Cotton Board and the National Peanut Board). This system supports university research that supplies breeding stock to seed companies who in turn sell seeds to farmers. An analogous system in forestry would be the collection of a check-off fee at the mill gate based on delivered tonnage. The cost of tree improvement would then be paid by the landowner and/or manufacturer who arguably benefit most directly from investments in productivity improvement. This solution, however, is extremely unlikely for forestry as it would require legislation.

Conclusions_

Forestry could follow another structure made possible because our production model is somewhat different. We are simultaneously the research arm, the seed company, and in many cases, also the farmer. A breeders association could raise funds based on a voluntary metric, such as the amount of outside seedling sales, and redistribute this income to support breeding and progeny testing and the necessary community resources listed in Table 1. Regardless of the model the forestry community ultimately chooses, we believe it should incorporate the following elements:

1. Those that benefit from tree improvement should pay for it. While the ultimate beneficiary is the consumer and the society to which he/she belongs, this is too nebulous to be practical. The points in the value chain where money changes hands determine the logical places where value can be captured. Possible links in the value chain are: 1) sale of seeds; 2) sale of seedlings; 3) sale of stumpage; 4) sale of a manufactured product; or 5) taxes raised due to increased economic activity.

- 2. Additional funds over and above the in-kind support currently provided by the participants in tree improvement cooperatives (Figure 1) must be generated so that the infrastructure and community resources necessary to support a modern tree improvement program can be funded. The implication of the need for additional sources of funding is that participation of defectors will have to be enforced.
- 3. An infrastructure should be created so that money raised goes to those who add value. Since this must be viewed as fair by the participants, a pay-forperformance system seems appropriate. The organizations that add value are those that create intellectual property in the form of ever better genetics, that is, those that do the actual breeding and progeny testing. We propose that the Cooperative staffs hosted at the universities should continue to raise their funds as they do now, by selling services to support the breeding and testing organizations.
- 4. Intellectual property developed through collaborative breeding programs must be actively managed to parse responsibilities, rights, and rewards fairly. This is necessary to encourage and value participation by organizations of vastly different capabilities by according them the rights and rewards that are consistent with their contribution. Simultaneously, cooperative breeding populations need to be available to proprietary breeding programs in a system that encourages and rewards innovative product development.

Historically, tree improvement has been one of the best investments a landowner could make. We have certainly only begun the process of crop domestication in three generations of breeding and stand to make even faster and more valuable progress in the future due to rapid improvements in the biological sciences. Whether these aspirations are realized will depend largely on the organizational choices we make. Our future is up to us.

Acknowledgments

Most of these ideas presented here were first aired at an *ad hoc* committee formed at the behest of the Southern Group of State Foresters. This committee's charge was to discuss the future of tree improvement. While no consensus was reached, the discussion was extremely worthwhile. The committee included Paul Belonger, Plum Creek Timber Company; Thomas D Byram, Western Gulf Forest Tree Improvement Program, Texas Forest Service; Barbara Crane, USDA Forest Service; George Hernandez, USDA Forest Service; Dudley Huber, Cooperative Forest Genetics Research Program, University of Florida; Steve McKeand, Cooperative Tree Improvement Program, North Carolina State University; Russell Pohl, Georgia Forestry Commission; Kenneth Roeder, North Carolina Division of Forest Resources.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

Historical Forest Seedling Production in the Southern United States: 2008 to 2009 Planting Season

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Enebak SA. 2011. Historical forest seedling production in the southern United States: 2008 to 2009 planting season. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 19-34. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p065.html

Abstract: Seedling production across the southern US for the 2008 to 2009 planting season was 1.05 billion seedlings, a decrease of 53.6 million (5%) from the 2007 to 2008 planting season. The vast majority (90%) of reduction in conifer seedling production from 2008 was bareroot loblolly (*Pinus taeda*) and slash (*P. elliottii*) pine. Hardwoods were about 1% of regional seedling production, a decrease of 2.7% from the 2007 planting season. Only 1% of all seedlings were grown in containers, with longleaf pine (*P. palustris*) comprising the majority of this production. During the past 80 years of tree planting, seedling outplanting peaked at 1.1 million ha (2.7 million ac) in 1991 and has declined annually. In addition to fewer hectares planted and a reduction in seedlings produced, more than 25 forest seedling nurseries have ceased operations since 1995.

Keywords: bareroot nurseries, container nurseries, conifer seedlings, hardwood seedlings

Introduction _

Seedling production and planting numbers in the southern United States have historically been collected by the USDA Forest Service and forest agencies of individual states. These data have been published in several types of reports that include single-year and multiple-year summaries (for example, Williston 1980; Moulton 1999; Moulton and Hernandez 1999; Georgia Forestry Commission's annual Planting Report). To supplement these data, in 2002, Auburn University Southern Forest Nursery Management Cooperative (SFNMC) began conducting a southern United States annual survey to determine production numbers for the previous planting season.

Seedling production data from 2002 to 2009 was obtained through a questionnaire mailed in June of each year to more than 200 plant nurseries in 12 southern states: Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia. The questionnaire was two pages in length and requested production numbers (not sales) for the nursery season for major pine and hardwood species. We attempted to contact all nurseries, regardless of affiliation or ownership, including those not associated with the SFNMC. The survey was followed by phone contact until the information from all nurseries was received. Additionally, The Longleaf Alliance, headquartered in Andalusia, AL, was contacted, and longleaf pine (*Pinus palustris*) seedling production by type was compared among both organizations to ensure that the smaller seedling producers that were not members of the SFNMC were included.

2008 to 2009 Seedling Production ____

Conifer Seedling Production

A total of 851 million bareroot (Table 1) and 124.7 million container (Table 2) conifer seedlings were produced during the 2008 to 2009 season, for a total conifer production of 975.7 million (Table 3). This was a decrease of 10% in bareroot production and an increase of 79% in container conifer production over last year. Overall, there was a decrease of 4.2% across the region in seedling production from 1.02 billion conifers produced in 2007 to 2008. Loblolly pine (*P. taeda*) was the most commonly grown species in the region, accounting for 81% of all conifer production, followed by slash pine (*P. elliottii*) at 9%, and longleaf pine at 8%. These three species accounted for 98% of all conifers produced in 2008 to 2009. Baldcypress (*Taxodium distichum*) was the fourth most important species in terms of production (0.3%), followed closely by white pine (*P. strobus*), shortleaf pine (*P. echinata*), sand pine (*P. clausa*), Virginia pine (*P. virginiana*) and Fraser fir (*Abies fraseri*) (Table 3).

	_	**%	15	12	4	16	3	10	9	٢	17	-	12	3	
	Tota		129,213	106,143	34,841	138,799	24,609	87,219	50,459	5,041	144,743	4,781	101,794	23,365	851,007
	Pine	*%	0	0	0	0	0	0	2	0	0	3	0	4	0
	White		0	0	0	0	0	0	1,200	0	72	123	0	950	2,345
	nia e	*%	0	0	0	0	0	0	0	-	0	ю	-	٦	0
	Virgi		79	0	81	170	70	0	110	64	48	141	598	200	1,561
	ine	*%	5	0	70	27	22	0	0	0	ю	0	10	0	10
	Slash p		6,056	0	24,233	37,764	5,429	264	0	0	3,748	0	9,905	0	87,399
	eaf è	*%	0	-	0	0	0	0	0	-	0	9	0	1	0
	Shortl pine		0	550	0	40	0	0	200	69	0	304	0	175	1,338
	oine	*%	0	0	4	0	0	0	0	0	0	0	0	0	0
	Sand p		0	0	1,373	0	0	0	0	0	0	0	0	0	1,373
	ß		0	0	3	0	0	0	0	4	-	2	0	0	0
,	Other		0	0	1097	200	51	0	30	216	896	80	0	0	2,570
	eaf	*%	0	0	14	-	2	0	0	0	-	0	0	0	-
	Longle pine		227	0	5,048	2,035	370	0	100	0	1,908	0	0	0	9,688
	oine	*%	95	66	9	71	75	100	95	92	95	84	06	94	87
	Loblolly p		122,769	104,772	2,214	98,320	18,339	86,814	48,000	4,649	137,737	4,021	91,291	22,000	740,926
	er fir	*%	0	0	0	0	0	0	-	0	0	0	0	0	0
	Frase		0	0	0	0	0	0	744	0	0	0	0	0	744
	ress	*%	0	-	2	0	٢	0	0	1	0	2	0	0	0
	Baldcyp		82	821	795	270	350	141	75	43	334	112	0	40	3,063
	State		Alabama	Arkansas	Florida	Georgia	Louisiana	Mississippi	North Carolina	Oklahoma	South Carolina	Tennessee	Texas	Virginia	Region

Table 1. Bareroot conifer seedling production by state for the 2008 to 2009 planting season across the southern US for various species (in thousands).

*Percentage of state production **Percentage of regional production

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	al	**%	e	0	ი	75	0	n	6	•	-	0	0	0	
	Tot		3,986	0	11,405	93,121	0	4,220	11,030	8	926	0	-	0	124,697
ite	Je	*%	0	0	0	0	0	0	0	0	0	0	0	•	0
١W	pi		0	0	0	0	0	0	0	0	0	0	0	0	0
inia	ы	*%	0	0	0	0	0	0	0	13	0	0	0	0	0
Virg	pii		0	0	0	15	0	0	0	-	0	0	0	0	16
	pine	*%	0	0	9	с	0	0	0	0	0	0	0	0	e
	Slash		0	0	650	3161	0	0	0	0	0	0	0	0	3,811
tleaf	Je	*%	0	0	0	-	0	0	0	0	0	0	0	0	-
Shor	pir		0	0	0	825	0	0	0	0	0	0	0	0	825
	pine	*%	0	0	0	0	0	0	0	0	0	0	0	•	0
Sand	Sand		0	0	35	0	0	0	0	0	0	0	0	0	35
	(0		0	0	4	0	0	0	~	63	0	0	0	0	-
Others	Others		0	0	460	177	0	0	770	5	0	0	0	0	1,412
af		*%	34	0	89	56	0	73	50	0	66	0	0	0	59
Longle	pine		1,351	0	10,161	52,145	0	3,062	5,500	0	920	0	0	0	73,139
Ŋ		*%	66	0	0	40	0	27	42	25	-	0	0	0	36
Loblo	pine		2,635	0	-	36,793	0	1,158	4,600	2	9	0	0	0	45,195
	vr fir	*%	0	0	0	0	0	0	~	0	0	0	0	0	0
	Frase		0	0	0	0	0	0	160	0	0	0	0	0	160
	ress	*%	0	0	-	0	0	0	0	0	0	0	100	•	0
	Baldcyp		0	0	98	5	0	0	0	0	0	0	~	0	104
	State		Alabama	Arkansas	Florida	Georgia	Louisiana	Mississippi	North Carolina	Oklahoma	South Carolina	Tennessee	Texas	Virginia	Region

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*Percentage of state production **Percentage of regional production

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							Longle	af					Shortl	eaf			Virgi	nia				
State	Baldcyp	ress	Frase	er fir	Loblolly p	oine	pine		Other	s	Sand p	ine	pine	0	Slash p	ine	pir	e	White <b>F</b>	oine	Total	
		*%		*%		*%		*%		*%		*%		*%		*%		*%		*%		**%
Alabama	82	0	0	0	125,404	94	1,578	-	0	0	0	0	0	0	6,056	5	79	0	0	0	133,199	14
Arkansas	821	-	0	0	104,772	66	0	0	0	0	0	0	550	-	0	0	0	0	0	0	106,143	11
Florida	893	2	0	0	2,215	5	15,209	33	1557	n	1,408	e	0	0	24,883	54	81	0	0	0	46,246	5
Georgia	275	0	0	0	135,113	58	54,180	23	377	0	0	0	865	0	40,925	18	185	0	0	0	231,920	24
Louisiana	350	-	0	0	18,339	75	370	2	51	0	0	0	0	0	5,429	22	70	0	0	0	24,609	e
Mississippi	141	0	0	0	87,972	96	3,062	с	0	0	0	0	0	0	264	0	0	0	0	0	91,439	6
North Carolina	75	0	904	-	52,600	86	5,600	6	800	-	0	0	200	0	0	0	110	0	1,200	2	61,489	9
Oklahoma	43	~	0	0	4,651	92	0	0	221	4	0	0	69	-	0	0	65	-	0	0	5,049	۲
South Carolina	334	0	0	0	137,743	95	2,828	5	896	-	0	0	0	0	3,748	n	48	0	72	0	145,669	15
Tennessee	112	2	0	0	4,021	84	0	0	80	2	0	0	304	9	0	0	141	3	123	3	4,781	0
Texas	٢	0	0	0	91,291	06	0	0	0	0	0	0	0	0	9,905	10	598	٦	0	0	101,795	10
Virginia	40	0	0	0	22,000	94	0	0	0	0	0	0	175	-	0	0	200	٢	950	4	23,365	2
Region	3,167	0	904	0	786,121	81	82,827	8	3,982	0	1,408	0	2,163	0	91,210	6	1577	0	2345	0	975,704	

Table 3. Conifer seedling production by state for the 2008 to 2009 planting season across the southern US for various species (in thousands).

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*Percentage of state production **Percentage of regional production

The conifer category "others" (pitch pine [P.rigida], Atlantic white-cedar [*Chamaecyparis thyoides*], and pitch × loblolly) made up just under 4 million of other conifer species grown in 2008 to 2009.

Container seedlings comprised 12.7% of conifer production, up from 6.8% of the conifers produced in 2007 to 2008. This is an increase (55 million) over last year, primarily consisting of longleaf and loblolly pine at 73.1 million and 45.1 million seedlings, respectively. Container production of longleaf seedlings for the 2008 to 2009 growing season was 88%, up from 83% for 2007 to 2008. Georgia was, by far, the primary producer of container conifer seedlings at 93.13 million, or 75% of all container conifer seedlings produced in 2008 to 2009.

All surveyed states produced conifer nursery stock. The amount ranged from 231.9 million in Georgia to about 4.7 million in Tennessee. Georgia produced 24% of all conifer planting stock in the southern US. In terms of total conifer production, the order was: Georgia, South Carolina, Alabama, Arkansas, Texas, Mississippi, North Carolina, Florida, Louisiana, Virginia, Oklahoma, and Tennessee (Table 3).

#### **Hardwood Seedling Production**

In the southern region, 28.9 million bareroot (Table 4) and 321,000 container (Table 5) hardwood seedlings were produced during the 2008 to 2009 season; total hardwood seedling production was 29.2 million (Table 6). This is a reduction of more than 10 million (or 25%) hardwood seedlings from 2007 to 2008, and 23 million less than the 52.4 million produced in the 2006 to 2007 growing season (McNabb 2007). Of those species being produced, Quercus was by far the most important genera, comprising 61% of all hardwood production (17.8 million). This is followed by "others" at 26%, green ash (Fraxinus pennsylvanica) at 4%, sweetgum (Liquidambar styraciflua) at 3%, pecan (Carya illinoensis) at 2%, dogwood (Cornus spp.) at 2%, yellow poplar (Liriodendron tulipifera) at 1%, sycamore (Platanus occidentalis) at 1%, black walnut (Juglans nigra) at 1%, and cottonwood (Populus deltoides) at 0.3%. Hardwoods were grown in all states surveyed, ranging from 9.1 million in Arkansas to 10,000 in Texas. The top five hardwood producing states in the region were Arkansas, South Carolina, Florida, Georgia, and Louisiana (Table 6).

### Production by Ownership Category

With the reclassification of nursery production from industry (International Paper) to private (ArborGen), there was a major shift of seedling production by ownership category (Table 7). A "private" nursery is defined as private ownership but not part of an organization or company that operates a wood processing facility, that is, "non-industrial." In 2007 to 2008, the majority of seedling production occurred within industrial nurseries (767.5 million, or 73%), followed far behind by private nurseries (150.0 million, or 14%), with state nurseries a close third (140.5 million, or 13%). In 2008 to 2009, there was a switch from last year's survey in terms of the proportion of total seedling production by owner category, with private nurseries produced 503.7 million seedlings (50%), followed by industrial nurseries at 377.1 million (38%), and state nurseries at 123.9 million (12%). Private nurseries supplied 80% of container conifer planting stock (99.9 million), followed by industry at 12% (15.3 million), and state nurseries at 8% (9.4 million) (Table 8). With the reclassification of the nursery producers, it is difficult to determine overall trends in container versus bareroot production. Overall bareroot seedling production is down 10%, and container seedling production is up an astounding 79% from last year's growing season. So while some nurseries cut back on bareroot seedling production, there were some nurseries that significantly increased container production (primarily longleaf pine) during the last year.

In the 2007 to 2008 growing season, state nurseries had the largest proportion of hardwood seedling production (17.6 million, or 45%) when compared to either the industry (12.6 million, or 32%) or private (8.7 million, or 22%) producers (Table 9). In 2008 to 2009, private nurseries produced more hardwood seedlings (13.0 million, or 45%) than both state (12.7 million, or 44%) and industrial nurseries (3.0 million, or 11%). While some of this increase in private over industrial production may be due to the reclassification of nurseries as described above, this is the first time in 7 years that state nurseries did not lead in hardwood production.

Seedling production for all stocktypes (container or bareroot) and tree type (conifer, hardwood) by forest agency (state, private, industry) and by state is shown in Table 10. Overall, private forest seedling nurseries produced 504 million or 50% of all seedlings grown. This was followed by industrial nurseries at 377.2 million (38%) seedlings and then state nurseries at 123.9 million (12%) seedlings.

# State Ranking and Changes from 2007 to 2008

A comparison of state-by-state ranking is provided in Table 11. The 5% decline in total seedling production from last year is not distributed evenly across the region. While a few states had a moderate reduction in seedling production (that is, Arkansas down 2%; Georgia down 5%; and Oklahoma down 5%), four states had significant reductions in seedling production from last year's growing season (Mississippi down 12%; North Carolina down 9%; Virginia down 26%; and South Carolina down 14%). Florida was the only state to increase their seedling production from last year, by 39%. Four states, Alabama, Louisiana, Tennessee, and Texas, remained unchanged in 2008 to 2009 from the 2007 to 2008 growing season. Despite the rather large swings in seedling production in an individual state, it does not necessarily indicate a large change in regional production, as seedling production is down region-wide from last year.

#### **Total Seedling Production**

Collectively, the forest seedling nurseries surveyed in the southern US produced 851.0 million bareroot conifers, 124.7 million container conifers, 28.8 million bareroot hardwoods, and 321,000 container hardwoods during the 2008 to 2009 growing season. The total forest seedlings produced in 2008 to 2009 was 1.01 billion seedlings. This is 5% (53.6 million) fewer seedlings than were produced in 2007 to 2008 (Tables 1, 2, 3, 4, and 11).

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ar	%	5	0	-	0	0	-	4	0	-	-	0	0	-
Yello	-	41	10	28	ß	0	15	30	0	42	17	0	2	190
nt	%	0	0	0	0	0	0	5	с	0	œ	0	-	-
Walr		0	0	-	0	10	0	40	30	13	92	0	30	216
Jore	%	-	0	2	0	-	0	5	4	2	0	0	e	-
Svcan		12	ъ	71	ъ	20	0	20	32	79	0	0	64	308
unp	%	0	с	8	0	e	0	0	0	2	5	0	0	e
Sweet		0	237	289	0	67	0	с	0	72	60	0	0	728
_	%	0	4	0	0	2	-	0	5	2	-	0	0	2
Peca		0	377	2	12	50	7	0	48	77	15	0	0	592
۶	%	ю	7	50	17	20	0	34	70	41	10	0	65	26
Othe		25	613	1,892	423	437	0	285	631	1,742	113	0	1,377	7,538
	%	89	81	34	79	69	96	45	13	45	70	0	21	61
Oak		803	7,418	1,307	2,012	1,500	1,000	375	117	1,886	796	0	440	17,654
ash	%	2	5	e	e	e	-	4	e	4	4	0	4	4
Green		21	452	96	72	60	12	30	24	177	42	0	80	1,066
poo	%	0	0	с	-	0	0	9	2	з	0	0	9	2
Dody	>	e	34	117	8	0	0	50	8	112	0	0	130	498
poowu	%	0	0	0	0	2	0	0	0	0	0	0	0	0
Cotto		0	40	0	0	45	0	0	0	0	0	0	0	85
State		Alabama	Arkansas	Florida	Georgia	Louisiana	Mississippi	North Carolina	Oklahoma	South Carolina	Tennessee	Texas	Virginia	Region

^{*}Percentage of state production **Percentage of regional production

	_	 	 _

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able 5. Container hardwood seedling production by state for the 2008 to 2009 planting season across the southern US for various species (in thousands).

State	Cotto	poowu	Dogv	poov	Gree	h ash	Oa	¥	Othe	rs	Pec	an	Sweet	aum	Syci	amore	Wal	nut	Yell pop	ow Iar	Tot	al
		%		%		%		%		%		%		%		%		%		%		%
Alabama	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arkansas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Florida	0	0	9	5	-	-	66	59	36	32	-	-	-	-	0	0	0	0	0	0	111	35
Georgia	0	0	0	0	0	0	80	4	0	0	0	0	116	59	0	0	0	0	0	0	196	61
Louisiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mississippi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
North Carolina	0	0	0	0	0	0	7	50	5	50	0	0	0	0	0	0	0	0	0	0	4	-
Oklahoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
South Carolina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tennessee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Texas	0	0	0	0	4	40	9	60	0	0	0	0	0	0	0	0	0	0	0	0	10	3
Virginia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Region	0	0	9	7	S	2	154	48	38	12	-	0	117	36	0	0	0	0	0	0	321	

*Percentage of state production **Percentage of regional production

low Total	0/0	0/	5 <b>305</b> 3	7.0         905         3           0         9,186         31	70         70         70         70         70         70         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71         71 <th71< th=""> <th 71<="" <="" th=""><th>70         905         3           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9</th><th>No         Sold         S</th><th>No         Solution         S</th><th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9           0         2,189         7           1         1,038         4           4         837         3</th><th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9           0         2,759         9           1         3,914         13           1         1,038         7           1         1,038         4           1         1,038         3           0         2,189         7           1         1,038         4           0         900         3</th><th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           1         3,914         13           0         2,759         9           7         3,914         13           1         3,914         13           1         1,038         4           1         1,038         4           4         837         3           0         900         3           1         4,200         14</th><th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           1         3,914         13           0         2,759         9           0         2,189         7           1         1,038         4           1         1,038         4           1         1,038         3           0         2,189         7           1         1,038         4           1         1,038         3           1         1,038         3           1         1,038         3           1         4,200         3           1         1,135         4</th><th>$\begin{array}{c ccccccccccccccccccccccccccccccccccc$</th></th></th71<>	<th>70         905         3           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9</th> <th>No         Sold         S</th> <th>No         Solution         S</th> <th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9           0         2,189         7           1         1,038         4           4         837         3</th> <th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9           0         2,759         9           1         3,914         13           1         1,038         7           1         1,038         4           1         1,038         3           0         2,189         7           1         1,038         4           0         900         3</th> <th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           1         3,914         13           0         2,759         9           7         3,914         13           1         3,914         13           1         1,038         4           1         1,038         4           4         837         3           0         900         3           1         4,200         14</th> <th>70         70         70           5         905         3           0         9,186         31           1         3,914         13           1         3,914         13           0         2,759         9           0         2,189         7           1         1,038         4           1         1,038         4           1         1,038         3           0         2,189         7           1         1,038         4           1         1,038         3           1         1,038         3           1         1,038         3           1         4,200         3           1         1,135         4</th> <th>$\begin{array}{c ccccccccccccccccccccccccccccccccccc$</th>	70         905         3           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9	No         Sold         S	No         Solution         S	70         70         70           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9           0         2,189         7           1         1,038         4           4         837         3	70         70         70           5         905         3           0         9,186         31           1         3,914         13           0         2,759         9           0         2,759         9           1         3,914         13           1         1,038         7           1         1,038         4           1         1,038         3           0         2,189         7           1         1,038         4           0         900         3	70         70         70           5         905         3           0         9,186         31           1         3,914         13           1         3,914         13           0         2,759         9           7         3,914         13           1         3,914         13           1         1,038         4           1         1,038         4           4         837         3           0         900         3           1         4,200         14	70         70         70           5         905         3           0         9,186         31           1         3,914         13           1         3,914         13           0         2,759         9           0         2,189         7           1         1,038         4           1         1,038         4           1         1,038         3           0         2,189         7           1         1,038         4           1         1,038         3           1         1,038         3           1         1,038         3           1         4,200         3           1         1,135         4	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
		41	10	28	5	0	15	30	0	42	17	0		
Inut	%	0	0	0	0	0	0	2ı	ო	0	∞	0	-	
Wa		0	0	~	0	10	0	40	30	13	92	0	0	
more	%	-	0	~	0	-	0	2	4	7	0	0	c	
Syca		12	2	71	5	20	0	20	32	79	0	0	č	
tgum	%	0	e	2	4	n	0	0	0	7	2	0	6	
Swee		0	237	290	116	67	0	m	0	72	60	0	-	
an	%	0	4	0	0	~	-	0	ъ	7	-	0	6	
Pec		0	377	e	12	50	£	0	48	77	15	0	6	
S	%	e	7	49	15	20	0	34	70	41	10	0	L C	
Othe		25	613	1,928	423	437	0	287	631	1,742	113	0	110 1	
	%	89	81	35	76	69	96	45	13	45	70	60	2	
Oak		803	7,418	1,373	2,092	1,500	1,000	377	117	1,886	796	9	077	
ash	%	2	S	2	e	e	-	4	e	4	4	40	-	
Green		21	452	97	72	60	12	30	24	177	42	4	0	
poov	%	0	0	e	-	0	0	9	2	e	0	0	9	
Dogv		e	8	123	8	0	0	50	18	112	0	0	120	
poom	%	0	0	0	0	2	0	0	0	0	0	0	-	
Cotton		0	40	0	0	45	0	0	0	0	0	0	-	
State		Alabama	Arkansas	Florida	Georgia	Louisiana	Mississippi	North Carolina	Oklahoma	South Carolina	Tennessee	Texas	Virginia	

Table 6. Hardwood seedling production by state for the 2008 to 2009 planting season across the southern US for various species (in thousands).

^{*}Percentage of state production **Percentage of regional production
Table 7. Species production for the 2008 to 2009 planting season across the southern US by ownership category (in thousands). Private = nurseries owned by companies or individuals that do not own wood processing facilities; Industry = nurseries owned by companies that have wood processing facilities; BR = bareroot; C= container.

		State	)	Priva	te	Indust	ry	
Туре	Species		%*		%*		%*	Total
	Baldcypress	1,071	35	1,697	55	295	10	3,063
	Fraser fir	744	100	0	0	0	0	744
	Lobiolly	68,877	9	325,409	44	346,640	47	740,926
Ì	Longleaf	5,710	59	3,751	39	227	2	9,688
	Others	1,250	49	1,320	51	0	0	2,570
BR	Sand	223	16	1,150	84	0	0	1,373
	Shortleaf	1,338	100	0	0	0	0	1,338
	Slash	19,434	22	56,367	64	11,598	13	87,399
	Virginia	783	50	778	50	0	0	1,561
ĺ	White	2,345	100	0	0	0	0	2,345
	Total	101,775	12	390,472	46	358,760	42	851,007
	Baldcypress	0	0	104	100	0	0	104
Ì	Fraser fir	160	100	0	0	0	0	160
	Lobiolly	408	1	36,899	82	7,888	17	45,195
	Longleaf	7.020	10	58,706	81	7.413	10	73.139
	Others	1 225	87	187	13	0	0	1 412
С	Sand	15	43	20	57	0	0	35
	Shortleaf	0		825	100	0	0	825
	Sloch	600	16	2 211	04	0	0	2 044
	Vincinio	000	10	3,211	04	0	0	3,011
	Virginia	1	6	15	94	0	0	16
	White	0	0	0	0	0	0	0
	Total	9,429	8	99,967	80	15,301	12	124,697
	Cottonwood	45	53	40	47		0	85
	Dogwood	228	46	267	54	3	1	498
	Green ash	454	43	586	55	26	2	1,066
	Oak	8,013	45	6,686	38	2,955	17	17,654
	Others	3,101	41	4,387	58	50	1	7,538
BR	Pecan	365	62	197	33	30	5	592
Ì	Sweetgum	142	20	586	80	0	0	728
	Sycamore	157	51	139	45	12	4	308
	Walnut	206	95	10	5	0	0	216
	Yel, Poplar	62	33	109	57	19	10	190
	Total	12.773	44	13.007	45	3.095	11	28.875
	Cottonwood	0	0	0	0	0	0	
	Dogwood	0	0	6	100	0	0	6
	Green ash	0	0	5	100	0	0	5
	Oak	2	1	152	99	0	0	154
	Others	2	5	36	95	0	0	38
С	Pecan	0	0	1	100	0	0	1
	Sweetgum	0	0	117	100	0	0	117
	Sycamore	0	0	0	0	0	0	0
	Walnut	0	0	0	0	0	0	0
	Yellow Poplar	0	0	0	0	0	0	0
	Total	4	1	317	99	0	0	321
All	Region	123.981	12	503.763	50	377.156	38	1,004.900
	5	,				,		, ,

*Percentage of species production for that ownership class

Table 8. Conifer seedling production for the 2008 to 2009 planting season across the southern US by ownership category (in thousands). Private = nurseries owned by companies or individuals that have wood processing facilities. Percentages are calculated for each stocktype within a state.

		-	Bareroot c	onifer				ŭ	ontainer co	onife	-			Con	ifer seedlinç	g prod	uction		
State	State	%	Private	%	Industry	%	State	%	Private	%	Industry	%	State	%	Private	%	Industry	%	Total
Alabama	0	0	60,245	45	68,968	52	0	0	105	0	3881	e	0	0	60,350	45	72,849	55	133,199
Arkansas	6,450	9	44,587	42	55,106	52	0	0	0	0	0	0	6,450	9	44,587	42	55,106	52	106,143
Florida	10,415	21	24,426	48	0	0	4,665	6	6,740	22	0	0	15,080	30	31,166	70	0	0	46,246
Georgia	13,883	9	119,786	52	5,130	2	0	0	93,121	40	0	0	13,883	6	212,907	92	5,130	2	231,920
Louisiana	24,609	100	0	0	0	0	0	0	0	0	0	0	24,609	100	0	0	0	0	24,609
Mississippi	0	0	0	0	87,219	95	0	0	0	0	4220	5	0	0	0	0	91,439	100	91,439
North Carolina	8,459	15	0	0	42,000	72	3,830	7	0	0	7200	7	12,289	21	0	0	49,200	79	61,489
Oklahoma	5,041	100	0	0	0	0	8	0	0	0	0	0	5,049	100	0	0	0	0	5,049
South Carolina	4,772	ю	39,634	27	100,337	69	926	-	0	0	0	0	5,698	4	39,634	27	100,337	69	145,669
Tennessee	4,781	100	0	0	0	0	0	0	0	0	0	0	4,781	100	0	0	0	0	4,781
Texas	0	0	101,794	100	0	0	0	0	1	0	0	0	0	0	101,795	100	0	0	101,795
Virginia	23,365	100	0	0	0	0	0	0	0	0	0	0	23,365	100	0	0	0	0	23,365
Region	101,775	10	390,472	40	358,760	37	9,429	-	99,967	11	15,301	-	111,204	11	490,439	51	374,061	38	975,704

	Total	905	9,186	3,914	2,759	2,189	1,038	837	900	4,200	1,135	10	2,123	29,196	
	%	25	20	0	0	0	100	0	0	0	0	0	0	11	
duction	Industry	224	1,833	0	0	0	1,038	0	0	0	0	0	0	3,095	
ng pro	%	75	29	100	74	0	0	0	0	95	0	100	0	46	
ood seed!	Private	681	2,698	3,914	2,031	0	0	0	0	3,990	0	10	0	13,324	
Hardwo	%	0	51	0	26	100	0	100	100	5	100	0	100	44	
	State	0	4,655	0	728	2,189	0	837	900	210	1,135	0	2,123	12,777	
	%	0	0	0	0	0	0	0	0	0	0	0	0	0	
po	Industry	0	0	0	0	0	0	0	0	0	0	0	0	0	
ardwo	%	0	0	3	7	0	0	0	0	0	0	100	0	-	
ontainer h	Private	0	0	111	196	0	0	0	0	0	0	10	0	317	
Ŭ	%	0	0	0	0	0	0	0	0	0	0	0	0	0	
	State	0	0	0	0	0	0	4	0	0	0	0	0	4	
	%	25	20	0	0	0	100	0	0	0	0	0	0	1	
po	Industry	224	1,833	0	0	0	1,038	0	0	0	0	0	0	3,095	
ardwo	%	75	29	97	67	0	0	0	0	95	0	0	0	45	
Bareroot h	Private	681	2,698	3,803	1,835	0	0	0	0	3,990	0	0	0	13,007	
	%	0	51	0	26	100	0	100	100	5	100	0	100	44	
	State	0	4,655	0	728	2,189	0	833	006	210	1,135	0	2,123	12,773	
	State	Alabama	Arkansas	Florida	Georgia	Louisiana	Mississippi	North Carolina	Oklahoma	South Carolina	Tennessee	Texas	Virginia	Region	

Historical Forest Seedling Production in the Southern United States: 2008 to 2009 Planting Season

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Table 10. Seedling production for the 2008 to 2009 planting season across the southern US by ownership category (in thousands). Private = nurseries owned by companies or individuals that do not own wood processing facilities; Industry = nurseries owned by companies that have wood processing facilities.

			Tota	I seed	ling productior	ı		
State	State	%*	Private	%*	Industry	%*	Total	%**
Alabama	0	0	61,031	46	73,073	54	134,104	13
Arkansas	11,105	10	47,285	41	56,939	49	115,329	11
Florida	15,080	30	35,080	70	0	0	50,160	5
Georgia	14,611	6	214,938	92	5,130	2	234,679	23
Louisiana	26,798	100	0	0	0	0	26,798	3
Mississippi	0	0	0	0	92,477	100	92,477	9
North Carolina	13,126	21	0	0	49,200	79	62,326	6
Oklahoma	5,949	100	0	0	0	0	5,949	1
South Carolina	5,908	4	43,624	29	100,337	67	149,869	15
Tennessee	5,916	100	0	0	0	0	5,916	1
Texas	0	0	101,805	100	0	0	101,805	10
Virginia	25,488	100	0	0	0	0	25,488	3
Region	123,981	12	503,763	50	377,156	38	1,004,900	

*Percentage of state production **Percentage of regional production

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Table 11. Change in seedling production from the 2005 to 2006 nursery season to the 2008 to 2009 season (in thousands).

			% Change from			% Change from			% Change from			% Change from
State	2008 to 2009 Production	Rank	previous vear	2007 to 2008 Production	Rank	previous vear	2006 to 2007 Production	Rank	previous vear	2005 to 2006 Production	Rank	previous vear
Alabama	134.104	с	, 	134.693	r.	6-	147.435	5	ې ب	152.365	e C	-13
Arkansas	115,329	4	-7	117,366	4	0	107,425	S L	μ	112,876	5	10
Florida	50,160	80	39	36,206	80	-38	58,202	80	4	56,199	8	4
Georgia	234,679	~	ې ب	248,143	~	4	257,481	<del>.                                    </del>	36	188,826	~	-30
Louisiana	26,798	<b>б</b>	~	26,515	10	-21	33,600	10	Ņ	34,128	10	-14
Mississippi	92,477	9	-12	104,902	5	13	92,927	9	ဖု	98,444	9	-12
North Carolina	62,326	7	<del>ර</del> -	68,075	7	-12	77,162	7	12	68,740	7	-2
Oklahoma	5,949	1	ч	6,286	1	ကု	6,470	1	282	1,694	12	-33
South Carolina	149,869	N	- 14	175,102	2	21	145,036	с	-12	164,949	0	-2
Tennessee	5,916	12	<del>ر</del>	5,877	12	11	5,305	12	-24	6,980	1	-47
Texas	101,805	Ð	<del>ر</del>	100,461	9	-13	115,865	4	Ņ	118,141	4	<u>,</u>
Virginia	25,488	10	-26	34,646	0	-12	39,507	6	с	38,304	6	14
Total	1,004,900		ų	1,058,271		ņ	1,086,415		4	1,0	41,646	-1

#### Historical Seedling Production Trends_____

The combined data for seedling production in the southern US are shown in Figure 1. After a stagnant level of seedling production and the effects of World War II, some of the more interesting points within the graph begin in 1955 when approximately 202,000 ha (500,000 ac) were planted across the 12 states in the southern US. Seedling outplanting increased steadily to 0.7 million ha (1.7 million ac), mostly due to the Soil Bank Program that started in 1957 and ended in 1960. From the early 1960s to the 1980s, seedling production climbed rapidly, peaking in the late 1980s with 1.1 million ha (2.7 million ac) planted in either conifer or hardwood seedlings. While the forest industry had a large part in the upward trend, the large peak was mostly due to the Conservation Reserve Program that tapped into non-industrial private landowners who were converting agricultural land to forest plantations. During the following 5 years (until 1995), seedling production and outplanting decreased rapidly, when only 0.7 million ha (1.7 million ac) were planted. There was a sharp spike and increase in 2001, when approximately 0.9 million ha (2.2 million ac) were planted. This third peak in seedling outplanting was due to cost-share programs such as the Conservation Reserve Program on longleaf pine. Seedling production and outplanting, however, has steadily decreased to 405,000 ha (1 million ac) in 2009 at levels not seen since the mid-1970s.

#### Nursery Contraction _

With help from SFNMC members, data has been gathered on the number of nursery closures as well as the amount of land area taken out of seedling production. However, because many smaller nurseries escape detection, this dataset is not complete. Since 1995, through mergers, shuttering, and cessation of state reforestation programs, at least 25 nurseries have closed in the 12 southern states (Figure 2). By estimating nursery size and historical production at each of those nurseries, the capacity loss is calculated at 579 million seedlings. Yet much of this reduction in capacity was offset by nurseries coming online (for example, Plum Creek Timber Company, Pearl River Nursery, Hazlehurst, MS) and increasing capacity at nurseries still in operation. While the overall effect of seedling production may be minimal, certainly there are many fewer nurseries in operation in 2009 than in 1995.



Southern Seedling Planting all Owners & all States: 1925-2009

Figure 1. Historical outplanting trends in the southern US for all forest land owners and all states from 1925 to 2009.



Figure 2. Forest seedling nurseries closed and potential production area lost since 1995.

#### Discussion

One of the shortcomings of this particular survey tool is that the numbers reported do not necessarily translate into acres planted within either the state or land-ownership category. Data is collected as production, so any information on actual seedling sales or seedlings outplanted by state or land-ownership category is simply not available. What these numbers do provide is a fairly good estimate of seedlings (species, planting stock, and so on) that probably were outplanted by non-industrial land-owners, forest industry, or real estate investment trusts during the 2008 to 2009 planting season. A simple estimate of the area planted across the region could be made by dividing the number of seedlings produced by 1480 seedlings/ha (600 seedlings/ac) for a total of 0.7 million ha (1.7 million ac) planted. While this figure is close to the area reported in Moulton (1999), it is about 18.7% less than what was reported for the 1997 season (Moulton and Hernandez 1999). With about 264 million fewer seedlings produced in 2009 than 1997, one could infer a corresponding decrease in area planted across the region over the past 12 years (Figure 3). At 1480 seedlings/ha (600 seedlings/ac), it could be inferred that approximately 178,000 fewer ha (440,000 fewer ac) were replanted across the region in 2009 than were planted in 1997.

#### Acknowledgments_

The author thanks member and non-member nurseries for sharing their seedling production information with the Southern Forest Nursery Management Cooperative, and Elizabeth Bowersock for her efforts in conducting and compiling the survey data.



### Seedling Production & Planting: 1997-2009

Data from USFS, GFC and Nursery Cooperative

Figure 3. Seedling production and acres planted in the southern United States from 1997 to 2009 (1 ac = 0.41 ha).

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# What's New with Nurseries and Reforestation Projects at the Missoula Technology and Development Center?

#### **Bob Simonson**

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**Abstract:** The USDA Forest Service Missoula Technology and Development Center (MTDC) offers technical expertise, technology transfer, and new equipment development to federal, state, and private forest nurseries. Current and recently completed projects at MTDC include a front and mid-mount tractor evaluation, ATV-pulled mechanical tree planter, greenhouse snow remover, freeze chamber, brush cleaner improvements, greenhouse crop mower, non-chemical vegetation control, pine seed screening, evaluation of single seed planters, wireless soil moisture monitors, herbicide shield for spraying irrigation pipelines, and rotary lasers.

Keywords: nursery equipment, freeze chamber, seed screening, moisture monitoring, herbicide

#### Introduction _

USDA Forest Service Missoula and San Dimas Technology and Development Centers (MTDC and SDTDC) help solve problems identified by field employees of the USDA Forest Service (USFS). For 60 years, both centers have been evaluating existing technology and equipment, developing equipment prototypes, and conducting technology transfer through their reports, Web sites, videos, and DVDs. The reforestation and nurseries program is located at MTDC in Missoula, MT. The principle focus of the nurseries program is to develop new equipment or technology to improve nursery operations and processes. The program is sponsored and funded by the USFS Forest Management staff group at the Washington Office (Washington, DC) and through State and Private Forestry. Our focus is applied technology and technology transfer. We do not conduct research, but sometimes we apply research findings to help solve on-the-ground problems. Projects typically last from 2 to 4 years

depending on their complexity. Equipment-based projects are field tested and fabrication drawings are made so the equipment can be duplicated by other nurseries. We document our projects through printed reports or journal articles that are available from MTDC. You can find our drawings and reports on our website (URL: http://www. fs.fed.us/eng/t-d.php).

Following are some current nursery projects that may be of interest to you.

#### Front and Mid-Mount Tractor Evaluation

Project leader Gary Kees is evaluating a Saukville diesel tractor for replacement of the old Allis-Chalmers Model G tractor. It has been fitted with a belly mower and S-tine cultivator. A basket weeder and sprayer are being adapted to the tractor. Field testing is ongoing in 2010 and 2011.



Figure 1. Saukville diesel tractor.

#### ATV-Pulled Mechanical Tree Planter

Project leader Gary Kees tested ATVs to pull a mechanical tree planter at USFS Lucky Peak Nursery (Boise, ID). He found that a UTV was needed to pull the transplanter. Modifications to a Holland hand transplanter (Holland, MI) is in the design-and-build phase. Field testing is planned for fall 2010.



Figure 2. ATV-pulled mechanical tree planter.

#### Greenhouse Snow Remover_

USFS JW Toumey Nursery (Watersmeet, MI) currently uses a long-handled broom with a styrofoam block to pull the snow off of their greenhouses. MTDC project leader Mary Ann Davies is investigating alternatives. IceClear[®], a biobased propylene glycol, was sprayed onto the greenhouse plastic, but the results were not satisfactory. The use of mechanical vibrators was examined but it was decided this would be too expensive and could damage the greenhouse structure. In winter 2010, a heating cable attached to the top of the greenhouse that heats the air space between the two layers of plastic will be tested.



Figure 3. USDA Forest Service, JW Toumey Nursery greenhouse in winter.

#### Freeze Chamber_

Project leader Mary Ann Davies has partnered with Oregon State University College of Forestry (Corvallis, OR) to design and build an on-site freeze chamber to simulate freeze events on bareroot seedlings. The data generated will provide a better understanding of expected damage and recovery potential, and aid in determining optimum lifting windows for harvest and outplanting.



Figure 4. An *in situ* freeze chamber developed to simulate freeze events on bareroot seedlings.

#### **Brush Cleaner Improvements**

Project leader Keith Windell developed an improved brushcleaning comb for the USFS Bend Seed Extractory (Bend, OR). The improved combs keep awns from clogging the rotating brushes in their Westrup seed cleaning machine (Huntsman, Incorporated, Twin Falls, ID) (Barner and Windell 2010).



**Figure 5.** Brush-cleaning combs keep awns from clogging rotating brushes in a Westrup HA-400 (Huntsman, Incorporated, Twin Falls, ID).

#### Greenhouse Crop Mower _____

Project leader Keith Windell designed a top pruner for trimming greenhouse container seedlings. Successful field tests at USFS Lucky Peak Nursery trimmed uniform tops while collecting the trimmings. Engineering drawings, an operator guide, and Tech Tip will be published.



Figure 6. A top pruner designed for trimming container seedlings.

## Non-Chemical Vegetation Control

Some greenhouse managers would like to reduce their reliance on synthetic herbicides for controlling weeds. Project leader Keith Windell helped USFS Dorena Genetic Resource Center (Cottage Grove, OR) test a prototype propane weeder cart and a wet steamer. Neither system was as effective as hand or mechanical weeding.



Figure 7. Testing a wet steamer to control weed growth.

#### **Pine Seed Screening**

Project leader Keith Windell partnered with the USFS Region 8 Resistance Screening Center (Asheville, NC) to increase seed testing rates. A one-time-use factory sterilized dish was found to replace the use of ethanol for sterilization germination trays. This eliminated a cleaning step and the use of hazardous chemicals. Seed crushing was improved with an upgrade from a 25-seed capacity to 53-seed capacity that can be autoclave sterilized. The seed crusher was also improved with a mechanical arbor press. Testing will be performed during summer 2010.



Figure 8. An arbor press and plates for pine seed screening.

