Tree Planters' Notes

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***Tree Planters' Notes* (TPN) is dedicated to technology transfer and publication of information relating to nursery production and outplanting of trees and shrubs for reforestation, restoration, and conservation.**

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Editor: Diane L. Haase

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Dear TPN Reader

Spring greetings! This issue of *Tree Planters' Notes* (TPN) may be the lengthiest one ever published! The nine articles contained herein provide a variety of information for anyone interested in growing and planting trees anywhere in the world.

I'm pleased to include articles from Pennsylvania and Louisiana as part of the ongoing TPN series to highlight past and present tree planting activities in every State. In the Louisiana article, there is mention of Phil Wakely's and James Barnett's pivotal work to develop nursery technology for southern pines. It happens that James Barnett also submitted an article for this issue of TPN in which he provides even more detail about the history of reforestation technology for southern pines.

A unique aspect of TPN is its emphasis on practical information that is readily useful to the practitioner. This journal provides a home for publications that instruct, inform, describe, and provide perspective on current programs, research, and technologies in a manner that is factually and scientifically sound while still being easily understood. This issue is no exception. It contains useful guidelines for collecting and stratifying common juniper seed, a detailed description of the steps necessary for successful controlled crosses of coastal Douglas-fir, a comparison of planting tools for longleaf pine seedlings, an examination of methods to increase seed germination of an important species in tropical dry forests, a summary of a trial to determine the effects of a short-day treatment on black spruce seedling quality, and a study on the effect of storage on pathogen development on red pine seedlings.

I encourage you to submit your article for publication in TPN, as well as to offer suggestions for future article topics or potential authors. If you have a project or program that would be a good subject for a TPN article but have never written an article before, there is no need to worry! Many articles arrive in a rather rough form but I am happy to assist authors in making necessary revisions so that the final article is clear, concise, and informative.

Best wishes for a great 2013 planting season!

Diane L. Haase

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The State of Penn's Woods

Tina M. Alban and Edward Dix

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Abstract

Pennsylvania has a legacy of rich forest resources. During the late 1800s, Pennsylvania supported the Nation's largest lumber industry, which led to overharvesting, uncontrolled wildfires, and heavy soil erosion. The Pennsylvania Legislature authorized a Forestry Commission and Forest Reserve System in 1897 to rehabilitate the State's decimated forest and water resources. In 1902, the agency's first forest nursery at Mont Alto produced more than 10,000 eastern white pine seedlings. This organization evolved into today's Department of Conservation and Natural Resources with a Bureau of Forestry (BOF) and Bureau of State Parks managing 2.2 million ac (890,000 ha) of State forest lands and 120 State parks. Annual seedling production peaked in 1950 with three BOF nurseries distributing more than 29 million seedlings. Additional public and private nurseries produced millions more. Today, the State has 16.6 million ac (6.7 million ha) of predominantly Appalachian oak and northern hardwood forests, making this combination the dominant land cover across the entire 28.7 million ac (11.6 million ha) of the State. Of the original four State nurseries, Penn Nursery is the only BOF nursery still operating; it provides in excess of 1 million seedlings of more than 40 different Pennsylvania native species to State forest and park lands for reforestation, diversity, water quality protection, and wildlife habitat. Penn Nursery also manages 22 tree-improvement seed orchards, which include a variety of hardwood and conifer species.

Introduction

Pennsylvania means "Penn's woods." The State's name combines the name of the colonial founder William Penn with the Latin word for woods—*silva*.

Pennsylvania's location, landforms, and climate favor the development of mixed hardwood forests statewide. Its 44,820 mi^2 (116,080 km^2) of land area rise in elevation from sea level on the Atlantic Coastal Plain near Philadelphia and climb north and westward to the Allegheny Plateau and Laurel Highlands. The highest point is 3,213 ft (980 m) on Mount Davis in Somerset County. From that point, the plateau slopes downward to the Ohio River basin to the west and Lake Erie to the northwest.

The statewide annual precipitation averages 41.2 in (105 cm). The central counties in the rain shadow east of the Allegheny Plateau are slightly drier than the eastern and western borders.

Prehistoric pollen deposits indicate that this region supported tundra and spruce woodlands as the last glaciations ended 14,000 years ago. About this time, the first people to explore Pennsylvania left stone spear points and scrapers in rock shelters and seasonal campsites across the postglacial landscape. Over the next several thousand years, the climate warmed. New tree species —oaks, chestnut, hickories, pines, and hemlocks—migrated north, providing a wealth of new forest resources. The descendants of hunter-gatherers developed village life and subsistence agriculture.

European settlers arriving in the 1600s described the land as primarily forested but broken by rivers, wetlands, natural barrens, and Native American village clearings. "Indian fields" were kept open, using fire to manage the landscape. Forests covered more than 90 percent of Pennsylvania's 28.7-million-ac (11.6-million-ha) land area.

The newcomers' consequent use of forest resources dramatically changed the land cover of Pennsylvania. European Americans cleared the southeastern counties for agriculture and urban settlements. In the 1760s, tall, straight eastern white pines (*Pinus strobus* L.) suitable for ship masts were harvested in large quantities from northeastern Pennsylvania counties and rafted down the Delaware River to Philadelphia. Harvests of lumber, fuelwood, charcoal, tannins, and wood chemicals reduced the forest area from southeast to northwest as settlement spread farther from the Coastal Plain.

Pennsylvania's location linked the waterways of the Great Lakes and Ohio River with the Atlantic seaports on the Delaware River. The State became a center of early urbanization and industrialization. For a brief period between 1870 and 1880, Pennsylvania had the Nation's largest lumber industry centered around Lock Haven, Jersey Shore, and Williamsport on the Susquehanna River. Steam sawmills turned massive quantities of mixed hardwoods, eastern white pine, and Canadian hemlock (*Tsuga canadensis* [L.] Carrière) into furniture, barrel staves, shingles, window sashes, door framing, and other construction lumber. Rough timber became

railroad ties and props for the roofs and walls of coal mines. Wood chemical factories produced methanol, acetate lime, wood alcohol, and tannic acid.

When settlers cut large numbers of trees, they left behind piles of unusable slash. As a result, millions of acres burned in uncontrolled fires. Fire became increasingly destructive in the remnants of cut forests and spread into standing forests. By 1895, much of Pennsylvania's woodlands had been reduced to stumps and ashes (figure 1). In that year, the State established what has become today's Department of Conservation and Natural Resources (DCNR), Bureau of Forestry (BOF) to develop a fire protection program and acquire land for reforestation and watershed protection.

Figure 1. Eroded hillside in Potter County, PA. (Photo from Pennsylvania Department of Conservation and Natural Resources, 1918)

In 1900, Pennsylvania had 224,000 farms, although 55 percent of its 6.3 million inhabitants lived in cities and towns. Industrial timber harvesting and agricultural land clearing had diminished the forest land base to only 9.2 million ac (3.7 million ha), about 32 percent of the State's land area. Because most of the population shifted from rural to urban areas, abandoned farmland reverted to forest. Forest acreage increased steadily through the 20th century as trees reclaimed old fields. Forest cover increased in every inventory conducted from the 1930s through the 1980s. Pennsylvania's current forest cover of 16.6 million ac (6.7 million ha) is the dominant land cover, at 58 percent of the total State area.

The most recent forest inventory data show large, contiguous patches of forest extending across the Allegheny Plateau in the north-central portion of the State (figure 2). In central Pennsylvania, forest land distribution follows the topographical contours of the ridges that divide agricultural valleys. Smaller, more fragmented blocks of forest land occur in more urban and agricultural regions, especially across the southern-tier counties.

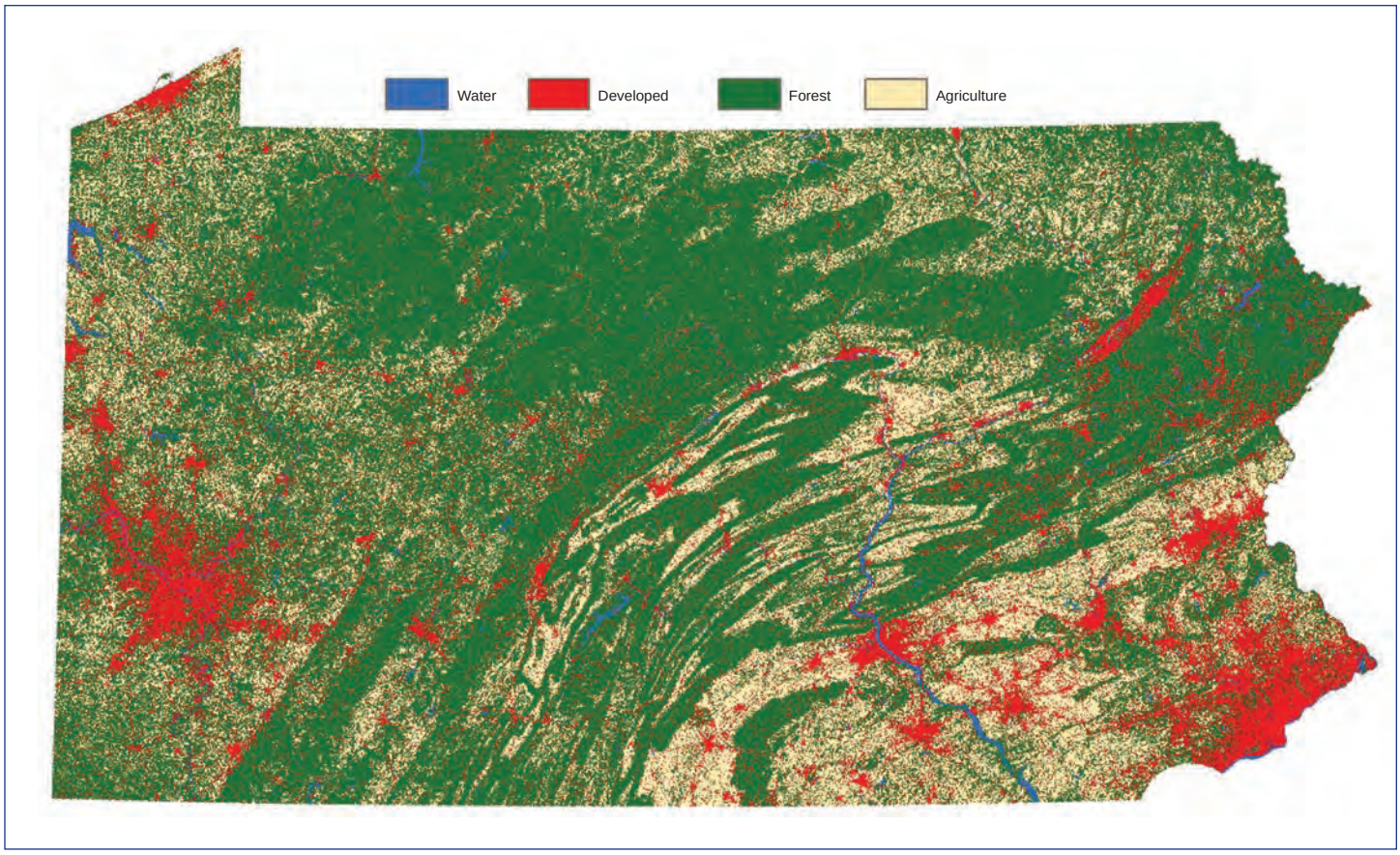

Figure 2. Forest land cover in Pennsylvania. (Data source: National Land Cover Dataset, 2006)

Statewide, Pennsylvania's net forest acreage is stable. More than 660,000 ac (267,000 ha) of forest land were lost from 1989 to 2004, mostly to residential or industrial development. Over the same period, however, a 617,500-ac (250,000-ha) gain was made, largely from reforested agricultural land (McWilliams and others 2004). More than one-half of the forest land in the State is privately owned by families and individuals (figure 3). Most of the 4.8 million ac (1.9 million ha) of public forest land is in State forests, State game lands, State parks, and municipal watersheds. Federal forests are limited to 611,100 ac (247,300 ha), mostly in the Allegheny National Forest.

Pennsylvania's 20 State forest districts comprise 2.2 million ac (890,300 ha) managed by the DCNR BOF. These forests amount to 13 percent of the State's total land area. The Pennsylvania State Forest System is one of the largest certified as "well managed" by third parties under the Forest Stewardship Council standards.

The 10 most abundant tree species by volume cataloged by the ongoing U.S. Department of Agriculture (USDA), Forest Service Forest Inventory and Analysis program are red maple (*Acer rubrum* L.), black cherry (*Prunus serotina* L. Ehrh.), northern red oak (*Quercus rubra* L.), sugar maple (*A. saccharum* Marshall), chestnut oak (*Q. montana* L.), Canada hemlock (*Tsuga canadensis* [L.] Carrière), tuliptree (yellow-poplar) (*Liriodendron tulipifera* L.), white ash (*Fraxinus Americana* L.), white oak (*Q. alba* L.), and sweet birch (*Betula lenta* L.). The most extensive forest type classifications are mixed oak, sometimes called Appalachian oak, and northern hardwoods.