## Evaluation of Single-Seed Planters

Project leader Gary Kees evaluated four commercially available single-seed planters. Each planter was tested in its ability to plant whitebark pine (*Pinus albicaulis*), lodgepole pine (*P. contorta*), and Douglas-fir (*Pseudotsuga menziesii*) seed. The planters were tested in previously prepared seedbeds at the USFS Coeur d'Alene Nursery (Coeur d'Alene, ID) and in a field setting at MTDC. They all proved acceptable, but durability was questioned (Kees and Campbell 2010).



Figure 9. Single-seed planters (from left to right, Hatfield Transplanter, Almaco Hand Jab, Stand 'n Plant, and Seed Stick).

#### Wireless Soil Moisture Monitors

Project leaders Mary Ann Davies and Ted Etter worked with the Coeur d'Alene Nursery to test the HOBO Micro Station weather logger (Onset[®], Bourne, MA) and its ability to monitor soil moisture and soil temperature remotely. Data was communicated wirelessly from the field to a base station allowing the monitoring of soil moisture at four plots as far as 0.8 km (0.5 mi) from the nursery headquarters. Remote monitoring saved staff time by eliminating frequent trips to the field to manually check irrigation needs. After one growing season, with watering based on the wireless monitors, seedlings looked healthier than seedlings grown the previous year. Additionally, weeds were not as common as they had been in previous years when plots were more likely to be overwatered. Evaluation is ongoing. Stations cost about US\$ 1200 (Davis and Etter 2009).



**Figure 10.** This wireless soil moisture monitoring station used a gel cell battery charged by a solar panel.

#### Herbicide Shield for Spraying Irrigation Pipelines

USFS nurseries find it difficult to spray weeds growing along irrigation pipelines because the spray kills seedlings in nearby beds. Project leader Gary Kees developed a tractoroperated system that uses adjustable shields, two spray nozzles, and a 12-volt battery-powered pump to spray weeds along irrigation pipelines (Kees 2008).



**Figure 11.** A sprayer shield assembly is shown mounted to a threepoint hitch sprayer designed specifically for spraying around irrigation pipelines.

#### Rotary Lasers_

Project leader Gary Kees developed a system where selfleveling lasers were used to help lay out irrigation pipe and nursery beds in a straight line. Kees (2008) describes the use of the Spectra Precision HV401 laser and Spectra CR600 and AGL MR360R receivers by the Coeur d'Alene Nursery.



**Figure 12.** A rotary laser, used vertically, projects a beam of light to help field personnel lay out irrigation pipelines and nursery beds in a straight line.

#### **Additional Information**

A complete listing of the nursery projects completed over the years is available electronically to USDA Forest Service and USDI Bureau of Land Management employees at the MTDC intranet site (URL: http://fsweb.mtdc.wo.fs.fed.us/ programs/ref/). Drawings and reports are also available to the public in electronic format (URL: http://www.fs.fed. us/t-d/).

Paper copies of MTDC reports and drawings are available from:

USDA Forest Service, MTDC Attn: Publications 5785 Highway 10 West Missoula, MT 59808 Phone: 406.329.3978 Fax: 406.329.3719

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## Update on Soil Fumigation: MBr Alternatives and Reregistration Decisions

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Enebak SA. 2011. Update on soil fumigation: Mbr alternatives and reregistration decisions. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 40-45. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p065.html

**Abstract:** This article gives a brief history of the importance of methyl bromide in the production of forest seedlings in the southern United States and the timeline for the Montreal Protocol and Clean Air Act to phase out ozone-depleting compounds. In addition, the process, steps, and potential for continued MBr use under the Critical Use Exemption and Quarantine Pre-shipment articles within the treaty are discussed. A summary of the re-registration decisions proposed by the US Environmental Protection Agency for the re-registration of all soil fumigants under the Food Quality Protection Act is outlined as well as current status of MBr alternatives available for the production of forest seedlings in the southern United States.

Keywords: Montreal Protocol, Critical Use Exemption, Quarantine Pre-shipment, Reregistration Decisions

#### Introduction _

In the early 1980s, a consensus emerged in scientific circles that the concentration of stratospheric ozone was declining and that chlorinated fluorocarbons (CFCs) were the cause. To address the ozone hole, the Montreal Protocol on substances that deplete the ozone layer was signed in 1987 to bring about the eventual phase-out of all CFCs. In 1991, methyl bromide (MBr) was added to the list of ozone-depleting compounds, and the amount of MBr produced and imported in the US was reduced incrementally until it was phased out by 1 January 2005 under the *Montreal Protocol* and the Clean Air Act (CAA). Allowable exemptions to the phase-out of MBr included the Critical Use Exemption (CUE) and the Quarantine and Preshipment (QPS) exemption, both designed for agricultural users with no technically or economically feasible alternatives.

#### Methyl Bromide _

MBr is an odorless, colorless gas that has been used as a soil fumigant in most southern forest seedling nurseries to control a wide range of soil-borne pests and enhance seedling production (Carey and McNabb 1996). MBr has proven to be a reliable pesticide for the past 50 years, and has been the industry standard in every pest management program in forest seedling nurseries. The use of MBr to control nursery pests has reduced the demand for more specific herbicides, fungicides, and insecticides. Prior to the MBr phase-out in 2005, 96% of southern forest seedling nurseries used soil fumigation, and 90% of that fumigation was done with MBr (Jang and others 1993). Generally, MBr was applied once every 3 to 4 years, based on 2 to 3 years of pine production followed by 1 to 2 years of cover crop. The total amount of MBr used is estimated at 73,000 kg (161,000 lb) and was approximately 0.33% of the estimated 22 million kg (49 million lb) used for soil fumigation in the US in 1990 (Anonymous 1993). The extensive use of MBr in forest seedling nurseries across the southern US was the best indication of its consistent effectiveness across a wide range of soil and environmental conditions.

#### Critical Use Exemptions ____

CUEs are permitted under Section 604(d) of the Clean Air Act and the Montreal Protocol. Under Decision IX/6 of the Protocol, the use of MBr should qualify as critical use only if the nominating Party (the US, for example) determines that: a) the specific use is critical because the lack of availability of MBr for that use would result in a significant market disruption;

and b) there are no technically and economically feasible alternatives or substitutes available to the user that are acceptable from the standpoint of environmental and public health and are suitable to the crops and circumstances of the nomination.

Beginning in 2004, the US Environmental Protection Agency (EPA) requested applications for CUEs from growers that continue to need and use MBr in their production systems. Generally, CUE applications for MBr use is by consortium or groups of growers/users. A CUE application includes a number of questions on current MBr use, production data, pest issues, research and efficacy on alternatives, methods to reduce MBr emissions, and so on that can be used by EPA to determine the "critical use." An onerous document, the 2009 Southern Forest Nursery Management Cooperative's CUE application was 77 pages in length. After reviewing the CUE applications, EPA develops a Methyl Bromide Usage Numerical Index (BUNI/BUNNIE) for each consortium/group requesting MBr that takes into account each request, subtracts double reporting and quarantine pre-shipment uses, and nominates an amount of MBr for that consortium to the State Department. From the various BUNI/BUNNIEs, the US Government requests authorization for those critical uses from the Parties (Methyl Bromide Technical Options Committee [MeBTOC]) to the Montreal Protocol. Once the Parties of the Protocol authorize the request for a critical use and an amount of MBr for those critical uses, EPA publishes a rule in the Federal Register allowing for the additional production of MBr for that critical use in that year. Each application for a Critical Use round takes up to 3 years and is conducted annually. Thus, for those forest seedling nurseries that use CUE MBr in 2010, the application process began in July 2007 and the current 2010 CUE application is for the 2013 growing season.

As growers adopted different pest management systems, the number of Critical Users has decreased over time. In 2010, there were 11 pre-plant and 3 post-harvest users/ growers authorized to use MBr under the CUE process as outlined under the Montreal Protocol. Within the pre-plant users are the Forest Nursery Seedlings groups that include six different forest seedling consortiums throughout North America approved to use MBr in their production systems. Some of the other critical users include commodities, orchard replant, sweet potato slips, and fruit, nut, and flower nurseries. The primary objective of the Montreal Protocol and the Clean Air Act was to reduce, and eventually eliminate, the use of all ozone-depleting compounds, including MBr. Thus, since the first CUE in 2005, the amount of MBr requested by US growers, the amount authorized by the State Department, and amount approved by the Parties has steadily declined from 9.4 million kg (20.8 million lb) in 2005 to 2 million kg (4.5 million lb) in 2011 (Figure 1).



## U.S. CUE End-User Applications

**Figure 1.** United States methyl bromide nominations and United Nations-approved methyl bromide use under the Critical Use Exemption process for 2005 to 2012.

## Quarantine and Pre-shipment Exemption

As part of the Montreal Protocol, the QPS rule implements an allowable exemption for production and consumption of MBr for quarantine and pre-shipment purposes. Article 2H, Paragraph 6 of the Montreal Protocol states that "the calculated levels of consumption and production under this Article shall not include the amounts used by the Party for quarantine and pre-shipment applications." The EPA agreed to the Montreal Protocol's definitions of quarantine and preshipment. The QPS exemption is based on self-certification of the individual Parties as described in UNEP (2003).

Quarantine applications are treatments to prevent the introduction, establishment, and/or spread of quarantine pests (including diseases), or to ensure their official control, where: a) official control is that performed by, or authorized by, a national plant, animal, or environmental protection or health authority; or b) quarantine pests are pests of potential importance to the areas endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

An example of a quarantine application of MBr is the fumigation of a commodity, such as potatoes in Idaho, that are subject to infestation by a specific and officially recognized quarantine pest, such as the pale cyst nematode (*Globodera pallida*), when the fumigation is conducted before transport of the commodity to meet official quarantine requirements. The purpose of quarantine fumigation is to prevent the introduction of specific quarantine pest(s) into a defined geographical area, such as an importing country. "Pre-shipment applications" are those non-quarantine applications that are within 21 days of export that need to meet the official requirements of the importing country or the existing official requirements of the exporting country. Official requirements are those that are performed by, or authorized by, a national plant, animal, environmental, health, or stored product authority.

As part of the CUE application and approval process, when EPA develops the BUNI/BUNNIE for each critical user, they routinely deduct a percentage of the MBr requested for each user for QPS. For example, in 2006 the Southern Forest Nursery Management Cooperative requested 111,600 kg (246,000 lb) of MBr for use in 2009 for all forest seedling producers in the southern US. From that amount, EPA deducted 66% (37,650 kg [83,000 lb]) for QPS uses, and submitted 74,000 kg (163,000 lb) to MeBTOC for CUE approval. Since the phase-out of MBr use in 2005, there has been an increase in the amount of MBr assigned as "QPS MBr" (Figure 2). Correspondingly, there has been a push by the European Union (EU) nations to significantly reduce QPS use worldwide. There have been some claims made by other nations that the US is playing games with EU and that pre-plant uses lack efficacy data to adequately get control based on EU standards. Thus, at the International Plant Protection Convention, there were plans to rework definitions as outlined in the Montreal Protocol. At the heart of the matter is that the EU claims that state boundaries, as listed and used by the US, do not qualify for usage as QPS and that the definitions as outlined in the Montreal Protocol were for International Boundaries. Specifically, any rule put into place in the US after 1993 does not count based on international rules.



Data from UNEP and MeBTOC: Methyl Bromide: Quarantine and Preshipment Uses.

Figure 2. Worldwide use of methyl bromide classified as Quarantine and Pre-shipment use for 1990 to 2005. (Methyl Bromide: Quarantine and Pre-shipment Uses [UNEP 2003; page 15]; 1 tonne = 1.1 ton).

In early 2010, as Director of the Southern Forest Nursery Management Cooperative (SFNMC), I was contacted by representatives within the EPA, USDA Animal and Plant Health Inspection Service (APHIS), and the US State Department to clarify the role the Nursery Cooperative plays in the CUE application process as it pertains to QPS. The question posed to me was, "If the production of forest seedlings falls under the QPS umbrella for MBr use, why does the Nursery Cooperative even file the request for a CUE MBr use?" To that end, copies of the 12 southern State Plant Pest Requirements for Pest-Free Certification on forest-tree seedling production were forwarded to those agencies for their use in negotiating CUE and QPS MBr use with the EU and MeBTOC.

The CUE and the QPS amendments were not intended to be a permanent solution for continued MBr use. While there is no "cut-off" date for either of these programs (there are still a few chlorofluorocarbons (CFCs) in use 15 years after their phase-out), the overall objective of the Montreal Protocol and the Clear Air Act was to eventually phase out and stop all uses of MBr. In July 2010, EPA announced that the agency was considering ending the CUE program by 2014, with 2013 as the last year MBr would be available under the CUE process. That has provided US growers with an additional 6 years beyond the 2005 phase-out of MBr to implement ozone-safe alternatives. According to EPA, production and consumption of methyl bromide has "declined significantly over the last 20 years," particularly since the substance was phased out in 2005. The CUE since that time was meant to give affected industries time to develop viable alternatives to ozone-depleting substances. Developing countries have until 2015 to phase out MBr. The US was one of only five countries to request the critical use exemptions for methyl bromide in 2010. Israel has announced it will end its critical use program after 2011, while Japan has said it will no longer request the exemptions after 2013.

#### **MBr Alternatives**

It is an understatement to mention that significant time, effort, and dollars have been spent within the agricultural community in an attempt to identify an economical and technical alternative to MBr. Since 1991, when the SFNMC began to look, in earnest, for a replacement, over US\$2 million of its annual dues have been spent on research to find an alternative to MBr. In early 1991, the choices for MBr replacement were chloropicrin, 1,3-dichloropropene, dazomet, and metam/potassium sodium, either alone or in combinations (Carey and McNabb 1996). Since that time, data collected from numerous trials on seedling production, pest control, and application issues have narrowed that list to just chloropicrin and 1,3-dichloropropene (Telone[®]), alone or in combinations. Fortunately, there has been new chemistry developed and these new soil fumigants include Pic + (chloropicrin + a solvent), dimethyldisulfide (DMDS = Palidin[®]), and methyl iodide (MI; iodomethane = Midas[®]). A few compounds that are currently under examination in other crop systems that use MBr, but not yet tested by the SFNMC, include sulfuryl fluoride, phosphine, halosulfuron, furfural, and napropamide.

None of the soil fumigants tested, however, has performed equally in all nurseries in all situations. While producing decent seedling characteristics, Palidin[®] (DMDS + chloropicrin) has significant odor issues that last long into the growing season. Unless the odor is eliminated, adoption of this particular alternative is doubtful. Since its labeling in 2008, restrictions on the availability and application of Midas[®] have limited research to one study in one nursery in 2009. Studies with other alternatives have shown that soil type, pest pressures, cropping history, and nursery location affect the efficacy of soil fumigants (Starkey and Enebak 2008; Quicke and others 2009a,b; Quicke and others 2010a,b). More studies with this compound in other nurseries and soils are needed. Data collected in 2005, prior to the label approval of Midas[®], showed that iodomethane produced decent seedling characteristics, a significant reduction on Trichoderma spp., but poor weed control (Starkey and others 2006). The soil fumigant Pic + (chloropicrin + a solvent) has been one of the better MBr alternatives across a wide range of soils and nurseries where it has been tried (Starkey and Enebak 2008; Quicke and others 2009a,b; Quicke and others 2010a,b). Weed control issues have occurred in some nurseries with this compound. This is not surprising, as chloropicrin is not known for efficacy in weed control (Carey and McNabb 1996; South 2006). The eventual loss of MBr is going to result in individual nurseries needing to fine-tune their seedling production and pest control treatments more carefully, because MBr allowed for a larger margin of error.

#### Reregistration Eligibility Decisions

Superimposed on the CUE process, the QPS rules, and the agencies that fall under the Montreal Protocol and the Clear Air Act is the enactment of the Food Quality and Protection Act (FQPA) of 1996. With the passage of the FQPA, congress presented EPA and all producers and users of pesticides with the challenge of implementing the most comprehensive and historic overhaul of the major requirements include stricter safety standards, especially for infants and children, and a complete reassessment of all existing pesticide tolerances for all uses and users, applicators, handlers, and bystanders.

In 2006, EPA began the process of reviewing the safety of all compounds that are used as soil fumigants in an attempt to mitigate bystander exposure, taking into consideration application methods, soils, compounds, rates, crops, and so on, and develop rules on usage and application methods as part of the reregistration of each soil fumigant. The compounds examined in this reregistration process included chloropicrin, dazomet, metam/potassium sodium, methyl bromide, 1,3-dichloropropene (Telone[®]), methyl isothiocyanate (MITC), and iodomethane as a group to ensure that similar risk assessment tools and methods were used for all, and risk management approaches were consistent.

It would be an understatement to suggest that the first proposed rules of the EPA in February 2007 were a blow to nearly 15 years of MBr alternative research in the forest seedling arena. At a meeting in Crystal City, VA, I mentioned to the EPA personnel who had agreed to meet with a few stakeholders (forest seedlings, potatoes, orchard replant, strawberries) on the newly proposed re-registration decisions (REDs) that "these rules were a punch in the stomach to all those who had been trying to identify and locate an alternative to MBr." For example, using the newly proposed EPA rules for soil fumigants, a 4-ha (10-ac) block (nursery average) fumigated with 160 kg (350 lb) chloropicrin under a High Density Plastic, the best alternative to MBr (see South and others 1997; South 2006) would require a buffer zone of 1400 m or 1.3 km (4200 ft or 0.8 mi). Along with the other proposed rules, the SFNMC estimated that 50% of the forest seedling nurseries would have ceased operations due to a loss of production areas within 3 years with the remaining nurseries significantly increasing seedling costs (SFNMC 2007). It turns out that the best "alternative" to the 2007 proposed soil REDs was the soil fumigant MBr, because this compound required a much smaller buffer zone than straight chloropicrin. For someone who has been working on soil fumigants since 1985 (Enebak and others 1990a,b,c), the irony of identifying an alternative to MBr under the Montreal Protocol and the 2007 Soil REDs was simply a bitter pill to swallow.

Fortunately, after a number of EPA "comment periods" that included new soil flux data, information on seedling production systems, identification of high barrier tarps, evaluation of new technologies, and shareholder input, a revised and amended Soil RED was released in May 2009. These new rules will affect all aspects of soil fumigation for years to come and will require that producers, applicators, and users play a role in the safe and proper application of soil fumigants for the production of forest seedlings. These steps include buffer zones, posting requirements, agricultural worker protection, applicator and handler training programs, tarp perforation and removal, good agricultural practices, application methods/practices and rate restrictions, new restricted use designation for Dazomet, site-specific fumigation plans, emergency preparation and response requirements, compliance assistance and assurance measures, and community outreach and education programs. A complete listing of all the requirements outlined in the Final Soil RED can be accessed at EPA (URL: http://www.epa.gov/ opp00001/reregistration/soil fumigants/#background). All of these measures are going to take a lot of time, effort, and money on someone's part to comply. Thus, the cost to use soil fumigants in the production of forest seedlings is going to increase more than it already has.

Prior to the implementation of the Montreal Protocol and the phase-out of MBr, the average cost to fumigate nursery soil was just over \$US 3700/ha (US\$ 1500/ac) (Figure 3). After 2005, there were two sources of MBr (CUE and QPS);the cost was less for QPS than CUE MBr. These two sources of MBr have increased over time to US\$ 4450 and 7160/ ha (US\$ 1800 and 2900/ ac) in 2010 for CUE and QPS MBr, respectively. No one (producers or applicators) has any idea of what these new rules will do to the price of any of the soil fumigants (chloropicrin, MBr, Telone[®], and so on) available for 2011 and beyond.



#### Year Since MBr Phase-out

Figure 3. Source of methyl bromide and relative cost per acre to apply in forest seedling nursery settings: 2001 to 2010.

While these new rules will change the way nurseries use soil fumigants, the lifting of the buffer zone overlap restrictions to 24 hours, the incorporation of the new soil flux data into the buffer tables, new plastic tarp technologies that allow the gluing of high barrier plastics (virtually/totally impermeable films [VIF/TIF]), and other soil credits should allow nurseries to continue their use of soil fumigants in the production of forest seedlings with minimal disruptions and loss of production acreage. Without these changes, many forest seedling nurseries would have ceased to exist, unable to comply with the bystander safety restrictions. Slated for enforcement in 2010, as of July 2010 many of these requirements have not yet been agreed upon by the registrants and EPA. Full enforcement of all new soil rules and corresponding pesticide labels is scheduled for 2011. That should give producers, applicators, and users a couple of years to work out the kinks as EPA plans to consider the soil fumigants together (all over again) during the Registration Review that begins in 2013.

#### Summary

The continued availability and use of MBr for the production of forest-tree seedlings is limited to those who have access to a Critical Use Exemption or fall under the Quarantine Pre-shipment rules. Both of these MBr sources are limited and under scrutiny by a number of US governmental and international organizations. A number of soil fumigants have been examined as alternatives to MBr; none has proven to be a drop-in replacement as each has its own unique properties and challenges that will need to be tweaked by individual nursery managers under their own production systems. The new Soil Fumigation REDs will require a concerted effort by producers, applicators, and users to ensure the safety of bystanders and document each application of soil fumigant. While the costs to do so will probably increase, at least the rules allow the continued use of soil fumigants in the unique production systems that are forest seedling nurseries.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

### **Rehabilitating Afghanistan's Natural Resources**

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**Abstract:** The Soviet Union invaded Afghanistan in late 1979. During the next 23 years, the war between the Mujahideen Resistance and the Soviet forces, the ensuing civil war, and eventual take over by the Taliban caused enormous harm to the natural resources of Afghanistan. In 2003, the USDA Forest Service (USFS) was asked by the USDA Foreign Agricultural Service to provide technical assistance to the Afghan Government to rehabilitate tree seedling nurseries. The past 7 years have seen severe drought that has significantly reduced agricultural production. The work of the USFS National Reforestation, Nurseries, and Genetic Resources has been to help the Afghan government rebuild their natural resources.

Keywords: Agricultural Development Teams, Afghan Conservation Corps, Afghanistan, natural resources

#### Afghanistan Resources

Afghanistan is a landlocked country bordered by China, Russia, Iran, and Pakistan. It has had the misfortune of lying in the crossroads where Europe and Asia meet. During the past 1400 years, it has been invaded by most major world conquerors, including Alexander the Great, Attila the Hun, Genghis Khan, the Moguls, the British, and the Soviets.

Afghanistan can be compared to Texas in many ways. The area of Afghanistan is  $648,000 \text{ km}^2 (250,000 \text{ mi}^2)$  and the area of Texas is  $694,000 \text{ km}^2 (268,000 \text{ mi}^2)$ ; the population of Afghanistan is 24.6 million and the population of Texas is 32.7 million. In Afghanistan, the average rainfall is 33 cm (13 in), while in west Texas, the average rainfall is 25 cm (10 in). A major difference between Afghanistan and Texas is the amount of the population employed in agriculture. In Afghanistan, 80% of the population is employed in agriculture.

#### Afghan Conservation Corps_

The 1979 invasion by the Soviet Union destroyed much of the fabric of Afghan civil society, but caused only limited damage to the natural resources. As the fighting between the Mujahideen Resistance and the Soviets increased, the Soviets began to remove trees from urban areas and along road sides in an effort to protect themselves from ambush. Over time, Kabul was transformed from an urban green oasis to an urban combat zone. During the civil war that broke out after the Soviet pullout, large areas of Afghanistan were reduced to rubble. War lords destroyed areas that they could not control as a means of denying their adversaries any advantage. By the time the US military invaded Afghanistan in 2002, Kabul and most other urban areas resembled the post-apocalyptic scenes found in Hollywood movies.

Prior to the US invasion, Afghanistan had endured 5 years of drought. Agricultural production had been drastically reduced as a result of this drought. Afghanistan endured this drought despite having lost almost 50% of its irrigated land between 1979 and 2002. Rain-fed cereal production fell to about 10% of production in a normal rainfall year and approximately 50% of orchards were lost due to the severe drought. This drought severely tested the resilience of rural Afghans. A comparison can be made between the situation in Afghanistan in 2003 and the situation the US faced as a result of the "Dust Bowl" that occurred between 1929 and 1941 at the height of the Great Depression.

To recover from its Dust Bowl, the US Department of Agriculture (USDA) developed a comprehensive soil conservation program that was implemented by the Civilian Conservation Corps (CCC). The CCC was a job creation program for unemployed men, providing vocational training through the performance of useful work related to conservation and development of natural resources. Between 1933 and 1942, the CCC outplanted more than 3 billion trees, built more than 156,000 km (97,000 mi) of roads, erected 3,700 fire towers, and restored 34.2 million ha (84.4 million ac) of agricultural land. They developed and improved state and national parks, built fish hatcheries, and are considered the start of modern conservation practices. The CCC was so successful that it has served as the inspiration for the Job Corps and Ameri-Corp, and many states have since developed their own Conservation Corps.

In 2003, the US State Department offered funding to the Afghan government to develop an Afghan Conservation Corps (ACC). When the Afghan government accepted this offer, the US State Department requested the US Secretary of Agriculture to provide technical assistance to the Afghans to develop and operate an ACC. The Foreign Agricultural Service (FAS) is the USDA agency charged with fulfilling State Department agricultural and forestry technical assistance requests. The FAS asked the USDA Forest Service (USFS) to provide forest nursery experts to assist with the development of the ACC. This request came 70 years after the USFS had been asked by the USDA to develop and operate the CCC.

#### **Technical Assistance to the ACC**

In March 2003, members of a team composed of USFS and FAS specialists travelled to Kabul and met with members of the Afghan Ministry of Irrigation Water Resources and Environment (MIWRE) and the Afghan Ministry of Agriculture and Animal Husbandry (MAAH). The UN Office for Project Services (UNOPS) was asked to provide operational management and support to the project. The combined team of MIWRE, MAAH, UNOPS, USFS, and FAS conducted a natural resource assessment in order to develop objectives and a training plan for the ACC.

In July 2003, the USFS and FAS team returned to Afghanistan to help plan and facilitate the start-up of the ACC. The ACC Inception Workshop was designed to provide participants with the training they would need to complete job creation projects in their provinces. Participants from 17 provinces received training in project development, seed handling, nursery management, and tree planting. During the workshop, participants were assisted with writing project proposals that were evaluated by UNOPS. As a result, 56 projects from 17 provinces were selected and funded and, by December 2003, contracts for 346,000 labor days had been paid.

The USFS and FAS team returned to Afghanistan in November 2003. During this technical assistance visit, the team helped the ACC develop goals and objectives, a work plan, and a strategy to meet their goal and objectives. Additionally, the team determined the technical expertise the ACC needed to implement nursery production and tree-planting projects. A training and development plan was developed to ensure that the ACC staff had the necessary skills they would need to be successful. Training for MIWRE and MAAH personnel who would be providing project oversight was also developed.

The objectives that were established for the ACC were: to provide jobs to thousands of Afghans; to implement conservation actions, especially reforestation; and to establish and foster a commitment to conservation in the Afghans who participated in ACC projects. The team developed a proposal for how the USDA would deliver needed technical expertise to the ACC and sought funding from the State Department to complete this training.

In March 2004, the USFS team returned to Kabul to provide training to the key MIWRE and MAAH personnel that were providing oversight to the ACC. The training of the ACC staff began after the ministry training was completed. The ACC staff travelled to the US where the training focused on seed processing, nursery production, tree planting, and conservation education. The majority of the training took place in the Great Basin. This region of the US is geologically and climatically similar to the Central Plain of Afghanistan. Both of these regions are located between two mountains ranges that run north to south. The annual precipitation in both of these regions comes as winter snow, with light rain occurring in the spring. The ACC staff was guite surprised to find that the topography, climate, and soils of the Great Basin were so similar to those found in Afghanistan. One of the highlights of the training was a visit to the USFS Lucky Peak Nursery located east of Boise, ID. The Lucky Peak nursery was selected to visit because the soils and physical characteristics are very similar to those found at the Paghman Nursery located southeast of Kabul.

In March 2005, the staff of the ACC returned to the US for additional training. The focus of this training was on conservation education and an examination of the conservation corps programs in the US. The three types of conservation corps that were visited included: a program operated by a non-governmental organization; a state program that was operated as an alternative method of completing high school; and a live-in program were trainees lived on a center.

The USFS team developed training materials that were translated into Dari and Pashto, the two dominant languages used in Afghanistan. Two training manuals were also developed—*Raising Forest Seedlings in Afghanistan* and the *Afghan Forest Nursery Manual*. The USFS team also worked with ACC staff to develop seed harvesting and seed testing protocols, guidelines for the deployment of native Afghan species, and nursery management procedures.

#### ACC Operations

By summer 2009, the ACC had completed 355 projects. They had helped farmers establish over 750 orchards; rehabilitated 21 nurseries that produced 3.5 million seedlings; worked with local officials to organize tree-planting programs in which citizens planted 1.5 million seedlings; distributed 500,000 fruit trees; and worked with farmers to direct seed 81 ha (200 ac) of pistachios. ACC workers have improved 6 km (3.7 mi) of trails and developed tourism infrastructure at Lake Band-e-Amir near Kabul. They have started and continue to support 15 projects for women. Because no training facilities existed at many locations where they initiated projects, the ACC constructed 6 training centers. The lack of sufficient water is the major limiting factor to farmers and villagers, so the ACC planned and completed 150 watershed projects.

#### **Technical Assistance to Task Force Yukon**

In June 2009, the FAS asked the USFS to provide technical assistance to the US Army Airborne Division that operated Forward Operating Base Salerno, located in Khost Province in eastern Afghanistan. The training was focused on small-scale projects to rehabilitate degraded watersheds and provide local employment. This 4-day workshop addressed a variety of basic soil and water conservation techniques that were deemed to be useful in the rehabilitation of degraded watersheds. The course included a combination of lecture and discussion, in class exercises, and demonstrations for each training module. The training was well received and Task Force members contacted the USFS training team via the internet with follow-up questions for several months after the training. The USFS team has subsequently been asked to provide training for the Army National Guard Agricultural Development Teams prior to their deployment to Afghanistan.

There is still a lot of work to do to return Afghanistan's natural resources to pre-war condition. The USFS team feels strongly that their contributions have helped Afghan farmers and citizens rebuild their country.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

## The Use of Proline[®] (Prothioconazole) to Control Pitch Canker, *Rhizoctonia* Foliage Blight, and Fusiform Rust in Forest Seedling Nurseries and Efforts to Acquire Registration

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Starkey TE, Enebak SA. 2011. The use of Proline[®] (prothioconazole) to control pitch canker, *Rhizoctonia* foliage blight, and fusiform rust in forest seedling nurseries and efforts to acquire registration. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 49-57. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p065.html

**Abstract:** Laboratory, greenhouse, and field trials have shown Proline[®] to be efficacious against three fungal pathogens that cause damage and seedling mortality in forest seedling nurseries. Disease control using Proline[®] has been obtained at 365 ml/ha (5 fl oz/ac) for the control of fusiform rust (*Cronartium quercuum* f.sp. *fusiforme*) on loblolly pine (*Pinus taeda*) in both greenhouse and field trials. In greenhouse trials, a biweekly application at 365 ml/ha (5 fl oz/ac) controlled pitch canker (*Fusarium circinatum*) on longleaf pine (*P. palustris*) and shortleaf pine (*P. echinata*), and resulted in a significant increase in seedling production over non-treated seedlings. *In vitro* studies using Proline[®]-amended agar resulted in 100% fungicidal control against *Fusarium circinatum* at all 5 rates used: 0.0625x, 0.125x, 0.25x, 0.5x, and 1x the recommended label rate. A biweekly application of Proline[®] at 402 ml/ha (5.5 fl oz/ac) in nursery field tests significantly reduced *Rhizoctonia* foliar blight on loblolly pine when compared to applications of azoxystrobin and the non-treated, azoxystrobin, and Proline[®], respectively. A second trial was conducted applying Proline[®] every 3 weeks. The monetary loss per acre was US\$ 2142, 1235, and 1 for non-treated, azoxystrobin, and Proline[®]-treated seedlings were significantly larger and appeared greener than non-treated seedlings. Proline[®] did not affect longleaf, loblolly, slash (*P. elliottii*), or shortleaf pine seed germination.

Keywords: loblolly pine, longleaf pine, slash pine, shortleaf pine, chemical control, fusiform rust, pitch canker, Rhizoctonia foliar blight

#### Introduction

The availability of fungicides to control specific forest seedling nursery diseases is either nonexistent, limited, or faces possible loss of US label registration. Of the many insects and diseases that occur in forest seedling nurseries, three fungal pathogens stand out as problematic in southern US nurseries. These diseases include fusiform rust, pitch canker, and *Rhizoctonia* foliar blight. The most important disease of loblolly (*Pinus taeda*) and slash (*P. elliotti*) pine seedlings is fusiform rust caused by *Cronartium quercuum f.* sp. *fusiforme*. Since 1980, formulations of Bayleton[®] (triadimefon) have been the primary chemical used to control this disease (Carey and Kelley 1993) and have consistently provided excellent cost-effective control as both seed treatments and foliar sprays (Snow and others 1979; Carey and Kelley 1993; Carey 2004).

In July 2007, Bayer CropScience received US Environmental Protection Agency (EPA) cancellation order for Bayleton[®]. While most of the food and non-food crops such as apples, pears, grapes, and raspberries were removed from the US label, its use on pine seeds and seedlings was still allowed. However, the availability of Bayleton[®] remains unsettled, resulting in nurseries having difficulty locating and obtaining the product; an alternative is needed.

Pitch canker, caused by the fungus *Fusarium circinatum* (=*Fusarium subglutinans*), can cause significant seed and seedling mortality in nurseries and later after outplanting in the field (Dwinell 1978; Dwinell and Barrows-Broaddus 1981; Kelley and Williams 1982; Barrows-Broaddus and Dwinell 1984; Blakeslee and Rockwood 1984; Lowerts and others 1985; Carey and Kelley 1994). In the southern US, infection and seedling losses have been reported on loblolly, slash, longleaf (*P. palustris*),

shortleaf (*P. echinata*), and Virginia (*P. virginiana*) pines. The fungus is also considered one of the most threatening diseases in many areas of the world, particularly the South African nurseries (Viljoen and Wingfield 1994; Storer and others 1998). Unlike fusiform rust, there are no fungicides registered for the control of pitch canker on either seeds or seedlings, and nursery growers are forced to use either bleach or hydrogen peroxide to disinfect seeds. Many of the fungicides registered for use in forest seedling nurseries indicate that they control fungi in the genus *Fusarium*. However, the degree of control of *F. circinatum* was insufficient to justify the cost of application (Runion and others 1993).

Longleaf and loblolly pines are particularly susceptible to *Rhizoctonia* foliar blight. The disease is caused by a species of Rhizoctonia, or binculeate forms of sexual states belonging to the genera Thanatephorus or Ceratobasidium. Rhizoctonia foliar blight can cause significant pine mortality in nursery beds and typically occurs in late July when the seedling canopy closes (Carey and McQuage 2003). Symptoms of dead and dying needles and seedling mortality appear in patches within the bed where moisture and temperature favor infection. The disease is often not observed until seedlings are top-clipped to maintain seedling shoot-to-root ratios and heights. Varying degrees of resistance among seedling families can be found, with US Gulf Coast seedlots more susceptible than Piedmont sources, and the disease is rarely observed on slash pine (McQuage 2009). Rhizoctonia foliar blight generally is not distributed uniformly throughout a nursery and is limited to foci within nursery beds. The disease is also more severe in second crop, post-soil fumigated fields. While there are fungicides registered for *Rhizoctonia* foliar blight, they are not always efficacious (Carey and McQuage 2004).

In an attempt to find an alternative for the control of fusiform rust, trials examining numerous fungicides have been underway at the Southern Forest Nursery Management Cooperative (SFNMC) since 2004. In 2008, Proline[®] 480 SC (41% prothioconazole, Bayer CropScience) was examined as it had a broad spectrum systemic control of ascomycetes, basidiomycetes, and deuteromycetes on numerous field crops. Prothioconazole belongs to the new chemical class of triazolinthiones (Mauler-Machnik and others 2002) and inhibits the demethylation process at position 14 of lanosterol or 24-methylene dihydrolanosterol, that are precursors of sterols in fungi. Prothioconazole efficiently stops many steps of the fungal infection chain like appressoria and haustoria formation, mycelial growth, as well as spore formation. Currently Proline[®] is registered in 44 countries and in the US for food crops including peanuts, barley, wheat, sugar beets, beans, soybeans, and rapeseed.

Although Proline[®] is not currently registered for commercial use in US forest seedling nurseries, these studies examined Proline[®] in laboratory, greenhouse, and field trials to determine if the fungicide was efficacious against the three fungal pathogens that are capable of causing significant damage and seedling mortality in forest seedling nurseries. Data collected from such studies has been used in an attempt at obtain a full-use label from Bayer CropScience and US EPA for disease control in forest seedling nurseries in the southern US.

#### Methods

#### **Fusiform Rust Greenhouse Trials**

**Seed Treatments**—In 2006, 2007, 2008, and 2009, loblolly pine seeds were stratified for 4 weeks, and then treated with fungicides prior to sowing (Table 1). For dry formulation fungicides, seeds were first moistened in a seed tumbler, and the fungicide was added at the rate of 25 g/10 kg (2 oz/50 lbs) of seeds. For liquid fungicides, approximately 26 ml (2 fl oz) of the product were used per 10 kg (50 lbs) of seeds, and slowly added to pine seeds in a tumbler. The fungicide and seeds were tumbled until dry. All treated seeds, as well as non-treated seeds for both positive and negative controls, were double sown in Ray Leach containers (164 ml [10 in³], Stuewe and Sons, Tangent, OR) and then thinned to one seedling per cell as they germinated.

		Foliar tre	eatment1	Seed treatment
Treatments	Active ingredient	1X	2X	1X
Check (water)				
Bayleton [®]	tridimefon 50%	560 ml/ha (8 oz/ac)		25 g/10 kg seeds (2 oz/50 lb seeds)
Folicur [®]	tebuconazole 38.7%	292 ml/ha (4 fl oz/ac)	584 ml/ha (8 fl oz/ac)	
Provost [®] 433 SC	prothioconazole 12.9% tebuconazole 25.8%	621 ml/ha (8.5 fl oz/ac)	1.24 l/ha (17 fl oz/ac)	25 g/10 kg seeds (2 oz/50 lb seeds)
Proline [®] 480	prothioconazole 41%	365 ml/ha (5 fl oz/ac)		25 g/10 kg seeds (2 oz/50 lb seeds)

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Table 1	. Fungicide rates,	actual product	per unit,	used in 2006,	2007, and 2008.

¹ Based upon 280 I water/ha (30 gal water/ac)

**Foliar Treatments**—Loblolly pine seeds were stratified for 40 days and then double sown to Ray Leach containers. Following germination, seedlings were thinned to one seedling per container and then randomly assigned fungicidal treatments. Seven weeks post-sowing, seedlings were treated at the Auburn University Pesticide Research Facility (Auburn, AL). A Bayleton[®] and a water check were included for both positive and negative controls, respectively. Application rates for each fungicide included a 1x as listed in Table 1. Provost[®] was tested in 2007 and 2008. Proline[®] was only tested in 2008 and 2009. After spraying, seedlings were returned to the greenhouse to dry.

**Inoculations**—One day following the foliar fungicide application, the seedlings were transported to the USDA Forest Service Rust Screening Laboratory in Asheville, NC. Seedlings were allowed to acclimate to the new growing conditions for 5 to 7 days, and then challenged with 20,000 basidiospores/ml of *Cronartium quercum f.sp. fusiforme* (collected from Zone 7 inoculum area) using standard inoculation protocols for the laboratory. Seedlings remained under the care of the laboratory for the duration of the growing season. At 3 and 6 months post-inoculation, seedlings were evaluated for swellings along the main stem, an indication of basidiospore infection.

#### **Fusiform Rust Field Trials**

In 2008, two nurseries (South Carolina Forestry Commission Nursery in Trenton, SC and Arborgen Nursery in Shellman, GA) participated in testing Proline[®] operationally on several nursery blocks. Proline[®], Provost[®], and Bayleton[®] were compared to a non-treated control. At each nursery, a randomized complete block design was used with treatments replicated 3 times at one nursery (SC) and 5 times at the other (GA), with plot sizes of 0.24 ha (0.6 ac) and 0.405 ha (1.0 ac), respectively. Each replication/treatment was applied to either 3 adjacent nursery beds or a 9-bed nursery section using standard nursery spray equipment. Proline[®] and Provost[®] were applied at a rate of 365 ml/ha (5 fl oz/ac) and 621 ml/ha (8.5 fl oz/ac), respectively, as well as the standard Bayleton[®] application. At the end of the growing season (December 2008), seedlings were collected from each treatment plot and examined for rust infection and measured for seedling quality. In addition, seedlings were collected from the nursery in February 2009 and outplanted at a site near Auburn, AL, to monitor for any long-term effects of the fungicides on seedling survival.

In 2009, Proline[®] and Bayleton[®] were operationally field tested at the Arborgen Nursery in Shellman, GA. Experimental design, rates, and application methods were similar to those described above.

#### **Pitch Canker Laboratory Trials**

Fungal growth studies were conducted in the laboratory to determine if *F. circinatum* was able to grow on agar media amended with of Proline[®] and Pageant[®] - BASF (Table 2). Potato Dextrose Agar (Difco[®] PDA) was amended with each fungicide after autoclaving and just before pouring the plates. Twenty plates of each fungicide concentration and 20 non-amended PDA plates as a control were used. A #4 cork-borer (8 mm) plug of F. circinatum, taken from a 2-week-old culture, was placed at the center of each plate. The radial growth of the fungus was measured over a period of 11 days and recorded. To determine if the treatments were either fungicidal (killed the fungus) or fungistatic (stopped fungal growth) 11 days after placing onto the amended media, the agar plugs within each treatment were removed and plated onto non-amended media. Fungal growth on the non-amended media was recorded for another 5 days.

Fungicide	Active Ingredient	Rate	ppm
Proline [®] 480 SC	prothioconazole – 41%	1x = 365 ml/ha (5 fl oz/ac) ¹	1300
		0.5x = 183 ml/ha (2.5 fl oz/ac)	650
		0.25x = 91 ml/ha (1.25 fl oz/ac)	325
		0.125x = 46 ml/ha (0.625 fl oz/ac)	162
		0.0625x = 23 ml/ha (0.321 fl oz/ac)	81
Pageant®	pyraclostrobin 12.8%	1x = 104.8 g/100 l (14 oz/100 gal)	1100
Ū.		0.5x = 52.4 g/100 l (7 oz/100 gal)	550
	boscalid 25.2%	0.25x = 26.2 g/100 l (3.5 oz/100 gal)	225

Table 2. Fungicide, active ingredient, and rate used in Fusarium circinatum-amended media trial.

¹ Based upon 280 I water/ha (30 gal water/acre)

#### **Pitch Canker Greenhouse Trials**

In 2008 and 2009, longleaf pine seeds were stratified for 10 days and sown to Ray Leach containers in the greenhouse in May. In 2009, slash, loblolly, and shortleaf seeds were stratified for 21, 40, and 45 days, respectively, and sown into Ray Leach containers in the greenhouse in May. Only the loblolly and shortleaf seeds were confirmed to be infested with F. circinatum. To increase fungal pressure, an 8 mm agar plug from a 2-week-old stock culture of F. circinatum was added to half of the container cavities at the time of sowing. After sowing longleaf pine seeds, all cavities were covered with a thin layer of coarse perlite and misted. In addition to the fungal plug of F. circinatum, half of the containers were sprayed with Proline[®] at sowing and every 2 weeks throughout the study. There were 20 container sets sown to longleaf pine, each container set had 20 cavities for each treatment as follows: treatment 1 = F. *circinatum* and no Proline[®] spray; treatment 2 = F. *circinatum* and Proline[®] spray; treatment 3 = no F. *circinatum* and no Proline[®] spray; treatment 4 = no F. circinatum and Proline[®] spray. Following germination, seedling counts were measured weekly for 4 weeks and then once per month until October. Dead seedlings were later assayed to establish the cause of death.

#### Rhizoctonia Foliar Blight Laboratory Trials

Fungal growth studies were conducted in the laboratory to determine if Rhizoctonia solani was able to grow on agar media amended with Proline[®] at 1x, 0.25x, and 0.0625x the label rate of 365 ml/ha (5 fl oz/ac). Potato Dextrose Agar (Difco[®] PDA) was amended with Proline[®] after autoclaving and just prior to pouring the plates. There were 20 PDA plates of each fungicide concentration and 20 non-amended PDA plates used as a control. A #4 cork-borer (8 mm) plug of Rhizoctonia solani taken from a 12-day old culture was placed at the center of each plate. The radial fungal growth was measured over a period of 7 days and recorded. To determine if Proline[®] was fungicidal (killed the fungus) or fungistatic (stopped fungal growth) 7 days after placing the plugs onto the media, the agar plugs were removed from the amended agar media and placed onto a non-amended agar plate. Fungal growth on the non-amended agar plate was recorded for another 5 days.

#### Rhizoctonia Foliar Bight Field Trials

In 2008, a forest seedling nursery tested Proline[®] at a rate of 402 ml/ha (5.5 fl oz/ac) and Heritage[®] (50% azoxystrobin) at a rate of 1.68 kg/ha (24 oz/ac) operationally for the control of *Rhizoctonia* foliar blight. A randomized block design with four replications was used in a nursery section growing its second seedling crop following soil fumigation. Each replication plot was 12 x 18 m (39 x 59 ft) with a non-treated plot (6 m x 18 m [20 x 59 ft]) left as the disease control. Fungicides were applied on a 2-week interval beginning 15 July 2008 using a Hardee 400-gal (1532-L) sprayer (EVH Manufacturing Company, Loris, SC) with a 9-bed spray boom with nozzles on 0.5 m (1.6 ft) centers. A total of eight applications of both

fungicides were made. Temperature and relative humidity 25.4 cm (10 in) above the seed bed were recorded using a HOBO data logger (Onset[®], Bourne, MA).