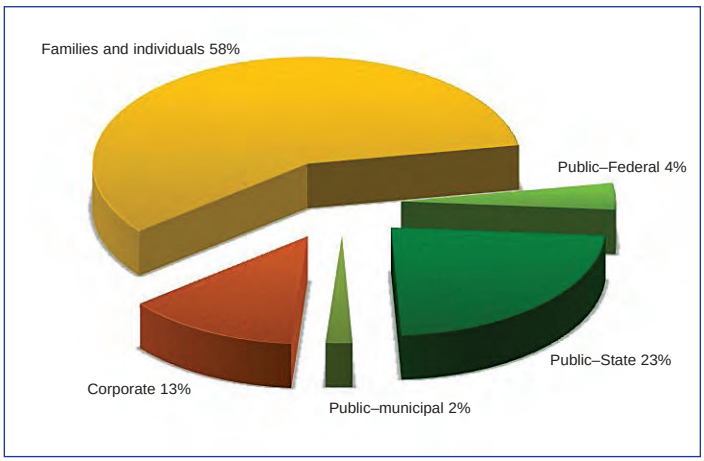

Figure 3. Pennsylvania forest land ownership. (Data source: Pennsylvania's Forest, 2004)

Replanting Penn's Woods

At the turn of the 20th century, demand boomed to acquire lands in Pennsylvania for reforestation. Political leaders recognized the necessity to restore productive conditions to land rendered unproductive by removal of the original forest cover (Rothrock 1902).

On May 25, 1897, Governor Daniel H. Hastings formed the Forestry Commission, headed by Joseph T. Rothrock (figure 4), to purchase land for Pennsylvania's new Forest Reserve System and to protect and manage those lands. As its responsibilities changed over the next century, this agency would be called the Department of Forestry, the Department of Forests and Waters, and, most recently, the DCNR BOF. In this historical narrative, we simply refer to "the agency."

Rothrock recognized the need to plant trees as part of rehabilitating the land. Before 1901, seedlings were purchased from private nurseries for planting on the forest reserves. Seedling demand outpaced availability, however. State Forester George Wirt, influenced by nurseries he had seen in Germany, established the first State-run forest tree nursery in Mont Alto, PA. Land was cleared in 1902, 6 lb of eastern white pine seed was sown, and 10,000 white pine seedlings

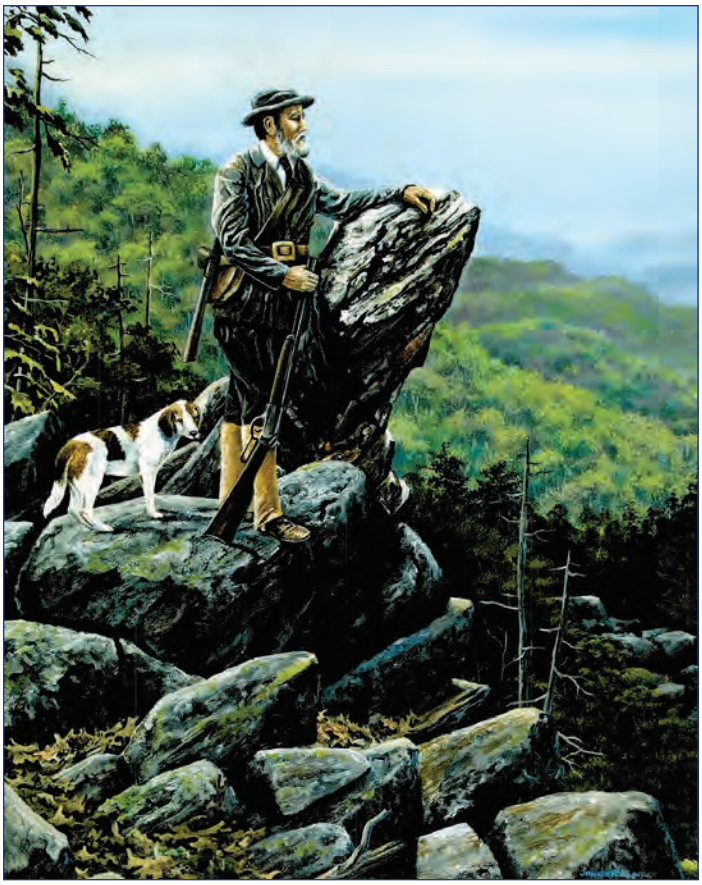

Figure 4. Joseph T. Rothrock, an early leader in Pennsylvania's reforestation efforts. (Painting by John Sidelinger, 2005)

from a private nursery were transplanted into nursery beds (Wirt 1902). The nursery size increased as demand for planting grew (figures 5 and 6).

Additional nurseries were developed to keep pace with demand for planting stock. By 1907, the agency managed three large nurseries with a combined annual production of 2.25 million seedlings. The State legislature authorized distribution of trees to farmers and private landowners with the passage of Public Law 115 in April 1909. Eastern white pine, Scots pine (*Pinus sylvestris* L.), European larch (*Larix deciduas* Mill.), Norway spruce (*Picea abies* L.), and balsam fir (*Abies balsamea* [L.] Mill.) were produced, along with a small amount of oak and hickory. Seedling production costs of $3.11/thousand, which were considered outrageous at the time, were due to the initial startup costs of land clearing, fencing, and labor (Conklin 1907).

Figure 5. Workers covering seed at Mont Alto Nursery in the 1950s. (Photo from Pennsylvania Department of Conservation and Natural Resources, Bureau of Forestry, year unknown)

Figure 6. Workers lifting seedlings at Mont Alto Nursery in the 1950s. (Photo from Pennsylvania Department of Conservation and Natural Resources, Bureau of Forestry, year unknown)

Wirt supported the idea that each forest reserve would benefit from having a local nursery. By 1915, the agency operated as many as 50 "ranger" nurseries, so named because, in general, the ranger in charge of the reserve also served as nursery manager. Nearly all these ranger nurseries closed by 1920 when World War I resulted in a lack of available nursery personnel. Demand for seedlings was still high, however, so cooperative agreements with local State institutions and asylums were formed to establish 12 cooperative nurseries. The hope was that these nurseries would produce seedlings at low cost and provide healthful employment for inmates. Because of a lack of forester supervision, the effort was not successful, and all the nurseries were closed within a few years, except for the Western Penitentiary Nursery at Rockview, which still operates today (Meek 1936).

During the 1930s, tree planting again increased significantly when Pennsylvania became home to 113 Civilian Conservation Corp (CCC) camps. The CCC, authorized by President Franklin D. Roosevelt, brought relief to a Nation reeling from the effects of the 1929 stock market crash and subsequent depression. CCC workers in Pennsylvania made extensive improvements to the State forest and park systems—building roads, erecting facilities, and planting millions of trees—until the program ended with the start of World War II.