In early December 2008, seedling densities, disease incidence, severity, and seedling loss were calculated in two subplots within each treatment plot. From each subplot, 30 seedlings were hand-lifted and later measured to determine seedling quality, root collar diameter, height, dry weight, and root morphology for each treatment.

In 2009, the identical study was established at the same nursery using the same experimental design and application methods. However, the fungicides were applied every 3 weeks instead of every 2 weeks to determine the minimal spraying time interval for disease control.

#### Results and Discussion _

#### **Fusiform Rust Greenhouse Trials**

The SFNMC has tested many fungicides for an efficacious alternative for Bayleton® (Carey 2004; Starkey and Enebak 2008). One fungicide that provided disease control equal to or better than Bayleton[®] was Provost[®] (Figure 1), which contains prothioconazole and tebuconazole (Table 1). However, when Folicur[®] (containing only tebuconazole) was tested, 50% of the seedlings developed fusiform rust galls. It was later determined that disease control achieved with Provost[®] was due to the prothioconazole portion within that fungicide. A technical representative suggested testing Proline[®] (prothioconazole), which was registered in the US in 2007. In subsequent greenhouse trials, Proline[®] provided control of fusiform rust on loblolly pine equal to or greater than Bayleton[®] as a foliar spray (Figure 1, Table 3). In addition, when tested as a seed treatment prior to sowing for disease control, there was no affect on seed germination, and Proline[®] had disease control equal to that obtained with the current standard Bayleton[®] (Table 4). If registered for forest seedlings, Proline[®] will provide a second efficacious fungicide for the control of fusiform rust in the southern US.

#### **Fusiform Rust Field Trials**

At the South Carolina Forestry Commission Nursery, there was no rust infection in the control plots, so Proline[®] could not be evaluated. By the end of the growing season in December 2008 at the Arborgen Nursery, 54% of the seedlings in the control plots had developed main stem swellings or galls. In contrast, no stem swellings or galls were observed on seedlings in any of the Proline[®]-, Provost[®]-, or Bayleton[®]treated plots. There were no differences in the seedling quality (RCD, biomass) among the treatments except for seedling heights and root mass. Seedlings in the control plots were significantly taller than seedlings grown in the three fungicidal treatment plots. This was due to control plots not getting top-clipped, because the nursery was not going to sell non-treated, infected seedlings. Proline[®]-treated seedlings had significantly longer roots and a larger number of root tips than seedlings in the non-sprayed control plots (Table 5).



Figure 1. Three-year average fusiform rust control on loblolly pine using foliar applications of fungicides.

Table 3. Foliar treatment rates and mean percentage of fusiform rust infection in 2008².

Foliar treatment		
fungicides	Foliar rate ¹	% Infection
Bayleton [®]	560 g/ha (8 oz/ac)	7.1% ^a
Provost [®] 433 SC	621 ml/ha (8.5 fl oz/ac)	2.5% ^a
Proline [®] 480 SC	365 ml/ha (5 fl oz/ac)	6.9% ^a
USFS Check Seedlings		45%

¹Based upon 280 I water/ha (30 gal water/ac)

 2  Within column means followed by same letter do not differ at  $\alpha\text{=}0.05$  using Duncan's Multiple Range Test.

0.63^a 53.4 0.04 0.21 105 Isd

Proline®

Provost[®]

Control

Bayleton®

Table 4. Seed treatment rates, germination, and mean percentage of fusiform rust infection in 2008¹.

Seed treatment		
fungicides	% Germination	% Infection
Bayleton®	92%	0.0% ^a
Provost [®] 433 SC	96%	0.0% ^a
Proline [®] 480 SC	96%	1.0% ^a
USFS Check Seedlings		45%

 1  Within column means followed by same letter do not differ at  $\alpha \text{=}0.05$  using Duncan's Multiple Range Test.

To determine if treatments had any long-term affect on seedling growth and/or survival, seedlings from the Proline® and Bayleton  $^{\it \$}$  plots were outplanted following the 2008 growing season in a randomized complete block design in an area north of Auburn, AL. During the 2-year evaluation, there was no difference between Proline[®]- and Bayleton[®]-treated seedlings with respect to seedling height and survival.

¹ Within column means followed by same letter do not differ at  $\alpha$ =0.05 using Duncan's Multiple Range Test.

Table 5. Root length, average root diameter, root volume, and number

Root

volume

(cm³)

0.89^a

0.88^a

0.82^a

0.76^a

Number of

root tips

854.1^a

827.3^a

798.1^a

683.6^b

of root tips for each fungicide treatment¹.

Average

diameter

(mm)

0 59^a

0.61^a

0.60^a

Total root

length

(cm)

320.7^a

304.3^a

287.8 ab

241.4 ^b

#### **Pitch Canker Laboratory Trials**

In vitro fungal growth on agar amended with Proline[®] resulted in 100% fungicidal control against *F. circinatum*. Fungal growth did not occur on any of the Proline[®]-amended PDA plates for any concentration examined for the 11-day experiment (Figure 2). On some Proline[®]-amended plates, the fungus grew from the original 8-mm plug for several mm, but never touched the agar surface. The appearance was that of a mushroom cap suspended over the soil. F. circinatum, while somewhat inhibited on Pageant[®]-amended agar, grew on all concentrations tested. F. circinatum growth on the non-amended control plates was significantly greater than either Pageant[®]- or Proline[®]-amended plates.

After 11 days, the agar plugs containing F. circinatum were removed from each of the amended media and put onto non-amended agar. Mycelia of F. circinatum did not resume growth when returned to non-amended agar. The lack of growth on non-amended media indicates that Proline[®] was fungicidal to F. circinatum. However, agar plugs from the Pageant[®]-amended medium did resume growth on the nonamended agar, indicating that Pageant[®] was fungistatic to F. circinatum.



Figure 2. Radial growth of Fusarium circinatum on fungicide-amended and non-amended agar.

The fungicidal activity of Proline[®] on *F. circinatum* indicates that repeated applications throughout the season in a nursery may not be needed. Once the initial source inoculum has been controlled, repeated applications of Proline[®] may not be needed. Pitch canker losses occur either from external seed-borne fungi (early season) or later in the season from seeds infected internally. Further research needs to determine if several applications of Proline[®] early in the season will also control late season mortality.

#### **Pitch Canker Greenhouse Trials**

A biweekly application at 365 ml/ha (5 fl oz/ac) on longleaf and shortleaf pine to control pitch canker (Fusarium circi*natum*) resulted in an 17% and 50% increase in seedling production over non-treated seedlings, respectively (Table 6). Most of the mortality in longleaf pine occurred early in the season, whereas, the greatest losses with shortleaf pine were later in the season. Not all shortleaf pine mortality was attributed to pitch canker. Rhizoctonia spp. was also isolated from dead shortleaf pine and slash pine late in the season. The application of Proline[®] to these pine species was effective in controlling both fungal pathogens in this study. The percentage(Table 6) of seedlings produced in treatments that did not get additional disease pressure (fungal plug) and no Proline[®] applied is what a nursery sowing these same seedlots would expect to obtain without any fungicidal control. Longleaf and shortleaf seedlings receiving Proline[®] and no fungal plug had significantly smaller root collar diameter that was due to seedling density. Seedling size generally increases with a decrease in seedling density (Landis 1990). The use of Proline[®] resulted in significantly greater seedling biomass for longleaf, shortleaf, and slash pine.

#### Rhizoctonia Blight Laboratory Trials

Agar media amended with  $Proline^{\circledast}$  resulted in 100% control against *Rhizoctonia solani* as fungal growth did not occur on any of the Proline[®]-amended PDA plates for any concentration used for the 7-day experiment (Figure 3). After 7 days, the plugs were removed from the amended media and placed onto non-amended agar media and the mycelia of *R. solani* resumed growth, indicating that Proline[®] was fungistatic.

The fungistatic activity of Proline[®] on *R. solani* indicates that repeated applications throughout the period of peak infection in a nursery may be needed. New sources of inoculum can continually be reintroduced into a nursery bed through wind and soil/debris movement on machinery.

#### Rhizoctonia Blight Field Trials

When Proline[®] and Heritage[®] where sprayed at label rates at 2-week intervals, disease incidence, severity, and number of seedlings lost in the Proline[®]-treated plots was significantly lower than in the Heritage[®] and non-treated control plots (Table 7). An estimate of the potential loss (assuming similar incidence and severity throughout the acre area) indicated that losses from Proline[®] were negligible (0%). There were no significant differences in either seedling quality or root morphology between fungicides tested, although the controls had numerically fewer seedlings. The potential monetary loss in Table 7 reflects the seedling loss in the test plot, not the whole nursery, because *Rhizoctonia* foliage blight tends to occur in isolated foci in susceptible seedlots.

Table 6. Fill percentage and I	longleaf, shortleaf, slash	, and loblolly pine seedling	quality in greenhouse	: pitch canker
study ¹ .				

		Percentage fill				Final		
Pine species	Treatment	W	/eek 5	Week 17	RCD (mm)	HT (cm) ²	Biomass (gm/ft ² )	
Longleaf	Proline [®] + No Fungal Plug	8	88.3 ^a	88.3 ^a	4.8 c	14.1 ^{ab}	80.6 ^a	
-	No Proline [®] + No Fungal Plug	8	83.1 ^a	71.7 ^b	5.7 ^a	14.4 ^a	60.9 ^b	
	Proline [®] + Fungal Plug	8	85.8 ^a	85.3 ^a	5.3 ^{ab}	13.6 ^b	77.7 ^a	
	No Proline [®] + Fungal Plug	-	74.4 ^b	66.1 ^b	5.2 ^b	14.5 ^a	57.7 ^b	
		lsd	6.1	7.2	0.4	0.5	6.7	
Shortleaf	Proline [®] + No Fungal Plug	9	93.9 ^a	93.6 ^a	2.9 ^b	23.0 ^a	54.7 ^a	
N	No Proline [®] + No Fungal Plug	8	84.2 ^b	43.3 ^c	3.1 ^a	21.0 ^b	20.1 ^c	
	Proline [®] + Fungal Plug	9	93.1 ^a	92.8 ^a	3.0 ^{ab}	22.9 ^a	58.3 ^a	
	No Proline [®] + Fungal Plug	8	87.8 ^b	60.6 ^b	3.1 ^a	21.4 b	38.6 ^b	
		lsd	4.6	10.4	0.1	1.2	6.7	
Slash	Proline [®] + No Fungal Plug	(	91.9 ^a	91.7 ^a	3.7 ^{ab}	26.8 ^a	92.3 ^a	
	No Proline [®] + No Fungal Plug	8	86.4 ^a	72.5 ^b	3.6 ^b	24.4 ^b	64.0 ^b	
	Proline® + Fungal Plug	(	91.1 ^a	91.1 ^a	3.7 ^{ab}	25.5 ^{ab}	84.6 ^a	
	No Proline [®] + Fungal Plug	8	83.3 ^b	74.4 ^b	3.8 ^a	25.6 ^{ab}	66.3 ^b	
	0 0	lsd	5.9	8.2	0.1	1.4	8.5	
Loblolly	Proline [®] + No Fungal Plug	(	91.4 ^a	91.4 ^a	3.1 ^b	25.4 ^c	77.7 ^a	
,	No Proline [®] + No Fungal Plug	9	90.6 ^a	88.3 ^a	3.4 ^a	29.3 ^{ab}	75.8 ^a	
	Proline [®] + Fungal Plug	(	93.6 ^a	93.6 ^a	3.1 ^b	30.2 ^a	78.2 ^a	
	No Proline [®] + Fungal Plug	9	91.6ª	90.3 ^a	3.3 ^a	28.5 ^b	78.9 ^a	
	0	Isd	4.6	5.9	0.1	1.0	7.0	

¹ Within column and within species means followed by same letter do not differ at  $\alpha$ =0.05 using Duncan's Multiple Range Test. ² 1 cm = 0.4 in



Figure 3. Radial growth of Rhizoctonia solani on fungicide-amended and non-amended media.

 Table 7. Seedling density and disease loss as measured by incidence¹, severity², and seedling loss³ per ft² and potential loss per hectare caused by Rhizoctonia foliage blight (1 ft² = 0.09 m²).

Seedling density/ ft ²	Disease incidence	Disease severity	Seedling loss/ft ²	Potential loss/Ac (\$US)
22.9	0.354	0.182	3	\$1,735
23.6	0.162	0.083	1.2	\$373
23.7	0.003	0.001	0.01	\$0
0.7762	0.0004	0.0004	0.0031	_
16.8	0.509	0.213	3	\$2,142
20.5	0.344	0.149	2.6	\$1,235
19.7	0.01	0.005	0.05	\$1
0.51	0.0008	0.007	0.0013	—
	Seedling density/ ft ² 22.9 23.6 23.7 0.7762 16.8 20.5 19.7 0.51	Seedling density/ ft2Disease incidence22.90.35423.60.16223.70.0030.77620.000416.80.50920.50.34419.70.010.510.0008	Seedling density/ ft2Disease incidenceDisease severity22.90.3540.18223.60.1620.08323.70.0030.0010.77620.00040.000416.80.5090.21320.50.3440.14919.70.010.0050.510.00080.007	Seedling density/ ft2Disease incidenceDisease severitySeedling loss/ft222.90.3540.182323.60.1620.0831.223.70.0030.0010.010.77620.00040.00040.003116.80.5090.213320.50.3440.1492.619.70.010.0050.050.510.00080.0070.0013

¹ Incidence = proportion of bed area within a 0.4 m2 (4 ft²) frame with Rhizoctonia foliar blight.

² Severity = proportion of tissue affected by Rhizoctonia foliar blight.

³ Seedlings loss= seedling density x incidence/drill x severity /drill.

⁴ Controls were not included in the statistical analysis due to lack of replication among blocks.

When the interval between fungicide sprays was increased to 3 weeks, Heritage[®] had a disease incidence of 34% compared to 1% for the Proline[®]. When comparing the two studies, the disease intensity more than doubled for the Heritage[®] applications, and the potential loss per acre increased by greater than three times when applied every two weeks rather than three weeks. When using Heritage[®] for *Rhizoctonia* foliage blight control, the interval between spray applications should be kept to the minimum as recommended on the label. This study suggests that the time interval between Proline[®] sprays using suggested label rates is not as critical as with Heritage[®]. It is possible that maintaining a 2-week spray schedule with a reduced level of Proline[®] may achieve the same economic level of control.

This particular nursery reported that within these susceptible seedlots, total seedling mortality to the disease would be less than 0.5%. Proline[®] was effective in reducing seedling mortality due to *Rhizoctonia* that normally would occur. In years when the environmental parameters do not favor spread of the fungus through the seedling beds, Heritage[®] may provide a suitable level of control.

In summary, laboratory, greenhouse, and field trials have shown Proline[®] to be efficacious against three important fungal pathogens that cause damage and seedling mortality in forest seedling nurseries. Disease control of all three fungi using Proline[®] was obtained using rate of 365 ml/ha (5 fl oz/ac), that is within the current Proline[®] range of 183 to 416 ml/ha (2.5 to 5.7 fl oz/ac) for registered crops. There is also an annual maximum use rate for each crop and these laboratory studies show that Proline[®] is capable of controlling fungi *in vitro* at rates much lower than 365 ml/ha (5 fl oz/ac). The key to any fungicide application is to apply the minimum rate necessary to control the disease, and caution should be used when applying laboratory results to field or greenhouse studies.

#### Label Registration Efforts

Over 1 billion hardwood and conifer seedlings are produced in southern US forest seedling nurseries each year (Enebak 2009) on approximately 1012 ha (2500 ac). Despite the large number of seedlings produced, most chemical companies consider forest seedlings to be a low profit, minor crop and tend to avoid marketing products for such a small acreage. When the cost of discovery, development, and registration of a new pesticide exceeds US\$ 180 million (Whitford and others 2006), it is easy to understand why chemical companies focus their marketing efforts on commodities such as wheat, soybeans, and peanuts that will insure a profit from sales.

In the 1970s and 1980s, the SFNMC tested the efficacy of both registered pesticides and numbered compounds as provided and requested by chemical companies. Over time, due to the increased scrutiny by state and federal agencies, the cooperative found the registration of numbered compounds increasingly difficult to obtain and ceased testing compounds that were not currently registered for use in the United States. Currently, only registered pesticides are tested by the SFNMC with the hope of obtaining the necessary registration for use in forest seedling nurseries.

Part of the SFNMC mission statement is to bring new pesticide chemistry to its members. One of those new chemistries was prothioconazole, the active ingredient in Proline[®]. In early 2009, as a result of various experiments during several years, and in cooperation with Bayer CropScience, an application was filed with the US EPA in six southern US states for a Proline[®] 24(c) label. The intended special use label was for the control of pitch canker and Rhizoctonia foliar blight in loblolly and longleaf pine. Approval for its use had been received in five of the six states when in March 2009, US EPA requested Bayer CropScience pull the approved 24(c) labels. The US EPA determined that forest seedling nursery use is a "new non-food use" that requires a separate ecological risk assessment, and the existing data on file for Proline[®] only supports food crops. In response to US EPA request to pull the approved labels, Bayer CropScience requested the continued use under the Section 24(c) based on: 1) the minor acreage involved; 2) the use pattern is only for nursery and not forestry; 3) the proposed use pattern has a similar application method and exposure as the already registered crop use; and 4) the proposed use pattern poses no greater risk (or lower risk) compared to the currently registered uses. However, in the end, the US EPA did not change their ruling and Proline[®] is not yet available for forest seedling nurseries. Several other labeling efforts (for example, IR4) were explored but found not feasible with a non-food crop.

In November 2009, after a number of conversations with both US EPA and Bayer CropScience, we were informed that our registration request for Proline[®] in forest seedling nurseries could be considered under the Pesticide Registration Improvement Renewal Act (PRIA) of 2007 under the category of "additional use, non-food; outdoor" (PRIA code R230). Bayer CropScience agreed to allow the request to go forward if the SFNMC were to pay the PRIA fee of US\$ 22,827.

In late December 2009, the US EPA acknowledged the Proline[®] registration package from Bayer CropScience for an additional use. The examination of Proline[®] for this additional, non-food, outdoor use is expected to take EPA about 15 months. Once this process has been completed, we anticipate a full label for Proline[®] to be registered for use on nursery seeds and seedlings of shortleaf, loblolly, slash, longleaf, and other pines, and other conifers and hardwoods. Until this is complete, nurseries are allowed under FIFRA rules to test a pesticide on areas less than 4 ha (10 ac) as long as they are collecting data for future use. Therefore, small trials testing this product under the different environmental conditions that occur in nurseries are warranted (and encouraged) prior to becoming operational.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

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> Portland, Oregon August 24 to 26, 2010



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Ponderosa pine drawing by Lorraine Ashland, College of Natural Resources, University of Idaho.

### The Target Plant Concept—A History and Brief Overview

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Landis TD. 2011. The target plant concept—a history and brief overview. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 61-66. Available at: http://www.fs.fed. us/rm/pubs/rmrs_p065.html

**Abstract:** The target plant concept originated with morphological classification of conifer nursery stock in the 1930s, and the concept was enhanced through physiological research and seedling testing towards the end of the century. Morphological grading standards such as shoot height, stem diameter, and root mass are the most common use of the target plant concept, and some physiological grading standards are also being operationally implemented by nursery workers and seedling users. Since 2000, the concept has been expanded to include all types of plant materials, including seeds, cuttings, or wildlings, as well as traditional nursery stock. Because these native plant materials are being outplanted on harsh, severely disturbed sites, this more comprehensive native plant materials concept also involves environmental conditions on the project site.

Keywords: nursery, reforestation, restoration, seedling, native plant

The term "target seedling" has become a standard part of nursery and reforestation jargon, and this is the second symposium on this subject. Although we don't know the exact time when the target seedling was first used, the term has undergone constant refinement over the years. In researching published literature, we can examine the development of the target seedling concept in three chronological phases.

#### Phase 1: Morphological Specifications

Since the early 1900s, foresters and nursery managers have attempted to measure the quality of nursery stock by morphological characteristics such as shoot height and stem diameter, oven-dry weight, and relative size comparisons, such as shoot-to-root ratio. These morphological targets have helped growers manage their crops and fine tune cultural practices; in addition, these physical attributes served as grading specifications after harvesting. In the 1930s, a visionary USDA Forest Service scientist named Phil Wakeley proposed three morphological grades of southern pine (*Pinus* spp.) seedlings, and developed a system of seedling quality testing by monitoring survival and growth after outplanting (Wakeley 1935). One of the morphological measurements most consistently related to survival and growth after outplanting was stem diameter at the root collar (Figure 1A). After years of testing, however, he realized that grading seedlings using morphology alone was often ineffective in predicting outplanting performance (Wakeley 1954). Morphological grading specifications are still the most common application of the target plant concept and stem diameter at the root collar ("caliper") is the most consistently correlated to outplanting performance (Mexal and Landis 1990).

#### Phase 2: Physiological Research Leads to Seedling Quality Testing

Wakeley's research prompted him to develop the concept of physiological grades and to conclude that mineral nutrient content, stored carbohydrates, or water tension were the most likely differences between the grades (Wakely 1948). This observation showed amazing foresight in describing the phenomenon that we now call "root egress" and consider essential to outplanting success.



80 cm

**Figure 1.** (A) Wakeley developed a series of morphological grades for southern pines by relating them to survival and growth after outplanting (modified from Wakeley 1954). (B) Root growth capacity was used to create physiological grades of ponderosa pine seedlings; these morphologically identical seedlings had significantly different amounts of new root growth (modified from Stone and Jenkinson 1971).

30 cm

High physiological quality of southern pine seedlings seems to improve survival principally by insuring that the water intake of the seedlings immediately after planting equals or exceeds their water loss. The probability is great that in many cases it insures this favorable water balance by enabling the seedlings to extend new root tissue into the soil of the planting site within the first few days after planting (Wakeley 1954).

The first person to develop an actual seedling testing procedure was Edward Stone, a forestry professor at the University of California at Berkeley. He pioneered the root growth capacity test (RGC) in the 1950s and first presented the idea of seedling quality testing to a western forest nursery association meeting (Stone 1954). He observed that the ability to grow new roots after outplanting was somehow related to seedling quality (Figure 1B) and the current RGC test is the result. RGC was later used to develop a series of physiological grades for bareroot ponderosa pine (*Pinus ponderosa*) seedlings (Stone and Jenkinson 1971).

In the 1970s and 1980s, there was a tremendous surge in the amount of research on seedling physiology. The first symposium on planting stock quality was held in New Zealand in 1979 and produced some of the classic articles on seedling quality testing (Gadgil and Harris 1980). It was at this symposium that one of the most concise definitions of seedling quality was coined, that is, seedling quality is "fitness for purpose" (Ritchie 1984). This idea that plant quality is defined on the outplanting site and not at the nursery is one of the pillars of the target seedling. The proceedings from a workshop on evaluating seedling quality at Oregon State University have become one of the primary references for seedling quality testing (Duryea 1985).

This phase culminated in the first Target Seedling Symposium (Rose and others 1990), which included over 25 articles on both morphological and physiological aspects of seedling quality. By this time, the term target seedling was well accepted in the nursery and reforestation field and was most commonly used to designate planting stock specifications, especially plant height, stem diameter, and shoot-to-root ratios. More attention was also being paid to the root system and techniques such as root volume were being tested (Haase and Rose 1990). While seedling quality tests such as RGC and plant moisture stress were commonly used, there was still no operational use of seedling physiological grades. This symposium also introduced the physiological treatment of short-days or "blackout" (Eastham 1990), which has subsequently been specified in growing contracts for container seedlings.

In the 20 years since the first Target Seedling Symposium, the list of seedling quality tests has steadily increased and several firms have offered testing on a fee basis (Landis and others 2010). Traditional tests, such as RGC and cold hardiness, are still the most popular with both nurseries and seedling users (Figure 2). Testing plant moisture stress at different stages of the harvest-to-outplanting process can ensure that plant stress is minimized. Chlorophyll fluorescence and root electrolyte leakage tests may be used immediately after unexpected stresses. Cold hardiness testing can be done to determine proper harvesting windows and to ensure that stress resistance is still high prior to outplanting. In reality, a combination of two or more seedling quality tests may prove to better predict


Figure 2. Seedling quality tests can be done by both nursery managers during the production cycle or by nurseries and seedling users during harvesting, shipping, and outplanting (from Landis and others 2010).

outplanting performance. For example, an index using RGC and chlorophyll fluorescence proved to be highly correlated with survival and growth of conifer seedlings (L'Hirondelle and others 2007).

### Phase 3: Expanding the Target Plant Concept to Restoration of Disturbed Lands with Native Plants _____

Starting in the 1990s, nurseries began to produce a wider variety of native plant species for ecological restoration projects. Existing forest and conservation nurseries expanded their product line from a few traditional tree species to include grasses, forbs, woody shrubs, and non-commercial trees (Landis and others 1993). To accommodate this new emphasis, the target seedling concept was expanded to include all types of plant materials, that is, seeds, seedlings, cuttings, and even plants salvaged from project sites (wildlings). The target plant concept was one of the key driving forces used to develop the Roadside Revegetation Manual (Steinfeld and others 2007) and subsequent training sessions. Initially, the target plant concept was defined by six aspects (Landis 2001); because these native plants would be outplanted on harsh, severely-disturbed sites, site evaluation and mitigating measures were added (Figure 3).

#### **Reforestation or Restoration Objectives**

The reason non-commercial native plant materials are being used has an overriding influence. In traditional reforestation, commercially valuable tree species that have been genetically improved for fast growth are outplanted with the ultimate objective of producing saw logs or pulp. The fact that restoration projects use a different variety of plant materials radically changed the target plant concept. Restoring severely disturbed lands generates a new list of project objectives, including soil erosion prevention or the elimination of exotic weeds.



Figure 3. Considering all types of native plants for disturbed site restoration, the target plant materials concept consists of eight aspects.

#### Site Evaluation—Soil, Climate, Plants

Whether for reforestation or restoration, the project site should be comprehensively described early in the process. Using a map of the project area, the soils should be evaluated and an overlay made of the various soil types. Soil survey maps are available from the USDA Natural Resources Conservation Service Web Soil Survey (URL: http://websoilsurvey.nrcs. usda.gov/app). Climatic information can be obtained from local weather stations or online from the Western Regional Climate Center that has 2800 weather stations in the western US (URL: www.wrcc.dri.edu). A trained botanist should conduct detailed surveys of which plants are currently found on disturbed and undisturbed reference sites in the project area. A wealth of botanical information can be found on-line; Ecoshare is one example that is a joint effort of the USDA Forest Service and the USDI Bureau of Land Management (URL: http://www.reo.gov/ecoshare/index.asp).

#### **Limiting Factors**

After all site information is compiled and evaluated, the next step is to determine which environmental factors on the project site are most limiting to plant establishment and growth. Because they are typically severely disturbed, restoration sites are particularly difficult to revegetate due to soil loss or damage. A whole array of atmospheric and edaphic factors can be limiting, but soil moisture and temperature are the most common factors to consider. There are typically more than one limiting factor, and they should be ranked in order of severity. Limiting factors are cumulative and sequential, that is, once one factor is overcome, another will typically become limiting (Figure 4).

#### Mitigating Measures

Once limiting factors have been identified and ranked, the best and most cost effective way to mitigate their effects must be determined. Many mitigating measures will affect more than one limiting factor, and their effectiveness will depend on the site characteristics and project objectives. For example, mulches of organic matter are often used to prevent surface soil erosion as well as retard soil moisture loss. With roadside revegetation trials, hydromulch was found to be most effective on the western side of the Cascade mountains where there was plenty of precipitation. In contrast, fiber mulches worked better in eastern Oregon where soil moisture is especially limiting (Steinfeld 2010).

#### **Genetics—Species and Source**

The question of which native plant species should be used on a restoration project is usually dependent on project objectives as well as the results of vegetation surveys of similar sites. "Workhorse" species are locally adapted native plants that are locally common, have broad ecological amplitude, and are relatively easy to propagate (Steinfeld and others 2007). Once the species have been selected, the question becomes one of source; local sources are preferred to ensure that plants are adapted to the environment on the project site. Seed zones are available for most commercial tree species, but guidelines for shrubs, grasses, and forbs are still being developed. Most restorationists, therefore, require that plant materials be collected at or near the project site. When working with cuttings of dioecious species, such as willows or cottonwoods, the sex of the plant material is also a serious consideration to ensure that a good mix of male and female plants is established (Landis and others 2003).



Figure 4. The idea of limiting factors is critical to the target plant materials concept because it helps characterize environmental conditions on the outplanting site.

#### Plant Materials—Seeds, Cuttings, Plants

Compared to the original target seedling, this more expansive target plant materials concept includes all types of propagules used to establish plants on the project site. Seeds of grasses, forbs, and some woody shrubs are directly sown onto the site, whereas larger woody plants are typically grown as nursery stock and outplanted. In some cases, wildlings are harvested from the local area and transplanted onto the project site. In riparian restoration, both unrooted and rooted hardwood cuttings are extensively used. Cuttings collected on the project site are the primary propagules used to produce nursery stock, whereas streambank bioengineering utilizes live stakes and branched cuttings for structures such as wattles or vertical bundles (Hoag and Landis 2001).

#### **Outplanting Tools and Techniques**

Unfortunately, many inexperienced foresters or restorationists don't consider how they are going to get their plants in the ground until the last minute. Seeds can be broadcast sown, drilled into the soil surface, or applied through hydroseeders. Unrooted cuttings are planted with dibbles or specialized equipment like the waterjet stinger (Hoag and others 2001). A wide variety of hand tools have been used successfully to outplant nursery stock. All too often, however, foresters or restoration specialists develop a preference for a particular implement because it has worked well in the past. Professional planters will choose the implement that gets plants into the ground as quickly as possible. Although this obsession with productivity is understandable, it can lead to serious problems with survival and growth. For example, dibbles work reasonably well on sandy soils, but they create a compacted soil layer that inhibits root egress in clay soils (Landis and others 2010). The pattern and spacing of outplanted seedlings is also a reflection of project objectives. Industrial forestry projects, where timber production is the primary objective, outplant the maximum number of trees per area in a regularly spaced pattern. Where ecological restoration is the objective, however, installing plants randomly or in random groups is more representative of natural vegetation patterns (Landis and Dumroese 2006).

#### **Outplanting Windows**

Timing of the outplanting project is the final aspect of the target plant concept to consider, and it should be considered during the planning stage. The outplanting window is the period of time during which environmental conditions on the outplanting site are most favorable for survival and growth of the plant material. The main idea is to get the seeds, cuttings, or plants installed when the normally limiting soil moisture and temperature are at ideal levels. For instance, in the Pacific Northwest of the US, nursery stock is typically outplanted during the rains of winter or early spring. Fall outplanting is preferred on project sites where access is limited during the winter or spring. Summer and autumn outplanting with container plants is becoming more common at high elevation or latitudes, but the stock must undergo special cultural conditioning to ensure hardiness (Landis and Dumroese 2006).

Basic precepts of the target plant materials concept can be summarized as follows:

- Nursery stock can be described by both morphological and physiological characteristics, but must be related to outplanting performance.
- The most common application of the target plant concept is the use of morphological attributes, such as shoot height and stem diameter, as grading specifications.
- Target plant characteristics can only be described on the outplanting site, not in the nursery.
- Plant users must be involved in establishing objectives and setting specifications.
- Target plant specifications must be tested in the field and results of outplanting trials used to refine the concept.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# Producing the Target Seed: Seed Collection, Treatment, and Storage

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**Abstract:** The role of high quality seeds in producing target seedlings is reviewed. Basic seed handling and upgrading techniques are summarized. Current advances in seed science and technology as well as those on the horizon are discussed.

Keywords: target seeds, seed quality, optimizing seed performance

#### Introduction _

"First the seed" is an old saying among seed workers. It implies that high quality seeds are the first step in producing a good crop and in producing target seedlings. High purity, high germination, and high vigor are basically the targets for high quality seeds. These factors affect seed sowing efficiency, the amount of thinning or transplanting, and the number of empty cells in a container nursery. In other words, these factors affect the quality and cost of seedlings.

## **Defining High Quality Seeds**

High germination is a relative term that depends on such factors as species, seed availability, and seedling production methods. For example, pines (*Pinus* spp.) often have higher germination rates than true fir (*Abies* spp.). Therefore, 95% might be high and 75% low for pine, but 75% might be high for some species of fir. Similarly, 90% might be high in a bareroot nursery, but possibly not in a container nursery. Table 1 illustrates how lower seed germination can affect seedling production costs in a container nursery. If germination is 98% to 100%, there is little wasted production space and no seeds would be wasted. Even at 95% germination, however, a significant number of wasted cells start to occur. Because empty cells cost money and offer no return, their presence reduces nursery profitability and perhaps even the ability to meet production goals. Various strategies exist for dealing with empty cells such as, sowing two seeds per cell, sowing extra cavities, or transplanting germinants into empty cells. These strategies give some relief, but increase costs in seeds, materials, time, and perhaps even quality of seedling. The higher the germination, the better the chances are of producing the desired seedling at the best possible cost (Figure 1).

Vigor is also a relative term and, despite much research, remains difficult to define objectively for native plants. Nevertheless, it is a very useful concept for handling seeds and comparing the performance of seedlots. High vigor can be defined in two ways. The first is the ability of seeds to germinate under adverse conditions or to germinate rapidly. Personal communication with nursery managers indicates these are critical factors in getting a seedling crop through the fragile stage of germination and on to target seedling specifications. The second way to define high vigor is the ability of seeds to perform well after storage. High germination and high vigor often go together, but not always. Low germination could occur due to weak seeds or dormancy. Figure 2 illustrates how, as a seedlot ages, vigor is lost earlier and faster than germination. As a consequence, a seedlot that germinated well a few years ago might suddenly germinate very poorly in the nursery. A current germination test, not more than 6 months old, will help identify seedlots that are losing viability and vigor.

Thinning required; higher seed costs

Germination	Seeds/cell (#)	Filled cells (%)	Double seedlings (%)	Some consequences
100	1	100	0	Optimal
98	1	98	0	Good
95	1	95	0	5% space lost/cost per seedling up
90	1	90	0	10% of growing space is lost
90	2	99	81	Thinning required; higher seed costs
85	1	85	0	15% space lost/cost per seedling up

72

98

 Table 1. Results and consequences of lower seed germination.



2

Figure 1. Higher germination gives a better chance of producing the desired seedling at the lowest possible cost.



**Figure 2.** As a seedlot declines in quality, so does total germination and vigor. Vigor is lost quicker than germination.

## Obtaining High Quality Seeds ____

High quality seeds are obtained by following basic seed handling procedures and upgrading the seeds as needed. Basic seed handling involves collecting at physiological maturity, handling seeds after harvesting in a manner that does not lead to deterioration, and extracting and cleaning without damaging the seeds but still removing trash and empty seeds. Upgrading seeds involves removing insect or fungus damaged seeds, small amounts of trash, mechanically damaged seeds, and weeds. Both basic cleaning and upgrading require physical differences among the seeds as well as between the seeds and trash; differences can include thickness, length, weight, surface texture, roundness, or color. A detailed discussion on seed cleaning and upgrading machines and techniques can be found in Bonner and Karrfalt (2008).

Cleaning and upgrading seeds can be a multistep process because undesirable seeds and particles can vary in at least two characteristics, weight and size. Fungal or insect damaged seeds (Figure 3 and 4) usually can be removed with air cleaners or gravity tables that separate particles of different weight, but not until the seeds are divided into fractions that are uniform dimensionally. Figure 5 shows that damaged larger seeds can weigh the same as smaller undamaged seeds, and can sort out together by weight until they are separated from each other with screens. Some species with wings do not remove uniformly, so screen sorting prior to weight separation is less effective. As a consequence, these species may not be as thoroughly upgraded. True firs and longleaf pine (*Pinus palustris* Mill.) are two examples of such species.

Some techniques sort seeds using fluids. The Prevac procedure (Simak 1984) (Figure 6) uses water flotation and vacuum to remove mechanically cracked seeds. Seeds are placed in a chamber, such as an Erlenmeyer evacuation flask, on water. A vacuum is then drawn on the chamber to break the surface tension between the water and the seeds, causing the cracked seeds to rapidly take up water and sink, and leaving the uncracked seeds to float. The Imbibe, Dry, Separate (IDS) procedure (Simak 1984) relies on the principal that dead seeds will dry and lose weight faster than alive seeds. By carefully controlling the drying process, dead seeds can be floated away using a chamber similar to Figure 7. These procedures can be used for Sitka spruce (Picea sitkensis (Bong.) Carriere), Scotch pine (Pinus sylvestris L.), lodgepole pine (Pinus contorta Douglas ex Louden), and some white spruce (Picea glauca (Moench) Voss).

85



Figure 3. X-ray image of fungal damaged pine seeds.



**Figure 6.** An evacuation flask can be used to perform the Prevac procedure (Simak 1984) to mechanically remove cracked seeds.



**Figure 4.** Good (left) and insect-damaged (right) *Lomatium* spp. seeds. Insect holes are visible in damaged seeds.



**Figure 7.** A large plastic soda bottle can be used to separate seeds in the Imbibe, Dry, Separate (IDS) procedure.

**Figure 5.** The weight of individual seeds from four seed sizes: number 16 heavy (16H); 16 light (16L); 18 heavy (18H); 18 light (18L).

# Optimizing Good Seed Performance

The first step in optimizing seed performance is using good seed. The second step is to use seed wisely. It is important to know the germination requirements for the target species. Seeds have an optimum temperature for germination. Sowing too early might result in temperatures too low for good germination, while sowing too late might result in temperatures being too high for germination. Other factors can also lead to germination problems. For example, in one nursery, the use of irrigation water from a snow-fed stream, with water temperatures near the freezing point, germination was very slow despite using high germination seeds. Apparently the cold water was retarding germination.

Sowing depth is another important factor in optimizing seed performance. Most native plant seeds germinate on or near the surface of the soil. It is usually easiest to obtain a correct planting depth by surface sowing the seeds and mulching to a specified depth. Some species, mountain hemlock for example, require dark to germinate. Some provision must be made, therefore, to exclude light to start the germination process. In some cases, seed size can ultimately affect seedling size; therefore, using screens to sort seed by size is another tool that optimizes seed performance. Karrfalt (2004) found that acorn size effected oak (*Quercus* spp.) seedling size (Figure 8). Many studies indicate the same is true for other species. A critical factor in sizing seeds for better performance is to establish enough size criteria. Seeds have three dimensions and, therefore, sorting only according to one of them can result in a large amount of weight variation within one size.

Cold stratification has proven to be a very powerful tool to improve seed performance. Extended periods of cold stratification, or the use of cold stratification on non-dormant seeds, have been found to increase the ability of seeds to germinate faster and over a wider range of temperatures. In short, cold stratification increases realized seed vigor. Edwards and El Kassaby (1996) (Figure 9) found stratification improved the speed of germination of mountain hemlock (*Tsuga mertensiana* (Bong.) Carriere). Gosling and Rigg (1990) (Figure 10) showed that extended cold stratification promoted germination at suboptimal temperatures for Sitka spruce. Unpublished data at the National Seed Laboratory showed the same to be true for loblolly (*Pinus taeda* L.), slash (*Pinus elliottii* Engelm.), and longleaf pines.



**Figure 8.** White oak (*Quercus alba*) seedlings produced from a smaller acorn (size 30) and a larger acorn (size 38+).



**Figure 9.** Stratification improves seed vigor for western hemlock (*Tsuga mertensiana*) (Edwards and El Kassaby 1996). Lower values of R50 and R50' indicate high seed vigor, while higher values of PV and GV indicate high seed vigor.



**Figure 10.** Seed germination occurred over a wider range of temperatures (for example, seed vigor increased) for Sitka spruce following cold stratification (Gosling and Rigg 1990).

Seeds can sometimes germinate in cold stratification, especially if the treatment is extended. However, using a few simple treatment techniques, premature germination can be minimized. When extended stratification times are used, it is necessary to keep temperatures in the 1 to 3 °C (34 to 37 °F) range and restrict the amount of moisture. Prior to stratification, seeds should be soaked to full imbibition (generally overnight) and surface dried to remove free water; the seed coats should look damp. Gosling and Rigg (1990) treated Sitka spruce in this manner and saw no germination up to 20 weeks in cold stratification. Gosling and Rigg (1990) also examined the optimal moisture content for stratification

(Figure 11). They found an interaction with moisture content and length of cold treatment. At 25% moisture content, the stratification was effective if held sufficiently long; a 20% moisture content was not as effective. These results are compatible with the work of Edwards (1981) and Leadem (1986) on the stratification re-dry technique for *Abies* spp. In this procedure, seeds are fully imbibed, stratified for 30 days, dried to about 35% moisture content, and returned to stratification at this reduced moisture content for extended periods until a maximum germination is obtained. The germination results are superior to a stratification treatment of fully imbibed seeds for an equal length of time.



Figure 11. Sitka spruce germination at 10 °C (50 °F); seed moisture content by stratification period interaction.

Edwards (2008) has stated that he is not sure 35% moisture content is optimal because the procedure does not work well for all seedlots. This might be explained in the relationship between moisture content and water activity. Figure 12 shows that seeds with similar moisture contents have different equilibrium relative humidity (ERH). For that reason, ERH more accurately reflects the water status of the highest quality seeds in a seedlot by showing how tightly bound the water is. Equilibrium relative humidity readings, however, do not work well for seeds of high moisture contents, as there is much free water in seeds at or near full imbibition. Another means of accessing the seed moisture status, such as osmometry, might work better for this purpose.



Figure 12. Seed moisture content of green ash seeds plotted against equilibrium relative humidity.

#### Summary

Producing seedlings economically and efficiently starts with high quality seeds. Defining the characteristics of the target seed is just as important as defining the target seedling. Factors such as nursery production system and species will determine what target seeds will be. Applying good seed handling and upgrading techniques usually will produce target seeds. Once target seeds are acquired, it is necessary to optimize their performance. Prechilling has been the most widely used method to optimize seed germination. Properly applied, prechilling can improve total germination, speed of germination, and the capacity of seeds to germinate in adverse conditions. Seeds must also be planted at the proper depth and at the correct season.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# Morphology Targets: What Do Seedling Morphological Attributes Tell Us?

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Pinto JR. 2011. Morphology targets: what do seedling morphological attributes tell us? In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 74-79. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p065.html

**Abstract:** Morphology is classically defined as the form and structure of individual organisms, as distinct from their anatomy or physiology. We use morphological targets in the nursery because they are easy to measure, and because we can often quantitatively link seedling morphological traits with survival and growth performance in the field. In the 20 years since the Target Seedling Concept was developed, morphological targets remain some of the most commonly used attributes to link seedling quality and field performance. Traits such as height and root-collar diameter are still standard attributes, but others have also proven their worth despite being more difficult to measure.

Keywords: Target Plant Concept, seedling performance, performance potential, stocktypes, seedling quality

#### Morphology Targets _

In the context of biology, morphology describes the form and structure of an organism and gives a sense of how that form contributes to function. Morphology, in the context of seedlings, conjures up an image of how a seedling looks after nursery culturing; a stocktype designation also does the same. Measuring morphology in the nursery has been standard practice for quite some time because it is an easy way to track growth and describe a seedling at harvest. Seedling morphology has, therefore, evolved into classification that correlates seedling survival and growth with specific morphological traits.

Over time, morphological traits became targets that drove nursery culture and were also species-specific. For example, a target loblolly pine (*Pinus taeda*) seedling produced in a bareroot nursery 20 years ago was defined as having a height of 20 to 25 cm (7.9to 9.8 in), a root-collar diameter (RCD; also referred to as stem diameter and caliper) of > 4 mm (0.16 in), mostly secondary needles, a single prominent stem, a well-developed bud with resinous scales, a minimum of six first-order lateral roots, fibrous roots, mycorrhizae, a root volume of  $\geq 3.5 \text{ ml} (0.2 \text{ in}^3)$ , and high root growth potential (Rose and others 1990) (Figure 1). The targets defined grading specifications at harvest; if any seedling did not meet minimum specifications, it was culled. Grading specifications in line with morphological targets ensured planted seedlings would have the best chance for survival and growth.

Just over 20 years ago (1990), the Target Seedling Symposium was held in Roseburg, Oregon. The objectives were to discuss the "latest methods of describing and measuring the ideal seedling for reforestation purposes." Within this discussion, the Target Seedling Concept was introduced to unify the thought that specific morphological and physiological seedling characteristics could be quantitatively linked to reforestation success. By this time, it was pretty well understood that morphological traits were tied to seedling success in the field; therefore, the emphasis shifted more towards further understanding of seedling physiology. Despite the considerable emphasis on physiological tests for seedling quality and performance, the fact remained that morphological traits were still the easiest and quickest to measure on an operational basis. Furthermore, morphology is a general reflection of physiological quality in that a seedling with poor physiological quality can often have morphological attributes that do not meet targets. With a few basic pieces of equipment, most nurseries could obtain a quick morphological estimate on how well they grew their seedlings, and how well they might perform in the field as a result.



Figure 1. Some of the most common morphological measurements and targets include height, root-collar diameter, root size, and the presence of a well-developed bud (from Landis and others 2010).

The most important morphological traits discussed during the 1990 symposium are still the ones that are discussed today. Height and RCD are two of the most commonly used measurements for specifying seedling targets (and subsequent grading) as well as indicating outplanting performance potential. Thompson (1985) and Mexal and Landis (1990) provide great discussions and reviews on measurement, standards, and quality prediction of height and RCD. In addition to these two common characteristics, other morphological traits have been used as targets, grading criteria, and quality predictors. Thompson (1985) also reviews weights (biomass), root size, bud length, color, secondary needles, mycorrhizae, shoot-to-root ratio (S:R), sturdiness (the quotient of height in cm divided by RCD in mm), and several others.

#### Morphological Ties to Seedling Field Performance_____

Many morphological traits and targets have been wellcorrelated with seedling performance after outplanting. Despite being a physical descriptor of a seedling, morphology has implications toward physiology. Essentially, morphological attributes have become a proxy for physiological processes that occur once a seedling has been outplanted. The key to this assumption is that the actual physiology and vigor of the seedling is of the same high quality as the target morphology. In reality, physiology and vigor can change significantly between harvest and outplanting while morphology tends not to change during that time. However, most correlations stemming from morphology have met the assumption of matched, high quality physiology.

In general, seedlings that are tall at the time of outplanting keep their height advantage in the field over time (Figure 2A). In some cases, initial seedling height may not always indicate a seedling will grow more. For example, Pinto and others (2011b) found that seedlings of different stocktypes (from 60 to 166 ml in container size [4 to 10 in³]) maintained their relative height differences over time because the actual height growth among stocktypes was the same (Figure 2B). Height is closely linked with needle number, and therefore, approximates photosynthetic capacity and potential transpirational surface area. Because other factors, such as S:R and outplanting site conditions, can influence seedling growth, however, it is important to remember that tall seedlings may not always be the desired target. For example, a tall seedling with high transpiration potential is likely not the target seedling for a dry site, nor might it be recommended for a site with lots of wind.

RCD is often linked with seedling survival in the field. Typically, large RCDs mean higher survival rates (Figure 3). With container seedlings, however, RCDs can become too large, indicating the seedlings have become root bound. South and others (2005) found that container-grown longleaf pine (P. palustris) showed decreasing survival with RCDs exceeding 9 mm (Figure 4). This correlation was linked to a root bound index (RBI) value that divides RCD by the container cell diameter. Longleaf pine RBIs with a value greater than 30% indicated seedlings were likely root bound and would consequently exhibit decreased survival. Conversely, bareroot seedlings, suffering no bounds by container walls, tend to survive well with continuously increasing RCD (South and others 2005). The physiological correlate for RCD is that it represents the main plumbing line for piping water from the soil to the leaves and vice versa for piping the products of photosynthate to the roots. Larger RCD values tend to imply well-developed root systems for satisfying transpirational demand.

Root size has been used as an index to predict outplanting success. Many methods for measuring root size exist, and for the purposes of this paper will only be covered briefly. For more detailed information on target root specifications and relationships, see Haase (2011). As mentioned, many methods exist for measuring root size. Root volume, mass, length, area index, and number of first-order laterals are all possible ways to measure and classify root morphology. In bareroot nursery stock, Rose and others (1997) found that larger root volumes led to higher survival in ponderosa pine seedlings after 8 years. For container nursery stock, Pinto (2009) demonstrated that larger container volumes produced taller seedlings after two growing seasons in the field (Figure 5). The importance of a functional and vigorous root system in seedling survival and growth cannot be over emphasized. Physiologically, roots are the first and main connection in the moisture status of the plant and the first point of resistance in the soil-plant-air continuum (Grossnickle 2005).

A few other target morphological traits have been used to predict outplanting success. While the presence of a welldeveloped bud is desirable, bud length has shown some predictability on shoot height. Kozlowski and others (1973) observed that longer buds meant greater shoot length in red pine (P. resinosa). Seedling S:R is a morphological attribute that describes the balance between above and below ground biomass. The ratio quantifies the balance between potential transpiration from the shoots to potential absorption from the roots. In general, values of less than 2.5 are beneficial. Cregg (1994) found that a S:R of 1.7 was ideal among several genotypes of ponderosa pine (*P. ponderosa*). The color and form of a seedling also has some bearing on outplanting performance. Seedlings that are pale green to yellow indicate a mineral nutrient deficiency and are likely to perform poorly on the outplanting site. Similarly, seedlings that appear damaged or malformed are also likely to show decreased field performance.



**Figure 2.** Initial seedling height corresponds well to absolute seedling height (1 cm = 0.4 in) after outplanting (A) (from Pinto 2009). When differently sized stocktypes are planted, and similar growth occurs among stocktypes, absolute heights can remain stratified over time (B) (adapted from Pinto and others 2011b).



**Figure 3.** For outplanted Engelmann spruce, seedlings with larger root-collar diameters (1 mm = 0.04 in) exhibited better survival after 2 years (modified from Hines and Long 1986).



**Figure 5.** Heights of ponderosa pine seedlings (1 cm = 0.4 in) grown in six different container volumes ranging in size from 60 ml (3.3 in³) to 166 ml (10.0 in³). Seedlings were measured after planting (spring 2007), after one growing season (fall 2007), and after two growing seasons (fall 2008) (modified from Pinto 2009).



Figure 4. Longleaf pine seedlings can become too large for their containers, thus becoming root bound; consequently, root bound seedlings with large root-collar diameters (RCD; 10 mm = 0.4 in) can show reductions in survival after outplanting. Because bareroot seedlings are not limited by container environments, increasing RCD values continue to show increases in outplanting survival (modified from South and others 2005).

Most morphological targets are determined because they can be quantitatively linked with outplanting success. Although these morphological attributes describe physical characteristics, they are often tied to physiological processes that aid a seedling's establishment on the outplanting site. The model of seedling establishment proposed by Burdett (1990) outlines this link and forms it into a positive feedback loop. Any interruption in the loop will decrease survival or growth (Figure 6).

#### Seedling Morphology and Stocktypes _____

In early seedling production, nursery managers learned how to manipulate seedling characteristics to maximize survival and growth for the benefit of the practicing forester. The dialogue between the two helped develop specific targets for species and outplanting sites. Eventually, nursery managers began expanding their products, offering various types of bareroot, bareroot transplant, and container seedlings. Since then, the number of seedling stocktypes has increased dramatically, thereby expanding the range of target seedlings—and morphological attributes—for practicing foresters. The range of stocktypes has not only benefited foresters, but has also aided restorationists. In fact, restoration projects have greatly increased the diversity of stocktypes because of the broad range of species and unique outplanting sites being managed.



**Figure 6.** The model of seedling establishment proposed by Burdett (1990). Morphological attributes that contribute to photosynthesis (that is, seedling height) and potential new root growth (that is, root size) are part of the feedback relationship and contribute to seedling establishment, survival, and growth (Pinto and others 2011a).

#### The Target Plant Concept_

"The Target Seedling Concept means to target specific physiological and morphological seedling characteristics that can be quantitatively linked with reforestation success" (Rose and others 1990). Over the years, this concept has evolved to include other types of plant materials and is now termed the Target Plant Concept (TPC) (Landis 2009). The concept unifies the three ideas that: 1) you start at the outplanting site; 2) the nursery and client are partners; and 3) the emphasis is on seedling quality. From these ideas, the target plant materials are defined in six-interrelated steps: 1) what are the project objectives?; 2) what are the type(s) of plant material needed?; 3) are there genetic or sexual considerations?; 4) what are the limiting factors on the outplanting site?; 5) what is the outplanting window?; and 6) what are the best outplanting tools and techniques for the outplanting site? For greater, in-depth coverage of the TPC, see Landis (2009, 2011) and Landis and others (2010).

To define the target morphology of a seedling, the outplanting objective must be known. Second, a major consideration is the limiting factors on the outplanting site. Once these two variables of the TPC are characterized, the nursery manager and client can better define the type of plant material needed. The subsequent steps including genetics, outplanting timing, and tools will also be better sorted out once the plant material is known. In essence, the target morphology for the target plant becomes the target stocktype. Because of the increasingly unique circumstances of reforestation and restoration-drastically disturbed sites, mining, road construction, invasive species, and changing conditions due to climate change-stocktypes are constantly evolving. With this new development, testing needs to validate not only stocktype use, but also target morphology with the stocktype. Properly executed trials will identify key performance advantages that help overcome site limiting factors (see Pinto and others 2011a).

#### Summary

The morphological attributes of height and RCD are the most commonly used to define seedling targets and infer seedling quality. These attributes are the easiest to measure lending to their popular use in operational settings. Morphological attributes are physical in nature and tend not to change appreciably between harvest and outplanting. Therefore, despite not being direct measures of seedling physiology, inferences to seedling quality assume optimum physiology and vigor. When this assumption is met, morphological attributes have been shown to predict performance potential in most circumstances. Morphological targets and stocktypes are intimately related. The Target Plant Concept provides a framework for defining the target plant material.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

## **Seedling Root Targets**

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**Abstract:** Roots are critical to seedling performance after outplanting. Although root quality is not as quick and simple to measure as shoot quality, target root characteristics should be included in any seedling quality assessment program. This paper provides a brief review of root characteristics most commonly targeted for operational seedling production. These are: root mass (with special attention to shoot:root), root fibrosity, root length, root form, and root growth potential.

Keywords: root mass, root-to-shoot ratio, root fibrosity, root growth potential

A functional nursery root system, that can supply adequate water and produce new roots after outplanting, is critical for successful seedling establishment, growth, and survival. Roots are the primary mechanism for water and nutrient uptake and provide structural support for the plant. However, plant specifications at the nursery are most often based on height and stem diameter of the seedling shoot. Although stem diameter is an indicator of root system size, root characteristics are rarely assessed beyond a cursory inspection at the time of packing. Obviously, root quality cannot be assessed as quickly and easily as shoot quality; but it should be considered an important factor in determining overall seedling quality and field growth potential.

Following is a brief overview of root characteristics most commonly measured for operational evaluation of seedling quality. For each of these, specific targets should be developed based on stocktype, species growth habit, seed source, and the expected environment at the designated outplanting site.

## Root Mass

Studies show that seedlings with larger root mass at the time of outplanting have greater growth and survival compared to those with smaller root mass, even if they have smaller stem diameters (Omi and others 1986; Blake and others 1989; Rose and others 1997; Jacobs and others 2005). Douglas-fir (*Pseudotsuga menziesii*) seedlings with large initial root volumes had greater terminal growth, stem diameter growth, and needle length across a range of soil water contents compared to seedlings with smaller initial root volumes (Haase and Rose 1993). Those with larger root volumes also had higher nutrient concentrations, suggesting that increased root biomass per unit of soil area may result in greater nutrient use, supply, uptake, and storage (Haase and Rose 1994). The effects of initial root system size can have lasting effects on seedling growth. Sundström and Keane (1999) found initial root system size to be correlated with diameter at breast height (DBH) and shoot fresh weight on excavated 10-year-old Douglas-fir trees.

Root mass is usually measured by weight or by volume. Root weight can be assessed on fresh or dry plant tissue. However, because water content in plant tissues varies considerably, dry weight is a more consistent measure of plant mass than fresh weight. Dry weight is measured on plant parts following oven drying for a minimum of 48 hours at 68  $^{\circ}$ C (154  $^{\circ}$ F). Root volume is easily measured via water displacement (Harrington and others 1994) and, unlike dry weight, it is a non-destructive measurement requiring very little time. Not surprisingly, root volume and root dry weight tend to correlate well with one

another. It is important to note that root mass is not necessarily a reflection of root fibrosity (see section below); a highly fibrous seedling with many fine roots may have a root mass similar to a seedling with a large tap root and low fibrosity.

Setting targets for root mass is dependent on the stocktype, seed source, and species. For instance, a quality 1+0 bareroot, loblolly pine (*Pinus taeda*) seedling may have an average root volume of 3 to 5 cm³ (0.18 to 0.24 in³), whereas a quality plug+1 bareroot, coastal Douglas-fir seedling may average 25 to 35 cm³ (1.5 to 2.1 in³).

#### Shoot:Root_____

When setting targets for root mass of a specific seedling crop, it is imperative that those targets be set relative to the shoot mass. A quality seedling must have an appropriate balance between its capacity to absorb water (roots) and its transpirational area (shoot). The ratio between the shoot to root (shoot:root) is calculated from shoot and root volumes or dry weights. A typical shoot:root target is 3:1 or less for bareroot seedlings and 2:1 or less for container seedlings. These targets are particularly important for seedlings designated for outplanting sites where water is limiting; seedlings that are "top heavy" may perform poorly on dry sites if the root system is inadequate to supply the water needs of the shoot.

#### Root Fibrosity ____

Seedlings with a proliferation of lateral roots (fibrosity) generally perform better than those with few lateral roots (Wilson and others 2007). A fibrous root system has a relatively high surface area with many root apices. Determination of fibrosity is often by visual inspection of the "moppiness" of the roots or by counting the number of lateral roots originating from the tap root, (referred to as first-order lateral roots [FOLR]). For seedlings that are strongly tap rooted (such as many pines and hardwoods), the number of FOLR >1 mm (0.04 in) diameter can be a useful target characteristic for quantifying fibrosity and predicting growth (Davis and Jacobs 2005; Gould and Harrington 2009). Schultz and Thompson (1996) found greater survival and growth for red oak (Quercus rubra) seedlings with at least five FOLR and black walnut (Juglans nigra) seedlings with at least seven FOLR.

#### Root Length ____

Root length is often determined by culturing and pruning methods in bareroot seedlings or by the container depth and dimensions for container seedlings. For bareroot seedlings, target specifications for final root length are met through culturing in the nursery beds (undercutting and wrenching) and by root pruning at the time of packing (based on target specifications agreed upon by the grower and the customer). Root length targets are dictated by the desired balance of the plant (that is, shoot:root), conditions of the outplanting site (soil texture, depth, and moisture), and planting method.

#### **Root Form**

The criteria for root form quality are based on ease of handling and influence on future growth. Container seedlings rarely have issues with root form because the root systems develop in a defined space and shape. The main concern with container seedling roots is that they fill the container and create a firm, but not root bound, plug. Conversely, bareroot seedlings may have stiff lateral roots or other deformities that make them difficult to pack in storage containers or carry in a planting bag—these seedlings are generally culled. Additionally, deformities such as root sweep (often caused during transplanting) are culled because they may result in poor structural support after outplanting.

#### Root Growth Potential

Root growth potential or capacity (RGP or RGC) of a seedling is its ability to initiate and elongate new roots when placed into an optimal environment. RGP is usually measured in late winter or early spring by either potting a sample of seedlings or placing them into a hydroponic tank. Subsequent root growth is quantified following a specified duration in an environment favorable for root growth (typically 2 to 4 weeks in a greenhouse environment where plants are kept moist and temperatures are 65 to 75°F [18 to 24°C]). New roots (distinguishable by their white tips) are then counted and measured. Quantification can either include all new roots or only those that are greater than 1 cm (0.4 in) long. Burdett (1979) developed an index of six RGP classes as follows:

Class	Description
0	no new root growth
1	some new roots but none over 1 cm long
2	1 to 3 new roots over 1 cm long
3	4 to 10 new roots over 1 cm long
4	11 to 30 new roots over 1 cm long
5	more than 30 new roots over 1 cm long

Because RGP is assessed in a favorable environment for new root growth, the same growth may not be realized when the seedling is outplanted to field conditions with cool soil temperatures. RGP tests are also a reflection of physiology at a single point in time; a seedling's physiological quality is subject to change from harvesting right up until outplanting. As such, RGP is not necessarily a good predictor of early field performance (Simpson and Ritchie 1997). In a study comparing 1+1 bareroot and 1+0 container seedlings, container seedlings had higher RGP than bareroot seedlings prior to outplanting as well as more root and shoot growth during the spring after outplanting; in the fall following outplanting, however, bareroot seedling survival and root growth were similar to container seedlings and subsequent long-term performance was expected to be comparable between the two stocktypes (Rose and Haase 2005).

Despite not being able to predict field performance through a range of conditions, RGP may be useful for identifying seedling viability. Using Douglas-fir, van den Driessche (1987, 1991) demonstrated that new root growth is dependent on current photosynthesis. The connection between new root growth and photosynthesis is dependent on many physiological functions including stomatal regulation, plant moisture status, photosynthate translocation, and so on; a high RGP result, therefore, will indicate a seedling is alive and well at the time of testing. Conversely, if a seedling tested very low, it is likely an indication that one of these systems is compromised.

#### Summary

When developing target characteristics for seedling quality, root morphological parameters such as mass, fibrosity, length, and form are important to include. Targets for root size should always be made relative to the shoot size to ensure a proper balance between the plant's water supply (roots) and demand (shoots). Root growth potential is another characteristic that may be considered, although its link to field performance can be uncertain. As with any quality characteristics, determining specific root target ranges is based on stocktype, growth habit of the plant species, and environmental conditions of the outplanting site.

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# Repetitive Reaction and Restitution (R³) Induction of Drought Hardiness in Conifer Container Seedlings

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Hodgson J. 2011. Repetitive reaction and restitution (R³) induction of drought hardiness in conifer container seedlings. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 83-86. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p065.html

**Abstract:** Planting failures are often attributed to unexpectedly harsh conditions after planting. Characterization of soil water at the planting site, along with associated influences of site preparation and soil texture, is recommended. Additionally, tree planting technique and seedling biology should be targeted to site conditions.

A nursery regime for induction of drought hardening is proposed for hot-lift summer- and fall-planted container conifers.

Keywords: seedling hardiness, nursery culturing, drought stress

#### Introduction

Nursery growers, researchers, and field foresters have improved forest seedling quality, planting technology, and site preparation with a corresponding increase in survival and growth after outplanting. This expertise has been disseminated worldwide through publications and conferences. In the 1970s and 1980s, average survival for planted seedlings was 50-75% (Cayford 1978; Mexal and others 2008). Today we expect survival of 90% or greater, although particularly harsh sites may need to be planted more than once due to poor seedling survival where water is a limiting factor (Mexal and others 2008; Regan and Davis 2008).

## Characterizing the Site _

Site preparation treatments such as vegetation control, scarification, and cultivation, along with annual weather patterns, result in a range of site conditions for outplanting. Lantz (1996) describes North Carolina standards for planting categories as normal, critical, and severe based temperature (Table 1), relative humidity, wind, and soil moisture on the day of planting. To further characterize the site, soil texture must be considered; the ability to hold water varies among soils (Table 2; Figure 1). When soil water potential is below -60 to -70kPa (-0.6 to -0.7 bar), root elongation ceases (Grossnickle 2005). In order to characterize the planting site for outplanting dates beyond the traditional outplanting windows, a soil moisture stress curve should be estimated based on a progression of moisture conditions at selected depths (Figure 2) using standard methods for determining soil hydraulic conductivity. This curve will suggest the moisture stress level likely to be encountered by a newly planted seedling before new root growth penetrates to a new source of moisture.

	Normal	Critical	Severe		
Temperature	2 to 24 °C (35 to 75 °F)	24 to 29 °C (76 to 85 °F)	> 29 °C (> 85 °F)		
Relative Humidity	>50%	30-50%	<30%		
Wind	<10mph	>10mph	>15mph		
Soil moisture buildup	<30	30-80	>80		

Table 1. North Carolina planting categories (Lantz 1996).

Table 2. Storage capacity for different soil texture classes (inches water/
feet depth). (1 in = 2.54 cm; 1 ft = 0.3 m)

Texture class	Field capacity	Permanent wilting point
Sand	1.2	0.3
Fine sand	1.4	0.4
Sandy loams	1.9	0.6
Fine sandy loams	2.6	0.8
Loams	3.2	1.2
Silt loams	3.4	1.4
Light clay loam	3.6	1.6
Clay loam	3.8	1.8
Heavy clay loam	3.9	2.1
Clay	3.9	2.5



Figure 1. Volumetric water content of three soils at different matric suctions (from Black 1968).



Figure 2. Matric suction versus distance from root in bulk sandy loam soil at -5 and -15 bars (Black 1968).

#### The Root/Soil Interface

Kramer and Kozlowski (1960) note that pines (Pinus spp.) have few growing root tips during winter, yet can absorb considerable quantities of water. Suberized roots can be a major route of water uptake in tree root systems, through lenticels on the surface of the root and by discontinuities in the periderm (bark) plates although unsuberized (white) root tips on growing roots have the highest uptake per unit of surface area (Carlson and others 1990). New root growth leads to an increase in daily minimum water potential  $(\Psi)$ for newly planted seedlings (Figure 3) (Grossnickle 2005). Given that the newly planted seedling may encounter a soil moisture stress condition that precludes new root growth, survival depends upon creating the water continuum with the existing root system-the hydrostatic system (Kramer and Koslowski 1960) or the soil-plant-atmosphere continuum (SPAC) (Grossnickle 2005).

Grossnickle (2005) provides an excellent discussion of the importance of root-soil contact in the planting of seedlings and points out that poor contact will increase the resistance to water movement from the soil to the plant. Landis and Dumroese (2009) emphasize the importance of ensuring the planting stock is fully hydrated at the time of planting. When the soil is dry or the soil texture is coarse, the South African "Puddle-Plant" technique may be employed, with the addition of 3 to 5 L (0.8 to 1.3 gal) of water per plant (Viero 2000). Seedling water demand in the first few days after outplanting can be estimated from irrigation studies in the nursery immediately before lifting. When outplanting takes place outside the traditional outplanting window, however, hydration at the time of outplanting may be insufficient to meet moisture needs until the next rainfall event. Water deficits can inhibit physiological processes such as photosynthesis with a consequent reduction in root growth (Joly 1985).



Figure 3. Diurnal y of newly planted and established lodgepole pine seedlings. Insert of vapor pressure deficit (Grossnickle 2005).

Water uptake can occur via an apoplastic or a symplastic pathway (Figure 4), both of which are influenced by solute concentrations and osmosis. As the distance from the root increases, soil moisture can be expected to increase. Water content in close proximity to the roots, however, can be increased due to production of mucigel. The peripheral cells of the root cap and the epidermal cells of the root produce and secrete large amounts of mucigel, a slimy substance made by dictyosomes. Mucigel is a hydrated polysaccharide containing sugars, organic acids, vitamins, enzymes, and amino acids (Walker and others 2003; Earley 2010). A prominent role of mucigel is to provide continuity between root surfaces and soil moisture.

## Proposed R³ Protocol ____

In the nursery, mild moisture stress (-1.5 MPa [-15 bars]) is often used to induce dormancy, but foliar injury can occur at higher levels (-1.8 to -2.0 MPa [-18 to -20 bars]) (Landis 1999). Lopushinsky (1990) notes that drought hardiness can be increased by exposure to moderate water stresses (-0.5 to -1.0 MPa [-5 to -10 bars]) and cites work by Christersson showing that pot-grown Scots pine (Pinus silvestris L.) and Norway spruce (Picea abies [L] Karst.) seedlings could increase drought tolerance to -3.5 MPa (-35 bars), compared to -2.5 MPa (-25 bars) for unhardened seedlings. Joly (1985) suggested that the turgor pressure that leads to observable wilt probably changes as a result of water stress conditioning, and Stocker (1960) describes the "time course of changes in the plasmatic viscosity" as comprising two phases-Reaction phase (destruction of the plasma) and Restitution phase (reparation of the damage).

#### **Reaction Phase**

At the beginning of the hardening regime, seedlings are leached with water (to reduce growing medium solution EC) and then dried down to the "Just Open Limit" (50% stomatal closure; temporary wilting). This increases respiration, decreases transpiration and phytosynthesis, and reduces growth. The expected  $\psi$  is approximately -2500 kPa on these temporarily wilted seedlings.

#### **Restitution Phase**

Seedlings are re-wetted with hardener fertigation. The irrigation system must be adequate to cool the entire crop within minutes. This increases photosynthetic rate. This regime is continued for 1 to 2 weeks.

#### Repetition²

Reaction and Restitution phases are repeated. The temporary wilt will likely be observed on those seedlings that did not wilt on the first dry-down. Photosynthetic capacity may become greater after successive wilting periods (Stocker 1960).

#### Repetition³

Reaction and Restitution phases are repeated again. The temporary wilt will likely not be observed on this final dry down.

Once the steps have been completed, seedlings are rewetted with nutrient loading solution, then harvested and shipped.



Figure 4. Water uptake pathways (Earley 2010).

Similar drought pre-conditioning protocols have been investigated over the past several decades with varying results. Any change to culturing regimes should first be tried on a small scale for each species, seed zone, and stocktype in question to determine its influence on subsequent crop quality and outplanting performance.

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## Seedling Mineral Nutrition, the Root of the Matter

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**Abstract:** Plants have the marvelous ability to take up inorganic mineral nutrients as atoms or simple molecules and process them into proteins, enzymes, and other organic forms. This paper reviews the 14 essential mineral nutrients, their roles within the plant, their target concentrations in tree seedling nursery culture, and their effects on seedling growth and performance after planting. Three areas of active research in tree nutrition over the past 20 years with relevance to the Pacific Northwest are discussed: steady state nutrition, exponential fertilization and nutrient loading; adaptation to nitrogen form in forest soils and organic nutrient uptake; and variation in nitrogen uptake within species.

Keywords: nutrient targets, nutrient translocation, steady state nutrition, nutrient loading

#### **Essential Mineral Nutrients**

Plants are the basis for life on earth, not only because they use energy from the sun to capture carbon dioxide  $(CO_2)$  and convert the carbon into carbohydrates, but also because they capture nitrogen (N), phosphorus (P), potassium (K), and other minerals from the soil and convert them into forms that heterotrophs (like us) can use. More than 60 mineral elements have been identified in plants, including gold, silver, lead, mercury, and arsenic, but only 14 elements are considered "essential" in most plants (Raven and others 2005). A formal definition of an essential element was published by Arnon and Stout (1939), and this definition is widely cited to the present day, including in the 1990 Target Seedling Symposium proceedings (Bigg and Schalau 1990). Epstein and Bloom (2005) modified the definition, stating that an element is essential if it fulfills either one or both of two criteria: 1) it is part of a molecule that is an intrinsic component of the structure or metabolism of a plant; or 2) plants deprived of this element exhibit abnormalities in growth, development, or reproduction.

The 14 essential elements make up a small fraction of plant dry mass, but they are truly essential. On average, a tree is 60% to 90% water, depending on the tissue; thus 10% to 40% is dry matter. Of this dry matter, 90% to 96% is carbon from  $CO_2$ , oxygen from  $O_2$  and  $CO_2$ , and hydrogen from water. These elements are also essential, but they do not come from minerals and so are not considered to be mineral nutrients. The remaining 4% to 10% of plant dry weight is made up of mineral elements. The 14 essential mineral elements are divided into macro- and micronutrients depending on whether their average concentration is greater than or less than 100 ppm (mg/kg) in dry matter, respectively.

The essential mineral elements are presented in Table 1 in descending order by average concentration and range of concentration in crop plant dry matter. Nickel (Ni) is the most recent addition to the list. Its essentiality was not proven until the 1980s (Epstein and Bloom 2005). Ni is required to activate urease, an enzyme found in higher plants, including conifers (Chalot and others 1990; Todd and Gifford 2002). Urease is associated with the conversion of urea to ammonium. It is very difficult to eliminate Ni from experimental nutrient solutions and induce deficiency (Epstein and Bloom 2005). Ni deficiency in the field has been shown only in pecan trees (*Carya illinoensis*) on the Gulf Coast Plain of the US (Wood and others 2003). Although only needed by some plant species, four other mineral elements have been shown to be essential. Silica (Si) is required at a mean tissue concentration of 0.1% to strengthen and protect the epidermis in some algae, horsetails, and grasses. **Table 1.** The 14 essential mineral elements for plants, presented in descending order by average concentration and range of concentration in crop plant dry matter (from Epstein and Bloom 2005).

Element	Symbol	Average Concentration	Range of Concentration
Macronutrients (%	%)		
Nitrogen	, N	1.5	0.5-6
Potassium	К	1.0	0.8 -8
Calcium	Са	0.5	0.51-6
Magnesium	Mg	0.2	0.05-1
Phosphorus	P	0.2	0.15-0.5
Sulphur	S	0.1	0.1-1.5
Micronutrients (p	pm)		
Chlorine	CI	100	10-80,000
Iron	Fe	100	20-600
Manganese	Mn	50	10-600
Boron	Bo	20	0.2-800
Zinc	Zn	20	10-250
Copper	Cu	6	2-50
Molybdenum	Мо	0.1	0.1-10
Nickel	Ni	0.05	0.05-5

Sodium (Na) is required at a mean tissue concentration of 10 ppm in some halophytes (for example, saltlover [*Halogeton glomeratus*]) and in plants with C4 and CAM photosynthesis to regenerate phosphoenolpyruvate. Cobalt (Co) is required (0.1 ppm) for an enzyme complex in N-fixing bacteria associated with some plants, including leguminous trees and alder (*Alnus* spp.). Selenium (Se) may be essential for some plants from Se-rich soils (for example, *Astragalus* spp.).

Mineral nutrients in plants are usually measured as a concentration (percentage or parts per million [ppm] in dry matter). A number of methods exist to measure these elements, but generally the plant material to be analyzed is dried and then combusted or digested to remove the C, H, and O. The ash or digest is then analyzed for the elements of interest by various methods. Results may also be presented as nutrient content. Nutrient content is the total amount (weight in g or mg) of an element in a given amount of plant tissue (for example, 1 g dry matter or 100 needles). Percentage concentration can be calculated from content by dividing the element weight by the sample dry weight and multiplying by 100. It is important to distinguish between content and concentration, as fast-growing seedlings may have low nutrient concentrations due to dilution but may have high nutrient contents due to their large size.

Some of the essential mineral nutrients play many roles in plants, but others play only one role. An exhaustive description of the functions of each element can be found in most plant physiology texts; therefore, only brief descriptions of the major functions of each element are presented in Table 2.

### What Are the Targets for Nutrients?_____

We know that seedlings require essential nutrients, but how do we know they contain sufficient quantities of nutrients? Severe deficiency of a given element results in characteristic deficiency symptoms detailed in most plant physiology texts. Moderate deficiency, however, can limit growth but otherwise not be obvious. Moderate nutrient deficiencies are

Table 2. Major functions of essential mineral elements (adapted from Epstein and Bloom 2005).

#### Integral in carbon compounds

- N a constituent of all amino acids and proteins, amides, nucleic acids, nucleotides, and polyamines.
- S a constituent of several amino acids and, thus, proteins, and coenzymes.

#### Integral in energy acquisition and utilization and in the genome

P plays a key role in nucleotides and nucleic acids, and in all metabolites dealing with energy acquisition, storage and utilization—sugar phosphates, adenosine phosphates (AMP, ADP, ATP).

#### Associated with the cell wall

- Ca contributes to cell wall stability, but is also important in signaling and regulating some enzymes.
- B contributes to cell wall stability

#### Integral constituents of enzymes

- Cu in metalloenzymes
- Mn in superoxide dismutase enzymes, part of the water-splitting complex of photosystem II; also activates some enzymes
- Mo in nitrogenase and nitrate reductase
- Ni in urease
- Zn in metalloenzymes and activates some enzymes

#### Activate or control enzyme activity

- CI part of the water-splitting complex of photosystem II
- Fe activates several enzymes and is also a part of heme proteins, ferredoxin, and Fe-S proteins
- K activates many enzymes and is a major cellular osmoticum
- Mg activates more enzymes than any other other nutrient; part of chlorophyll

thus difficult to diagnose. The classic plant stress response curve can be modified to show response of any growth parameter (for example, height or biomass) to any nutrient (Figure 1). At low internal nutrient concentrations, growth is severely limited; as concentrations rise, growth increases to the critical or optimum point where maximum growth is achieved with minimum nutrient concentration (Figure 1). As nutrient concentrations in the growing medium rise further, the plant has reached its genetically programmed maximum for growth, and so accumulates nutrients in a phase called "luxury consumption". Eventually, nutrient supply and internal nutrient concentrations may become so high as to be toxic, and growth declines (Figure 1).

Depending on the mobility of the element, nutrient deficiency symptoms will manifest differently among plant tissues. Some elements like N, P, and K are easily recycled and retranslocated within the plant. Deficiencies of these mobile nutrients become evident in older foliage, but not until the deficiency is well developed. Immobile elements like Ca and some micronutrients exhibit deficiency symptoms in new tissues, and these symptoms are observed at an earlier stage.

Landis (1985) defined a standard range of target values for mineral nutrient concentrations in needle tissue of conifer container and bareroot nursery stock that are still in use (Table 3). The critical concentration will vary by species, age of tissue, stocktype, and growing medium. Critical concentrations have been defined more narrowly for some species and age classes.



Increasing Tissue Nutrient Concentration-



Table 3. The standard range of values for mineral nutrient concentrations in conifer needle tissue of container and bareroot
nursery stock (Landis 1985), the adequate range for white spruce container stock (van Steenis 2002), and the
minimal nutrient concentration in whole seedlings for optimum growth of 10- to 16-week-old Douglas-fir and
white spruce (van den Driessche 1989).

	Standard Range		Adequate Range	Minimal Concentrations	
Element	Container	Bareroot	White spruce	Douglas-fir	White spruce
Macronutrients (%)					
N	1.3-3.5	1.2-2	2.04	2.2	2.46
Р	0.2-0.6	0.1-0.2	0.33	0.24	0.39
K	0.7 -2.5	0.3-0.8	1.12	0.89	1.46
Са	0.3-1.0	0.2-0.5	0.51	0.12	0.2
Mg	0.1-0.3	0.1-0.15	0.15	0.12	0.1
S	0.1-0.2	0.1-0.2	0.14	0.2	0.13
Micronutrients (ppm)	)				
Fe	40-200	50-100	98	39	50
Mn	100-250	100-5,000	326	35	100
Zn	30-150	10-125	63	30	33
Cu	4-20	4-12	7	11	15
В	20-100	10-100	26	52	46
Мо	0.25-5.0	0.05-0.25	1.4	4	5
Cl	10-3000	10-3000			

Critical concentrations are typically defined for single elements with all other nutrients at optimum concentration. This situation is rarely found in nature, and a limitation in one nutrient may negate the response to increasing concentrations of another element. Thus nutrients should not only be supplied in adequate concentrations, but also in balanced proportions. This concept underlies the diagnosis and recommendation integrated system (DRIS) developed by Beaufils (1957) and reviewed in the 1990 Target Seedling Symposium proceedings (Bigg and Schalau 1990). Ingestad (1970) defined optimum nutrient ratios for birch as N 100: K 50: P 8.4: S 9: Ca 6: Mg 6: Fe 0.7: Mn 0.4: B 0.2: Cu, Zn, Cl 0.03: Mo 0.007. In young Douglas-fir seedlings provided with free access to nutrients in aeroponic culture, Everett (2004) defined similar optimum macronutrient ratios of N 100: K 45: P 15: S 9: Mg 2: Ca 1. The Ca proportion was surprisingly low, but was likely due to the small proportion of cell wall in the young (8-week old) seedlings used in that study.

In British Columbia, most stock is grown in containers, and liquid fertigation is the norm. Target tissue nutrient levels in nurseries are based on experience, and the targets stated by Landis (1985) are well known. Regular monitoring maintainstargets (for example, foliar N during active growth is 2% to 3% of dry weight) (van Steenis 2002). In Washington and Oregon, more seedlings are outplanted as transplants or bareroot stock. Pre-plant fertilizers are incorporated prior to sowing or transplanting, followed by liquid fertilization. Target nutrient concentrations are often those defined by Landis (1985). Some growers have a target growth curve for each stocktype and species, and nutrients are adjusted to keep the seedlings on the target growth trajectory. All growers surveyed use different fertilizer regimes for different species and stocktypes. Most start the season with high levels of N, decrease N supply over the growing season, and monitor foliar nutrient concentrations frequently (biweekly to monthly). More complex methods of analysis, including vector analysis (Timmer and Armstrong 1987), and DRIS as described by Bigg and Schalau (1990), are not widely used.

#### Why Do Nutrient Targets Matter? Effects of Nutrients After Outplanting

**Shoot-to-root Ratio**—Optimum nutrient content and balance will result in optimal physiological functioning and growth in the nursery, and will have a major influence on survival and growth of planting stock once it leaves the nursery. Shoot-to-root ratio is determined, to a large degree, by fertilization in the nursery. High N fertilization, especially with ammonium, can result in excess shoot growth relative to root growth. A greater proportion of nitrate encourages lateral branching of roots (Everett and others 2010). The shoot-to-root ratio can affect seedling survival after outplanting, particularly on dry sites. A large, transpiring shoot may create an unsustainable demand for water on a small root system.

**Nutrient Retranslocation**—Since N, P, and Kare mobile nutrients within the plant, and root establishment may be slow after outplanting, high nutrient content in seedling shoots will allow retranslocation of mobile nutrients to support new growth before uptake begins. In young conifers, 32% to 40% of N used in new leaf growth is remobilized N from older tissues, mostly older needles (Millard 1996), but seedlings need to accumulate nutrient reserves before retranslocation can support new growth. Nutrient loading (see below) promotes accumulation of nutrients for retranslocation, and remobilization of N in black spruce (*Picea mariana*) has been increased 569% by nutrient loading (Salifu and Timmer 2001). Retranslocation rates have been correlated with shoot growth rate in radiata pine (Pinus radiata), and in this fast-growing species, retranslocation has been observed from needles as young as 2 months old (Nambiar and Fife 1991). Slow-growing, shade-tolerant species may conserve nutrients in older needles for a longer time to maintain functioning of these leaves (Hawkins and others 1998). In young seedlings of Douglas-fir (Pseudotsuga menziesii) and Pacific silver fir (Abies amabilis), retranslocation was not observed from older needles until the third growing season (Hawkins and others 1998). These results support the suggestion of Munson and others (1995) that retranslocation efficiency is species-specific and is related to both plant growth potential and short-term imbalances in nutrient supply. Under conditions of low nutrient availability, retranslocated N from stems and roots of deciduous species can make up almost 100% of the N found in new leaves (Salifu and others 2008).

**Mycorrhizae**—High levels of fertilizer in the nursery can reduce root colonization by mycorrhizae or alter the species of fungal partner. In a meta-analysis of 31 studies involving N fertilization and 20 studies of P fertilization, mycorrhizal abundance decreased 15% under N fertilization and 32% under P fertilization, on average (Treseder 2004). Decreased mycorrhizal colonization may have negative effects on performance after outplanting, depending on the tree species and planting environment (for example, Teste and others 2004; Ósakarsson 2010).

**Storage Molds**—When seedlings are in cold storage over winter, the environment is moist and encourages the growth of *Botrytis* spp. and storage molds. If foliar N is high, seedling susceptibility to storage mold is increased. van Steenis (2002) suggests that foliar N concentration should be less than 2% in stored seedlings to reduce the development of storage molds.

**Cold Hardiness**—Many studies have investigated the influence of various mineral elements on cold hardiness of tree seedlings. In general, nutrient deficient plants are less cold hardy than nutrient sufficient plants (Bigras and others 2001); fertilization, however, has also been associated with fall frost damage or early release of dormancy, flushing, and increased risk of frost damage the following spring (Colombo and others 2001). Individual mineral nutrients can have different effects on cold hardiness. K fertilization has been shown to increase frost hardiness in a number of conifer species (Bigras and others 2001; Colombo and others 2001). The effects of N fertilizer on cold hardiness, however, may be positive, negative, or none because results depend on the timing and amount of N application. Fertilizing with high levels of N in the mid to late growing season may delay bud induction, favor continued growth in indeterminate species (for example, western hemlock [Tsuga heterophylla] and western redcedar [*Thuja plicata*]), or lead to lammas growth in determinate species (for example, Douglas-fir). Prolonged growth can have a negative effect on seedling cold hardiness in the fall and winter (Hawkins and others 1995). High N applied in early fall during the hardening phase, however, can increase winter hardiness. In field-grown trees, foliar N has been shown to be positively correlated with frost hardiness in September (Hawkins and Stoehr 2009).

**Herbivory**—Nursery fertilization can influence the susceptibility of seedlings to herbivory by mammals or insects after outplanting. Plants with high levels of N (which often correlates with lower levels of tannins or phenolics) have been shown to be more damaged by browsing in some studies (for example, Close and others 2004).

# Developments Since the 1990 Target Seedling Symposium

The heyday of applied tree seedling nutrition research in the Pacific Northwest could be considered to be the 1970s to 1990s. The successful extension of research results over this period, and the facilitated communication between nursery managers and scientists, led to improved nursery practices, seedling health, and, ultimately, seedling survival. In British Columbia, seedling survival rates in plantations increased from 54% in 1982 to 87% in 1990 (Brown 1993). During the past 20 years, there has been increasing interest in ecophysiological questions in tree nutrition and the interactions between soil processes and tree nutrient uptake. In the following section, three active areas of research in tree nutrition will be discussed.

#### Steady State Nutrition and Nutrient Loading: Growing Regimes to Mimic Natural Growth and Optimize Nutrient Uptake

The techniques of steady state nutrition, exponential nutrition, and nutrient loading originated in the seedling nutrition work of Torsten Ingestad at the Swedish University of Agricultural Sciences in the 1970s and 1980s. Ingestad aimed to develop growing regimes that would induce seedlings to grow in natural developmental patterns and optimize nutrient uptake (Ingestad and Lund 1986; Ingestad 1987). Theoretically, unshaded tree seedlings grow at an exponential rate. The more branches or roots there are, the more lateral branches and roots will be formed. Exponential root growth allows seedlings to exploit an ever-increasing volume of soil, thus an ever-increasing pool of nutrients. As the plant increases in size, the root area over which nutrient uptake occurs increases accordingly, and nutrient concentrations remain at a steady state within the plant.

Seedlings in containers or nursery beds are not able to develop a naturally spreading root system. According to Ingestad, the optimum fertilization method should mimic natural exponential growth by adding nutrients at an exponentially increasing rate, matching plant relative growth rate. Thus low amounts of fertilizer are added when seedling root systems are small and unable to take up large quantities of nutrients, then fertilizer application is increased exponentially as seedlings increase in size. The proof of optimum nutrition is constant internal plant nutrient concentrations over time, called steady state nutrition. This contrasts with the declining internal concentrations observed when plants increase in size but nutrient application remains constant. Steady state nutrition is very difficult to achieve when seedlings are grown in pots in nutritional studies, and results of these studies have been criticized based on the episodic nature of nutrient application.

The concept of exponential nutrition can be extended to exponential nutrient loading, where nutrients are supplied at an exponentially increasing rate exceeding seedling growth rate. Extra nutrients are stored in the seedling for retranslocation after outplanting. There is evidence that seedlings can accumulate greater stores of nutrients with exponential compared to constant-rate nutrient loading (Timmer 1997).

The concepts of exponential fertilization and nutrient loading were tested in Ontario in the mid-late 1990s and early 2000s by Dr Vic Timmer at the University of Toronto and his students and colleagues. Results of many experiments with black spruce, white spruce (*Picea glauca*), red pine (*Pinus resinosa*), larch (*Larix occidentalis*), *Eucalyptus* spp., and China fir (*Cunninghamia lanceolata*) were published, showing greater growth, nutrient uptake, and mycorrhizal colonization after outplanting, particularly on nutrient-deficient sites (reviewed in Hawkins and others 2005). Timmer (1997) has also shown improved nutrient retranslocation and reduced planting shock in exponentially fertilized trees.

Two experiments testing the exponential nutrition and nutrient loading concepts have been conducted with species from the Pacific Northwest, western hemlock and Douglas-fir. Hawkins and others (2005) compared conventional versus exponential nutrition in western hemlock. Seedlings were grown in Styroblock[™] 410A containers (80 cm³ [4.9 in³]) and were fertilized twice per week over the growing season with 20N:20P₂O₅:20K₂O all-purpose fertilizer applied in three treatments: constant rate (100 mg N/L, total 83 mg N/ seedling), 2% per day exponential to a maximum of 250 mg N/L (total 134 mg N/seedling), and 3% per day exponential to a maximum of 559 mg N/L (total 236 mg N/seedling). Seedlings from each nursery treatment were outplanted the following spring after cold storage with or without a 9-g (0.3-oz) 26N:12P₂O₅:6K₂O Silva Pak slow release fertilizer package (Reforestation Technologies International, Salinas, CA).

At the end of the summer season in the greenhouse (1 September), the 3% exponential treatment increased N concentration in all plant parts by approximately 10% compared to the constant rate treatment. By December, there were no significant differences in N concentration among treatments due to continued growth after fertilization ended in early September. One year after outplanting, N concentrations were highest in seedlings fertilized at outplanting followed by 3% exponential seedlings; after that time, foliar N concentrations did not differ significantly among nursery or fertilized-at-outplanting treatments (Hawkins and others 2005).

At the time of lifting in December, there was no significant effect of exponential versus conventional fertilization treatment on height, biomass, or root-to-shoot ratio. As well, there was no significant effect of treatment on duration of shoot growth. Three years after outplanting, greenhouse treatment had no significant effect on height, but root-collar diameter and height increments were 10% greater in the 3% seedlings compared to conventional seedlings. This was supported by results of a pot experiment, where seedlings from the three nursery treatments were planted in pots with 10 or 100 mg/L N supply and grown for a second summer. In the pot experiment, biomass of new roots was increased by exponential fertilization. Seedlings fertilized at the time of outplanting had greater height, height increment, and rootcollar diameter (RCD) 3 years after outplanting than those not fertilized at outplanting. The effects of post-outplanting fertilization outweighed any effects of nursery fertilization (Hawkins and others 2005) (Figure 2).