By 1935, the agency managed four nurseries: Mont Alto, Dague, Greenwood, and a smaller transplant nursery called Penn Nursery. This small but efficient ranger nursery had a humble beginning in a potato patch behind Ranger George L. McKinney's home on the Seven Mountains Reservation (figure 7). Penn Nursery operated as a transplant nursery, providing larger conifer and shade trees for roadside

Figure 7. George L. McKinney served as a ranger on the Seven Mountains Reservation and operated a nursery behind his home. (Photo from Pennsylvania Department of Conservation and Natural Resources, Bureau of Forestry, year unknown)

beautification of Pennsylvania's highways until 1928, when the Highway Department started its own nursery in Milton, PA (figure 8).

Penn Nursery has repeatedly adapted to changing demands and still provides high-quality planting stock for State, Federal, and private lands. Concurrently, Pennsylvania's original Forest Reserve System has grown to more than 2 million ac, forming today's Pennsylvania State Forest and Park Systems (figures 9 and 10).

Pennsylvania State Nurseries

The DCNR BOF has distributed hundreds of millions of seedlings to many organizations, agencies, and companies since 1899 (figures 11 and 12). As the agency's only forest tree nursery, Penn Nursery produces more than 1 million seedlings annually from more than 40 different Pennsylvania native species. The nursery distributes these seedlings to State forest and park lands for reforestation, diversity, water-quality protection, and wildlife habitat.

Figure 8. Workers line up seedlings in transplant boards for planting at Penn Nursery. (Photo from Pennsylvania Department of Conservation and Natural Resources, Bureau of Forestry, year unknown)

Since the establishment of DCNR nurseries, staff members have collected seed and distributed seedlings within the same genetic conservation zone whenever possible (figure 13). Each seed lot is assigned a number, which includes information

Figure 9. Workers lifting seedlings at Penn Nursery. (Photo by Tina Alban, Penn Nursery, May 2007)

Figure 10. Workers sowing acorns at Penn Nursery. (Photo by Tina Alban, Penn Nursery, April 2008)

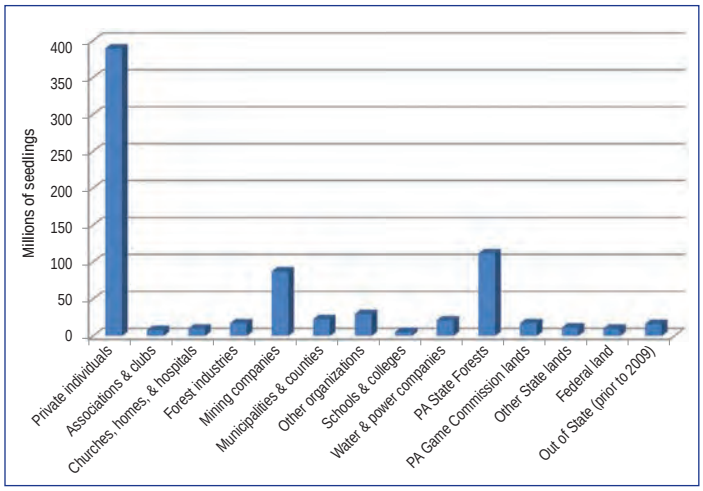

Figure 11. DCNR BOF seedling distribution by classification of planter, 1899–2012. (Data source: Penn Nursery, 2012)

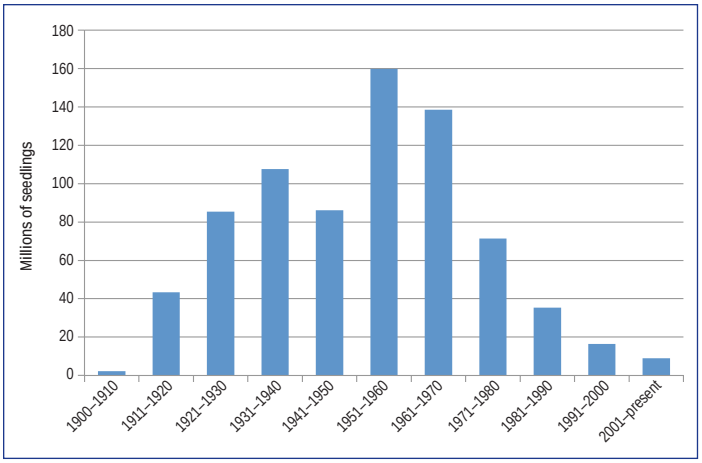

Figure 12. Seedlings distributed by DCNR BOF nurseries. No seedling production figures for other nurseries in Pennsylvania were available. (Data source: Penn Nursery, 2012)

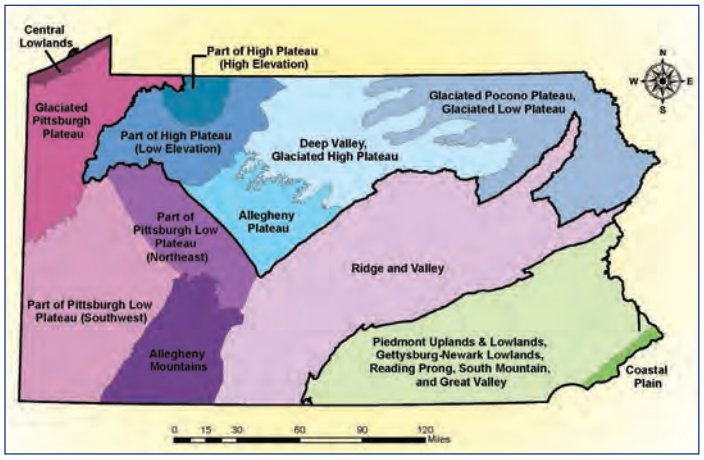

Figure 13. Plant genetic conservation zones of Pennsylvania. (Data source: Pennsylvania Department of Conservation and Natural Resources, 2007)

about its genetic zone of origin. The seed lot number remains with that seed until it is permanently established on public lands as a seedling. In addition to managing local seed collection, Penn Nursery maintains 22 tree-improvement seed orchards established by BOF forest geneticists and through cooperative projects with the USDA Forest Service and universities.

Another successful State government nursery is Howard Nursery, managed by the Pennsylvania Game Commission (PGC). In 1939, this nursery was established in Howard, PA, by the USDA and managed by the Soil Conservation Service. By 1947, the USDA had leased the nursery to Pennsylvania's BOF to produce planting stock for distribution to landowners for reforestation and farm woodlots. The PGC took over the lease in 1954 and, a few years later, the land was permanently transferred to the PGC. The Howard Nursery currently produces 2.3 million seedlings of more than 50 different species, including those that produce the most desirable food and cover for wildlife, with an emphasis on native species.