The study with relatively fast-growing, indeterminate western hemlock did not show the same degree of positive response to exponential fertilization seen in studies with other species. Any gains in N concentration and RCD due to exponential fertilization were in the order of 10% (reviewed in Hawkins and others 2005), whereas gains in height or RCD from fertilization at outplanting were in the range of 15% to 20%. A likely reason for the limited relative response to exponential fertilization treatments in western hemlock is the high rates of fertilizer applied in all treatments. Large seedlings with high growth rates call for high rates of fertilization, so even constant-rate seedlings received high levels of N. In many studies with slower growing trees, the maximum N application rate in constant-rate trees is 40 mg N, and may be as low as 10 mg N (reviewed in Hawkins and others 2005). Seedlings from British Columbia nurseries typically get 80 to 125 mg N per seedling and have 2% to 2.6% foliar N, so conventionally fertilized stock performs well.

The study with western hemlock showed constant-rate fertilization can produce seedlings that are equal to exponentially fertilized seedlings if the rate of N application is high. The next question is then, "Can exponential fertilization produce seedlings of similar quality to conventionally fertilized seedlings using similar quantities of N?" To address this question, an experiment was conducted with interior Douglas-fir, a species that does not have the complication of semi-indeterminate growth seen in hemlock.



**Figure 2.** Mean ( $\pm$  S.E.) height and root-collar diameter of western hemlock seedlings three growing seasons after outplanting in the field. Seedlings were grown with one of three nursery fertilization treatments (constant rate, 2% exponential, 3% exponential) and outplanted with (+FP) or without slow-release fertilizer. Treatments indicated in the figure are: constant rate seedlings outplanted without fertilizer (CR); 2% exponential seedlings outplanted without fertilizer (3%); and constant rate seedlings outplanted with fertilizer (CR+FP) (adapted from Hawkins and others 2005).

Everett and others (2007) compared conventional versus exponential fertilization in interior Douglas-fir grown in StyroblockTM 412 A containers (125 cm³ [7.6 in³]) in an operational nursery. Two fertilization treatments were applied. Fertilizer was applied once per week in a 19N:4P₂O₅:15K₂O formulation at a constant rate (100 or 150 mg N/L, total 40 mg N/seedling) or 2% per day exponential to a maximum of 403 mg N/L (total 54 mg N/seedling). Seedlings were lifted and cold stored and outplanted the following spring. Seedlings from the conventional treatment, only, were outplanted with or without a 10-g (0.4-oz) 16N:8P₂O₅:8K₂O Planter's Pak of slow-release fertilizer.

Twenty-five percent more N was applied to exponentially fertilized seedlings; at outplanting, however, the exponential seedlings had only slightly greater mean foliar N concentration than conventionally fertilized seedlings (1.85% versus 1.7% N; Figure 3). Exponentially fertilized seedlings had smaller dry mass but greater root-to-shoot ratio than constant-rate treatment seedlings at outplanting. After 2 years in the field, exponential fertilization did not confer any significant benefits to Douglas-fir seedlings; these seedlings, however, still had the greatest root-to-shoot ratio. Seedlings fertilized at outplanting had greater dry mass and height than those not fertilized at outplanting (Everett and others 2007) (Figure 3).

Both studies with relatively fast-growing conifer species from the Pacific Northwest reached the conclusion that exponential fertilization did not confer any dramatic benefits over adequate constant-rate fertilization, and that fertilizer at outplanting outweighed any differences in nursery fertilization treatment. Everett and others (2007) suggested that fast-growing species may be unable to take advantage of exponential fertilization over a whole growing season because they are self-shading and accumulate more non-photosynthetic biomass. There may also be a danger of setting the initial level of N supply at too low a level, inhibiting early growth to such an extent that the seedlings never catch up. The combined results suggest that it is not the method of fertilizer application in the nursery that has the greatest influence on outplanting success, but rather the quantity of nutrients in the seedlings.



**Figure 3.** Mean (± S.E) whole plant dry mass and N concentration of Douglas-fir seedlings at the time of outplanting (May 2004), and 5 and 14 months after outplanting in the field. Seedlings were grown with one of two nursery fertilization treatments (conventional; 2% exponential) and outplanted with (+FP) or without slow-release fertilizer. Treatments indicated in the figure are: conventional rate seedlings outplanted without fertilizer (Conventional); 2% exponential seedlings outplanted without fertilizer (2% Exponential); and conventional seedlings outplanted with fertilizer (Conventional+FP) (adapted from Everett and others 2007).

#### Adaptation to N Form

Plants take up N as ammonium, nitrate, and in organic form; in forest soils, however, these N sources are generally found in low concentrations. In a study on soils near Jordan River, British Columbia, a maximum of 4.5 µmol nitrate, 5 µmol ammonium, and 8 µmol amino acid-N per g soil was measured in mid-summer (Metcalfe 2008). The fact that plants can take up organic forms of nitrogen, primarily amino acids or small peptides, has been known for over 60 years. It was not appreciated until the last 20 years, however, that amino acids can comprise a substantial proportion of the N absorbed by forest plants, particularly in cold soils. This work has been mainly led by scientists working in the boreal environments of Sweden and Alaska (reviewed in Näsholm and others 2009). Plants that take up organic N can bypass the N mineralization step in decomposition and compete with soil microbes for access to soil N. Advantages of organic N uptake for plants include a competitive advantage over other plants through earlier access to N released by decomposition. Theoretically, organic N uptake may also be metabolically "cheaper," as the first step in assimilation of inorganic N into organic compounds is bypassed.

Based on the evidence of organic N uptake in natural environments, researchers in Sweden have developed an organic N fertilizer called arGrow based on the amino acid arginine. Arginine is positively charged, and therefore binds to soil cation exchange sites, reducing N runoff compared to negatively charged nitrate. Results of trials comparing arGrow to ammonium nitrate fertilizer indicate that seedlings grown with arGrow have better root systems with more fine roots, larger stem diameter, and higher foliar N levels (Ohlund and Näsholm 2002). These improvements have resulted in 40% greater volume after 7 years in the field in trees grown with arGrow compared to trees grown with ammonium nitrate in the nursery. Holmen Skog (Gideå and Friggesund, Sweden) is fertilizing more than half their seedlings (>15 million) with arGrow, and other Swedish forest companies are evaluating its use (Confederation of Swedish Enterprise 2010).

Soil temperature and pH influence the form of N in the soil. Cold, acid soils have a greater proportion of N as ammonia, whereas warm, neutral soils have more N as nitrate. In warmer soils where the N mineralization cycle is more rapid, plants take up more N in inorganic form. Disturbed soils have a relatively high proportion of nitrate due to higher rates of decomposition. Agricultural soils have much higher proportions of nitrate than forest soils. There is growing evidence during the past 15 years that some plants have adapted to preferentially take up the N form most common in their environment.

Kronzucker and others (1997) measured uptake of radiotracer  13 N-labelled ammonium and nitrate by white spruce. They found that uptake of ammonium was up to 20 times greater than uptake of nitrate, and that assimilation of ammonium was more efficient than that of nitrate. Preference for ammonium had been observed before in conifers, but Kronzucker and others (1997) went on to attribute the failure of conifer plantations on disturbed sites, in part, to the lower ammonium-to-nitrate ratio on these sites relative to undisturbed forest soils. This interpretation of the results was hotly debated, but the Glass and Kronzucker labs con-

tinued to publish a volume of excellent work on ammonium and nitrate uptake in trees. They have provided substantial evidence that late-successional species, such as white spruce, have a "preference" for ammonium, while faster-growing early successional species, such as Douglas-fir and trembling aspen (Populus tremuloides), have higher rates of nitrate uptake and may exhibit futile cycling of ammonium from root cells (for example, Min and others 2000; Kronzucker and others 2003; Britto and Kronzucker 2006). Work with microelectrodes has also shown greater nitrate than ammonium net uptake along the roots of Douglas-fir and lodgepole pine (*Pinus contorta*) (Hawkins and others 2008) (Figure 4); most whole seedling studies, however, show best growth with a mixture of ammonium and nitrate. Everett and others (2010) showed that at pH 4, Douglas-fir seedlings grew best and had stable internal N concentrations with an NH₄:NO₃ ratio of 40:60 or 20:80.





**Figure 4.** Mean net fluxes of  $NO_3^-$ ,  $NH_4^+$ , and  $H^+$  (nmol m⁻² s⁻¹) (± S.E.) at various distances from the primary root tip of Douglas-fir (A) and lodge-pole pine seedlings (B). Negative flux values indicate efflux (adapted from Hawkins and others 2008).

#### Variation in N Uptake within Species

Genetic variation exists among species in nutritional characteristics such as nutrient uptake and utilization, but variation also exists within species in N-uptake efficiency and N-use efficiency. A number of studies published in the past 20 years have looked for genetic variation in the efficiency of nutrient uptake and have asked the question, do fast-growing families have higher rates of nutrient uptake or greater nutrient uptake efficiency than slow-growing families?

In studies with Douglas-fir and interior spruce (Picea glauca (Moench) Voss X Picea engelmannii Parry ex. Engelm.) grown in containers or in aeroponic culture, fast-growing families were shown to exhibit greater plasticity in biomass allocation to roots versus shoots in response to N availability than slow-growing families. Most plants respond to a high N supply by increasing biomass allocation to shoots, whether this is due to an accelerated growth trajectory (for example, Coleman and others 2004) or a true allometric shift. Fast-growing families of Douglas-fir (Hawkins 2007) and interior spruce (Miller and Hawkins 2003) grown with a high N supply allocate proportionally more biomass to shoots, but more to roots at low N supply than slow-growing families. Fast-growing seedlings also have greater rates of N uptake, greater N productivity (more biomass produced per unit of N supply), and higher N utilization indices (more biomass produced per unit of plant N concentration) than slow-growing seedlings (Figure 5).

An examination of N uptake rates by roots of fast- and slow-growing interior spruce families using N depletion measured with macroelectrodes showed that fast-growing families had greater rates of  $NH_4$  uptake, particularly at high  $NH_4$  concentration, and greater rates of  $NO_3$  uptake, on average (Figure 6) (Miller and Hawkins 2007). In Douglas-fir roots, mean family net influx of ammonium ( $\rm NH_4^+$ ), measured with microelectrodes in high- and low-nutrient treatments, was significantly correlated with measures of mean family biomass (Hawkins 2007). These results indicate that efficient nutrient uptake and utilization contributes to higher growth rates of trees.

Further afield, a study examining variation in nutrient concentration and growth in six clones of radiata pine (*Pinus radiata*) planted across New Zealand on a range of site qualities showed some clones differed significantly in their nutritional characteristics (Hawkins and others 2010). Clones with consistently high N or P uptake across a range of sites were identified. These results suggest that selection of families or clones with efficient nutrient uptake or nutrient use should be considered for inclusion in tree breeding programs.

With the rapidly developing genomics resources for some tree species, the ability will come to select or even create genotypes with particular characteristics. Work is under way to identify and characterize nitrate and ammonium transporters, as well as genes for enzymes involved in nitrogen assimilation in trees. Potentially, genes that result in high rates of N uptake or assimilation could be identified. Genotypes with these genes could then be selected or the genes inserted into individuals with desirable growth rates or wood characteristics. Public resistance to genetically modified organisms may be less for non-food crops, particularly if these plants can be made sterile. These technologies are being employed in Sweden, China, and other countries where transgenic trees are being created that grow more quickly or have greater fiber length, more biomass, greater drought or salt tolerance, higher energy content, or improved bioenergy properties.



Figure 5. Mean (± S.E.) applied nitrogen uptake (percentage of the applied nitrogen taken up) and nitrogen utilization index (dry mass produced per unit plant N concentration) of slow- and fast-growing families of interior spruce in three fertility treatments after 175 days (adapted from Miller and Hawkins 2003).



**Figure 6.** Mean ( $\pm$  S.E.) short-term ammonium and nitrate uptake rates of slow- and fast-growing families of interior spruce measured at two concentrations of each ion. Bars surmounted by the same letter indicate no significant difference in the means within each concentration of each ion (from Miller and Hawkins 2007).

## Conclusion

Forest renewal management techniques in the Pacific Northwest have improved dramatically during the past 30 years. In British Columbia, the greatly improved seedling survival rates have been attributed to a greater diversity of stocktypes (including more container stock), increased seedling vigor (based on improved nursery techniques, including nutrition), improved stock handling, and more site preparation and brushing (Brown 1993). A major factor driving these changes was increased funding for research (Brown 1993). When a good measure of success has been achieved, it is easy to become complacent and to forget the effort and investment that enabled this achievement; but there is still much to be done in forest research to continue to build on the successes of the past. In tree nutrition, we need to learn more about growing regimes to optimize the performance of valuable, genetically improved stock. With more focus on restoration, we need to understand the nutrition of a greater diversity of tree species. There is still much work required to understand the relationships between tree species and their mycorrhizal partners, both in the nursery and in the field. The interaction of nutrition and cold hardiness, disease resistance, and browsing are also areas where more research is needed to maximize survival after outplanting. Overarching all of these challenges is the very real possibility of climate change that may have great impact on long-lived forest trees. Now, more than ever, we need research and sound science to make the best decisions for the future.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# The Role of Plant Water Relations in Achieving and Maintaining the Target Seedling

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**Abstract:** Water management is one of the most important factors in achieving the target seedling. Water is required for cell growth, nutrient transport, cooling through transpiration, and in small amounts for the photosynthetic reaction. Furthermore, judicious use of limiting water availability during the hardening phase can induce budset and increase seedling cold hardiness. Nursery managers typically measure seedling moisture status with the pressure chamber and medium water status using the block weight method. Newer soil moisture sensors, such as time domain reflectometry (TDR) units, offer increased control over irrigation scheduling. Few growers utilize climatological data to estimate evapotranspiration and schedule irrigation based on demand. An example of how these data can be used is explored, as well as the consequences of inadequate monitoring during the hardening phase. Proper water management will help achieve the target seedling as well as maintain the target seedling during the hardening phase.

Keywords: irrigation scheduling, evapotransipiration, soil moisture, plant water potential

#### Introduction _

Of all the resources that must be supplied to nursery crops, water is required in the greatest amount. Seedlings "consume" 400 to 700 g (14 to 25 oz) of water through transpiration in order to "capture" a single gram of biomass through photosynthesis. At the same time, that same plant may absorb less than 20 mg of nitrogen, the next most important constituent. To say water is important almost trivializes the critical role in both meeting and maintaining the target seedling. Water transports nutrients from the soil (or soilless growing medium), through the roots, to the leaves; provides positive pressure for cell enlargement; cools leaf surfaces through transpiration; and is a reactant in the photosynthetic process.

Colombo and others (2001) categorized the morphological, physiological, and chemical attributes of seedlings needed for successful reforestation. Every attribute, including the genetic components of seedling quality, are regulated or affected by water availability. Water, or lack thereof, can result in genes being expressed or repressed. Thus, water is important to defining the target seedling at the most fundamental and basic level.

Water management is also an important responsibility of the nursery manager. Overwatering increases pumping costs of unnecessary water, fertilizer costs as nutrients are leached through the soil/medium profile, pest management costs to combat moss, *Botrytis*, and insect outbreaks (for example, fungus gnats). Furthermore, overwatering increases the risk of environmental problems from runoff either to groundwater or surface waters. Supplying insufficient water during the growth phase can result in failure of seedlings to reach target size specifications, and that could decrease production and create employment risks. Consequently, most nursery managers tend to overwater and confront the cost issues brought about by overwatering (Carles and others 2005). The objective of this paper is to review the role of water in achieving and maintaining the target seedling and to discuss technologies currently in use or available to the nursery manager.
#### Survey Results_

A brief survey conducted at this Target Seedling Symposium was completed by 16% of the participants; 58% use English units, while 42% prefer metric units. This likely represents the split between US and Canadian participants completing the survey. Most nursery managers were container growers (64%), while 21% grew both container and bareroot seedlings, and only 14% operated bareroot nurseries exclusively. Most (75%) of the container nurseries had travelling boom irrigations systems for at least part of the nursery, while 25% had only fixed irrigation systems.

Over 90% of respondents used the block weight method to schedule container irrigation during the growth phase (Figure 1a). This is higher than earlier surveys reported (McDonald 1978; Landis and others 1989), and likely indicative of improved grower expertise. Grower experience was the second most important resource for scheduling irrigation. During the hardening phase, medium moisture monitoring was the most important tool, followed by block weight (Figure 1b). For both growth and hardening, seedling moisture status was the least important characteristic to monitor. Bareroot seedling monitoring techniques were less clear cut because of the small sample size. Nevertheless, bareroot seedling moisture status appeared to be the most important criteria for both growth and hardening, followed by soil moisture monitoring. While it appears that container and bareroot growers use different criteria to schedule irrigation, those growers that grew both seedling types tended to prefer seedling water status over other criteria; again, this is an improvement over past practices (Johnson 1986).

#### Definitions

The status of water in a system is defined by its water potential. Water potential  $(\psi)$  is the chemical free energy of water, or, basically, the ability of water to do work. The units of water potential are the units of force per unit area, or Pascal. Thus, pure free water would have a water potential of 0 Pascals. For reference, the traditional term of field capacity can be represented in the following units: -30 kPa, -0.03 MPa, or -0.3 bars; while permanent wilting point can be expressed as -1500 kPa, -1.5 MPa, or -15 bars (note that bars are often used to denote plant moisture stress [PMS], in which case they are expressed in positive units; -1.0 MPa = 10 bars). Lowering or reducing the water potential makes the water potential more negative while increasing water potential makes the number more positive, or closer to zero. In the case of reforestation seedling production, almost anything (for example, adding fertilizer) done to water will lower the water potential (see Supplement I).

Water moves in response to a gradient in water potential, that is, from high water potential to lower water potential in order to equalize the system. Water moves from the soil solution at relatively high water potentials into the roots, through the plants, and ultimately through the stomata into the atmosphere as water vapor. Water in the soil is not quite at 0 MPa (or 100% RH) because of dissolved salts (osmotic or solute potential,  $\psi_s$ ) and adsorption to the surface of soil particles (matric potential,  $\psi_m$ ) (Table 1). As soil dries, the salts concentrate, thereby decreasing  $\psi_s$ . Furthermore,  $\psi_m$  also increases as the remaining water is more tightly bound to the soil particles. Finally, as the soil dries, the larger pores drain and the path water travels through the soil becomes more tortuous, increasing the resistance to movement to the root.



**Figure 1.** Container seedling survey results, Respondents were asked to rank the importance of varying factors (with 1 being least important and 5 being most important) for scheduling irrigation during a) growth or b) hardening.

#### Supplement I Rules of Thumb—Water Water flows from high concentration to low

- concentration.Water flows from high humidity to low humidity.
- Water flows from areas of low salt concentration to higher concentration.
- The steeper the gradient, the faster the water movement.
- "A pint's a pound, the world around" [whatever that means].
- $1 g = 1 mL = 1 cm^{3} (cc).$
- 1 mL of water applied to 1 m² = 1kg = 1 L [useful in estimating block weights or water flow].
- ETo ≈ 0.75 ETp.
- ETg ≈ 0.60-0.80 ETo_(outside) [depending on composition and age of covering material].
- Latent heat of vaporization (cooling) and fusion (frost protection) work only when there is freely available water (liquid).
- Under full canopy coverage, all plants consume readily available water at about the same rate (i.e. ETc = ETo).
- Plants differ only in their response to reduced water availability.
- Most water (>99%) is used in the transpiration process cooling the leaves and carrying nutrients to the leaves. Less than 0.3% is actually hydrolyzed in the photosynthetic reaction.
- Evapotranspiration is highly correlated with net radiation and vapor pressure deficit, but less so with temperature.

By the time soil dries to the permanent wilting point, the relative humidity of the soil is still 99% (Kohnke 1968). At 93% RH,  $\psi$  is below -9.8 MPa, and at an atmosphere RH of 50%, the  $\psi$  is below -980 MPa. Not only does the gradient drive water movement to and through the plant, but also the steepness of the gradient speeds the movement. Thus, the demand for water is greater at 20% RH (-3160 MPa) than when the atmosphere is relatively humid (50%). Consequently, nursery managers need to be cognizant of both available soil moisture and drivers of evapotranspiration, for example, humidity.

Evapotranspiration (ET) is the combination of soil evaporation (E) and plant transpiration (T). ET varies as a function of soil moisture, stage of plant development, and climate. Potential evapotranspiration (PET) is the ET of a crop under non-stressed conditions. However, since the crop is not specified, the term PET is being supplanted by reference evapotranspiration (ETo or ETr). ETo is the evapotranspiration of a reference crop (usually a perennial grass) under non-stressed conditions. ETo is estimated from climatic variables using the Penman-Monteith equation (Allen and others 1998). The predominant variables regulating ETo are vapor pressure deficit (VPD), net radiation, and temperature. The variables are highly correlated, especially VPD and radiation, with ETo. The relationship for greenhouse crops is similar, albeit with simpler calculations (Seginer 2002). Thus, ETo varies with latitude, elevation, and proximity to coastal influences. For example, the annual ETo for coastal San Diego, CA, is 118 cm/yr (46 in/yr) while that of Calexico in the interior of southern CA is 182 cm/yr (72 in/yr) (CIMIS 2010). In contrast, the annual ETo for coastal Brookings, OR, near the California border is 91 cm/yr (36 in/yr), whereas the ETo of Lakeview, OR, is 134 cm/yr (53 in/yr) (US DOI 2010). The ETo of southern Oregon is about 75% of southern California, and the ETo of the interior stations is about 50% greater than the coastal stations. Consequently, the water required to produce the same seedling would be greater where the ETo is greater.

In order to schedule irrigation using the Penman-Monteith equation, typically a conversion factor (crop coefficient = kc) is needed to convert ETo to the ET of the crop in question (ETc). Unfortunately, kc varies by species and growth stage. Consequently, many growers are reluctant to adopt this seemingly complicated tool. Nevertheless, ETo can be used not only for field grown crops, but also for greenhouse grown crops. The variables of temperature, vapor pressure deficit, and incoming radiation (filtered through the greenhouse covering) individually account for nearly 80% of the variation in evapotranspiration of greenhouse-grown (ETg) garden cucumbers (*Cucumis sativa*) (Shibuya and others 2010). Thus, an instrument or service that estimates ETo is a potentially useful tool in irrigation scheduling.

Maximum or management allowable depletion (MAD) is the portion of plant available water in the soil profile allowed for plant use prior to the next irrigation (Welsh and Zajicek 1993). It is based on plant and management considerations,

Table 1. Comparison between relative humidity (%) and equivalent
water potential (MPa), and the maximum soil pore size
filled by water at that water potential, where ~FC is near
field capacity and PWP is permanent wilting point (after
Kohnke 1968).

Relative humidity	Water potential	Maxim water-fille	um ed pore
(%)	(MPa)	(mm)	(µ)
100.00	0	2	2,000
99.999	-0.001	0.2	200
99.99	-0.01 (~FC)	0.03	30
99.93	-0.10	0.003	3
99.00	-1.50 (PWP)	0.0002	0.2
98.00	-3.09	_	_
93.00	-9.8	0.00003	0.03
50.00	-980.0	na	na
10.00	-3,160.0	na	na

and typically reported as a percentage of available water (for example, 50% MAD). It is an especially important management tool in flood-irrigated agriculture. Where irrigation water is applied through sprinkler or drip systems, irrigation can be applied to meet the daily ET needs, thereby minimizing soil depletion and maintaining the crop under non-stressed conditions. However, if water is not applied daily to meet ET demands, MAD is crucial to avoiding the threshold seedling water potential, where photosynthesis and subsequent growth is reduced. For example, Dumroese (2009) found ponderosa pine (*Pinus ponderosa*) seedlings could tolerate a MAD = 40% with only a small reduction in growth. Furthermore, there was no difference in growth between 10% and 25% MAD in this study.

Water use efficiency (WUE) or biomass to water ratio (BWR) is the amount of water required to produce a unit of biomass, typically expressed as grams of water/gram of biomass (for example, 400 g/g). Generally, WUE can be increased through deficit irrigation. Plants under mild stress tend to be more efficient at fixing carbon. Until data and technologies are developed to demonstrate the benefits of deficit irrigation, however, a nursery manager should grow seedlings under non-stressed conditions to reach the target seedling. Growth (cell enlargement, biomass) is impacted much more severely and at a much higher  $\psi_{leaf}$  than photosynthesis.  $\psi_{leaf}$  must approach -1.5 MPa before photosynthesis is reduced 50%. Growth, however, can be reduced 50% by  $\psi_{\text{leaf}}$  as high as -0.25 MPa (Morison and others 2008). Finally, the economics of water conservation are dwarfed by the economic benefits of achieving target specifications for a high percentage of the crop.

## Measuring Water Status

Jones (2004) discussed the advantages and disadvantages of the numerous instruments used to assess plant or soil moisture relations. Many are currently inappropriate for use beyond research, either because of expense or complexity of use. There are several, however, that have application to the nursery industry (Table 2). Currently, the pressure chamber is the only instrument in operational use that directly measures plant water potential, either  $\psi_{xylem}$  or  $\psi_{\text{leaf}}$ . Typically, seedling  $\psi_{\text{xvlem}}$  is measured pre-dawn after the seedlings have fully recovered but before stomata open in the morning. Alternatively, seedlings can be wrapped in aluminum foil during the day and allowed to equilibrate for 1 to 2 hours with soil moisture before measuring (McCutchan and Shackel 1992). Pressure chamber measurements are precise, but time consuming and do not lend themselves to automation. A nursery can spend over 10 hours/week measuring seedling  $\psi_{xylem}$  (Khadduri 2010). As a consequence, some nurseries have abandoned this technique as a management tool.

Pressure chambers are used to monitor seedling water status, and irrigation is applied when a critical threshold  $\psi_{xylem}$  is reached (Figure 2). In these examples of bareroot 1+1 and 2+0 Douglas-fir (*Pseudotsuga menziesii*) seedlings,  $\psi_{xylem}$  is seemingly decoupled from ETo. That is, when ETo is high,  $\psi_{xylem}$  is also high, when the opposite would be expected. The reason for this is seedlings were irrigated when  $\psi_{xylem}$  reached about -0.7 MPa (actual irrigation records unavailable). As soil moisture was depleted,  $\psi_{xylem}$  could easily change

 Table 2. Characteristics of irrigation scheduling tools (after Jones 2004). Note this list does not include numerous plant monitoring techniques (for example, porometer, psychrometer, sap flow) that provide valuable research information, but limited operational application.

Instrument	Advantages	Disadvantages	
Pressure chamber	Direct measure of seedling $\psi_{\text{xylem}}$	Time consuming; training; expense; point-in-time measurement (predawn); unsuitable for automation	
Block weights	Measures growing medium available moisture; inexpensive	Point-in-time measurement	
Medium moisture sensor (tensiometer)	Inexpensive; ease of use; electronic recording	Soil contact is critical; soil heterogeneity requires numerous sensors (expensive)	
Medium moisture sensor (TDR)	Continuous readouts; precise electronic recording	Expense; new terminology (m ³ /m ³ ); soil heterogeneity requires numerous sensors	
Pan evaporation	Continuous readouts; electronic recording available	Overestimates ETo by ~33%	
Atmometer (for example,ETGage™)	Continuous readouts; electronic recording; small size	Must be calibrated with climate data	
Infrared Thermometry (for example, SmartCrop™)	Continuous readouts; precise electronic recording; wireless	Expense; line of sight communication; requires time and temperature thresholds	
Climate data-historic	Estimates crop ETo	Approximates current ETo; not as accurate as real time measurements; requires kc	
Climate data-current	Real time measure of ETo	Steep learning curve; requires kc	

0.1 MPa/day. Thus, careful monitoring is required if  $\psi_{xylem}$  is allowed to exceed the critical threshold affecting growth, which could be as high as -0.5 MPa for Douglas-fir seedlings (Blake and Ferrell 1977; Bond and Kavanagh 1999).

The most common container monitoring technique is the block weight method. A block or tray of seedlings are watered to saturation and weighed periodically until a predetermined weight is reached (often to 50% to 60% of original weight) (Dumroese 2009). The containers are then re-watered to runoff, which leaches salts from the growing medium. Khadduri (2007) monitored Douglas-fir seedling  $\psi_{xylem}$  during repeated dry down cycles to 50% (moderate) or approaching 40% (severe) of total moisture (Figure 3). Only after soil moisture dropped below 50% did  $\psi_{xylem}$  decrease below -0.6 MPa. Over this period, the moderate treatment had four cycles, while the severe had three cycles. Dry down to 50% required 3 to 6 days in each cycle, while the severe dry down usually required an additional day. Dumroese (2009) found container ponderosa pine in Idaho required 2 days for medium moisture to drop to 90% (MAD = 10%), 4.6 days to 75%, and 7.8 days to 60%. Furthermore, there was little impact to seedling quality under any irrigation regime, although the driest irrigation regime reduced seedling size (height, diameter, and biomass) by about 10%. There were no differences between 90% and 75% in seedling quality.



**Figure 2.** Comparison of pre-dawn water potential of Douglas-fir (*Pseudotsuga menziesii*) 1+1 and 2+0 seedlings to ETo for nearby Puyallup, WA in 2007, where 12.7 and 19.3 are rainfall events (mm) and  $\uparrow$ = scheduled irrigation (estimated) (Khadduri 2007).



Figure 3. Container block weight (■) vs xylem water potential (◊) during sequential moderate (50%) and severe (<50%) dry down cycles of container Douglas-fir (*Pseudotsuga menziesii*) (Khadduri 2007).

In the work by Khadduri,  $\psi_{xylem}$  was only weakly correlated with moisture block measurements of container seedlings (Figure 4). In fact, there was essentially no correlation as long as block weights were above 50%. This should not be surprising. Moisture contents above 50% have a  $\psi_m$  above field capacity (-0.03 MPa), and often above -0.01 MPa (Pinto and others 2009); whereas  $\psi_{xylem}$  often is below -0.2 MPa. Thus, the transpiration gradient is still strong even at relatively low block weights. This can be disconcerting to growers since seedlings may appear relatively insensitive to block weights. It should be viewed as a management opportunity to save water and labor without sacrificing seedling quality. This is where judicious use of MAD is important. Caution is nevertheless required, especially as moisture content approaches 50%.

Alternatives to block weights are instruments that directly measure soil moisture content, such as tensiometers or time domain reflectometry (TDR) tools (Murray and others 2000; Arguedas and others 2007; Van Iersel and others 2010). These instruments are finding increasing utility in agriculture (for example, Kallestad and others 2006), in part because the data can easily be downloaded to a computer and viewed graphically. Lamhamedi and others (2005) monitored irrigation uniformity with TDR units in an open-grown container nursery with a fixed sprinkler system (growing white spruce [*Picea glauca*]) (Figure 5). Unfortunately, application uniformity changed throughout the growing season, and crop height was only weakly correlated with soil moisture. The authors estimated the grower would need about 20 units to adequately characterize crop uniformity in the 240 m² (2580 ft²)nursery, at a cost of nearly US\$ 2000. Alternatively, the authors suggested tracking the growth of 4 seedlings would accomplish the same. It would appear additional development is warranted before widespread adoption by container nurseries is warranted.

Tools that directly measure soil moisture content are likely better suited for bareroot nurseries (Davies and Etter 2009). It is not that bareroot soil, or fixed sprinkler systems used in bareroot nurseries, are more uniform. Rather, in a container system, the sensor is measuring moisture content of a seedling completely isolated from other seedlings by the block or cell. The particular cell holding the sensor may not be representative of surrounding seedlings, as indicated by Lamhamedi and others (2005). The sensor in a bareroot



nursery simulates the block weight technique in that the sensor measures moisture content available to more than just one seedling. The main drawback is the equipment may have to be removed for field operations, which may damage sensors.

The above-mentioned tools measure the seedling after a night of recovery or the balance of plant-available moisture remaining in the soil. While these instruments measure actual seedling water status or medium moisture, they are point in time and place measurements. They require integration or estimation over the entire crop through multiple sampling, both in place and time. Furthermore, these require labor during the growing season that the manager might be unwilling or unable to allocate to moisture monitoring, regardless of the benefit (Thompson and others 2002). Thus, often grower experience, that is, "seat-of-the-pants," becomes the default irrigation method.

Unfortunately, none of the tools described above estimate the environmental parameters that actually drive ET, including radiation, VPD, and temperature that would provide some integration. Fortunately, these environmental variables can be easily estimated and used to schedule irrigation for the entire crop while avoiding issues of variability as discussed above. Tools such as a pan evaporimeters and atmometers integrate the impacts of radiation, VPD, and temperature on water evaporation that, in turn, can be used to approximate ETo. Pan evaporation (ETp) tends to overestimate ETo by about one-third (Snyder and others 2005), possibly explaining the lack of adoption of ETp data. Recent advances in atmometer design allow these instruments to more closely approximate ETo. Bauder (1999) found close agreement between ETgage[™] and the Penman ET equation.

One tool that is rapidly gaining popularity for scheduling irrigation is the use of climate data, historic or current, available from regional or state climatologists. The reason for the increased popularity is climatologists are finally developing tools or display images that are more user friendly to managers. Growers can access real time ETo calculations for the current year, past ETo data for a specific year, or long-term average for nearby meteorological stations (Figure 6). Given the myriad of duties during the growing season and the wide fluctuations in daily ETo, it may be impractical to check climate data and adjust irrigation schedules on a daily or even weekly basis. The irrigation schedule, however, can easily be adjusted on a bimonthly or monthly basis using either current ETo or historic ETo data. Scagel (2010) used real time climatological data to provide nursery managers with PET (ETo) data on a weekly basis. The success of the program, however, was limited. A possible reason was the wealth of data that was provided at a time when the manager may have simply wanted to know if the seedlings should be irrigated. This is a common refrain among producers that has limited the adoption of irrigation scheduling tools (Thompson and others 2002). When determining irrigation schedules, time or labor is a greater concern than the equipment expense.

#### Growing to Target ____

How can ETo data (historic in this example) be used to schedule irrigation of conifer nurseries? Using mature pecan trees (*Carya illinoiensis*) grown in New Mexico as our example, two points are obvious (Figure 7). First, there is reasonable similarity in ETc among 6 years and 2 orchards.



Figure 6. Comparison of real time ETo data for 2009 to long-term average for Forest Grove, OR meteorological station (http://www.usbr.gov/pn/agrimet/agrimetmap/agrimap.html).

Second, it is obvious ETc bears little relationship to ETo. Monthly ETo ranges from about 2.5 mm/day in January and December, peaks at 9.0 mm/day in June as temperatures rise, and declines as temperature declines and RH increases during the monsoon season. In contrast, pecan ETc is near 1 mm/day prior to budbreak in the Spring (Stage 1), increases linearly in Stage 2 as leaves and shoots expand, closely follows ETo (Stage 3) once canopy coverage is complete, and declines as leaves senesce and drop (Stage 4).

While pecan ETc may seem to have little relationship to ETo, the behavior of a mature pecan orchard does have many similarities to nursery seedling production, whether in a bareroot nursery or container greenhouse.

#### Stage 1

During the seedling emergence phase, ET consists entirely of evaporation (E). E is higher when readily evaporable water is at or near the soil surface, but much reduced as water moves slowly from soil depths to the surface (Allen and others 1998). Mulched nursery beds or container medium covered with grit have reduced evaporation. Thus, E is typically low, that is, less than 1 mm/day in bareroot nurseries and less than 0.5 mm/day in container nurseries. Evaporation occurs only from the exposed medium surface of containers, and that may constitute less than 50% of the exposed surface area; the container or block constitutes the balance of the exposed surface. Thus, E in containers would be less than expected.

A recent study by Pinto and others (2009) examined the irrigation frequency during emergence of lodgepole pine (*Pinus contorta*). Given the container type and irrigation frequency, ET ranged from 0.24 mm/day for the low irrigation frequency to 0.90 mm/day for the high treatment during the emergence phase. In all treatments, medium  $\psi_m$  remained above field capacity (-0.033 MPa). Nevertheless,

germination was positively correlated, albeit weakly, with moisture availability. While it is important to maintain a high medium  $\psi_m$ , there can be potentially negative impacts. In the study by Pinto and others (2009), misting three times daily reduced seedbed temperature by as much as 3 °C (5 °F), potentially below the optimum temperature for both emergence and growth. Irrigating to meet the E demands will save water without impacting emergence. While irrigating in the morning can reduce temperatures below optimum, irrigating in the afternoon can reduce temperatures down into the optimum range. Better management may actually improve emergence as well as water use.

#### Stage 2

ETc is a function of expanding leaf area (Asakura 1998). This is the most difficult stage to characterize or model. Nevertheless, there are common elements even in this stage. As a general rule, once crop canopy coverage reaches 65% to 70%, Etc = ETo (Wang and others 2007). Thus, Stage 2 encompasses the time from complete emergence to 65% canopy coverage. Stage 2 ETc can be estimated by estimating canopy coverage using digital photographs and software, such as Photoshop[®] (see Supplement II for examples of this technique). During this phase, irrigation should be incrementally increased as canopy coverage increases.

#### Stage 3

ETo = ETc when the percentage of canopy cover exceeds 65% under non-stressed conditions. Additionally, during this phase, E can be as little as 10% of ET and can be effectively ignored (Beeson 2010). This appears to be a general rule, regardless of crop species (Allen and others 1998). Work with pecan orchards (Sammis and others 2004), honey mesquite



Figure 7. Daily pecan ET compared to ETo in the Mesilla Valley, NM (Sammis and others 2004; Samani and others 2009).

# Supplement II Determining Percent Cover by the Pixel Counting Method.

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The picture on the PowerPoint slide (Figure below) was saved as an image and imported into an Image analysis software package (Adobe PhotoShop CS4 version 11.0, Adobe Systems Incorporated, San Jose, CA, USA) that can distinguish and count the number of pixels that were covered by the seedlings. The "Magic Wand" tool was used to select a green leaf. Then right click on the selected leaf and clicked on "Similar" (in most Photoshop versions) to select all seedling leaf pixels. Then, the "Histogram" function was used to obtain the number of pixels in the seedling cover (Figure A). With the Histogram box still open, deselect the leaf selection by right clicking anywhere in the selection and clicking "deselect", to see the total number of pixels in the Image (Figure B). There were 178,932 pixels in the entire image (not shown in entirety), and 55,006 pixels in the seedling cover. Dividing seedling cover pixels by the total pixels and multiplying by 100, estimates the percent seedling cover in the image to be 30.7%. When selections are made, care must be taken not to select large amounts of irrelevant material such as grit or soil. Small errors are to be expected, so use at least three selection points for more accurate results. (See more examples at right)

Figure. A. Selected image (only partially shown) with histogram box (55,006 pixels [circled]], B. Unselected imag with histogram box (178,932 pixels).



(Prosopis glandulosa) in landscapes (Levitt and others 1995), and creosote bush (*Larrea tridentata*) in containers (Saucedo and others 2006) has shown that all have similar water use under non-stressed conditions as the reference crop. That is, the crop coefficient (kc) = 1.0. This should be verified for conifers; if the hypothesis holds, this may greatly ease management of irrigation during growth phases.

#### Stage 4

Senescence applies only to deciduous trees entering dormancy. As leaves begin to senesce, transpiration declines as the plant remobilizes nutrients to the roots in anticipation of impending shortened photoperiods and cooler temperatures. Once leaf drop is complete, only E operates. Currently, there is little information about water use of dormant conifers. However, roots do not undergo a physiological dormancy (endodormancy) typical of meristematic tissues (buds), and must be protected from desiccation.

The stages described above apply directly to bareroot or open-grown nursery crops. For greenhouse-grown crops, however, managers must determine a correction factor. Generally, the protective covering of a greenhouse reduces incoming radiation up to 60% (Seginer 2002; Möller and others 2004; Mpusia 2006), but 10% to 20% is more typical. Wind is also decreased, but the effect of air movement is minor and can be ignored. Nevertheless, ETg is reduced below ETo, but the exact amount will depend on covering type and age. Conversions can be accomplished using atmometers, such as ETgage[®], or pan evaporation. Using this approach to irrigation scheduling should result in a crop grown under non-stressed conditions without excess water usage.

Using historic climate data rather than real time data introduces uncertainty about whether the crop is receiving sufficient water. Kallestad and others (2008) found long-term historic data accounted for about 90% of the variability in climate over a growing season, so the risks should be minor. As an example, a hypothetical open-grown nursery near Forest Grove, OR, meteorological station that was

sown in March would have low ETc demands (only E) until the crop completely emerged (Table 3). The ETc demands would increase until canopy coverage reached 65% to 70% in July, after which time ETc would equal ETo. While irrigation scheduling to exactly meet ETc requirements would not allow additional water for leaching salts from the medium, avoiding deficit irrigation may reduce the need for regular leaching of salts (Chartzoulakis and Drosos 1995). Periodic

 
 Table 3. Example of irrigation requirements for a conceptual outdoor nursery at Forest Grove, OR (see Figure 6).

Date	Activity	ETo (mm/day)	ETc (mm/day)	ETg/ETo	
Late March	Sow	2.70	0.8 (E only)	0.30	
May 15	Emergence complete	4.25	1.25	0.30	
June 15	40% canopy coverage	5.37	2.10	0.39	
July 15	50% canopy coverage	6.57	3.30	0.50	
August 15	75% canopy coverage	5.56	5.56	1.00	
September 15	100% canopy coverage	3.86	3.86	1.00	

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irrigations that supply 110% of ETc could be scheduled to leach excess salts that might harm seedling growth.

# **Maintaining Target**

Once seedlings have achieved the target size, managers may reduce irrigation frequency to maintain size, induce bud dormancy, or increase cold hardiness. Subjecting seedlings to moisture stress can have positive benefits from reducing growth and biomass accumulation, transpiration, and increasing carbohydrate accumulation (Table 4). Carbohydrates tend to accumulate because growth is more severely impacted by mild moisture stress before photosynthesis (Morison and others 2008). Not all the changes in seedling physiology, however, are beneficial. Factors such as osmotic adjustment, root-to-shoot ratio, cold hardiness, and dormancy might not be improved by subjecting seedlings to moisture stress. Consequently, subsequent root growth potential and subsequent survival can actually decline after conditioning (Table 4).

One possible explanation for these disparate results could be the difficulty of maintaining medium  $\psi_m$  in the range where growth is reduced, but the physiological components of the target seedling are not diminished. This can be challenging. Dinger and Rose (2009) presented an elegant study on the relationship between soil moisture  $(\psi_m)$  and seedling water potential  $(\psi_{xvlem})$  following outplanting (Figure 8).

 Table 4. Brief survey of the physiological effects of moisture stress conditioning on seedlings.

		1
Parameter	Effect	Reference
Photosynthesis/Biomass accumulation	Ļ	Cleary 1971; Havranek and Benecke 1978; Cregg 1994; McMillan and Wagner 1995; Nzokou and Cregg 2010
Transpiration	Ļ	Havranek and Benecke 1978; Seiler and Johnson 1985, 1988; Villar-Salvador and others 1999
Carbohydrate accumulation	<b>↑</b>	Villar-Salvador and others 1999
Osmotic adjustment	$ \stackrel{\uparrow}{\leftrightarrow} $	Seiler and Johnson 1985, 1988; Seiler and Cazell 1990; Villar-Salvador and others 1999
Root-to-shoot ratio	$\downarrow$	Seiler and Johnson 1988; McMillan and Wagner 1995
Cold hardiness/Dormancy	$\uparrow \\ \uparrow \leftrightarrow$	Timmis and Tanaka 1976; Blake and others 1979; Zaerr and others 1981; Almeida and others 1994
Root Growth Potential	Ļ	Vallas Cuesta and others 1999; Villar-Salvador and others 1999
Survival		van den Driessche 1991; Vallas Cuesta and others



**Figure 8.** Low soil moisture determines seedling: (A) predawn ( $\psi$ pd) and (B) midday ( $\psi$ md) xylem water potential for treatments receiving complete weed control (treated) and no weed control (control) (after Dinger and Rose 2009).

Neither predawn nor midday  $\psi_{xylem}$  were highly correlated with soil moisture above 0.3 m³/m³. Once soil moisture fell below  $0.25 \,\mathrm{m}^3/\mathrm{m}^3$ , however, both predawn and midday  $\psi_{\mathrm{xvlem}}$ decreased precipitously, and both were highly correlated with soil moisture. This likely could explain differences in seedling response to moisture stress in various seedling quality studies (Table 4). If moisture stress is not carefully monitored and maintained above the critical threshold. damage could result in decreased seedling performance. Work by Burr (1982, as cited in Landis and others 1989) found seedling  $\psi_{xvlem}$  could decrease from -0.05 MPa to -0.10 MPa by transpiring only 2.7 mm from Ray Leach containers (163 cm³ [10 in³]). This would take less than one-half day in August at our hypothetical Forest Grove nursery. Work by Khadduri indicated similar rapid responses (Figure 3). Thus, a manager that successfully grows to meet target specifications can actually lose components of that target (for example, root growth potential) during the conditioning phase if moisture stress is severe.