The nursery distributes PGC seedlings for planting on State game lands and private lands enrolled in Farm-Game, Forest-Game, and Safety Zone cooperator programs. It also distributes seedlings to other State agencies, schools, Boy Scouts, Girl Scouts, and other private-property owners.

A third State-owned nursery, operated by the Department of Corrections at Rockview, provides meaningful vocational training opportunities for inmates. Inmates can develop vocational skills in planting, transplanting, pruning, irrigating, fertilizing, and integrated pest management for the production of trees and shrubs. Rockview favors production of species native to Pennsylvania, although not exclusively. The nursery also has a greenhouse component in which inmates can sow a variety of annual flowers for bedding plants, hanging baskets, planters, and window boxes. Rockview propagates poinsettias, Easter lilies, chrysanthemums, and other seasonal flowers. Nursery products are used by the State Correctional Institution and other State agencies.

Current Tree Planting Programs and Challenges in Pennsylvania

Pennsylvania is rich in forest and park lands, and it is also rich in mineral resources, including coal, both bituminous and anthracite. Mining's destructive impacts on the land were widespread long before the Pennsylvania Legislature acted to regulate mining activity in the mid-1940s. Legislation passed in the early 1960s and 1970s provided further environmental protections and encouraged tree planting as a long-term, permanent cover to reclaim surface mine sites. In 1986 alone, the DCNR BOF shipped more than 2.5 million seedlings for surface mine reforestation. This figure is only a fraction of the seedlings produced for mineland reforestation by other State and private nurseries. In addition, the Department of Environmental Protection, Bureau of Abandoned Mine Reclamation provided funding between 1984 and 1995 to the DCNR BOF to plant trees on orphaned mine sites, resulting in more than 3 million seedlings being established on reclaimed areas (figure 14). During the past decade, use of seedlings has declined in favor of direct sowing of tree, shrub, and herbaceous seed directly onto orphaned mine sites. Direct sowing may be more cost effective, but the extent of successful reclamation has yet to be fully evaluated.

Other programs, such as the Appalachian Regional Reforestation Initiative and the creation of habitat for the endangered Indiana bat (*Myotis sodalis* Miller & Allen), encourage hardwood tree planting in addition to traditional regulatory plantings (figure 15).

Figure 14. Planting on strip mine near Dubois, PA. (Photo by Tina Alban, Penn Nursery, April 1994)

Figure 15. Northern red oak (*Quercus rubra*). (Photo by Tina Alban, Penn Nursery, October 2012)

The DCNR BOF cooperates with the USDA Natural Resources Conservation Service (NRCS) to assist private forest landowners with tree planting for a variety of purposes, including agroforestry, wildlife habitat, riparian buffer establishment, and native forest restoration. Planting projects are accomplished through technical assistance and Federal incentive program funding via the BOF's foresters and NRCS field staff.

Several commercial reforestation contractors working in Pennsylvania agree that large-scale, bareroot reforestation plantings have declined in the past decade. Although area plantings still occur, the number of acres being reforested has decreased. This decline may be due to the fact that many goals of the Conservation Reserve Enhancement Program (CREP) have been met and enrollment of new land has declined. Riparian buffer plantings have dominated planting contracts. Some report that changes in Federal laws regarding prevailing wages and securing laborers also present a challenge by increasing business costs.

In addition to CREP, the Wildlife Habitat Incentive Program and Environmental Quality Incentive Programs through NRCS, along with the Chesapeake Bay Foundation promotion of riparian buffer plantings, create demand for seedlings. Riparian buffers are also encouraged by the TreeVitalize Program, a broad-based, public-private partnership to encourage tree planting in communities across the State. To date, the program has established more than 340,000 trees since 2004. These plantings consist of seedlings for riparian buffers and large caliper trees for city streets, parks, and other public properties. The program also includes a rebate incentive for homeowners to purchase large caliper trees for planting on private property. Shrinking Federal and State grant monies are also affecting nonprofit groups.

Large-scale, bareroot tree planting may have declined, but tree planting overall during these economically challenged times remains strong across the State. Increases in educational programs to promote awareness of the importance of trees and technical assistance to private landowners continue to sustain demand for seedlings. Interest in planting trees for biomass fuels is also increasing. Willow (*Salix* spp.), poplar (*Populus* spp.), and alder (*Alnus* spp.) are a few of the species for which demand may increase for use as fuels (figure 16).

Forest tree mortality from exotic pests, such as gypsy moth (*Lymantria dispar* L.), emerald ash borer (*Agrilus planipennis* Fairmaire), and hemlock wooly adelgid (*Adelges tsugae* Annand), continue to threaten the health of Penn's woods. White-tailed deer (*Odocoileus virginianus* Zimmermann) and invasive exotic plants, including oriental bittersweet

Figure 16. Planting by Williams Forestry & Associates, Canton, PA. (Photo by Justin Ulanoski, Williams Forestry & Associates, spring 2011)

(*Celastrus orbiculatus* Thunb.), Japanese barberry (*Berberis thunbergii* DC.), Japanese honeysuckle (*Lonicera japonica* Thunb.), and Japanese stiltgrass (*Microstegium vimineum* [Trin.] A. Camus), make successful seedling establishment a challenge.

In recent years, many industrial forest products companies in Pennsylvania divested their timber- and pulp-producing lands. Although many acres were purchased by the State and other conservation organizations, most lands were bought by timber investment management organizations to be managed as commercial timber land.

Future impacts, such as climate change and invasive exotics, are uncertain, but Pennsylvania nurseries will be ready to shift seedling species and quantities to accommodate new changes.

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A Brief History of Reforestation and Restoration in Louisiana

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Abstract

Louisiana is rich in culture, politics, and ecosystem diversity, all of which have affected forested timber lands and their use over time. Landscape diversity and historic land use changes have also played a significant role in shaping Louisiana forests. Native Americans participated in small agriculture production areas and burned areas for clearing, crop production, and wild game pursuit. European settlements began in the 1700s, and with them came land clearing and draining, levee building, and logging. By the 1930s, the State was almost completely clear-cut. Then, a few forward-looking people introduced forestry and the legislation to support its practice to the State. The works of these leaders—along with the assistance of the U.S. Department of Agriculture, Forest Service; the 1904 establishment of the Louisiana Department of Forestry; and the work of the Civilian Conservation Corps program—eventually changed the face of the Louisiana timber industry from one of "cut-and-run" practices to one of vibrant, sustainable forests. Today, environmental and man-made factors threaten millions of acres of forest land. Much work is being done to address these threats, but much still remains to be done.

Introduction

Louisiana has a very diverse landscape, ranging from the rolling hills in the northwest to the marsh regions of the south. The highest elevation in the State is Driskill Mountain, with an elevation of 535 ft (163 m), and the lowest is 8 ft (2 m) below sea level in New Orleans. Forests are a vital part of Louisiana's economy and provide material for a thriving woods product industry, as well as for recreation, wildlife, and environmental enhancement. Louisiana's forests cover 14.0 million ac (5.7 million ha), about 50 percent of the State's land area. Louisiana has 148,000 forest landowners. Private, nonindustrial landowners own 81 percent of this forest land; the forest products industry owns 10 percent; and the public owns 9 percent (Louisiana Forestry Association 2011). Trees are Louisiana's No. 1 crop, with an economic impact of $3.0 to $4.0 billion annually, peaking at $5.4 billion in 1998. Total forest landowner income in 2010 was $396.8 million compared with a high in 1998 of $744.0 million (Louisiana Forestry Association 2011). Louisiana's forests support approximately 180 primary and 750 secondary wood-using industries (The Nature Conservancy 2007). The forest industry is second only to oil and gas in the State.

Known as the sportsman's paradise, in part because of the diversity of its ecosystems, Louisiana has 12 river basins containing a wide variety of bottomland hardwood forests. Among the river basins are rolling hills and bluffs that support upland hardwood-pine forests. These basins and their watersheds flow to the Gulf of Mexico and make up a system of Gulf coast marshes and prairies that comprise 40 percent of the lower 48 States' coastal wetlands (USGS 2012). This area includes the great Atchafalaya River basin, one of the last great bottomland ecosystems. The Mississippi River Alluvial Valley alone makes up more than 12,000 mi^2 (31,080 ha^2) of Louisiana's surface area (figure 1).

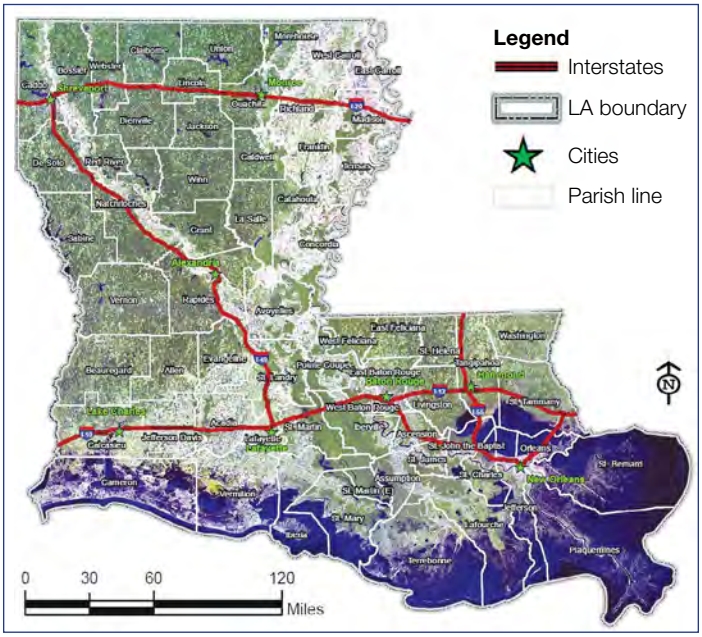

Figure 1. State of Louisiana map detailing the urban centers. (Map source: Louisiana Department of Forest and Agriculture, Landsat Image, 2002.)

Louisiana's Forests

Louisiana forests are quite diverse because of the nature of its topography. Its forests are composed of a wide variety of upland and bottomland hardwood species, along with five pine species (figures 2 and 3).

Timber Regions

The northwest corner of the State originally supported shortleaf pine (*Pinus echinata* Mill), a variety of oaks (*Quercus* spp.), other hardwood species such as sweetgum (*Liquidambar styraciflua* L.), blackgum (*Nyssa sylvatica*

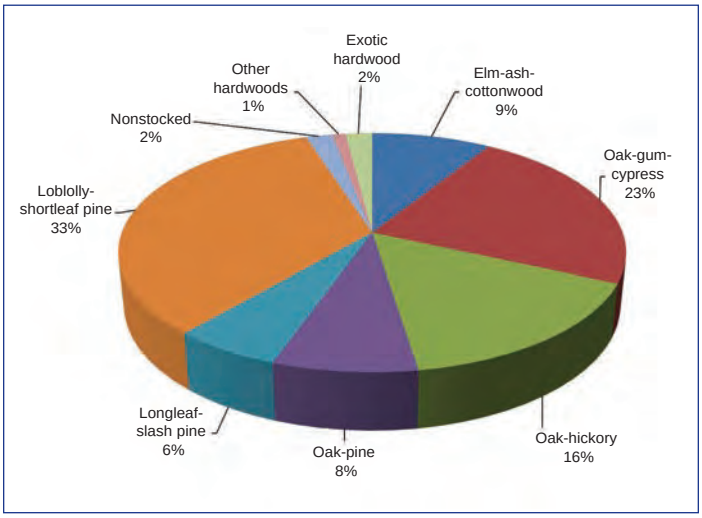

Figure 2. Relative abundance of Louisiana forest types; loblolly-shortleaf pine dominates with oak-gum-cypress a close second. (Data source: Oswalt and Johnson, 2012)

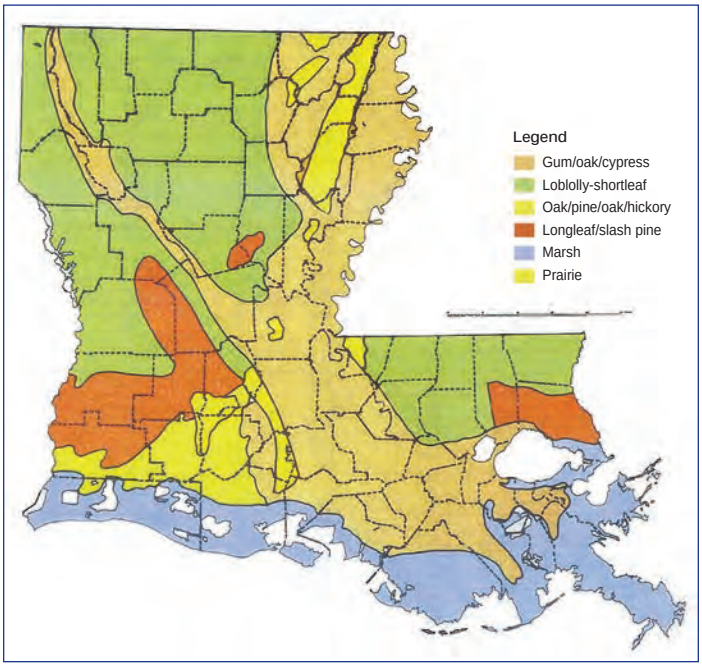

Figure 3. Geographic distribution of forest types in Louisiana. (Map source: Louisiana Department of Agriculture and Forestry, 2002)