## Conclusion

There have been many changes and challenges since the first Target Seedling Symposium 20 years ago. Nursery managers are still dedicated to providing a quality seedling at reasonable cost, and irrigation management is a critical component of management strategies. Growing under nonstressed conditions allows the crop to reach target size in the shortest amount of time at the least expense. Maintaining target specifications (morphological, physiological, chemical) during the conditioning phase also requires careful water management. Fortunately, the nursery manager has an expanding suite of tools (climate data, moisture sensors) that can facilitate both growing to target specifications and maintaining the target seedling.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# **Provenance Variability in Nursery Growth of Subalpine Fir**

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**Abstract:** Subalpine fir (*Abies lasiocarpa* [Hook] Nutt.) is a wide-ranging, high-elevation species in the interior of British Columbia. It is commonly harvested for lumber, but replanting of it is limited. Some reticence is based upon wood quality and rate of growth, but there are also seed and nursery culturing difficulties. This study investigated seedling growth traits of 111 provenances grown in four nurseries. Considerable variation in growth potential was found. The strength of nursery effects and correlations of nursery height to height growth at 5 years in the field are reported. Recommendations for use of genotypes more amenable to nursery culture are presented.

Keywords: subalpine fir, provenance effects, nursery culture

#### Introduction

Subalpine fir (*Abies lasiocarpa* [Hook] Nutt.) is a common lumber species for the interior region of British Columbia. It is found at higher elevations in the south, but the population increases at lower elevations in more northerly latitudes. It is very cold hardy and both shade and drought tolerant. Subalpine fir accounts for roughly 6% of products billed in the interior but only 1% of seedlings planted (BCMFR 2007). Part of the reason for this is the fact that wood properties of the species are considered less desirable than the congenial Englemann spruce (*Picea engelmanni* [Parry] Engelm.) and lodgepole pine (*Pinus contorta* var. *longifolia* [Dougl.] Loud.). In addition, subalpine fir is not as amenable to nursery culture as other species, with lower harvest observed on seedlings grown in StyroblockTM 410A containers (80 cm³ [4.9 in³]) at 7 cm (2.8 in) (BCMFR 2010).

In recent years, much of the lodgepole pine in the southern two thirds of the province below 1500 m (4920 ft) elevation has been decimated by mountain pine beetle. This event will mean the Interior cut is likely to move north and higher in elevation in search of green logs, resulting in more subalpine fir being harvested. At the same time, ecologists are recommending greater species richness in forests in order to produce more resilient stands (Campbell and others 2009). Both of these factors are likely to result in greater numbers of subalpine fir being planted; that will only exacerbate the nursery culturing problems with this species.

The nature of subalpine fir seed present several characteristics that make it difficult to harvest and process for nursery culture (Koletelo 1997). First, since *Abies* spp. cones are dehiscent, collectors tend to collect early so that cones do not disintegrate during operations. This often leads to seedlots where much of the seeds are immature, resulting in lower or abnormal germination and more variable germination speed. Second, seeds are prone to mechanical damage due to a thin seed coat with presence of resin vesicules. Third, dead filled seeds, where there is no embryo but a center containing dark material of similar density, are common. Even sound seeds are problematic because most are locked in deep dormancy, and some are contaminated with *Caloscypha fulgens* or *Fusarium* spp.

Koletelo (1997) described how thorough cone and seed processing can alleviate some of the problems. Cones are dried in a manner mimicking natural conditions, and care is taken to screen out impurities that might damage the seeds in the de-winging process. The removal of wings is done gently at lowered temperature and moisture contents that render the wings brittle and easily broken off. Non-viable seeds and fine debris are removed by cleaning with an air separator or gravity table, and may be upgraded by further density separation processing. Long (90-day) stratifications with an initial high moisture content (45%) phase, followed by a drying and lower moisture content (35%) phase, all at relatively high temperatures (22 to 28 °C [72 to 82 °F]), are effective in breaking deep dormancy. With the above thorough and time-consuming procedures, the species can average 70% germination; although, that is still low relative to the other species with which it is planted.

Nursery concerns for subalpine fir also abound. These include poor cavity fill due to low germination capacity (van Steenis 1997) and variable germination speed (Knapp and Smith 1982). This leads to lack of canopy closure in the containers, resulting in open-grown seedlings that are slower in height growth. Another problem is that crop uniformity is compromised by the tendency of the species to stall. Individual plants are prone to switch between leaf and bud scale initiation throughout the growing season, independent of the physiological state of adjacent seedlings. Though this may represent a preferred strategy in the harsh climates from which subalpine fir originates, it adds difficulties in greenhouse operation. A related problem is that there may be premature terminal budset in the season and problems with failed terminal budburst if a crop is held over as 2+0; holding over of subalpine fir is commonly done for summer planting or where lower cull was not achieved in the first growing season.

Van Steenis (1997) recommended earlier sowing dates and higher density stocktypes in order to achieve canopy closure. He states that this is vital because of its ameliorating effect buffering the extremes that may occur in the larger greenhouse environment. To try to balance growth and differentiation, stalls can be minimized by manipulations of the growing environment, including partial shade, daylight extension, higher temperatures, slightly positive differential between day and night temperature, and passive versus active venting. Many of these factors help to avoid vapour pressure deficit. Higher fertilization levels may be used to help push plants through the stall phases when they do occur.

The practices just described have been known for over a decade, but problems still persist. Growers have found much variability in the growth performance of different subalpine fir seedlots. Some seedlots grow almost as easily as spruce (*Picea* spp.), while others are plagued with the numerous problems described above. Because of these concerns, it is important to establish some baseline knowledge about nursery effects on provenance growth. This study compares the growth of 111 seed sources, quantifying differences between them, between different nurseries growing them, and the subsequent effects on field growth.

#### Methods

The provenance study encompassed 111 subalpine fir seed sources from Yukon Territory, British Columbia, Alberta, and from adjacent Sates of Washington and Idaho (Figure 1). The collections ranged in latitude from 48° 06' N to 60° 12' N, and in elevation from 200 to 1859 m (660 to 6100 ft). Three sources of corkbark fir (*Abies lasiocarpa* var. *arizonica* [Merriam] Lemmon) from within a degree of 34° N and from 2700 m (8860 ft) elevation in New Mexico were also included. The subalpine fir collection likely represents the largest provenance sampling for this species ever assembled.

Seedlots were sown at four British Columbia nurseries into standard StyroblockTM 410A containers (80 cm³ [4.9 in³]). All 111 provenances were sown at Woodmere Nursery in Telkwa BC; 104 provenances were sown at Sylvan Vale Nursery in Black Creek near Campbell River, BC; and 58 provenances were sown at both Skimikin Nursery (Tappen, BC) and Cowichan Lake Research Station (Mesachie Lake, BC). Each provenance was represented by 5 to 10 families or a bulked seedlot. Each of these appeared in each of four replications, except at Skimikin and Cowichan, where there were only two replications.

For each seedlot, the number of seeds sown per cavity was determined by germination tests prior to sowing. Lots were soaked, treated for fungi, and put into the long two- stage stratification as recommended by Koletelo (1997). Seedlings were cultured under the customary growing regimes for each facility, and heights were measured at the end of the first two growing seasons at each site. From this point, stock was lifted and randomized for farm field tests. Heights (5 years from sowing) were then measured at Prince George, Telkwa in the BC Interior, and at Black Creek on Vancouver Island.

#### Results ____

Provenance effects were found to be significant at all nurseries for heights at 1 and 2 years, with the best provenances yielding heights twice that of the overall mean. Surprisingly, there was no overall trend in growth by latitude (Figure 2). The correlation for the 111 provenances appearing on the Woodmere site was not significant at either age. A few data points are, however, of note. Seedlings from all 5 provenances from north of 59° were below average in height; in contrast, the 3 corkbark fir provenances were average or greater in seedling height. These outlying populations are irrelevant to our consideration of nursery culture, and it was clear that the fastest growing sources arose from a broad range of latitudes; the fastest growing seedlings from ten provenances ranged in source latitude from 50° to 59° N.

Second-year nursery height was weakly correlated with seed source elevation (R = -0.26), indicating seedlings from lower elevations had a slight advantage in growth properties (Figure 3). Despite this, the ten fastest growing sources originated from between 300 and 1800 m (985 and 5900 ft). These best performers came from a climate with a mean annual temperature ranging from -1 to 6 °C (30 to 43 °F) and mean annual precipitation ranging from 600 to 1200 mm (24 to 47 in).



Figure 1. Seed collection sites for the subalpine fir provenances tested and the location of nurseries used in culturing trials.



Figure 2. The relationship of seedling height and seed source latitude after 2 years growth at the Woodmere nursery (10 mm = 0.4 in).



**Figure 3.** The relationship of seedling height and seed source elevation after 2 years growth at the Woodmere nursery (10 mm = 0.4 in; 500 m = 1640 ft).

Conservative growth could be found in the coldest environments north of 59° and likely at the highest elevations had they been sampled. Since the range of the species drops in elevation to the north, the effects of elevation and latitude are confounded. Although fast-growing families could be found in any region, growth performance was strongest between 55° N and 59° N in the west at elevations under 1000 m (3280 ft).

Two and 5 year outplanting height data were compared at the Telkwa farm field site; seedlings at this outplanting site were from the Woodmere nursery. The correlation for height after 2 years in the nursery to height at 5 years in the field was moderate (R=0.6) and positive (Figure 4).



Figure 4. Correlation of nursery height at 2 years and height at 5 years in the field (1 cm = 0.4 in).

## Conclusions

Strong provenance level effects were found for nursery growth of subalpine fir. No correlations between nursery height at 1 or 2 years with latitude of seed source were observed, and only a weak but significant correlation to elevation of origin was detected. Since there was little influence of geographic factors on nursery growth, fast-growing provenances were found across the area sampled; finding acceptably adapted sources that meet seed transfer guidelines and grow well should not be problematic.

Whether the provenance will show a weak influence of geographic location of origin in long term field trials is still to be determined, but there were some indication that it may not. All three corkbark fir sources were average or above in nursery growth, but below average in the farm field tests at 5 years. In general, nursery and field performance were moderately positively correlated. Results among the nurseries were generally consistent, despite being in different growing regions; the best ranked seed sources performed well regardless of where they were grown. These materials might be made available if the superior sources in the wild can be selectively collected and seeds extracted for use. Eventually, parent trees could be collected in order to take advantage of better families within provenances.

Given strong provenance effects and wide variability in growth traits, faster growing wild stand seedlots could be selected to avoid some of the problems associated with nursery culture of subalpine fir. Field trials have been established with the same sources, and eventually genetic gains could be ascribed to the provenances with superior growth traits, further enhancing their value.

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# **Targeting Hardwoods**

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**Abstract:** Increasing demand for hardwood seedlings has prompted research to identify target seedling characteristics that promote hardwood plantation establishment. Operational establishment of hardwood plantations has typically emphasized seed collection from non-improved genetic sources, bareroot nursery seedling production, and spring planting using machine planters. The increasing diversity in objectives of hardwood planting projects, however, has led to identification of a wider range of management options to meet specific goals. Use of container hardwood seedlings may help to reduce transplant stress on harsh sites and improve seedling competitiveness; container production of hardwoods may be most effective using subirrigation. Greater use of genetically improved sources will help to promote hardwood plantations with better stem form, faster growth, and less incidence of disease or insect problems. On nutrient poor sites, exponential nursery nutrient loading may promote translocation of nutrients from reserves to new growth and improve establishment success. Fall outplanting may result in equivalent performance to spring outplanting, thereby extending the outplanting season. With use of larger stocktypes and alternative soil preparation treatments, hand planting may become increasingly common.

Keywords: seedling quality, stocktype, nursery fertilization, tree improvement, subirrigation

#### Introduction

This paper presents an overview of current initiatives led by the Hardwood Tree Improvement and Regeneration Center (HTIRC) at Purdue University and cooperators to identify target characteristics and improve the outplanting performance of hardwood forest seedlings. This current research initiative builds upon the extensive work of many scientists and practitioners that focused on seedling quality of conifer species, as well as some pioneering work done with hardwoods. Emphasis is on the Central Hardwood Forest Region of the US. This region differs from other forest regions in North America in that these forests are comprised almost exclusively of hardwood species (that is, oak [*Quercus* spp.], walnut [*Juglans* spp.], ash [*Fraxinus* spp.], cherry [*Prunus* spp.], hickory [*Carya* spp.], and so on) and are owned mainly by non-industrial private forest landowners. Commercial forest management focuses on development of high quality individual trees that may have value in excess of US\$ 10,000 (Figure 1).

Global production of conifer seedlings is much higher than that of hardwoods; therefore, relatively little attention has been given to seedling quality and grading research for hardwoods. Production of hardwoods, however, has increased in recent years to meet rising demand. Additionally, hardwood seedlings are generally larger and grown at wider spacing than conifer seedlings. They are usually more expensive to produce and may represent a significant portion of total nursery revenues in some regions (Wilson and Jacobs 2006). Additional research to improve quality and outplanting performance of hardwoods is justified.



Figure 1. High-value black walnut tree.

## Objectives of Outplanting Project

Motivations for afforestation differ for many hardwood outplanting projects in the Central Hardwood Region compared to those for conifer plantations. Most reforestation operations in major planting zones in North America (that is, southern or Pacific Northwest US and Canada) focus on establishment of productive commercial plantations. Timber production, however, ranks lower as a justification for most hardwood plantation projects that usually emphasize conservation values and creating wildlife habitat. For example, a recent survey of Indiana landowners found that timber production ranked fourth among reasons for afforestation (Ross-Davis and others 2005). These landowners mainly emphasized the importance of establishing hardwood plantations for the benefit of future generations; this likely reflects differences in typical commercial rotation ages between hardwood (>60 years) and conifer (15 to 60 years) plantations.

# Type of Plant Material

Bareroot seedlings are, by far, the dominant stocktype produced by nurseries in the Central Hardwood Forest Region, representing greater than 95% of production (Jacobs 2003). A survey of operational plantation establishment success of afforestation hardwood plantations (ages 1 to 5 years) in Indiana reported 65% survival and <50% of seed-lings deemed free-to-grow at age 5 (Jacobs and others 2004). This suggests that alternative stocktypes may provide new options to promote establishment success under specific site conditions.

During lifting of bareroot stock, disruption of root-soil contact and loss of fine roots may contribute to transplant shock (Figure 2). For instance, a study with three hardwood species on a well-managed outplanting site found that second-year growth of bareroot seedlings exceeded that of the initial year by 100% or more (Jacobs and others 2005), reflecting the time period required for root system re-establishment. Transplant shock may increase if shoot-to-root ratios become too high, emphasizing the need for proper root culturing and hardening in bareroot nurseries (Jacobs and others 2005).

Container seedlings offer an alternative to bareroot seedlings that may improve seedling establishment success under certain circumstances, such as harsh site conditions. Roots of container seedlings remain relatively undisturbed in medium at time of lifting and seedlings, and therefore, often show reduced transplant stress. For example, Wilson and others (2007) reported that although container northern red oak (*Quercus rubra*) seedlings were significantly smaller than that of bareroot seedlings at time of planting, they were statistically similar in size after one growing season.



Figure 2. Lifting of bareroot ash seedlings.

Despite the potential of container hardwood seedlings, some challenges have been identified in their production, mainly associated with the wide and spreading canopies characteristic of many hardwood species (for example, oaks; Figure 3). Traditional overhead watering under this circumstance often leads to poor uniformity, low water use efficiency, and foliar problems. Subirrigation (that is, delivering water from beneath the containers resulting in saturation of the plug via capillary rise) has been suggested as a method to overcome these limitations. This helps to keep foliage dry (thereby reducing risk of foliar disease), promotes high uniformity, and conserves water and fertilizer as irrigation is typically maintained in a closed-circuit system. A recent study with northern red oak reported equal or better performance of subirrigated seedlings versus those grown with overhead irrigation (Bumgarner and others 2008).



Figure 3. Canopy of container oak seedlings.

#### **Genetic Considerations**

In contrast to other important forest regions in North America, formal seed zones have not yet been developed in the Central Hardwood Forest Region. Typically, seed collection and transfer conforms mainly to state boundaries, although transfer between states occurs without regulation (Jacobs and Davis 2005). Only about 7% of hardwood stock in the eastern US originates from "genetically improved" sources (Jacobs and Davis 2005) and the majority of collection is done by nursery workers or people living adjacent to nurseries that prefer to collect from easily accessible trees with abundant seeds (that is, open-grown trees), or to collect from trees as they are harvested.

The tree improvement program at the HTIRC has helped to increase awareness and implementation of the benefits of using genetically improved sources in operation. Use of genetically improved black walnut (Juglans nigra) (that is, better stem form, fast growth, reduced anthracnose) has been shown to improve plantation productivity (Figure 4) and plantations of grafted black walnut have increased substantially in recent years. Additionally, HTIRC is now providing improved seeds of black walnut and other species to the Indiana Department of Natural Resources, and these are being sold directly to landowners. The HTIRC has also initiated programs to incorporate disease and insect resistance into threatened tree species, such as American chestnut(Castanea dentata), butternut(Juglans cinerea), and ash (Michler and others 2006; Jacobs 2007). It is expected that resistant sources of these species will be available for forest restoration in the future.



Figure 4. Plantation of genetically superior black walnut.

#### Jacobs

# Limiting Factors on the Outplanting Site _____

On most typical afforestation sites in the Central Hardwood Region, mortality and slow growth are mainly a function of browsing damage and competing vegetation (Jacobs and others 2004). This emphasizes the need for improvement in hardwood nursery stock quality and silvicultural techniques on the outplanting site to overcome these limitations. Research on these sites has shown the importance of welldeveloped root systems and well balanced root-to-shoot ratios to promote early growth and drought resistance (Jacobs and others 2005; Jacobs and others 2009).

An estimated 20% of hardwood seedlings in Indiana are planted onto mine reclamation sites. These sites can be extremely difficult to regenerate successfully due to adverse soil conditions (that is, nutrient limitations, extreme pH), alternating wet/dry conditions, and soil compaction. Use of container seedlings significantly reduced plant moisture stress of newly planted northern red oak seedlings compared to bareroot stock on a mine reclamation site in southwestern Indiana (Davis and Jacobs 2004), suggesting potential of this stocktype to promote early growth and survival on mine reclamation plantings. Exponential nutrient loading, whereby luxury nutrient uptake is achieved by gradually increasing nursery fertilizer application rates, represents another technique to overcome low soil fertility on mine reclamation sites. While past research in this area has focused primarily on conifers, recent reports have shown the applicability of these systems to northern red oak and white oak (Quercus alba) (Birge and others 2006; Salifu and Jacobs 2006). Nursery nutrient loading has been demonstrated to promote retranslocation of stored nitrogen to fuel current growth, with responses most prominent on nutrient poor soils (Salifu and others 2008; Salifu and others 2009b). This may help promote hardwood seedling establishment on mine reclamation sites (Salifu and others 2009a).

Nearly all hardwood seedlings in the Central Hardwood Forest Region are outplanted onto afforestation sites; natural regeneration is usually relied upon for reforestation. Decreasing abundance of natural regeneration of desirable hardwood species (for example, oaks) associated with high deer populations and current management techniques (mainly involving single tree selection harvests) has prompted interest in artificial reforestation. Deer browsing and competing vegetation (especially from yellow-poplar [Liriodendron tulipifera]) are primary limiting factors on these sites. A recent study by Morrissey and others (2010) found that use of relatively large container seedlings (that is, 12 to 20 L [3 to 5 gal]) promoted competitiveness of northern red oak seedlings compared to bareroot seedlings after 5 years under a range of harvest openings. A major advantage to these large seedlings is that their height is nearly above the deer browse level at time of outplanting (Figure 5).

### Timing of Outplanting Window

Nearly all hardwood outplantings in the Central Hardwood Forest Region are accomplished during spring after soil thaws. Fall outplanting has rarely been used mainly due to concerns that stock may not be sufficiently hardened

and because seedlings are then exposed to winter browsing. Fall outplanting, however, could be advantageous because seedlings may have an opportunity for root growth at two periods (fall and spring) when soil temperature and moisture are favorable. Additionally, fall outplanting may help to extend the outplanting period; this extended period is important because it is often logistically difficult to accomplish outplanting goals during only the spring period. Woolery and Jacobs (2011) reported that browsing during winter dormancy did not affect subsequent growth and development of northern red oak seedlings, while summer browsing was highly detrimental. Furthermore, a large-scale research trial with six hardwood species replicated over 2 years reported few differences in seedlings planted over a range of outplanting dates from November to July (Seifert and others 2006), further suggesting that the outplanting date for hardwoods can be extended beyond spring.

# Outplanting Tool or Technique_

The final consideration in targeting hardwoods is the type of planting tool or technique used to establish outplantings. In contrast to conifer production areas, about 90% of sites in the Central Hardwood Forest Region are machine planted (that is, tractor-hauled coulter with trencher and



Figure 5. Large container northern red oak seedling planted into gap opening.

packing wheels; Figure 6) (Jacobs and others 2004). This provides a relatively low-cost and efficient method to establish hardwoods on the flat and open afforestation sites most characteristic of this region. Under certain conditions, however, use of other options may be preferred or necessary. For example, trends toward increasing size of bareroot or container hardwoods that promote outplanting success on afforestation or reforestation sites (Jacobs and others 2005; Morrissey and others 2010) may necessitate use of hand planting with shovels or augers.

Mine reclamation sites represent another circumstance where outplanting operations may need to evolve toward increasing use of hand planting. Recent research in soil replacement options initiated in the Appalachians (and now expanding to the Central Hardwood Forest Region) has shown that loose dumping may be preferable to standard graded plots for mine reclamation (Figure 7). Loose dumping, however, is not conducive to machine planting due to steep residual piles; this represents a potential source of resistance to operational use of loose grading because hand planting is generally more expensive and difficult to coordinate. This reflects a classic example of when the traditional outplanting tool or technique should not dictate other aspects of the regeneration operation.



Figure 6. Machine planting of afforestation site.



Figure 7. Replacement of soil after mining.

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# Seedling Phenology and Cold Hardiness: Moving Targets

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**Abstract:** Phenology is the annual cycle of plant development as influenced by seasonal variations. Dormancy and cold hardiness are two aspects of the annual cycle. In temperate plants, the development of cold hardiness results in the ability to withstand subfreezing winter temperatures. Cold hardiness is also a reflection of overall stress resistance. In addition to describing cold hardiness and its use as a tool for understanding seedling quality and making management decisions in the nursery and in the field, this paper describes four tests used to determine cold hardiness: the whole-plant freeze test, freeze-induced electrolyte leakage, chlorophyll fluorescence, and genetic markers.

Keywords: seasonal variation, lifting window, seedling quality, freeze testing

#### What Is Phenology?

Phenology refers to periodic plant and animal life cycle events as influenced by seasonal variations in climate. In temperate species, the growth and dormancy cycle is an adaptation to prevent shoot growth during winter when such growth would be injured by freezing temperatures. As winter approaches, plants respond to environmental cues of decreasing photoperiod (daylength) and temperature by ceasing growth, setting buds, and developing the ability to withstand subfreezing temperatures with little or no damage (Figure 1). In addition to seasonal environmental variations, phenology patterns are strongly influenced by plant genetics (seed source) and plant vigor.

#### What Is Cold Hardiness?

Cold hardiness is defined as a minimum temperature at which a certain percentage of a random seedling population will survive or will sustain a given level of damage (Ritchie 1986). The term  $LT_{50}$  (lethal temperature for 50% of a population) is commonly used to define the cold hardiness level for a given seedlot on a specific date. Cold hardiness is the ability to withstand freezing stress; it is also an indicator of overall resistance to stresses such as those associated with lifting, packaging, storing, and outplanting (Ritchie 1986; Faulconer 1988; Burr 1990). Changes in  $LT_{50}$  are linked to the seedling dormancy cycle and are influenced by seed source, nursery practices, and environment.

The hardening process involves several physical and chemical changes within the plant tissues that enable them to resist freezing (Öquist and others 2001). The buds, stems, needles, and roots harden and deharden differently through the season (Rose and Haase 2002). Roots do not harden as much as shoots and are therefore less resistant to freezing and other stresses; hardiness levels can vary greatly among species and stocktypes. Furthermore, within species and stocktypes, there are many ecotypes which can vary significantly in their hardiness levels depending on local climate patterns of the seed source. Morin and others (2007) showed that carbohydrate concentration,  $LT_{50}$ , and local temperature were closely related for three oak species (*Quercus* spp).



Figure 1. Typical phenological cycle for temperate zone plants.

There is a common misperception that cold hardiness and bud dormancy reflect the same thing; deepest dormancy and maximum cold hardiness, however, do not occur at the same time. Bud dormancy is quantified by the length of time it takes for plants to resume growth in the spring. Once plants have entered rest in the fall (commonly October or November; Figure 1), they are at peak dormancy. In order to complete their dormancy cycle and resume growth in spring, seedlings require a period of chilling. The chilling requirement for Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) is 12 to 14 weeks (approximately 2000 hours). If not totally fulfilled at lifting, the chilling requirement may be met in cold storage (temperatures in freezer storage are considered below optimum for accumulation of chilling). On the other hand, cold hardiness continues to develop through the fall and reaches a maximum in winter (Figure 2). In the Pacific Northwest, conifer species tend to have maximum hardiness in January. As spring approaches, cold hardiness is quickly lost in response to longer photoperiods and warmer temperatures.



Figure 2. Cold hardiness (LT_{50}) and stress resistance are correlated, whereas dormancy intensity (days to budbreak) declines throughout the winter.

### How is Cold Hardiness Measured? _____

### The Whole-Plant Freeze Test

The whole-plant freeze test (WPFT) is the most common technique used to estimate cold hardiness. Unlike other available cold hardiness tests, WPFT is a simulation of an actual freeze event and takes the entire shoot into account. In this test, the entire seedling is potted, placed in a programmable freezer, and exposed to controlled, sub-freezing temperatures. After freezing, seedlings are placed in optimum growing conditions for 6 to 7 days, and then assessed for damage to the shoot tissues in order to quantify mortality at a given temperature (Tanaka and others 1997). The LT₅₀ is then determined by plotting survival percentage against temperature and assuming a linear relationship. Below are the WPFT steps in detail:

- 1. A minimum of 60 seedlings of a specific seed source and stocktype are randomly selected so that they provide good representation of the entire lot. The test should commence within 48 hours of sampling in order to accurately assess cold hardiness on the given sample date. If seedlings are to be shipped or stored prior to the test, they should be kept in cool conditions (1 to  $3 \ ^{\circ}C \ [34 to 38^{\circ}F]$ ).
- 2. The seedlings are randomly divided into four groups with 15 (or more) seedlings in each group. Each group of seedlings is potted into a light medium. Damp peat moss works best; high water content should be avoided because it will give off heat in the freezing chamber, thereby making it difficult to achieve target temperatures. Four-liter (1-gal) pots with five seedlings per pot works well. Large stocktypes (for example, plug+1)

may have to be potted at 3 to 4 per pot, whereas very small stocktypes (for example, 1+0 bareroot seedlings or seedlings grown in Styroblock TM 4 containers [66 cm³ or 4 in³]) can be potted at 7 to 10 per pot. It is important that the seedlings be upright and spread apart in the pot. Roots should be completely covered by the medium and shoots should not be too deep (medium should just cover the cotyledon scar). Stems planted too deep will be insulated from the freezing temperatures and, therefore, that section of stem will not be available for damage assessment. Container seedling plugs should be completely covered by the medium.

- 3. Each group of seedlings is assigned to a freezing temperature. Each pot should be clearly labeled with durable tags to identify the lot information and test temperature. Four target temperatures are chosen based on their expected ability to bracket the  $LT_{50}$  for that specific sampling date. As seedlings harden or deharden throughout the season, warmer or colder temperatures are used. Each group of seedlings is placed into a programmable chest freezer. Temperature to 0 °C (32 °F) at 20 °C (36 °F) per hour, then decreased to the target temperature at 5 °C (9 °F) per hour, held at the target temperature for 2 hours, then raised back to 0 °C (32 °F) at 20 °C (36 °F) per hour, at which point they are removed from the freezer (Figure 3).
- 4. After freezing, seedlings are placed into a greenhouse or other holding area with ambient photoperiod and an average temperature of about 20°C (68°F). Pots should be watered thoroughly as soon as the medium thaws, and be kept moist. Plants are held in this area for 6 to 7 days to allow for any damage to become evident.



Figure 3. Example of a whole-plant freeze test (WPFT) time sequence for a target temperature of -18  $^{\circ}$ C (0  $^{\circ}$ F).

5. After 6 to 7 days in the greenhouse or holding area, tissue damage is assessed on buds, cambium, and foliage as follows (see also Figure 4 for example data sheet):

Bud damage is determined on 5 to 10 randomly selected buds from throughout each seedling shoot. Each bud is cut in half and examined for evidence of browning. Buds that are questionable should be counted as "half dead." For example, if 8 buds are assessed and 4 are healthy (green),  $2\ are\ dead\ (clearly\ brown),\ and\ 2\ are$ questionable (slight browning), then the final total would be  $2 + \frac{1}{2} + \frac{1}{2} = 3$  out of 8 are killed for that seedling.

*Cambial damage* is evaluated by scraping the bark along the stem and examining for browning or drying in each third of the shoot (bottom, middle, top). Each third is rated as 0 (dead), 1 (healthy), or 2 (questionable).

Foliar damage is estimated visually as a percentage of brown or dry foliage. This can be done on an individual seedling basis or as an estimate of all seedlings in the group.

Stock/S	tock/Seedlot: <u>Doug-fir 1+1</u> Freezing Date: <u>December 10</u>						
Assessment Date: Freezing Temp: Freezing Temp:				<u>2°C</u>			
tree	ee # dead needle Cambium damage		ige				
#	buds	damage	bottom	middle	top	vitality	explanation
1	4/6	45%	2	2	1	0	> 50% of buds are dead
2	1/5		1	1	1	1	live
3	8/10		0	2	2	0	bottom cambium & >50% buds
4	0/6	•	1	1	1	1	live - no damage
5	3/7	-	2	2	2	2	questionable cambium damage
6	8/8	-	1	1	2	0	all buds killed
7	2/9	-	0	1	1	0	bottom cambium killed
8	1/6	-	2	1	1	2	bottom cambium questionable
9	0/5	-	1	1	2	1	live
10	4/9	-	1	2	1	2	middle cambium questionable
11	2/7		1	1	2	1	live
12	0/6		1	1	1	1	live - no damage
13	7/7		0	0	0	0	total kill - cambium and buds
14	2/6		0	2	0	0	bottom cambium killed
15	5/8		2	2	2	0	>50% bud kill
% KILLED:							
5 live seedlings (vitality = 1)					NOTE: ordinarily there would not be		
3 questionable seedlings (vitality = 2)				56.7%	so much variation in a test - this data		
7 dead seedlings (vitality = 0)					is for example purposes		
Total = 8.5 killed out of 15 total							

Key: cambium/vitality--O=dead; 1=healthy; 2=questionable

Figure 4. Evaluation of tissue damage is used to estimate seedling mortality at a given temperature.

- 6. Mortality is determined based on the extent of damage. If the cambium is damaged in the lower or middle third of the shoot, or if greater than 50% of the buds are damaged, then the seedling is considered non-viable. Just like the cambium ratings, vitality is rated as 0 (dead), 1 (live), or 2 (questionable). The percentage of foliar damage is only a determining factor when cambium or bud damage is borderline. Questionable trees are counted as "half dead" for determining the total mortality.
- 7. The final step is to plot survival percentage for each of the test temperatures (Figure 5). Assuming a straight line relationship, the  $LT_{50}$  is the temperature corresponding to the point where 50% of the seedlings were estimated to have been killed (Tanaka and others 1997).

#### Freeze-Induced Electrolyte Leakage

Another method for measuring cold hardiness is the freeze-induced electrolyte leakage (FIEL) test. It measures the amount of electrolytes that leak out of cell membranes when they are damaged by freezing. In the FIEL test, electrolytes are measured using an electrical conductivity meter and used to estimate the amount of damage in specific plant tissues as a result of freezing (Burr and others 1990; McKay and Mason 1991; Folk and others 1999). FIEL measurements are expected to be highest when seedlings are actively growing, and lowest when they are dormant due to the plants ability to withstand intracellular freezing.

For operational FIEL testing, tissue samples (for example, ten 1-cm [0.4-in] needle segments) are rinsed in distilled water (DI) and placed in vials. A small amount of DI is then added to each vial to prevent desiccation during freezing. Usually there are five to six sets of vials prepared (one set for each test temperature plus a control; there should be five to ten vials in each set). The control samples are placed in a refrigerator at 2 °C (36 °F). The samples designated for freezing are placed into a programmable freezer that is lowered from room temperature to 2 °C (36 °F) at 20°C (36 °F) per hour, and thereafter decreased at a rate of 5 °C (9 °F) per hour. When the chamber temperature reaches -2° C (28 °F), the vials are gently shaken to promote ice nucleation. Five pre-determined test temperatures are selected based on the expected hardiness of the samples. Upon reaching each test temperature as the freezer temperature is lowered, the samples designated for that test temperature are removed and placed in a refrigerator for thawing.

Once all the vials are thawed, more DI is added to each vial to promote a diffusion gradient for the electrolytes and vials are shaken for 2 hours. Following shaking, each is measured for initial conductivity (EC1), then autoclaved at 120 °C (248 °F) for 20 minutes and allowed to cool at room temperature. The purpose of the autoclaving is to achieve 100% electrolyte leakage in the samples. Once cooled, vials are shaken again for 2 hours and conductivity is measured again to determine total electrolytes (EC2). Electrolyte leakage of samples from each test temperature is expressed as a percentage of total electrolytes (EC1/EC2). LT₅₀ is derived by fitting the data set into nonlinear functions following a Gauss sigmoid model (Burr and others 1990).



**Figure 5.** By plotting mortality at each whole-plant freeze test (WPFT) temperature, the  $LT_{50}$  can be determined (intersection of the 50% mortality estimate and freezing test temperature).

#### **Chlorophyll Fluorescence**

Chlorophyll exhibits a characteristic fluorescence pattern when photosynthetically active tissue is exposed to saturating light. The attributes of the fluorescence curve reflect the efficiency with which light energy can be processed. Chlorophyll fluorescence measurements can provide information about the overall photosynthetic condition of the plant and its responses to disturbances (Rose and Haase 2002).

In species that grow in areas with extremely low winter temperatures, reversible photosynthetic inactivation in winter is an adaptive mechanism that provides protection against freezing damage. For these species, significant decreases in fluorescence can be measured and are associated with increases in freezing tolerance (Binder and Fielder 2006). On the other hand, coastal Douglas-fir and other temperate species exist in a geographic range that does not require complete photosynthetic inactivation for protection against winter cold. Mixed results have, therefore, been found regarding the relationship between chlorophyll fluorescence and cold hardiness. Chlorophyll fluorescence, however, has a good relationship with damage to tissues after exposure to freezing stresses (Rose and Haase 2002) and can be a useful tool for providing a rapid estimate of seedling vigor following freezing.

There are an assortment of fluorometers on the market for measuring chlorophyll fluorescence, including both portable and laboratory models. Measurements are accomplished on a representative sample of foliar tissue that is either kept in ambient light so that chlorophyll are at a steady state (for determination of quantum photosynthetic yield) or dark-adapted for 20 to 30 minutes so that all chlorophyll are at a ground state (for determination of photochemical efficiency). The sample is placed into a specially designed clip or chamber and fluorescence data are generated following exposure to pulses of saturating light.

#### Gene Expression

This newly developed molecular test for assessing cold hardiness in conifer seedlings was developed by NSure, a spin-off company from Wageningen University in The Netherlands. The test is based on measuring the activity level of a carefully selected set of genes. The condition of any plant, animal, or microorganism is reflected in the activity profile of its genes; all physiological responses are initiated and directed by genes switching on or off. Gene expression analysis is used to detect the level of activity of specific genes in order to evaluate plant responses to environmental triggers. The NSure cold-tolerance assays were made available for Scots pine (Pinus sylvestris L.), Norway spruce (Picea abies L. Karst), and European beech (Fagus sylvatica L.) following research that demonstrated a correlation with gene activity and cold hardiness (Joosen and others 2006). NSure tests on Douglas-fir seedlings showed a good relationship with results generated using the WPFT method. The correlation was consistent for seedlots from both high and low elevations, provided buds were used for the gene profiling; when needles were used, however, the correlation was poor (Balk and others 2007).

## Do-It-Yourself Freeze Assessment Following a Freeze Event _____

Knowing the cold hardiness level of a given group of plants at a given time is useful in making management decisions for culturing, lifting, storage, and planting. The biggest question that invariably arises, however, is how to determine whether or not stock is damaged following an unexpected freeze event, such as unseasonably low temperatures or a malfunction with the storage or transportation cooler. Following a freeze event, plants may not show damage or mortality while still in the nursery or in storage because cool temperatures will slow the development of symptoms (much the same as produce kept in our home refrigerators remains fresh for a period of time even though it is no longer a living plant).

The quick and easy way to determine if damage has occurred is to use some of the procedures from the WPFT:

- 1. Collect a random, representative sample of the affected stock (minimum 15 to 20 seedlings).
- 2. Pot seedlings (can be several per pot).
- 3. Place in a warm environment with ambient photoperiod (such as an office area); keep the medium moist.
- 4. Using a razor blade, evaluate the amount of browning in cambium and buds after 6 days.

# How Is Cold Hardiness Testing Used Operationally?

#### In the Nursery

Because cold hardiness is correlated with stress resistance, cold hardiness testing can yield data to help nursery growers make informed decisions regarding a specific seedling lot, especially those most vulnerable to freeze events. By knowing the hardiness level, growers can determine the need for frost protection, evaluate readiness for lift and storage, and estimate overall resistance to stresses incurred during handling. In a study with Douglas-fir and ponderosa pine (Pinus ponderosa Dougl. ex Laws.) seedlings, cold hardiness data was used to establish lifting windows that were shown to vary annually based on weather patterns and other factors (Tinus 1996). In addition, growers can track crop hardiness in response to dormancy induction treatments or other culturing activities. Jacobs and others (2008) showed that FIEL differed between Douglas-fir seedlings placed under differing day lengths following freezer storage. Similarly, Colombo and others (2003) found differences in FIEL among four hardening regimes for black spruce seedlings (Picea mariana [Mill.] B.S.P.).

#### In the Field

Cold hardiness data also helps reforestation foresters to assess seedling quality upon receiving their stock from the nursery. It can be very useful to have a baseline of seedling quality information at the time of outplanting to aid in understanding any issues that occur during the seedling establishment phase. In addition, cold hardiness can give an estimate of potential survival and establishment after outplanting. Simpson (1990) found that  $LT_{50}$  at lifting correlated well with first-year survival and shoot growth of conifer seedlings. It follows that cold hardiness data can help predict if seedlings are likely to have sustained damage following a freeze event, such as a late frost immediately after outplanting.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# Nursery Cultural Practices to Achieve Targets: A Case Study in Western Larch Irrigation

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**Abstract:** Nursery cultural practices are used to help growers achieve pre-determined size and physiological targets for seedlings. In that regard, irrigation is used to accelerate or slow growth and as a trigger for changing growth phase. In a case study highlighting the effects of irrigation on seedling development, western larch (*Larix occidentalis* Nutt.) seedlings were grown under three irrigation regimes to study seedling irrigation frequency, growth, and instantaneous water use efficiency (WUE₁). Seedlings were irrigated when daily container weights were reduced to 65% of saturated weight (SW), 85% SW, or at 85% SW for 8 weeks and then 65% SW for the remainder of the growing season. Mean irrigation frequency was once per 7.9, 4.6, and 3.8 days for the 65%, 85% to 65% and 85% treatments. Root-collar diameter (RCD) and height of all seedlings measured mid-way through the experiment revealed that seedlings receiving higher irrigation frequency (85%) were more variable in height than those receiving less irrigation. Irrigation regime did not influence final height or dry mass root:shoot. Mean RCD of seedlings in 85% moisture content treatments was only 2.4% larger than seedlings grown at 65% SW, and WUE₁ measured on five sample dates during moisture stress periods did not vary between irrigation treatments. Our results show that the environmental costs of increased nursery water use were not justified by a return of increased seedling size and that reduced irrigation decreased variability in seedling height.

Keywords: nursery water use, water use efficiency, seedling crop uniformity

#### Introduction _

Myriad factors influence plant growth: temperature, as a function of season; the form, quantity, and timing of precipitation; light quantity and quality; and nutrient availability being just a few. In a nursery setting, many of these factors are managed to manipulate seedling growth to meet objectives related to crop production and achieve morphological and physiological targets. These nursery cultural practices vary in their impact across species, timing, and intensity.

In the case of irrigation, the challenge is to strike a balance between supplying sufficient quantities of water to maintain and/or manipulate seedling growth at desirable rates to achieve target plant characteristics without being superfluous and wasteful. Frequency of irrigation depends upon the growth phase of seedlings (Landis and others 1989). Irrigation during the establishment phase should maintain sufficient moisture without creating anaerobic conditions that may inhibit seed germination; during the rapid growth phase, irrigation should saturate growing media to maintain high productivity and flush media to prevent the buildup of salts (Landis and others 1998). Practical determination of irrigation needs may be based upon daily sampling of container capacity weights expressed as a percentage relative to saturated weight (Timmer and Armstrong 1989).

In addition to initiating bud set and limiting late-season height growth, the hardening phase in container nurseries is intended to acclimate seedlings to environmental stresses (Landis and others 1998). Water uptake after outplanting is important for seedling growth and survival (for example, Mullin 1963), and nursery irrigation regime affects seedling performance both during nursery production and after outplanting (Duryea 1984; Seiler and Johnson 1985). Mild moisture stress conditioning during July and August increases winter cold hardiness in Douglas-fir (*Pseudotsuga menziesii*) (Blake and others 1979). Moisture stress conditioning during hardening is thought to acclimate seedlings to drought and improve their capability of withstanding additional water limitation after transplanting (Rook 1973). Seiler and Johnson (1985) found that severely moisture-stressed loblolly pine (*Pinus taeda*) seedlings, despite having smaller initial root and shoot volume, demonstrated better field performance than moderate or non-stressed seedlings, and hypothesized that physiological, rather than morphological, changes associated with stress treatment were responsible. Increased root growth and limitation of stem growth are morphological goals of the hardening phase in western larch (Larix occidentalis Nutt.) that, as a deciduous conifer, may invest less long-term photosynthates to foliage than other western conifers. Bassman and others (1989) found that irrigation of *Larix* spp. seedlings at 75% of saturated weight resulted in maximum root and stem growth.

As a case study in manipulating irrigation to achieve targets, we present an examination of western larch seedlings grown under three irrigation regimes. The objectives of this study were to: 1) quantify the number of irrigation events required to maintain minimum target moisture content container weights within a plausible range for containergrown western larch seedlings; 2) characterize water use efficiency (WUE) of seedlings grown under these irrigation regimes when provided with ample water (saturation) and across a range of dry-down stress values (container moisture contents); and 3) characterize the effects of these irrigation regimes on seedling morphology.

#### Methods _

Western larch seeds were sown in 1:1 (volume) peat: vermiculite growing medium in SuperblockTM (Beaver Plastics, Limited, Acheson, Alberta, Canada) 160/90 mL  $(5.5 \text{ in}^3)$  containers on 30 May 2007 at the University of Idaho Pitkin Forest Nursery (Moscow, ID). Medium was treated with Osmocote Classic Lo-Start 18N:6P2O5:12K2O slow release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH), and seedlings were grown for 4 weeks under normal irrigation. After 4 weeks, three irrigation treatments were applied. Seedlings were irrigated when container weight reached 65% of saturated weight, 85% of saturated weight, or at 85% for 8 weeks and then 65% for the remainder of the growing season. Blocks were weighed daily during the morning and saturated on days when weights were less than 65% or 85% thresholds. Reference saturated weights were determined monthly.

Two sets of intensive measurements and five additional sets of extensive measurements were conducted. Intensive measurements following saturation were taken for three seedlings selected using two uniformly distributed pseudo-random numbers (one for container column and one for row) from each container on 23 to 24 August and 10 September. Intensive measurements included seedling height and root-collar diameter (RCD), root and shoot dry mass after oven-drying for 48 hours at 60 °C (140 °F), net photosynthetic assimilation (A), and transpiration (E). A and E were measured using a LI-COR[®] LI-6400 Portable Photosynthesis System (LI-COR[®] Biosciences, Lincoln, NE). Additional extensive sampling of A and E was conducted on 1, 5, and 9 September, as well as 19 and 23 September in order to sample across the range of media moisture content (container weight %). Instantaneous water use efficiency  $(WUE_I, \mu mol CO_2 / mmol H_2O)$  was calculated as  $WUE_I =$ A / E (Lambers and others 1998). Final root and stem measurements were conducted on 16 December 2007. Twelve seedlings per container were sampled for height, RCD, and root and stem dry mass with needles off. Container weight data through 30 September were used for comparisons of irrigation timing and frequency.

Using the open-source statistical computing environment, R (R Development Core Team 2008), separate linear mixed effects models were fit to each response variable of interest (WUE, height, RCD, root-to-shoot dry mass) at each intensive measurement date and for the final measurements. While the initial study was established as a randomized complete block design with three treatment levels and three replicates, one of the replicates was removed mid-way through the experiment due to a high concentration of phosphoric acid inadvertently applied during irrigation. Separate linear mixed effects models of each response variable of interest were fit for post-saturation conditions for each intensive sampling date and final measurements.