Marsh), red maple (*Acer rubrum* L), and several varieties of hickories (*Carya* spp.). Most of the shortleaf pine in this region was replaced by loblolly pine (*P. taeda* L), partly because of loblolly pine's rapid growth and ability to reseed on denuded soils, along with extensive replanting efforts of the 1950s. The southwest and central portions of the State are considered the longleaf belt. In Louisiana, longleaf pine (*P. palustris* Mill.) historically occurred in the hilly region and on extensive flatland surfaces known as flatwoods. Today, this area consists of longleaf, slash (*P. elliottii* var. *elliottii* Little & Dorman), and loblolly pines. The many sloughs within the flatwoods area contain swamp blackgum (*N. biflora* Walt), water oak (*Q. nigra* L.), willow oak (*Q. phellos* L.), red maple, and green ash (*Fraxinus pennsylvanica* Marsh), along with baldcypress (*Taxodium distichum* [L.] Rich.) and tupelo gum (*N. aquatica* L.) on poorer drained areas. Spruce pine (*P. glabra* Walt.) occurs along the streams in the pine hills and on the higher parts of the Pearl River bottoms, with the most extensive stands occurring in parts of Livingston and Tangipahoa Parishes (Brown 1945).

Bottomland Hardwoods and Cypress Regions

The Mississippi River floodplain, as well as the deltas and floodplains of the Pearl, Red, Sabine, and Atchafalaya Rivers, and many streams have hardwood forests with large acreages of baldcypress trees. These floodplains consist of lakes, backwater swamps, old stream channels, natural levees, and levee slopes. The soil varies from sand to heavy clays. A difference of only a few inches in elevation here is often more influential on the plant community than is a hundred feet in other areas.

Baldcypress swamps also contain tupelo gum, swamp red maple (*Acer rubrum* var. *drummondii*), green ash, pumpkin ash (*Fraxinus profunda* Bush), and black willow (*Salix nigra* Marsh). Large areas of poorly drained, but a little drier, soil support growth of overcup oak (*Quercus lyrata* Walt), bitter pecan (*Carya aquatica* [Michx. F.] Nutt.), green ash, willow oak (*Q. phellos* L), water oak, and hawthorns (*Crataegus* spp.).

The areas closest to the river channels that receive sand and silts with each flood support growth of cottonwood (*Populus deltodides* Bartr.), sycamore (*Platanus occidentalis* L.), sweetgum, black willow, hackberry (*Celtis laevigata* Willd.), honey locust (*Gleditsia triacanthos* L.), and water locust (*G. aquatica*).

The old natural levees support sweetgum, cherrybark oak (*Quercus pagoda* Raf.), cow oak (*Q. michauxii* Nutt.), nuttall oak (*Q. taxana* Buckley), shumard oak (*Q. shumardii* Buckley), water oak, American elm (*Ulmus americana* L.),

winged elm (*U. alata* Michx.), pecan (*Carya illinoensis* [Wangenh.] Koch.), and persimmon (*Diospyros virginiana* L.). The higher and poorly drained portions of the floodplain contain willow oak, winged elm, nuttall oak, cedar elm (*U. crassifolia* Nutt.), and green ash.

The margins of old stream courses and meanders of the Mississippi River support baldcypress, water locust, and water elm. The adjoining natural levees, only a few feet higher, have sweetgum, overcup, bitter pecan, persimmon, hackberry, and cherrybark oak. In the lower portion of the flood plain south of Baton Rouge, live oak (*Quercus virginiana* Mill.) is found in areas above the height of normal floods (Brown 1945).

Extensive levee building after the great flood of 1927, along with draining of lands for agriculture in the 1800s, shifted the hydrology in many of these areas. These changes in the landscape also shifted the plant species in the second-generation bottomland hardwood forest. Many areas once dominated by nuttall oak, overcup oak, and bitter pecan are now cherrybark oak, willow oak, water oak, and shumard oak.

Upland Hardwoods

Many of the natural upland hardwood sites occur in small strips on bluffs above the floodplains. These sites include Chicot State Park, Grand Encore area in Natchitoches, and the west bank of the Ouachita River, just north and south of Columbia. Upland hardwoods also occur in areas of northwest Louisiana, which rise up out of stream bottoms into rolling hills. Tree species in these areas include white oak (*Quercus alba* L.), shumard oak, southern red oak (*Q. falcata* Michx.), post oak (*Q. stellata* Wang.), bitternut hickory (*Carya cordiformis* [Wang.] Koch.), shagbark hickory (*Carya ovata* Mill.), red maple, beech (*Fagus grandifolia* Ehrh), black cherry (*Prunus serotina* Ehrh.), dogwood (*Cornus florida* L.), and red bud (*Cercis canadensis* L.) (Brown 1945).

History of Louisiana Forests

Louisiana forests, much like the rest of the Nation's forests in the early years, were exploited. It took the hard work and dedication of many forward-looking people to save this great natural resource. They realized that with proper protection, management, and regeneration, these forests would be beneficial and productive for generations to come.

The Early Lumbering Era

The tremendous forest wealth of Louisiana was virtually untouched until the last two decades of the 19th century. The U.S. Decennial Census of 1880 estimated that Louisiana had more than 26.5 billion board feet of longleaf pine and more than 21.6 billion board feet of shortleaf pine (Burns 1968). Before 1880, small-scale lumbering had been confined to the mouths of a few streambanks and at New Orleans, where logs were floated down the Mississippi and across Lake Pontchartrain (Burns 1968). The first crude sawmill began production in 1716, and the first mechanized mill was established between 1803 and 1811 (Burns 1968). An 1809 newspaper advertisement placed by a steam-powered mill was seeking cypress logs. The 1810 census reported that Louisiana had only 34 sawmills (Burns 1968). Despite its continued growth, the New Orleans area reported only 11 sawmills by 1823, a relatively small number for that time (Burns 1968). The lack of fast and convenient transportation in Louisiana delayed intensive logging for some time. The 1880 census ranked Louisiana 30th in the Nation in lumber production, with only 175 sawmills (Burns 1968). As northern forests were depleted and the railroad system was established, however, lumber companies moved to the South's plentiful bounty of basically untouched timber lands. Louisiana was favorable because of its easily accessible terrain and large blocks of land available for purchase.