#### Results ____

From 8 July to 30 September, the frequency of irrigation events differed between irrigation treatment (Figure 1; P=0.0088). Maintaining 20% higher minimum container weight required 52.1% more frequent irrigation. Containers irrigated at 85% of saturated weight required irrigation once in every 3.8 (SE 0.4) day period, while those irrigated at 65% required irrigation once every 7.9 (SE 1.5) days. This higher frequency irrigation required 135% more total irrigations. Those irrigated 85/65 required watering every 4.6 days

WUE_{*I*} of seedlings after saturation of growing media did not vary between irrigation treatment levels after 8 weeks, after the first dry-down period, nor after the second dry-down period. WUE_{*I*}, however, increased over time in all treatments during the hardening phase (Figure 2). Seedling WUE_{*I*} on the five sample dates during dry-down periods did not vary between irrigation treatments levels. A simple linear, least-squares fit of WUE_{*I*} regressed upon container weight for all moisture stress sample dates, across all treatments, showed that container weight explained less than 3% of the variation in WUE in this study ( $R^2 = 0.028$ ).

After 8 weeks of treatment at alternative irrigation regimes, there were no differences in seedling height, RCD, or root:shoot dry mass between treatments. RCD and height of all seedlings measured in a full-tally inventory mid-way through the experiment (21 to 24 September) revealed that



Figure 1. Mean number of days between irrigation plus one standard error for each of three irrigation regimes (left). Difference in mean number of days between irrigation events (Tukey type) and 95% confidence interval for *Larix occidentalis* seedlings grown under three irrigation regimes (right).



Irrigation regime (container weight %)

**Figure 2.** Water use efficiency (WUE₁) of *Larix occidentalis* seedlings following saturation for three irrigation regimes (left) and at varying levels of media moisture content throughout the growing period (right).

heights of seedlings receiving higher irrigation frequency (85%) were more variable than those at lower treatment levels (Figure 3). When final seedling size measurements were conducted in late December, differences were detected in mean RCD (*P*=0.016; Figure 4), but not seedling height or dry mass root:shoot (data not shown).

#### Discussion

Irrigation at 85% minimum moisture content, rather than 65%, required more than twice as much water use in the nursery. Morphological differences due to alternative moisture regimes were only observed in final mean RCD, which was 2.4% larger in the 85% irrigation regime than



Figure 3. Root-collar diameter and height relationship of all seedlings for each of three irrigation regimes on 21 September 2008.



Figure 4. Western larch seedling root-collar diameter (RCD) following one season of nursery culture under three irrigation regimes (left); mean RCD plus one standard error (right). Difference in mean RCD is at the 95% confidence interval.

in the 65%. That final seedling height did not differ across irrigation regimes contrasts the findings of Royo and others (2001), who found differences in seedling height and RCD between moisture stress treatments in Aleppo pine (*Pinus halapensis* Mill.). That may be due to the growth habit of *Larix* versus *Pinus* spp. It should be noted, however, that well-watered seedlings exhibited the greatest variability, and thus lack of uniformity, of the three treatments at the end of the growing period (Figure 3). These impacts could lead to differences following outplanting, as lower relative height growth after field planting has been found in Douglas-fir seedlings given frequent irrigation treatments (van den Driessche 1992), in Douglas-fir and ponderosa pine (*Pinus ponderosa*) (Helgerson and others 1985), and in Allepo pine (Royo and others 2001). While we hypothesized that greater  $WUE_I$  would be observed in seedlings treated with less frequent irrigation during hardening, differences in  $WUE_I$  were not detected between treatments after seedlings received saturation, nor did  $WUE_I$  vary between irrigation regimes at varying levels of moisture stress during the two dry-down periods. The extent to which plants lose water relative to the amount of  $CO_2$  assimilated during photosynthesis at a given time is a measure of their  $WUE_I$  (Lambers and others 1998). This indicates that moisture stress was likely not limiting plant growth during the study period, a result that should be considered in designing irrigation regimes for western larch.

Our research provides evidence that seedling morphological and physiological gains from increased irrigation frequency in western larch did not justify the environmental or financial cost of added nursery water use. A post-hoc hypothesis of interest, based on our research and review of related earlier studies, is that the relationship of moisture stress during hardening and transplanting performance may depend more on fine details of root architecture than are exhibited either by root mass, the relationship of below- and aboveground mass, or WUE_I. It seems plausible, for example, that a mechanistic benefit of mean root diameter, or the distribution of root diameters, from adaptive root growth may facilitate equilibration with soil water potentials that are increasingly negative as moisture becomes tightly held in small pore spaces in drier soils.

# Conclusion and Future Directions

In order to maintain a 20% higher minimum block weight percentage of saturated weight, it was necessary to increase total irrigation by 135%. However, there was not a corresponding increase in seedling growth. The environmental and economic savings of using a lower minimum block weight as the point of irrigation was justified. Further studies are needed to identify specific seasonal irrigation patterns to optimize seedling production while minimizing inputs. Further research should focus on characterization of the relationship between fine root architectural relationships as they relate to the soil pore size-water potential continuum, growing media, and conductance in western larch.

This case study highlights that cultural practices, such as irrigation, can have short-term and lasting effects on seedling size. For western larch, growers might find that a significant reduction in water yields decreases height growth and/or increases uniformity, both of which are desirable for such a species.

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# Using Essential Oils to Control Moss and Liverwort in Containers

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**Abstract:** Liverwort and moss are economically significant weeds across a range of US container production sites, including forest seedling greenhouse culture in the Pacific Northwest. We have demonstrated the effectiveness of essential oils, or distilled plant extracts, in controlling liverwort and moss container weeds over three seasons of trials. When applied at the appropriate concentration and volumes, essential oils act as an effective contact herbicide. There is little or no residual, and under heavy pressure, liverwort and moss can re-establish within 2 weeks of knockdown. The applicator must pay close attention to method and application rates to successfully control weeds while avoiding crop damage, such as leaf or needle burn. Leaf wilt following the first drying cycle has occasionally been observed, with root damage suspected but not confirmed. This paper summarizes integrated pest management practices to limit the invasion and spread of liverwort and moss in containers, details postemergent trial results of directed-spray and over-the-crop herbicide applications, suggests guidelines for species and stocktype stages, and provides a checklist of procedures when applying essential oils.

Keywords: essential oils, liverwort, moss

# Liverwort and Moss in Container Nurseries _

A national survey of container and nursery growers and extension specialists ranked liverwort as the number two weed in horticultural production (IR-4 2009). This survey also identified liverwort, moss, and algae as the third largest "group" of weeds in container production, trailing only broadleaf plants and sedges. Liverwort and moss reduce crop growth and value by outcompeting crops for water and nutrients and by providing habitat and encouraging the development of fungus gnat populations. In the Pacific Northwest (and throughout much of the US), Marchantia liverwort (*Marchantia polymorpha*) is the liverwort best adapted to container culture, while silver-thread moss (*Bryum argenteum*) is the most likely moss to infest greenhouses.

Liverwort and moss are lower plants, meaning they lack a vascular system. They are the cockroaches of the plant world, with liverwort thought to be the first group of plants to make the transition from blue-green algae to successful land colonization. Liverwort and moss have several reproductive strategies, including asexual propagation through simple division as well as the unique ability of liverwort to develop gemmae cups. These cups fill with gemmae, small plant fragments, which can splash up to a meter away from the mother plant when hit at the right angle with a drop of water (Figure 1). Liverwort and moss also reproduce sexually, developing spore-releasing capsules.



Figure 1. Marchantia liverwort (Marchantia polymorpha) can develop gemmae cups. The gemmae fragments within can splash up to 1 m (3 ft) away from original plant.

#### **Pre-crop Sanitation**

Spore-release of liverwort and moss is widespread, so the primary goal of a nursery manager should be to kill spores between crops. Pre-crop sanitation may include the use of pressure washing to remove bulk debris and follow-up with a chemical treatment; any chemical treatment is always more effective with thorough pre-washing. Chemical treatments that are commonly used to reduce spore loads of liverwort, moss, and algae are quaternary ammonium chlorides, sold as products such as GreenShield[®] (Whitmire Micro-Gen Research Laboratories, St Louis, MO), Physan 20[™] (Maril Products Incorporated, Tustin, CA), and Triathlon[®] (OHP Incorporated, Mainland, PA). Other chemical treatments include hydrogen dioxide, chlorine dioxide, and chlorine bleach (Smith 2007).

Forest seedlings are commonly grown in Styroblock[™] containers (Beaver Plastics, Acheson, Alberta); along with other container styles, these may be a vector for liverwort and moss spore contamination from one crop to the next. Probably the most effective treatment to sterilize blocks, particularly for older blocks where old plant debris is very difficult to physically remove, is steam cleaning. Steam cleaning setups are expensive, but alternatives exist. At Washington Department of Natural Resources Webster Nursery (Olympia, WA), we use a hot-water dip tank to clean blocks. We run temperatures at 77 to 82  $^{\circ}\mathrm{C}$  (170 to 180 °F) for up to a minute, that is, just short of "warping" the blocks.

Additional pre-crop sanitation includes removal of any existing liverwort and moss adjacent to greenhouses. Baking soda, liberally sprinkled on liverwort in non-crop areas (complete coverage is not necessary), acts as an effective contact herbicide for initial eradication. Combinations of weed barrier and mulch, weed barrier alone, or pre-emergent herbicides such as flumioxazin or dimethenamid-P may be applied to limit liverwort and moss growth.

#### Sanitation During Propagation

Once a greenhouse crop cycle begins, the best liverwort and moss prevention strategy is to kill any spores that float into the greenhouse before they make it past the germination stage. Applying ZeroTol[™] (BioSafe Systems, Glastonbury, CT) (hydrogen dioxide, peroxyacetic acid, and stabilizers) at 1:450 as a post-rinse following each irrigation can successfully reduce spore loads (Thompson 2008). Another spore-killing strategy is to apply a constant ultra-low dose chlorine or copper treatment to kill spores through an in-line system.

#### Water Management

Greenhouse cultural practices are paramount to liverwort and moss control, with particular emphasis on water management; these weeds thrive in the presence of moisture. Choose mulches, such as screened grit, that are standardized in size, smooth-surfaced, and therefore drain well. Uniformly cover soil medium at time of propagation, as exposed surfaces will be become infested quicker.

Try to be a "dry" grower. To the extent possible, water by plant needs rather than human convenience. Up-front costs spent on determining when a crop actually needs irrigation will be rewarded with less water usage, lower pest pressure, and ultimately a higher quality crop. Once a decision to irrigate has been made, consider expediting crop drying with a spreader that breaks down water-surface tension, such as Sylgard[®] 309 or R-11[®] (Wilbur-Ellis, Fresno, CA). This practice is especially called for in late-day irrigations or irrigations in overcast weather. Use horizontal airflow fans in opposing patterns to break up boundary layers and monitor and reduce excessive relative humidity where possible. Again, liverwort and moss need moisture to thrive, in almost all cases more than the crop you are actually trying to grow. Starve them of excess moisture as a basic prevention technique.

#### Nutrient Management

Liverwort and moss love nutrients and, like many plants, thrive in nitrogen and phosphorus-rich environments. Growers may have a tendency to apply excessive amounts of fertilizer ("luxury consumption") to push seedlings to size simply because stock that does not meet minimum morphological specifications is less marketable. Seedling nutrient analysis can help a grower differentiate between hidden hunger, optimal growth, and luxury consumption, thereby targeting nutrient applications at levels that do not excessively stimulate liverwort and moss growth.
Since liverwort and moss are shallow-rooted (not really having roots at all, but rather root-like structures called rhizoids), they thrive in environments where nutrients are surface applied. Limiting surface-applied nutrient application generally means decreasing fertigation, the practice of applying fertilizer through the irrigation system. When a grower fertigates, they are applying nutrients to the soil surface, feeding liverwort and moss where they live. An alternative nutrient-delivery system is to focus at least partly on incorporation of controlled-release fertilizers (CRF) in the soil medium, where the majority of nutrients are located safely away from the soil surface. Use CRF formulations that cover the duration of the seedling's life in the container, as top-dressing of CRF returns a grower to a situation where liverwort and moss are fed where they live, that is, on the soil surface. Recent research, however, indicates that iron sulfate in CRF form top-dressed on containers has a strong inhibitory effect on liverwort, with reduced concern over nutrient toxicity due to the slow release of the product (Prine 2010).

#### Post-Emergent Options, or Lack Thereof

All the best management practices in the world may not prevent moss and liverwort outbreaks, particularly when cloudy, cool weather seems to work against you. Handweeding is an option, but that can damage the crop through disturbance of shallow roots or soil loss. Due to the tendency of liverwort to fragment, it sometimes feels like weeding only spreads the problem.

Terracyte[®] (BioSafe Systems, East Hartfort, CT) (sodium carbonate peroxy-hydrate) is a granular product labeled for post-emergent control of liverwort and moss. In the presence of water, the granules convert to 34% hydrogen peroxide, strong enough to burn any foliage it contacts. While the product may be applied safely on larger containers where foliar contact can be avoided, the granules tend to hang up in any sort of denser foliage. Dense foliage also tends to reduce the uniformity of application, which diminishes uniformity of liverwort and moss control.

Mogeton (Certis, Amesbury,Wiltshire, UK) or Gentry (Chemtura Corporation, Middlebury, CT) (quinoclamine) is a post-emergent originally used to reduce moss in rice paddies. Researchers have demonstrated excellent control over a range of species and stocktypes with considerable residual, but the parent company withdrew the product from a long EPA registration process in early 2008.

# Essential Oils—An Alternative Means of Control

Essential oils are distilled plant extracts. Some essential oils have insecticidal, fungicidal, bactericidal, herbicidal properties, or all of the above. Modes of action are poorly understood, but ancient Egyptians used thyme oil (with the phenolic compound thymol) to preserve mummies from fungal and bacterial attack. For weeds such as liverwort and moss, essential oils are thought to simply act as a contact herbicide. Thorough coverage of the intended target is necessary for proper membrane disruption and weed death to occur. A grower can take advantage of the limited or nonexistent cuticle of liverwort and moss to burn these plants while waxier surfaces, such as conifer needles, are relatively protected.

Growers in the Pacific Northwest first experimented with Cinnamate (cinnamon oil) (Acros Organics, Fairlawn, NJ), a product labeled for two-spotted spider mite control that turned out to control liverwort and moss as well. Due to factory safety issues, Cinnamate is no longer produced. Sporatec[™] (Brandt Consolidated, Springfield, IL; formerly sold as Sporan by EcoSMART Technologies Incorporated, Franklin, TN) is a product that consists of rosemary, clove, and thyme oil. The product is primarily labeled as a fungicide, but is now labeled for liverwort and moss as well. Sporatec[™] is a FIFRA 25(b) product, meaning it is exempt from EPA registration. There is no re-entry interval and no pre-harvest interval. Even so, essential oils like Sporatec[™] are concentrated oils and should be handled carefully. They can irritate skin, and are particularly troublesome to contact lens wearers. If you spray near harvest time, I strongly recommend that handlers wear gloves.

#### **Trials With Essential Oils**

The Stuff Actually Works!-During the past several years, we have tested a fair number of products with wideranging and astounding claims. Growers like to call these products snake oils, and Sporatec[™] certainly struck us as distilled snake oil at first blush. Nonetheless, we first tested the product on a liverwort and moss-infested crop of western redcedar (Thuja plicata) seedlings grown in Styroblock[™] 2A (313A) containers  $(40 \text{ cm}^3 [2.4 \text{ in}^3])$  in the fall of 2008. We conducted a replicated test at 0, 8, 15.5, and 31 ml/l (0, 1, 1)2. and 4 oz/gal) rates and, with each rate, included 0.03% spreader-sticker(Sylgard[®] 309) and 0.45% Hasten[®] penetrant (Wilbur-Ellis, Fresno, CA) based on general label recommendations to include these types of adjuvants to improve efficacy. The label encouraged thorough coverage, and we applied at a rate of  $2.3 \text{ l/m}^2$  (70 oz/10 ft²). While the 15.5 ml/l (2 oz/gal) rate showed some tip burning of the redcedar, from which it recovered, and the 31 ml/l (4 oz/gal) rate severely burned foliage, the 8 ml/l (1 oz/gal) rate showed no damage to the crop (Figure 2). Spraying 8 ml/l (1 oz/gal) not only did not damage the redcedar, but successfully controlled liverwort and, to a lesser extent, moss (Figures 3 and 4). We recorded 91% liverwort control and 75% moss control 9 days after treatment.

We followed this trial one week later with a second rate comparison over western redcedar, this time at 0, 4, 8, 12 ml/l (0, 0.5, 1.0 and 1.5 oz/gal) rates. We substituted Syl-tac[®] (Wilbur-Ellis, Fresno, CA), a combination of Sylgard[®] 309 and Hasten[®]; by keeping the Sylgard[®] rate the same, we decreased the rate of Hasten[®] penetrant in this combination product to one-quarter the rate of the first trial. Even though the same volumes of product were applied in fairly similar environmental conditions, control was not as thorough. Even the 12 ml/l (1.5 oz gal) SporatecTM rate in this second trial controlled only 81% and 62% liverwort and moss, respectively, 7 days after treatment (not shown). Since these were not head-to-head trials, we can only infer that the lower rate of penetrant led to the weaker control.



**Figure 2.** One replication of SporatecTM applied (from top to bottom) at 0, 8, 15.5, and 31 ml/l (0, 1, 2, and 4 oz/gal) rates at a volume of 2.3 l/  $m^2$  (70 oz/10 ft²). Photo is 9 days after treatment.

Oregon State University Test-Ed Peachey, an extension agent from Oregon State University, tested over-the-crop Sporatec[™] applications at a nursery in Independence, OR, in 2009. The nursery had 4-l (1-gal) pots of arborvitae (Thuja occidentalis) and Oregon grape (Mahonia aquifolium) coming out of dormancy in spring with thick liverwort infestations. Sporatec[™] was applied at rates of 8 and 12 ml/l (1 and 1.5 oz/gal), with and without various adjuvant combinations, including the combination and rates from our first trial. Applications were made in succession on 20 April and 24 June 2009. He found greater than 85% liverwort control up to 9 weeks following application at both rates, with some minor phytotoxicity to Oregon grape foliage. There did not appear to be increased performance with addition of any adjuvant combination, but there was a trend of less phytotoxicity when adjuvants were included. Franki Wai-Ki Lam of Brandt, Incorporated believes the spreader may have decreased crop phytoxicity by breaking down the surface tension of the oil droplets, thus lowering their tendency to burn broadleaf foliage.

Tests at Webster Nursery-In 2009, liverwort and moss growth was poor, but we did have some decent growth of liverwort and moss in some larger 4-l (1-gal) stock by fall. In early October 2009, we tested direct sprays on liverwort and moss beneath a range of species, including western hemlock(*Tsuga heterophylla*), Sitka spruce(*Picea sitchensis*), Douglas-fir (Pseudotsuga menziesii), western white pine (Pinus monticola), ponderosa pine (Pinus ponderosa), noble fir (Abies procera), grand fir (A. grandis), western redcedar, and Port-orford cedar (Chamaecyparis lawsoniana). We found no phytotoxicity when direct spraying rates as high as 15.5 ml/l (2 oz/gal) Sporatec[™]. While superficial spray coverage on conifer foliage did not result in any burn, needles tended to be quite waxy by this time of year. Roots were also suberizing by this time. Liverwort and moss were successfully knocked back, with 95% and 88% control, respectively, 3 weeks after treatment, and 93% and 76% control 6 weeks after treatment.



Figure 3. Comparison of healthy liverwort (untreated) with severely damaged liverwort treated at 8 ml/l (1 oz/gal).



Figure 4. At 9 days after treatment for the 8 ml/l (1oz/gal) treatment, we recorded 91% liverwort control and 75% moss control. Crop phytotoxicity was observed at 15.5 ml/l (2 oz/gal) rates and above.

**Irrigation Boom Trials** — All trials to this point involved some sort of hand-held spray apparatus. The next step was to chemigate the product through an irrigation boom, allowing for improved speed and accuracy. We also wanted to further test over-the-crop spraying, particularly over early- to midseason conifer "liners." For these trials, we used a Dosatron® injector (Dosatron International, Clearwater, FL), set at the strongest rate of 1:50. We further diluted this baseline 2% setting by making a pre-mix concentrate of one part oil to several parts water (Figure 5). This allowed us to constantly agitate the pre-concentrate, ideally creating a more uniform downstream application by minimizing settling of the product in the irrigation line. We did not include any adjuvants.

After a disastrous first trial where we over-applied volume of product, several additional trials allowed us to refine rates and volumes to the point where we were getting up to 90% control of liverwort and 75% control of moss, with no phytotoxicity to sensitive species like western hemlock in the middle of its growing season. Unfortunately, these reduced rates only temporarily knocked back the weeds for 10 to 14 days, and further trials are needed to see whether repeat applications on a shorter timeline can better control liverwort and moss in midseason crops.



**Figure 5.** For the boom irrigation trials, we started with a Dosatron[®] injector setting of 1:50. We further diluted one part oil to several parts water, allowing us to pre-agitate the product for better downstream uniformity.

Fine tuning of rates meant that 50% over-application might mean crop damage, where 50% under-application would result in no control. To prove to ourselves that we were accurately applying product downstream, we collected boom samples both between trials and within a trial (near beginning and near end of trial) and sent these off for analysis at the British Columbia lab where the product was developed. Lab analysis showed that our system was accurate to within +/- 15%, which is important when applying an herbicide over plants.

#### Suggested Guidelines

Consider these a starting point for rates to test in your nursery. Always test a small sample first.

#### Over-The-Crop Sporatec[™] Applications

Based on our trials, the following is a suggested guideline for over-the-crop SporatecTM applications that are applied with the intention of killing only the liverwort and moss:

- 1. For early to mid-season applications, apply 4.7 ml/l water (0.6 oz/gal water; 0.47%) SporatecTM with a volume of 4.9 l/m² (150 oz/10 ft²) or 5.8 ml/l (0.75 oz/gal; 0.59%) in a volume of 3.3 l/m² (100 oz/10 ft²).
- 2. For late-season over-the-crop sprays, apply 8 ml SporatecTM/l water(1.0 oz/gal water; 0.78%) in a volume of 3.3 to 4.9 l/m² (100 to 150 oz/10 ft²).
- 3. We noted a range of crop resistance, from toughest to most sensitive: ponderosa pine>true firs>interior Douglas-fir>coastal Douglas-fir> western redcedar> western hemlock>western larch (*Larix occidentalis*).
- 4. Use extreme caution when treating broadleaves. We noted at least some level of phytotoxicity on all broad-leaves we tested, including red alder (*Alnus rubra*), black cottonwood (*Populus trichocarpa*), and creeping Oregon grape. If you do apply over the top of broad-leaves, consider pre- and post-wetting the foliage.

#### Directed-Spray Sporatec[™] Applications

Season-long at 8 ml Sporatec TM/l water (1.0 oz/gal water). Where moss is present, consider using up to 1.2 ml/l (0.16 oz/gal) Syl-tac[®], as this may improve control.

Late-season at 12 ml SporatecTM/l water (1.5 oz/gal water). Again, consider using 1.2 ml/l (0.16 oz/gal) Syl-tac[®] where moss is present. Do not apply this rate to containers less than 98 cm³ (6 in³) or to plants that have not hardened off.

Season-long at 12 ml Sporatec  ${}^{\rm TM}/l$  water (1.5 oz/gal water) in the second season of a container crop.

One important note is to figure out the minimum volume of product you need for the stage of liverwort and moss in your crop and apply only that amount. It is relatively easy to over-apply when direct spraying. When going after a nasty infestation, remember that more volume equals more active ingredient!

#### Checklist for Success

When applying Sporatec[™], consider the following checklist:

- 1. Soften water to hardness <100 ppm. The label says 200 ppm, but the original developer suggests 100 ppm is a more appropriate cutoff.
- 2. Adjust water pH to 7.0 or lower.
- 3. Try to apply when temperatures are greater than 16  $^{\circ}C$  (60  $^{\circ}F)$  and less than 29  $^{\circ}C$  (85  $^{\circ}F).$
- 4. Remove contact lenses (preferably before application).
- 5. Maintain constant agitation throughout. Settling of oil will lead to uneven application rates.
- 6. A post-application water rinse 5 minutes after spraying may reduce phytotoxicity in sensitive species, particularly broadleaves.

#### Final Disclaimer

Many plants were harmed in the testing of this product! Always conduct small trials first; use extreme caution when testing with broadleaves, and remember that more is not always better!

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

## Tissue Culture of Conifer Seedlings—20 Years On: Viewed Through the Lens of Seedling Quality

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**Abstract:** Operational vegetative propagation systems provide a means of bringing new genetic material into forestry programs through the capture of a greater proportion of the genetic gain inherent within a selected tree species. Vegetative propagation systems also provide a method for multiplying superior varieties and/or families identified in tree improvement programs. Twenty years ago, a program at the Forest Biotechnology Centre, BC Research Incorporated (Vancouver, British Columbia, Canada), was initiated to apply somatic embryogenesis technology to conifer species with the intent of creating a commercially viable vegetative propagation system that could produce large numbers of somatic seedlings (then called emblings).

As this program was being initiated at the Forest Biotechnology Centre in the early 1990s, there was a perception that seedlings produced through somatic embryogenesis technology might have attributes unsuitable for large scale reforestation programs. To overcome this skepticism, a comprehensive seedling quality assessment program was designed to assess the performance of somatic seedlings. As the somatic embryogenesis technology for conifer seedlings improved, the application of quality practices to the production of somatic seedlings evolved into an approach that is comparable to the International Organization for Standardization (ISO) quality assurance programs now being applied across many industries. The following is a brief history of the evolution of this seedling quality approach (from the author's perspective) applied to conifer seedlings produced with the somatic embryogenesis technology.

Keywords: zygotic embryos, somatic embryos, seedling quality

#### Somatic Embryogenesis Tissue Culture Process _

Somatic embryogenesis tissue culture technology is the most recent vegetative propagation system to be implemented on an operational scale. In this tissue culture approach, proliferative embryo suspensor masses are established from nonmeristematic cells and subsequently cultured to produce organized somatic embryos possessing a shoot and root meristem. The term somatic refers to embryos developing asexually from vegetative (or somatic) tissue. Somatic embryogenesis technology was developed for conifer tree species in the late 1980s, originally on spruce species (*Picea* spp.) (Hakmann and von Arnold 1988; Webb and others 1989). Since then, somatic embryogenesis of tree species has expanded to encompass both conifer and hardwood species. Detailed examples of the application of somatic embryogenesis in woody plants can be found in Jain and others (1999).

#### Laboratory Steps

In general, the somatic embryogenesis process is divided into several laboratory steps that are performed under sterile conditions to prevent microbial contamination.

**Culture Initiation**—Mature zygotic embryos are dissected from seeds and placed onto semi-solid medium containing plant growth regulators.

**Proliferation**—Maintenance of 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 is followed by a multiplication step in which the tissue multiplies and develops as early-stage somatic embryos. Embryogenic cultures can be proliferated in a juvenile form for long periods of time to produce unlimited numbers of propagules from the same variety. Tissue can then be allowed to continue to grow, or it can be placed into long-term storage.

 $\label{eq:cryopreservation} \begin{array}{l} - Cryopreservation \ is \ a \ means \\ \ whereby germplasm can be stored. The embryogenic tissue \\ \ is treated with cryoprotectants, frozen to -35 °C (-31 °F) \\ \ under controlled freezing rate, and subsequently stored in \\ \ liquid nitrogen (-196 °C [-321 °F]). Cryopreserved tissue can \\ \ be stored indefinitely, and regenerated within a few weeks \\ \ after a simple thawing process. This long-term storage option offers a distinct advantage of somatic embryogenesis \\ \ tissue culture over rooted cuttings and organogenesis tissue culture. \\ \end{array}$ 

**Maturation**—Maturation advances the development of somatic embryos by exposing tissue to phyto-hormones and controlled environmental conditions. Within a period of a few months, they are transformed into mature somatic embryos that are analogous to zygotic embryos.

*In vitro* Germination—The final lab step, *in vitro* germination, takes place when embryos are placed on germination medium under controlled environmental conditions. *In vitro* germination occurs within a week and proceeds to the development of true needles. Somatic germinants can then be transferred to *ex vitro* nursery conditions.

#### **Nursery Production**

After somatic germinants are transferred to the nursery, they can be treated with cultural practices that are comparable to rooted cuttings (Dole and Gibson 2006). Once somatic germinants become established as young somatic seedlings, they are grown under standard seedling practices used by the forest seedling nursery industry.

# Seedling Quality and Somatic Seedling Stocktypes _____

Beginning in the early 1990s, it was important to show the forest industry that somatic embryogenesis technology could produce high-quality somatic seedlings in the nursery that would become established and grow rapidly after outplanting. As with any new technology, forest practitioners were skeptical that this tissue culture technology would provide seedlings with the desired benefits reported by the biotechnology industry. New seedling quality approaches were therefore, developed to provide foresters with enough information to understand how tissue culture technology could produce quality seedlings for outplanting. A program was initiated at the Forest Biotechnology Centre to develop a comprehensive seedling quality assessment procedure that "Measured the Product" to show the forest industry that seedlings produced from tissue culture practices would be a positive improvement for their reforestation programs.

#### **Measuring the Product**

Seedling quality assessment is based on the need for a better understanding of performance capabilities of nursery seedlings for outplanting on reforestation sites. Wakeley (1954) is usually recognized as the first person to identify the importance of morphological and physiological grading of seedlings prior to outplanting. As this concept began to take hold in the forest industry, seedling quality was defined as "fitness for purpose" (Lavender and others 1980) as it relates to achieving specific silvicultural objectives. Throughout the 1980s and 1990s, seedling quality assessment evolved to include both morphological and physiological tests (for example, Sutton 1979; Ritchie 1984; Duryea 1985a; Glerum 1988; Lavender 1988; Johnson and Cline 1991; Puttonen 1997). These seedling quality assessment procedures encompass both nursery development ("Monitor the Process"), and testing immediately before outplanting to determine probable field survival and/or field performance ("Measure the Product").

Conceptual Approach-Seedling performance on a reforestation site depends on the inherent growth potential and the degree to which environmental conditions of the field site allow growth potential to be expressed (Grossnickle 2000). The degree to which a seedling can adapt to site conditions immediately after outplanting influences its growth on the reforestation site (Burdett 1983). To determine the field performance potential, seedlings should be assessed in relation to anticipated environmental conditions at the site (Sutton 1982, 1988; Duryea 1985b; Grossnickle and others 1988, 1991a; Puttonen 1989; Hawkins and Binder 1990). Determination of seedling quality combines measurements of seedling properties that have been defined as material (that is, measure of a seedling subsystem) and performance (that is, subjecting whole seedlings to test conditions) attributes (Ritchie 1984). An array of morphological and physiological tests that examine factors important for determining field performance potential is required because seedling quality reflects the expression of a multitude of physiological and morphological attributes. Results from testing programs could be integrated to develop a means of expressing the overall physiological and morphological quality of seedlings (Grossnickle and others 1991b). An array of tests that simulate anticipated field environmental conditions would help forecast seedling physiological performance and potential for survival and growth on a reforestation site (Grossnickle and Folk 1993; Folk and Grossnickle 1997).

**The Stocktype**—An assessment of seedling performance was conducted in the early years of somatic embryogenesis technology for interior spruce (*Picea glauca* (Moench) Voss X *Picea engelmannii* Parry ex. Engelm.). This work indicated that somatic seedlings were smaller than zygotic seedlings, and this was related to the timing of integrating the tissue culture germinants into the nursery (Grossnickle and others 1994). The seedling quality testing program found that zygotic seedlings (because of their overall larger size) had better performance potential if they were to be outplanted on reforestation sites having optimum environmental conditions (Figure 1). On the other hand, zygotic and somatic seedlings had comparable performance potential if they were to be outplanted on sites under limiting cold (Figure 1) or drought conditions (Grossnickle and Major 1994a).

A subsequent field trial found that somatic seedlings could become successfully established on boreal reforestation sites (Grossnickle and Major 1994b), and had comparable physiological performance to zygotic seedlings in relation to reforestation site environmental conditions (that is, in both summer and winter). Both of these stocktypes had comparable growth over two field seasons, although the larger initial size of zygotic seedlings was still evident after 2 years. This trial work showed that somatic seedlings had comparable performance to zygotic seedlings at the nursery, in a seedling quality testing program, and in the field. It was felt that as long as the issue surrounding the timing of nursery planting could be rectified, this technology had the capability to produce high quality somatic seedlings that met the required standards of an operationally acceptable seedling.

During the mid 1990s, somatic embryogenesis technology improved and the Forest Biotechnology Centre developed the capability to produce somatic seedling crops of 350,000 seedlings (Grossnickle and others 1996). Trials conducted on somatic seedlings by other research groups during this timeframe found no major differences in either physiological



**Figure 1.** Performance potential index of interior spruce seedlings and emblings under optimum and cold reforestation site environmental conditions (from Grossnickle and Major 1994a).

or morphological attributes of spruce stocktypes produced through somatic embryogenesis technology compared to zygotic seedlings (Nsangou and Greenwood 1998; Lamhamedi and others 2000). Further testing of somatic spruce seedlings from the Forest Biotechnology Centre found that these seedlings met all of the criteria for an operationally acceptable seedling in British Columbia (Figure 2). This step was critical for the acceptance of somatic seedlings into plantation forestry programs requiring comparable performance to zygotic seedlings of similar genetic quality (Menzies and Aimers-Halliday 2004).

**Varietal Performance**—Testing field performance potential at the varietal level is required to determine varietal differences in somatic crops. This propagation procedure may lend itself to the selection of varieties with desired growth and stress resistance traits (Lamhamedi and others 2000). As with stocktype testing, this type of seedling quality testing was conducted with reference to environmental conditions of the outplanting site to get a more representative understanding of field performance capability (Grossnickle and Folk 1993; Folk and Grossnickle 1997).

A seedling quality program was conducted to determine the field performance potential for an array of varieties that made up an interior spruce somatic seedlot (Grossnickle and Folk 2007). The somatic seedlot was composed of 34 varieties from 12 full-sib families (one to six varieties per family). This somatic seedlot was the first of its kind to be registered for deployment in the Prince George seed zone in British Columbia during spring 1999 (seedlot number V4023), and to meet vegetative deployment guidelines regulated by the British Columbia Ministry of Forests. The somatic seedlot tested in this seedling quality assessment program met the operational criteria for a viable interior spruce seedlot that could be outplanted in reforestation programs in British Columbia (Grossnickle and Folk 2005). Field performance potential testing indicated that the 34 varieties comprising the somatic seedlot had a wide range of performance for measured parameters under optimum, nutrient-poor, cold, or drought conditions that simulated reforestation site conditions (Grossnickle and Folk 2007). Examples of the range of varietal performance are shown in Figure 3 in the shoot-growth potential under spring environmental conditions, as well as the varietal response of new shoots to a spring frost. This breadth of varietal performance is valuable to forest managers involved with varietal deployment and reforestation planning for two reasons: 1) it allows for the development of somatic seedlots with attributes for potential site conditions, with the possibility of field testing to verify findings from seedling quality tests; and 2) it ensures that somatic seedlots can be developed with a wide enough genetic base to minimize the vulnerability of plantations to environmental stress. This somatic seedlot approach for varietal deployment would allow for resilience to environmental stress and yet still confer benefits of clonal forestry.

#### **Measuring the Process**

During the past decade, significant progress has been made towards developing reliable, high-volume, cost-effective somatic embryogenesis production systems that can produce millions of seedlings. CellFor has begun to commercialize the production of loblolly pine (*Pinus taeda*) somatic seedlings (Grossnickle and Pait 2007). This has led to somatic seedling propagation technology being successfully integrated into both bareroot and container seedling production systems (Figure 4). This integration into standard nursery production systems has resulted in somatic seedlings consistently meeting required morphological standards for



**Figure 2.** Height, diameter, and root growth capacity of somatic (S) and zygotic (Z) seedlots of interior white spruce (1 cm = 10 mm = 0.4 in). Columns topped with a different letter indicate significantly different seedlots; errors bars indicate ± 1 standard error (Grossnickle and Folk 2005).





**Figure 3.** Varietal performance of a somatic seedlot (seedlot number V4023) to low soil temperatures in spring (that is, 10 °C [50 °F]) and frost events (new shoots exposed to -4 °C [25 °F]) that can typically occur on boreal reforestation sites (Grossnickle 2000). Measurements shown are shoot growth capacity (1 cm = 0.4 in) and freezing tolerance, with *P*-value for the performance difference between varieties (from Grossnickle and Folk 2007).

seedling production. In addition, reforestation site trials have tested the field performance of loblolly pine somatic seedlings and found these seedlings to have all of the traits that are desired in seedlings for use in forest regeneration programs (Figure 5).

# Application of Plant Quality Control in Industry _____

Commercial implementation of a novel technology, such as somatic embryogenesis, requires the ability to develop and implement a successful operational nursery production program. In addition, the quality of somatic seedlings produced during the nursery program needs to be monitored to ensure that they meet the required standards for a shippable seedling.

#### Understand Species Performance Capabilities

Each tree species has its own unique pattern of physiological response to environmental conditions. It must be recognized that environmental conditions change daily, seasonally, and yearly. Each species shows a specific physiological response pattern to these changing atmospheric (light, humidity, temperature) and edaphic (temperature, water, fertility) conditions throughout the year. Their physiological performance in response to the environment ultimately determines subsequent performance under nursery conditions. To develop an effective seedling monitoring program, the nursery needs to understand how species respond to cultural conditions. If growers understand the physiological response of a species to environmental conditions, they can create cultural



**Figure 4.** Loblolly pine somatic seedling nursery production of CellFor Incorporated: a) bareroot seedlings (Plum Creek Nursery, Jesup, GA; b) container seedlings (International Forest Company, Moultrie, GA).



**Figure 5.** Field performance of CellFor Incorporated bareroot loblolly pine seedlings growing on a reforestation site: a) 7 months following outplanting; b) 21 months following outplanting.

guidelines, or standard operating procedures, that will be the cultural plan of how to grow a quality seedling crop.

#### **Define the Process**

In growing a quality seedling crop, each step of the growing process must be defined and cultural guidelines developed. The use of these guidelines to define the cultural process in detail ensures that the growing of a quality crop can be repeated with each production season. This approach fits into the International Organization for Standardization (ISO) Quality Assurance program that is built on the principle of a controlled and consistent approach for the production of a product to ensure the effective operation of a program (Anonymous 2002). Within a nursery program, this means defining cultural practices related to planting, growing, integrated pest management, consolidation, hardening, storage, handling, and shipping in an easy-to-use format that can be quickly read and applied by growers and all other critical nursery personnel.

#### **Monitor the Process**

Once the cultural plan has been developed, it is important to track the crop to ensure the agreed-upon cultural guidelines are followed and a quality crop is grown. Monitoring the cultural process is necessary to ensure the crop is growing according to the crop plan. The ISO Quality Assurance program requires monitoring the production process to ensure achievement of the planned results (Anonymous 2002). The process for monitoring the production of seedling crops falls into three major areas of activity. **Tracking the Crop Environment**—Both optimum and limiting environmental conditions for crop performance need to be defined, and methods need to be developed to track the environment in real time. The capability to synthesize environmental information also has to be present so seasonal changes can be easily tracked and any deviations from the recommended environmental conditions defined in the cultural guidelines.

**Tracking the Plant Performance** — Important points in the development process of the crop must be defined. It is also important to select critical morphological and physiological parameters that give the grower a good understanding of the crop performance. Tools must be built that allow one to easily follow the plant performance throughout the entire crop cycle. An end-of-crop assessment needs to be conducted so shippable seedlings consistently meet morphological standards required for production of operational bareroot or container seedlings (that is, a simplified, one-time event of "Measuring the Product").

**Crop Diary**—A crop diary is required that defines operational and cultural adjustments to the crop plan. As with any plan to grow seedlings, the combination of seasonal environmental conditions, equipment capabilities and breakdowns, plus the "human factor" can result in deviations from the crop plan. These deviations need to be recorded so that when a crop review is conducted, one can understand where adjustments to cultural practices need to be refined to improve performance in future crop production cycles.

#### **Read and Respond to Signs**

Information on crop performance will not help in producing a quality crop unless there is a system in place to respond when the crop begins to deviate from the crop plan. To avoid deviation that can result in crop losses, the quality monitoring program needs to follow a number of simple steps: 1) weekly benchmarks for data collection must be established; 2) data must be tracked continuously and synthesized rapidly in an easy to read format; and 3) there needs to be a system for transferring incident reports into operational action plans.

#### Learn from The Past

One must continue to learn from the past to ensure that the quality of seedlings being produced improves with each production cycle. The quality system needs to track crop cycles and define the good, bad, and ugly patterns of crop performance. This information then needs to be synthesized from across crops and seasons to define poor crop performance patterns that need to be eliminated. In addition, there needs to be an integration of beneficial cultural practices into the cultural guidelines. In this way, the quality assurance program becomes a positive system of change and continued improvement in the cultural practices used to produce seedlings.

## Conclusions

In the early years of seedling quality programs designed to measure the performance of seedlings produced from somatic embryogenesis programs, the focus was on measuring the final product of the crop production cycle. This approach was important when validating a "Proof of Concept" for this new seedling product that needed to gain acceptance within the forest industry. Seedling quality systems have evolved as high-volume, cost-effective somatic embryogenesis production systems and have grown to the point of producing millions of somatic seedlings. Within a large scale production setting, a seedling quality program must focus on three central themes: 1) understand the species performance capabilities; 2) define the process to successfully grow plant material; and 3) monitor the process to ensure that quality somatic seedlings are being produced within every production cycle. This approach is applicable to the production of all commercial seedling crops, and it ensures that a nursery produces the best quality crop that meets the objective of quality seedlings for forest regeneration programs.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

## **Outplanting Strategies—GRO TRZ Consulting Incorporated**

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Farquharson D. 2011. Outplanting strategies—GRO TRZ Consulting Incorporated. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 147-149. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p065.html

Keywords: forest type, stocktype, planting methods

#### Introduction ____

The challenge for a silviculturist is the creation of a seedling microsite that is favorable enough for the seedling to not only survive, but thrive. The silviculturist must do this without irrigation, heat, glass, plastic, or daily monitoring and management. The silviculturist does this by understanding the needs of the forest seedlings and the shortcomings of the reforestation site and by using management techniques to bring them closer together.

There are, in fact, only four main things that young seedlings need: nutrition (food), water, soil, and sunlight. A good supply of these, in keeping with seedling needs, will ensure that the roots of the young seedling will grow well. If the roots grow well, the top will also grow well, as the top is largely a product of the roots. When the above-ground portion of our young seedling is healthy and growing well, it is able to better overcome the injuries and challenges it encounters.

With our management activities that lead up to outplanting seedlings, we are working to create a balance of soil air, water, and nutrition so seedling roots will grow quickly. At the same time, our management activities are designed to protect seedlings by minimizing or eliminating challenges we expect them to encounter, such as vegetation competition, root rots, snow creep, cattle damage, and occasional animal feeding (voles, rabbits, and deer).

#### Wet Forests ____

#### Challenge

Wet forests often have too much soil water and not enough soil air (micro-pore versus macro-pore balance).

#### Goal

Overcoming the soil pore imbalance requires creating a better balance of soil water and soil air in the planting microsite, resulting in improved seedling root egress.

#### **Management Alternatives**

- 1. Plant with shorter plug stock, such as Styroblock[™] 410 (10.4 cm [4.1 in] length) or 412 (11.7 cm [4.6 in] length) containers.
- 2. Plant raised microsites.
- 3. Reduce tree spacing to allow clumpy planting of raised microsites.
- 4. Plant undisturbed organic F- and H-layer microsites and set seedlings with a small amount of compaction of the organic soil.
- 5. Plant mineral/organic mixed soil raised microsites created by an excavator if the soil is not too wet.
- 6. Plant with western redcedar (*Thuja plicata*) or interior spruce (*Picea glauca* (Moench) Voss X *Picea engelmannii* Parry ex. Engelm.).

## Cold Forests_

#### Challenge

Cold forests often have too much soil water and not enough soil air in a cool climate, resulting in soils that are too cold for good root growth.

#### Goal

Improving the soil water/soil air balance will enable air to penetrate the planting microsite more easily and warm the planting soil.

#### **Management Alternatives**

- 1. Use excavator site preparation to create "raised screefs" made up of upper (more fertile) soil horizons (F, H, A, and B). Incorporating the organics with the mineral soil helps to aerate the soil as well.
- 2. Plant in late spring (mid June) or fall (mid September). Soil during summer planting (mid July) is often too dry, resulting in no root growth until the fall moisture arrives.
- 3. Plant interior spruce in shorter stock sizes, such as seedlings grown in Styroblock[™] 410 (10.4 cm [4.1 in] length) or 412 (11.7 cm [4.6 in] length) containers, to keep the roots in the preferred warmer, shallower soils.

## Heavy Snow Forests _____

#### Challenge

Physical damage can result from heavy snow and snow creep. Slow growth can be the result of cold, wet soils.

#### Goal

Planting seedlings with shelter may make them more robust before they experience snow creep. This will also improve the growing conditions so that the seedlings will grow faster and be stronger, enabling them to better repair themselves when they are damaged.

#### **Management Alternatives**

- Plant larger 2-year old stock, such as seedlings grown in Styroblock[™] 512 (220 cm³ [13.4 in³] volume) or 615 (336 cm³ [20.5 in³] volume) containers, and fertilize them (usually interior spruce).
- 2. Obstacle plant as many seedlings as possible beside stumps and downslope of anchored logs. Peel the bark off stumps adjacent to seedlings so the bark does not fall off onto seedlings.
- 3. If possible, use excavator site preparation to create raised screefs and place logs for planting obstacles.
- 4. Plant in late spring (mid June).

## **Rich Moist Forests**

#### Challenge

Heavy vegetation competition can result in physical damage from vegetation pressing the seedling to the ground in fall and winter. Seedlings may not be able to straighten up again the next spring. The lack of sunlight and, therefore, food resources will affect seedling form, and may reduce health and vigor.

#### Goal

Forecasting the type, extent, and timing of the vegetation competition can allow appropriate pre-emptive actions to be taken to reduce the vegetation competition. Reducing vegetation competition when it does develop will reduce the adverse effects on the young tree.

#### **Management Alternatives**

- 1. Site prepare the cut block as appropriate (depending on the type of vegetation competition complex expected) to improve the growth performance of the seedlings and reduce the influence of the vegetation competition for a period of time (a few years or more). Excavator-raised screefs will reduce the competition for a few years; the excavator can also root out vigorous shrubs when necessary.
- 2. Plant promptly prior to establishment of the competing vegetation.

- Plant larger stock, such as seedlings grown in Styroblock[™] 412 (125 cm³ [7.6 in³] volume), 512 (220 cm³ [13.4 in³] volume), or 615 (336 cm³ [20.5 in³] volume) containers, and fertilize them to improve strength, health, and performance.
- 4. Plant seedlings downslope of obstacles (stumps and logs) so seedlings are protected from upslope herbs and shrubs falling down onto them.
- 5. Herbicide or brush to release the seedlings and young crop trees from the competition. Implement appropriate strategies for each so as to be effective with the treatment while minimizing herbicide use.

## **Droughty Forests**

#### Challenge

A lack of moisture available to the seedlings during their sensitive establishment years can lead to mortality. As seedlings grow, their moisture demand increases; in a droughty year, moisture demand may exceed the supply, resulting in mortality.

#### Goal

Drought-challenged forest areas need to be identified prior to reforestation, and a strategy to establish and grow the best young forest possible needs to be implemented by planting into larger soil pockets or reducing moisture competition.

#### **Management Alternatives**

- 1. Ensure that the stocking expectation is reasonable for the degree of drought that the site will experience.
- Order planting stock grown in Styroblock[™] 410(10.4 cm [4.1 in] length) containers to facilitate proper planting in shallow soils.
- 3. Site prepare the area as possible to reduce moisture competition to the seedlings.
- 4. Teach planters where to look for deeper pockets of soil in rocky areas.

- 5. Plant as early as possible in the planting window to allow the longest moist period possible for seedlings to establish.
- 6. Do not replant the area unless you are sure that you made a mistake that led to the observed mortality; otherwise it will happen again.

## Douglas-fir Forest Type

#### Challenge

Interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) is challenging to reestablish and more costly than other local species; at the same time, it is a very common and relatively valuable species. It is also considered to be a keystone species for our region in the face of expected climate change.

#### Goal

Effective re-establishment of interior Douglas-fir and reduction of cost has become a goal in this forest type.

#### **Management Alternatives**

- 1. Some site preparation seems to help. The selection of planting locations with ample soil fertility, soil porosity, and soil moisture appear to be very important.
- 2. Douglas-fir reestablishment seems to be more effective with larger stocktypes, for example, seedlings grown in Styroblock[™] 412 (125 cm³ [7.6 in³] volume) containers.
- 3. Tea bag fertilization at the time of planting also helps. It is especially noticeable on the moist and wet areas. We use high nitrogen mixes with elevated levels of sulphur; our lack of natural sulphur restricts the utilization of nitrogen.
- 4. Manage competition throughout the establishment phase, until they are around 1.3 m (4.3 ft) tall. Once seedlings have reached this target, they seem to become more resilient and able to better deal with challenges.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# Target Seedling Strategies for Intensively Managed Douglas-fir Plantations in the Oregon Coast Range

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Wall M. 2011. Target seedling strategies for intensively managed Douglas-fir plantations in the Oregon Coast Range. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 150-156. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p065.html

**Abstract:** The target Douglas-fir seedling for outplanting on Roseburg Resources Company timberlands is a least-cost, large [stem] caliper 1- to 2-year old bareroot (>8 mm) or container (>6 mm) seedling with good form, high root growth potential, and the ability to withstand browse without the use of browse deterrents. This target is achieved through the use of large cell transplant plugs and low density spring or fall transplanting, or large cell low density container seedlings for outplanting.

Keywords: container seedlings, bareroot seedlings, target specifications, browse resistance

## **Roseburg Resources Company Timberlands**

Roseburg Resources Company (RRC) timberlands are located between Eugene, OR, and Roseburg, OR, and extend west to the coast. The company has been privately owned for more than 75 years, and sustainably manages approximately 190,00 ha (470,000 ac) of high quality timberlands in Oregon. The 50-year site indices on these lands range from 27 to 43 m (90 to 140 ft), with an average site index of 36 m (120 ft). Douglas-fir (*Pseudotsuga menziesii*) is the dominant tree harvested and planted on company lands. The emphasis of this discussion is on seedling strategies RRC employs on its higher site lands (>33 m [>110 ft]). These lands comprise the majority of RRC ownership. Different strategies are employed on the lower site timberlands that are touched on but not addressed in detail in this discussion.

## Target Seedlings _

RRC considers the target Douglas-fir seedling for outplanting to be a least-cost, large [stem] caliper 1- to 2-year old bareroot or 1- to 2-year old container seedling with good form, that is, good branch architecture, well-balanced fibrous roots with high root growth potential, and the ability to withstand browse without the use of browse deterrents.

#### Large Bareroot Seedlings

The target specifications for bareroot seedlings include calipers that are greater than 10 mm (0.4 in), with a minimum of 8 mm (0.3 in), and heights of 60 to 90 cm (24 to 36 in). On lower site index lands, the maximum seedling heights are usually reduced to 50 to 60 cm (20 to 24 in) with slightly lower minimum calipers.

#### **Base Stock**

1+0 Bareroot Seedlings—Although bareroot seedling culture is considered "old technology" by many foresters, it continues to have several advantages for use as stock designated for transplanting. Currently, bareroot 1+0 seedlings are relatively inexpensive to produce, costing approximately US\$ 110/thousand seedlings. In addition, field sowing usually results in

overruns, which can be advantageous when needs change on short notice and extra seedlings can be transplanted to supplement existing orders. Finally, the ordering process is simple because you are dealing with one nursery and one contract for all phases of seedling production.

Using 1+0 bareroot seedlings for base stock can confer some disadvantages. Direct sowing into seedbeds is often an inefficient use of seeds, since sowing calculations factor in higher losses than those found in greenhouse container culture. The resulting seedlings have a wide variety of both shoot and root sizes, so it is often difficult to achieve consistently large sized 1+0 seedlings from seedbed production. As well, seedlings in the seedbeds are susceptible to early frosts in the fall, and the lifting schedule is totally dependent on the weather. This last issue is becoming more of a concern with the widespread deployment of high gain orchard seeds that seem to be less tolerant of early freeze events in the fall.

*Container Seedlings*—Growing container seedlings destined for transplanting allows for very efficient use of seeds, with single and fractional sowing becoming more prevalent for the highest gain and most valuable orchard seeds. Efficient seed use not only lowers the overall cost per seedling, but also allows for broader deployment of valuable seeds. Growing transplant seedlings in containers can also result in a more uniform product that can be custom grown to a variety of target specifications, thereby providing the basis for larger more uniform transplant stock. If container seedlings are targeted for spring transplanting, they can easily be frost protected in the fall by remaining in, or being moved back into, greenhouses. In addition, lifting of these seedlings is not subject to weather delays.

Container transplant seedlings, however, are more expensive than bareroot transplant seedlings, ranging from US\$ 150 to 180/thousand seedlings. Logistical considerations can also be an issue if the base stock is grown at one nursery and designated for transplanting at another nursery. One of the big disadvantages to container stock in the US Pacific Northwest has been the limited opportunity for growing the stock in the region. Ten years ago, stock had to be ordered from container nurseries in Canada. The situation has changed in recent years, with more container production now occurring in the US Pacific Northwest.

Container transplant seedlings have become the preferred RRC base stock for outplanting—they currently account for almost 95% of base stock production. One primary reason is the consistency in producing large P+1 bareroot seedlings. Another reason is the flexibility in seasonal transplanting. Although most seedlings are transplanted in the spring, fall transplanting is also used for larger stock needs. In this case, the use of container seedlings for base stock makes fall transplanting more feasible with better yields over comparable 1+0 transplants. The preference for container transplants is not only due to the size but also because the bareroot nurseries that were best capable of producing a large fall 1+1 transplant seedling have gone out of business; additionally, the remaining nurseries struggle to produce 1+0 seedlings of the size required for large bareroot stock.

The justification for the use of more expensive container stock can be based almost solely on the cost of seeds (Table 1).

Table 1. Justification for cost of container versus bareroot base stock for transplant production
(All costs are in US $1 lb = 0.45 kg$ )

	Imp		
Stocktype	Styroblock™ 410A	Styroblock™ 411A	1+0
Order Amount	100,000	100,000	100,000
Cost of Seeds (\$/lb)	1500	1500	1500
Germination Rate	90%	90%	90%
Seeds/lb	36,000	36,000	36,000
Oversow Factor	1.15	1.15	2.2
Seeds/Cavity	1.2	1.2	n/a
Pounds of Seeds Required	3.8	3.8	6.1
Base Stock Cost/thousand (\$)	180	150	110
Total Cost (\$)	23,750	20,750	20,167
Cost/thousand (\$)	238	208	202
P+1/1+1 Final Yield	95%	95%	100%
Net Cost (\$)	250	218	202
	Woo	ds Run Seeds	
Stocktype	Styroblock™ 410A	Styroblock™ 411A	1+0
Order Amount	100,000	100,000	100,000
Cost of Seeds (\$/lb)	60	60	60
Germination Rate	80%	80%	80%
Seeds/lb	36,000	36,000	36,000
Oversow Factor	1.25	1.25	2.3
Seeds/Cavity	1.5	1.5	n/a
Pounds of Seeds Required	5.2	5.2	6.4
Base Stock Cost/thousand (\$)	180	150	110
Total Cost (\$)	18,313	15,313	11,383
Cost/thousand (\$)	183	153	114
P+1/1+1 Final Yield	95%	95%	100%
Net Cost (\$)	193	161	114

When woods-run seeds were used at a cost of approximately US\$ 133/kg (US\$ 60/lb), the nurseries could use all they needed for production. But with the cost of improved seeds exceeding US\$ 3300/kg (US\$ 1500/lb), efficient use of seeds becomes a critical part of the cost consideration when trying to figure out where to grow seedlings and at what cost. It is essential that foresters incorporate seed cost into their evaluation of seedling costs, and not just simply compare one stocktype price to another. The opportunity cost of not being able to deploy your highest gain seeds over more acres due to differences in efficiency is an additional consideration that can be quantified, if necessary, without too much effort.

RRC base container stock is grown in several nurseries located in both the US Pacific Northwest and British Columbia, Canada. RRC initially started with Styroblock[®] 311A and 313B containers  $(45 \text{ cm}^3 [2.6 \text{ in}^3] \text{ and } 65 \text{ cm}^3 [3.9 \text{ in}^3])$ , but has, over time, migrated to Styroblock[®] 410A (Figure 1) and, more recently, to 411B containers (80 cm³ [4.9 in³]). The increasing costs of producing 410A containers (US\$ 180+/ thousand seedlings) has led RRC to start to transition to 411B containers (US\$ 150+/thousand seedlings). They have the same volume as the 410A, but contain more cavities per block (144 versus 112), therefore, lowering greenhouse costs. The initial seedling orders we have gotten with this new stocktype meet comparable target specifications of the 410A stocktype with only minor reductions in caliper and no change in height specifications. While current production includes an even mix of both cavity types, RRC expects to migrate more production to the 411B stocktype over time.

Low Density Transplanting – RRC was interested in the effects of low density transplanting on seedlings and what the trade-offs in cost were as a result. Traditional transplant densities range from 24 to 32 trees/bed foot using 5 to 6 row transplanters. Research conducted by RRC quantified the caliper increases as a result of dropping transplant densities to as low as 12 trees/bed foot. As expected, there were diminishing returns as density was reduced. RRC concluded that when 410A/411B containers were used for base stock, a transplant density of 14 to 17 trees/bed foot provided the optimum density, and best value, target bareroot seedling.

Lower densities do increase seedling costs due to the increase in the amount of bed space needed. For example, a transplant cost of US\$ 200/thousand seedlings for 24 seedlings/bed foot would cost in excess of US\$ 340/thousand seedlings for 14 seedlings/bed foot. A transplant density of 14 to 17 seedlings/bed foot results in a final cost of US\$ 0.45 to 0.50/seedling depending on where the base stock is grown and which transplant nursery is used.

**Root Pruning**—The standard industry specification for root pruning of base stock is 20 cm (8 in). Because the rootto-shoot ratio needs to be considered, larger stock requires more roots. RRC, therefore, requires a 25 cm (10 in), or even 30 cm (12 in), root prune. One key point in ordering base stock, or any seedlings, is to be certain whether the measurement is taken at the root collar or at the first limb. The root collar can be an ambiguous point on the seedling; this is why RRC prefers to use the first live limb as the point of measurement.





Figure 1. Styroblock™ 410A large-cell container stock. Target speci-

fications for this transplant base stock are 3.5 mm caliper and 20 to

25 cm (8 to 10 in) height.



**Quantified Results for Large Bareroot Seedlings**— A trial based on initial caliper class at the time of outplanting was initiated in 1999. A total of 2000 seedlings were outplanted in 4 replications. The first of 4 replicates (500 seedlings) have recently been measured. Seedling performance was measured by calculating final tree volume and plotted it against initial caliper class (Figure 2). Our initial analysis shows that larger caliper class seedlings increased in volume at a faster rate than smaller seedlings, which supports our strategy of outplanting larger stock. The remaining replicates will be measured in the winter of 2011.

#### Large Container Stock for Outplanting

In 1999, RRC started experimenting with ouplanting large container stock, that is Styroblock[®] 515A (250 cm³ [15.3 in³]). Both root growth and field performance of these larger seedlings were encouraging, but browse resistance was a big concern. The initial assessment indicated there was not enough wood present to withstand ungulate browsing, and that seedling caliper was too small to survive mountain beavers. Working with several Canadian nurseries, the full range of large-sized plugs, from 615A (336 cm³ [20.5 in³]) through 1015A (1000 cm³ [61 in³]), were tested (Figure 3). As with bareroot seedlings, there are diminishing returns

as the densities in the blocks were lowered from the 515A (60 seedlings/block) to the 1015A (15 seedlings/block). If cost were not an issue, the 1015A plug would be preferred, but because seedlings cost US\$ 1.00+ each and transport logistics are difficult, use of these seedlings operationally is not realistic. Our current target specifications for higher site index, Douglas-fir container seedlings are a 5 mm minimum caliper (greater than 7 mm preferred) and height between 40 to 60 cm (16 to 24 in).

As with the bareroot stocktype, a container stocktype trial using initial caliper class at the time of outplanting was initiated in 1999. Seedling performance was measured by calculating final tree volume and plotting it against initial caliper class (Figure 4). Not surprisingly, the trend shows larger caliper class seedlings increasing in volume faster than the smaller seedlings.

**Browse Resistance**—The amount of browse resistance afforded by larger-sized plugs was surprising. In 2006, a trial to examine the effects of container size on browse resistance was established in an area known for heavy deer and elk browsing. Seedling volume (in cm³) was calculated at the end of the second growing season (Figure 5). Our data indicated larger plugs were more resistant to browse and were comparable to the 1+1 fall transplant seedlings from Humboldt Nursery that were used as a control.



Figure 2. Initial results from 1999 bareroot seedling outplanting study.



Figure 3. A) Douglas-fir seedling grown in a Styroblock[™] 815A; B) western redcedar (*Thuja plicata*) grown in a Styroblock[™] 1015A.



Figure 4. Initial results from 1999 container seedling outplanting study.

#### 2006 BROWSE RESISTANCE CONTAINER STOCK TRIAL YR 2 VOLUMES



Figure 5. Results from 2006 browse resistance outplanting study.

**Current Strategies**—Bareroot nursery capacity in the Pacific Northwest has been severely constrained due to nursery closures and impending regulations on the use of fumigants. The fumigation issue will increase bareroot costs due to the higher cost of alternatives, increased hand weeding, reduced yields, and reduced nursery capacity. The price differential and performance between large bareroot seedlings and comparable container stock is now closing quickly.

The use of large container stock for outplanting has both positive and negative economic considerations. On the positive side, green-up and adjacency requirements for harvesting are more easily met (Table 2); the need for browse deterrents is reduced; rotations can be shortened; and improved survival leads to fewer trees per acre planted and fewer acres that need to be replanted. One of the biggest negative economic impacts, however, involves logistical challenges. These challenges include: 1) coordination between nurseries with trucking schedules, handling schedules, and payment; 2) increased cooler space needs, with most cooler capacities based on 120+ seedlings/bag, and large stock packed at 40 to 80 seedlings/box; 3) field delivery considerations, including use of trailers and load capacity of delivery pickup trucks; and 4) planting tool considerations and getting trees onto the site. All of these logistical issues can result in increased seedling costs, planting costs, and transportation costs.

Table 2. The economic advantages of outplanting large container store	ck include
meeting green-up and adjacency requirements for harve	sting and
shortened timber rotations with greater ease. (All costs ar	e in US \$;
1 ac = 0.4 ha.)	

	Adjacency consideration scenario #1	Adjacency consideration scenario #2
Adjacent Stand Acres	120	40
Volume/acre	3030	
Stumpage Value (\$/m)	350	350
Total Value (\$)	1,260.00	420,000.00
Cost of Money	6%	6%
Cost of Holding 1 More Year (\$)	75,600	25,200
Plantation Acres	120	120
Small Stock Regime Cost/ac (\$)	551	551
Large Stock Regime Cost/ac (\$)	609	609
Difference (\$)	58	58
Total Cost Difference (\$)	6960	6960
Savings (\$)	68,640	18,240
Break-even Cost/Seedling Cost (\$)	1730	680

While RRC continues to experiment with larger container stock, the current focus is 1- and 2-year old seedlings grown in Styroblock[®] 615A systems ((336 cm³ [20.5 in³] at 45 cavities/block), and 2-year old seedlings grown in Styroblock[®] 815C systems ((440 cm³ [26.9 in³] at 35 cavities/block). RRC will order bareroot seedlings (1+1 or P+1) to meet 70% to 75% of anticipated needs 2 years out, then supplement this initial order with container seedling orders 12 to 18 months out as overall seedling needs become more apparent. This strategy also provides the opportunity to target the highest gain seedlots into container stocktypes to maximize seed efficiency and deployment acres.

The current practice of growing large 2+0P container stock has resulted in better sun versus shade needle development, better bud development, improved seedling hardiness earlier in the growing season, better caliper, improved root system, and more lignified stems and laterals to provide better browse resistance.

#### Summary

The lessons learned by RRC during the last decade have enabled the company to dramatically improve seedling outplanting survival and growth on its high site timberlands. The synergy of large stock, genetically improved seeds, and intensive site prep has thus far been impressive. The downstream cost savings of large planting stock more than overcome the initial extra costs and should be included in any discussion about seedling costs. Consider, for example, how much you might spend on browse protection per acre because you are planting a small tree and how much more you could spend on a larger seedling if you eliminated the browse protection and invested those dollars in a larger seedling that can withstand the browse.

The take-home lesson is to get to know your seedling growers and be involved in the crop development. In other words, learn to custom grow seedlings to meet your needs. Do not necessarily settle for the status quo, experiment with your current practices, continually strive for improvement throughout the entire process.

The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

# **Intertribal Nursery Council Annual Meeting**

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## Shrub-Steppe Species Germination Trials and Survival after Outplanting on Bare Soils

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**Abstract:** Work has been initiated to restore native vegetation on the soil and base gravel layers that were once underneath constructed facilities at the Umatilla Chemical Depot (UMCD) in eastern Oregon. Propagules were collected from native plant species found around the UMCD. Germination success ranged from 0% to 75% for the species tested. Ten species were successfully propagated in sufficient numbers to use in an outplanting study to monitor species survival. After three growing seasons, survival ranged from 100% for *Opuntia polycantha* (pricklypear) to 5.6% for *Lupinus sericeus* (silky lupine) with average survival over all species at 50%. Further testing is needed to determine what species are best adapted to local environmental conditions.

Keywords: ecological restoration, shrub-steppe, germination

#### Introduction

As the Umatilla Chemical Depot (UMCD) in eastern Oregon is decommissioned, planning is currently underway to restore the associated disturbances. Returning ecosystems to a natural, self-sustaining condition is complex. In the semi-arid shrubsteppe region, little knowledge has been published on how to restore soil disturbances to ecologically sustainable conditions dominated by native plants.

Because a large number of disturbances will be associated with the removal of facilities underlain by gravel beds, this study focused on a restoration site with characteristics similar to the gravel beds under the facilities. The study site was an old parking lot that had the asphalt removed, leaving a substrate composed of a mixture of sand and gravel.

The primary study objective was to implement a trial restoration project and determine outplanting success after 3 years. The results will help land managers plan larger-scale ecological restoration on similar sites. Our trial included collection of native shrub-steppe seeds, germination tests, production of viable plants, site preparation, outplanting, and monitoring plant survival.

The expected survival rate for establishing container seedlings in western ranges and wildlands can be highly variable by species. Stevens (2004) notes that bitterbrush (*Purshia tridentata*) are expected to be successful in about 40% of containergrown stock plantings, lupine seedlings (*Lupinus* spp.) in about 60%, and yarrow (*Achillea millefolium*) and bunchgrass nearly 100%. Other than these species, little is known about the germination and survival of many shrub-steppe species following restoration outplanting. Therefore, a secondary objective of this study was to investigate survival differences among the chosen species. Also, in accordance with US Department of Army Regulation 200-3 for preparation and implementation of integrated natural resource management plans, a third objective of this study was to assist the US Army in implementing ecological restoration efforts at the UMCD.

#### Materials and Methods

# Species Identification, Seed Collection, and Processing

Background information on the types and distribution of plant species native to the study area (TTEM 2002) was reviewed to select the appropriate target plants. From 30 March through 16 September 2007, seeds from a subset of these native species were collected at the UMCD. Fruits and seeds were collected by hand and stored in paper bags until they were manually cleaned and processed at the laboratory using a series of screens and various tools. Ease of seed extraction was highly variable among species.

# Seed Germination Tests and Plant Propagation

Two types of germination tests were implemented. In the first, seeds of 22 species (Table 1) were germinated in Petri dishes before being transplanted into containers (test #1). Thirty seeds from each species (except where noted otherwise) were placed in Petri dishes between a layer of paper towel and filter paper. Seeds were wetted with 3 ml (0.1 oz) of distilled water, covered, and kept at a constant temperature of 20 °C (68 °F) in the dark for 3 days before being exposed to natural light (approximately 10 hours per day) at 20 °C (68 °F). Each Petri dish was checked daily for radical emergence and recorded. Following emergence, germinants were transplanted into containers and survival was monitored.

The second test investigated seed germination of 14 species sown directly into container tubes (test #2). Seeds were sown directly into 164 cc³ (10 in³) containers filled with wetted potting medium consisting of 45:45:5:5 potting soil:washed coarse sand:perlite:vermiculite (v:v). Sowing date, number of seeds, and germination percentage were recorded (Table 2).

 Table 1. Seed collection dates and germination data for target restoration species at the Umatilla Chemical Depot. Seeds were germinated and counted in Petri dishes prior to being transplanted into pots. Survival was measured following transplanting in 2007.

Scientific name	Common name	Collection date	Sow date	Collection location	Number of seeds/ pads sown	Germination (%)	Transplant survival (%)
Achillea millefolium	Yarrow	6/29	6/29	UAD NE	30	50	50
Antennaria dimorpha	Pussytoes	3/30	4/6	Gazebo	30	0	0
,	,	5/11	6/14	UAD	30	0	0
Astragalus succumbens	Columbia milkvetch	6/29	6/29	C-1114 & 5	30	13	0
Balsamorhiza sagittata	Arrowleaf balsamroot	6/11	6/14	UAD	30	13	0
C C		6/29	6/29	Gazebo	30	0	0
Chrysopsis villosa	False goldenaster	3/30	4/6	UAD2	30	10	3
2	C	9/16	9/16	Study Plots	30	10	7
Chrysothamnus viscidiflorus	Yellow rabbitbrush	3/30	4/6	UAD2	30	0	0
Crepis atribarba	Slender hawksbeard	6/11	6/14	UAD4	15	33	0
Crocidium multicaule	Common spring-gold	3/30	4/6	UAD1	30	0	0
Elymus elymoides	Squirreltail	6/11	6/14	UAD1	30	73	63
		6/29	6/29	D-1281	30	23	23
Epilobium brachycarpum	Willowherb	9/16	9/16	D-1284	30	0	0
Fritillaria pudica	Yellow fritillary	6/11	6/14	UAD2	10	0	0
Hesperostipa comata	Needle and thread grass	6/11	6/14	UAD	31	6	6
Lomatium macrocarpum	Bigseed biscuitroot	6/11	6/14	UAD4	14	14	0
	-	6/29	6/29	D-1278	30	0	0
Lupinus sericeus	Silky lupine	6/11	6/14	UAD3	12	75	42
		6/29	6/29	UAD NE	30	0	0
Oenothera pallida	Evening primrose	6/29	6/29	D-1277	30	0	0
Opuntia polyacantha	Pricklypear	3/30	4/4	UAD1	5	NA	100
		6/11	6/16	UAD1	17	NA	100
		6/29	7/3	D-1277	15	NA	93
Phlox longifolia	Longleaf phlox	6/11	6/14	UAD1	32	75	69
		6/11	6/14	UAD4	30	7	7
		6/11	6/14	UAD2	30	54	43
Plantago patagonica	Woolly plantain	6/29	6/29	D-1294	30	43	43
Poa secunda	Sandberg bluegrass	5/11	6/14	UAD	30	0	0
Poa secunda		5/15	6/14	UAD	30	0	0
Psoralea lanceolata	Lemon scurfpea	6/29	6/29	D-1278	30	0	0
Pseudoroegneria spicata	Bluebunch wheatgrass	6/29	6/29	Gazebo	30	57	57
Purshia tridentata	Bitterbrush	6/11	6/14	UAD1	30	67	13
		6/11	6/14	UAD4	30	10	10
Sporobolus cryptandrus	Sand dropseed	9/16	9/16	D-1283	30	0	0

Table 2. Sow date, seed co	ollection location,	and germination	success of	species sown	directly
into tubes conta	ining medium.				

Species	Sow date	Collection location	Number of seeds sown	Germination (%)
Achillea millefolium	6/29/07	UAD NE	189	76
Astragalus succumbens	6/29/07	C-1114&5	133	8
Chrysopsis villosa	9/16/07	Study Plots	90	56
Crepis atribarba	6/11/07	UAD4	4	75
Elymus elymoides	6/11/07	UAD1	231	85
Hesperostipa comata	6/11/07	UAD	147	27
omatium macrocarpum	6/11/07	UAD4	49	0
upinus sericeus	6/11/07	UAD3	84	27
Phlox longifolia	6/11/07	UAD1	98	65
Plantago patagonica	6/29/07	D-1294	175	69
Poa secunda	5/15/07	UAD	196	26
Pseudoroegneria spicata	6/29/07	Gazebo	245	38
Purshia tridentata	6/11/07	UAD1	147	61
Sporobolus cryptandrus	9/16/07	D-1283	30	0
		Total	1818	49

Once germinants became established in containers, they were fertilized weekly with liquid fertilizer  $(12N:4P_2O_5:8K_2O)$  at a rate of 1.3 ml/l (fertilizer/water; 150 ppm N) during August, September, and October 2007. Fertilizing was discontinued when nightly temperatures in the greenhouse dropped well below freezing.

Pricklypear (*Opuntia polycantha*) was the only species not propagated from seeds. Cactus pads were harvested from the field and hardened for 5 days prior to planting into containers.

1940s (Figure 1). The soil at the site is a Quincy loamy fine sand with a gravelly substratum and the vegetation community is Bitterbrush-Sandberg Bluegrass-Cheatgrass (TTEM 2002). The asphalt had been removed from the planting area during the last week of December 2007. The study area was 19.5 m (64.0 ft) long, 9.2 m (31.2 ft) wide on the north side, and 7.2 m (23.6 ft) wide on the south side (trapezoidal in shape). Six replicated study plots were established; each plot was 8.2 m (27.0 ft) long and 3 m (9.8 ft) wide, with a buffer strip about 0.3 m (1.0 ft) wide between each plot.

#### **Study Site and Preparation**

The study area is located on the Umatilla Chemical Depot site in Morrow and Umatilla counties in northeast Oregon. The trial was conducted on an old parking lot built in the

#### Outplanting

A total of 508 plants of ten species were outplanted during 4 days in January and February 2008 (Table 3). Within each plot, seedlings were outplanted in different patterns, by



**Figure 1.** The outplanting site was located in a parking lot near the south entrance of the Umatilla Chemical Depot (UMCD).

Table 3. Native	plant s	pecies o	utplanted	at the	Umatilla	Chemical	Depot.
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Species	Outplanting date	Total seedlings planted	Seedlings planted per plot	Watering (ml)
Achillea millefolium	2/9/08	66	11	
Elymus elymoides	2/4/08	60	10	
Hesperostipa comata	2/12/08	38	6 or 7	100
Lupinus sericeus	2/6/08	17	2 or 3	
Opuntia polyacantha	2/4/08	36	6	
Phlox longifolia	2/4/08	60	10	
Plantago patagonica	2/6/08	58	9 or 10	
Poa secunda	2/6/08	29	4 or 5	
Pseudoroegneria spicata	2/12/08	60	10	100
Purshia tridentata small	2/12/08	42	7	100
Purshia tridentata large	1/18/08	42	7	

species, to facilitate their relocation. For example, pricklypear pads were planted in two rows of three pads each; *Plantago patagonica* (woolly plantain) was outplanted in two rows of five plants each. Larger species, such as bitterbrush, were widely dispersed in each plot.

Outplanting was done in two ways. Pricklypear and woolly plantain were planted close together to mimic their density in natural conditions. This was done by digging a narrow trench with a pick, inserting the seedlings at the edge of the trench, and then packing the soil back into the trench. All other seedlings were planted using a dibble with a planting blade 30 cm (11.8 in) long and 7.6 cm (2.8 in) wide.

Prior to outplanting, seedlings were extracted from the containers using one of several methods. The first involved rolling the container between the hands before gently removing the seedling. The second involved laying the container on the soil surface and gently tapping it until the seedling could be easily extracted. In some cases, larger seedlings, with long and extensive roots, had to be pulled out of the container by carefully pushing the roots through the open end of the tube. After being removed from the container, the seedlings were immediately placed in the planting hole and back-filled with soil. Care was taken to place the entire root system linearly in the hole without turning root tips up. Soil was packed around the seedlings to eliminate air pockets near roots. With the exception of the last planting date (12 February 2008), soils were adequately moist and did not require supplemental watering (Table 3).

#### **Plot characteristics**

Plots had variable cover of sand and gravel. Gravel cover percentage was visually estimated using a 0.5 m² (5.4 ft²) gridded frame (1 x 0.5 m [3.3 x 1.6 ft]) divided into 50 squares. This measure was multiplied by two to provide an estimate of percentage cover per m². Three sample locations, from the middle and each end of the plot, were used to compute an average cover percentage of gravel per plot.

#### Monitoring and Data Analyses

Seedling condition and survival were monitored for each species on four dates: 16 May 2008, 28 June 2008, 28 May 2009, and 21 July 2010. Linear and non-linear regression analyses were used to estimate seedling survival rates over time. Linear effects were described as:

$$S = b_0 + b_1 t \tag{1}$$

where *S* is seedling survival,  $b_0$  is the estimated survival at planting,  $b_1$  is the survival reduction rate, and t is the number of days since planting. Non-linear effects were described in two exponential decay functions:

$$S = b_0 e^{-\lambda t}$$
[2]

$$S = b_2 + b_3 e^{-\lambda t}$$
^[3]

where *S* is seedling survival,  $b_0$  is the estimated survival at planting,  $b_2$  is the long-term survival plateau level,  $b_3$ is the difference between initial survival and the plateau level, *t* is the number of days since planting, and  $\lambda$  is the decay constant.

The study plots had varied gravel cover allowing an examination of the effects of gravel cover on seedling survival. The relationship between survival and gravel cover were described linearly as:

$$\mathbf{S} = b_0 + b_1 g \tag{4}$$

where *S* is seedling survival,  $b_0$  is the estimated seedling survival with no gravel,  $b_1$  is the survival rate of change, and *g* is the amount of gravel.

Mean survival was analyzed using JMP software (SAS 2002). Survival percentage data were transformed (normalized) before statistical analysis (Steele and Torrie 1960). Survival data are presented using untransformed data with error bars for interpretation. Multiple range comparisons were done using the Tukey-Kramer HSD test at  $\alpha$ =0.05.

#### Results and Discussion ____

#### Germination and Propagation

The effect of stratification and scarification can be highly variable among species. Many species do not require any pretreatment to achieve high germination success, while others may require stratification, scarification, or both. In our germination efforts; we did not attempt any scarification or stratification prior to sowing. In part, because of this, we experienced low and variable germination success with some species. Germination success of seeds started in Petri dishes (test #1) ranged from 0% to 75% depending on the species, species population, time or location of seed collection, and time of germination trial (Table 1). For seeds that were directly sown into tubes (test #2), germination and establishment success ranged from 0% to 85% (Table 2).

**Achillea millefolium**-Yarrow (Achillea millefolium) had high germination in Petri plates and when directly sown into tubes and did not appear to need stratification or scarification (Luna and others 2008).

**Antennaria dimorpha** – Pussytoes (Antennaria dimorpha) did not germinate even with seed collected at different times and locations. Little work has been done on the germination characteristics of pussytoes, and it may need stratification to germinate.

Astragalus succumbens—Columbia milkvetch (Astragalus succumbens) seeds require scarification (Young and Young 1986); germination was therefore low in our study.

**Balsamorhiza sagittata**—Arrowleaf balsamroot (*Balsamorhiza sagittata*) had low germination in the greenhouse with 0% survival following transplanting. This result confirms the suggestion by Skinner (2004) that the species needs a long cold stratification to germinate well.

*Chrysopsis villosa*—False goldenaster (*Chrysopsis villosa*) had low germination in the Petri plates and low transplanting survival for both spring and fall dates; 56% germination, however, was obtained when fall-collected and fall-sown seeds were placed directly in the tubes. Our results are similar to Young (2001), who reported 50% germination without any pretreatment with seeds collected in the fall. During our study, false goldenaster germinated and grew slowly in the greenhouse and was not planted in the field.

*Chrysothamnus viscidiflorus*—Seeds of yellow rabbitbrush (*Chrysothamnus viscidiflorus*) are not dormant, but often have low viability (Young and Young 1986). This may help to explain why our yellow rabbitbrush did not germinate in Petri plates. To successfully use this species in restoration, it is possible that large numbers of seeds will need to be collected and germinated in order to obtain enough seedlings for outplanting.

*Crepis atribarba* – We successfully germinated slender hawksbeard (*Crepis atribarba*) in Petri plates, but none survived transplanting. Similarly, only three of four seeds planted directly in tubes became established, so it was not used in the outplanting portion of the study.

**Crocidium multicaule**—Common spring-gold (*Crocidium multicaule*) is an annual that must germinate every year in the field. Germination was attempted immediately following seed collection, but was not successful. We concluded that either the seeds were not viable or that they require physiological stratification to break dormancy. No attempt was made to germinate common spring-gold in tubes.

*Elymus elymoides*—Squirreltail (*Elymus elymoides*) showed variable germination by population, with some populations yielding high percentages, while other populations had

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little germination. Squirreltail had high germination in tubes and Petri plates for seeds collected at location UAD1, but relatively low germination in Petri plants for seeds collected at location D-1281. This result suggests that there may be population variation in germination success. Squirreltail germinates without seed pretreatment (Young and Young 1986).

**Epilobium brachycarpum**—Tall annual willowherb (*Epilobium brachycarpum*) seeds did not germinate in the Petri plates. While there is little published on the germination of willowherb, there is more on fireweed (Epilobium angustifolium). Skinner (2006) notes some populations of fireweed germinate better with stratification while others do not. More experimentation is needed to understand the germination requirements of willowherb.

*Fritillaria pudica*—Yellow fritillary(*Fritillaria pudica*) seeds did not germinate in the Petri plates. Seeds from this species require a long period (90 days) of cold, moist stratification to germinate, and are best prepared for propagation by sowing in flats and leaving them outside for the winter (Skinner 2007).

Hesperostipa comata – Needle and thread grass (Hesperostipa comata) seeds had only 6% germination in Petri plates and 27% germination when planted directly in tubes. Baskin and Baskin (2002) found that needle and thread grass seeds did not need pretreatment to germinate, and seeds will germinate at 27 °C (81 °F). However, Young and Young (1986) found that acid scarification increases germination success.

*Lomatium macrocarpum*—Bigseed biscuitroot (*Lomatium macrocarpum*) had very low to no germination without pretreatment in our study. Cold, moist stratification has been used to increase germination success in other Lomatium species (Parkinson and DeBolt 2005).

*Lupinus sericeus*—Silky lupine (*Lupinus sericeus*) showed variable germination by population. Silky lupine had significant germination success with seeds from the UAD3 location, but no germination with seeds from the UAD NE location. This variation suggests populations may differ in seed viability. Seeds should be collected from a number of sites to determine if there is an effect of population on seed viability. In addition, greater seed germination success may occur if seeds are scarified (Young and Young 1986).

**Oenothera pallida**—Evening primrose (*Oenothera pallida*) did not germinate in the Petri plates and germination was not attempted by planting seeds directly in tubes. Little is published on the germination of evening primrose, but other Oenothera species require cold, moist stratification for germination (Wick and others 2004).

**Opuntia polycantha**—Pricklypear pads nearly all survived transport from the field, 5 days of drying, and subsequent planting in tubes.

**Phlox longifolia**—Populations of longleaf phlox (*Phlox longifolia*) varied by collection site. Longleaf phlox germinated successfully from two of three populations and did not require pretreatment. This result contrasts with that of Ridout and Tripepi (2009) who found cold stratification was needed to elicit significant germination. Variation in seed viability may be due to population effects.

**Plantago patagonica**—Woolly plantain (*Plantago patagonica*) seeds germinated well in the Petri plates and in the tubes. Under our test conditions, we concluded that woolly plantain does not need pretreatment for increased germination.

**Poa secunda**—Sandberg bluegrass (*Poa secunda*) did not germinate in the Petri plates, even with seeds collected at different times and locations. However, 26% of seeds germinated when planted in tubes.

**Pseudoroegneria spicata**—Bluebunch wheatgrass (*Pseudoroegneria spicata*) germinated well in Petri plates and in tubes. Both Sandberg bluegrass and bluebunch wheatgrass seeds have not been found to need any pretreatment for germination (Young and Young 1986).

**Purshia tridentata** – Bitterbrush (*Purshia tridentata*) seeds germinated in the Petri plates and in the tubes, but the germination rate depended on location. These seeds were not pretreated, yet germinated relatively well. This is in contrast with the observation that bitterbrush seeds are quite dormant (Young and Young 1986).

**Sporobolus cryptandrus**—Sand dropseed (*Sporobolus cryptandrus*) did not germinate in Petri plates or in tubes. This species requires a pretreatment of 5 days of cold-moist stratification and then germination in light with potassium nitrate enrichment (Young and Young 1986).

#### **Outplanting Survival**

Outplanting survival was highly variable and strongly species dependent. By 28 June 2008, average survival of all outplanted seedlings was 81%. Survival of the four grass species was greater than 83%, while survival of perennials was averaged 79%. Pricklypear pads had 100% survival, while silky lupine had the lowest survival at 42%. Seven of 10 species had survival greater than 83%, with no significant differences observed between species (Figure 2A). The three species with the lowest survival (below 55%) were not significantly different from one another.

By 28 May 2009, the average survival of outplanted seedlings dropped to 67%. Three of the four grass species showed the same survival percentages, while squirreltail dropped to 51%. Survival of perennials averaged 67%. Pricklypear pads continued to have 100% survival, while silky lupine still had the lowest survival percentage, dropping to 14%. Survival of woolly plantain was not considered because it is an annual. Five species showed survival greater than 85%, all of which were not significantly different from one another (Figure 2B). Four species had survival between 43 and 52% and were not significantly different.

By 21 July 2010, the average survival of outplanted seedlings dropped to 50%. Survival ranged from 100% (pricklypear) to 5.6% (silky lupine). Grass seedling survival was 63%, while survival of perennials was 41%. By the third year of outplanting, only 3 species had survival greater than 80%; survival of these species was not significantly different (Figure 2C). Four species had survival between 40% and 59% and were not significantly different. Squirreltail, longleaf phlox, and silky lupine had the lowest survival.



**Figure 2.** Mean seedling and *O. polycantha* (pricklypear) survival at the UMCD restoration site as measured on 28 June 2008 (A), 28 May 2009 (B), and 21 July 2010 (C). Different letters indicate significant differences at a = 0.05; error bars represent one standard error of the mean.

In general, our survival results were similar to Davies and others (1999), who found variability in survival of transplanted plugs on a species-poor grassland in Britain. They observed high survival 3 months after transplanting, but survival dropped to between 20% and 40% after 3 years. Cabin and others (2002) also saw outplanting survival drop to 56% after 18 months in Hawaii; survival was highly varied by species, ranging from 23% to 91%. We observed a drop in overall survival from 81% to 50% after 28 months, with individual species ranging from 6% to 100% survival. Regression analyses showed that survival rate decreases were significant for 7 of the 10 species planted. According to the trends observed in our regression analyses most of the species in this study may continue to show reductions in survival while pricklypear, Sandberg bluegrass, and bitterbrush may have stabilized (Figure 3). Continued monitoring is needed to accurately determine long-term survival and success of this restoration effort. It appears that the three species that have stabilized could be used, with some confidence, for long-term survival, for other restoration sites under similar conditions.

The conditions of our test may limit the generality of our results. It is possible that survival was higher in our study than a typical water-limited, competitive environment. In the summers of 2008 and 2009, we observed high amounts of soil water, a likely contributor to high survival. In 2008, soils were wet to a depth of only 5 cm (2 in); in 2009, soils were wet to a depth of 7 cm (2.8 in). This gives us the indication that seedling roots were planted in ideal soil moisture establishment conditions. We hypothesize that the recent

removal of the asphalt and the lack of significant vegetation likely contributed to relatively high soil moisture conditions. It is also possible that the pre-existing parking lot became porous over the decades, thus allowing large amounts of soil water to accumulate in the underlying soil, making the study plots wetter than natural conditions.

Gravel cover ranged from 11% to 88% across the study plots and was a significant covariate for two of the species in the study area. From the cumulative survival data collected on 21 July 2010, a significant correlation was observed between percentage of gravel cover and survival percentage for squirreltail (P = 0.0322) and yarrow (P = 0.0246). The increase in gravel cover had a positive survival effect on squirreltail, while the effect was negative for yarrow (Figure 4).

The effect of gravel cover indicates that edaphic factors are significant for squirreltail and yarrow establishment. Variation in sand and gravel has been recognized as a significant factor in species establishment (Chambers 2000; Elmarsdottir and others 2003). Suzuki and others (2003) found higher survival with plants in finer soils and suggests this was associated with the greater water-holding capacity of a those soils compared to gravel. It is possible that greater water-holding capacity of the non-gravel surfaces improved success of yarrow, although we did not observe a strong gravel effect on soil moisture. Because there is a strong relationship between soil texture and soil moisture, it is possible that soils dried more in the gravel-covered areas later in the summer after we took our observations. It is also possible that factors other than water-holding capacity affected survival of the two species.



Figure 3. Linear and non-linear regression analyses describe changes in seedling survival over the 28 month study period.



**Figure 4.** *Elymus elymoides* (squirreltail) (solid circles, black line) exhibited a positive survival relationship with percent gravel cover (y = -4.16 + 0.758x;  $R^2 = 0.66$ ), while *Achillea millefolium* (yarrow) (empty triangles, dashed line) exhibited a negative survival relationship with percent gravel cover (y = 81.1 - 0.831x;  $R^2 = 0.70$ ).

## Conclusions_____

The purpose of this work was to investigate and begin the process of ecological restoration at the UMCD. Many areas in need of restoration have a sand and gravel base that may limit the types of species that can successfully establish. Our trial restoration project helped us determine how some species respond to planting into sands and gravels. Overall survival was 50% during the course of the experiment. Most of the species in this study may continue to show reductions in survival, while pricklypear, Sandberg bluegrass, and bitterbrush survival may have stabilized. Further investigation is needed to determine how other species found on the UMCD can be germinated and which ones will successfully establish on bare soils. This knowledge will increase the likelihood of restoration success using local native flora for all future projects.

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The content of this paper reflects the views of the authors, who are responsible for the facts and accuracy of the information presented herein.

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American chestnut drawing by Steven Morrison, College of Natural Resources, University of Idaho.
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