Mechanized logging and milling and the desire for large, speedy profits resulted in the clearing of enormous tracts of timber. Railroad spurs and mills dotted the landscape. The life expectancy of these mills, in general, was 20 years or less. As the lands were logged, they were sold off as junk, along with the mills. If not sold, these lands were abandoned and forgotten. In the rich alluvial valleys, farmers later turned this land into farms with row crops, such as cotton and sugar cane, or into pasture. In the poorer soils of the uplands, any lands that could not grow crops were left to regenerate naturally.

By 1914, Louisiana had become the greatest producer of lumber in the Nation. In 1899, Louisiana's production exceeded 1 billion board feet of lumber. That number doubled by 1904 and doubled again by 1916 (Burns 1968). The cutting necessary to produce so much lumber resulted in a total rout of the land. These lands remained idle for a generation, until forward-thinking foresters proved that the practice of forestry could be profitable (figure 4).

The Rise of Forest Management

"In 1939, southwest Louisiana had the dubious honor of containing 'the largest area of clear-cut longleaf land west of the Mississippi River of more than 1 million acres'" (404,694 ha) (Burns 1968, citing Cruikshank 1939). Even

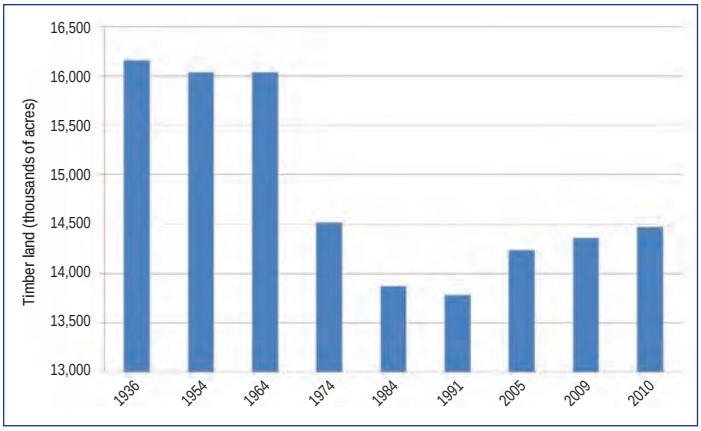

Figure 4. Change in timber land over time in Louisiana. Forested area has increased by 1.7 percent since 2005. (Data source: Oswalt and Johnson, 2012)

up to 1949, an estimated 43 percent of nonproductive forests were in the South, a result of severe logging practices and forest fires (Burns 1968).

Gifford Pinchot and Henry Graves are well-known pioneers who shaped the early years of forestry in the United States. In Louisiana, Henry Hardtner of Urania was instrumental in shaping the State's forestry profession. Hardtner's love of the pine forests and his desire to create a permanent mill with a sustainable timber base led him to put into practice the then-new ideas of reforestation, timber management, and sustained yield. From 1904 onward, he eagerly shared his knowledge with anyone who would listen and, eventually, became known as the Father of Forestry in Louisiana and the South. In 1913, Hardtner signed the first reforestation contract with the State; that date has been designated the birthdate of forestry in Louisiana and the South (Burns 1968). By 1954, the second forest survey showed the State's forest was growing at twice the rate it was being cut. This survey also showed the average volume per acre was higher in Louisiana than any other mid-South State (Burns 1968).

The Work of the Civilian Conservation Corp

The Federal Government established the first Civilian Conservation Corps **(**CCC) camp in Louisiana in 1933. In all, 27 camps were built, of which 20 were placed under the State forester's direction. The State Forest Service used roads, firebreaks, and telephone lines built under this program. CCC workers reforested approximately 185,000 ac (74,870 ha) of cutover timber lands in Louisiana. Camp crews built 18 fire lookout towers and spent more than 72,000 worker-days fighting fires (LDAF 2010).

The Effect of Legislation

As early as 1904, it was recognized that legislation would be the key to preserving the State's forests. The following summarizes critical legislation that had a significant effect on forest management in Louisiana:

- Louisiana Act 113 of 1904 established a Department of Forestry to provide for forest preservation within the State, suppression and prevention of forest fires, reforestation of denuded lands, proper forestry instruction in public schools, and penalties for the violation of this act (LDAF 2010).
- Louisiana Act 172 of 1910 created a permanent conservation commission (Burns 1968).
- Louisiana Act 196 of 1910 created a conservation fund derived from a severance tax to be used partly for fire protection (Burns 1968).
- Louisiana Act 261 of 1910 strengthened the forestry act of 1904 by designating an ex-officio State forester and an appropriation of $2,400 from the State severance tax. Act 261 is remembered today as the Timber Conservation Contract Act because of a provision in Section 13 that allowed an owner of denuded land worth $5 or less per acre to enter into a reforestation contract with the State (Burns 1968).
- Louisiana Act 127 of 1912 created a Conservation Commission appointed by the Governor, and enumerated all previous legislation over which the commission had authority including Louisiana's first forestry bill, Act 113 of 1904 (Burns 1968).
- The 1911 Weeks Act passed by the Federal Government granted matching State funds for forest fire protection. Louisiana did not participate until 1915, when the Conservation Commission voted for $2,000 to be matched by Federal dollars (Burns 1968).
- Louisiana Act 66 of 1916 amended Louisiana Act 127 of 1912 and created the Department of Conservation under control of the Commissioner of Conservation. Beginning in 1918, the forestry law of 1916 gave the Division of Forestry one-fifth of the severance tax on forest products (Burns 1968).
- In 1928, after the 1927 flood, the Federal Flood Control Act passed. This act placed flood control under the authority of the Federal Government. As a result, a system of levees was later constructed, under the authority of the U.S. Army Corps of Engineers (USACE), to harness the Mississippi River and its tributaries.

- Louisiana Act 179 of 1944 established the Forest Protection Acreage Tax. This funding source only accounts for approximately $800.00 per year and is used for the purchase of supplies and equipment utilized for wildfire suppression (LDAF 2010).
- Louisiana Act 328 of 1944 was voted on by the citizens of Louisiana and passed on November 7, 1944. The act established that, whereas the Commissioner of Wildlife and Fisheries and Commissioner of Conservation would still be appointed by the Governor, a seven-member forestry commission would name the State forester, thus eliminating politics from the process (Burns 1968). This approach prevailed until the mid-1980s, when the independent authority of forestry was legislatively merged with the Louisiana Department of Agriculture. The new Louisiana Department of Agriculture and Forestry, headed by its publicly elected commissioner, thereafter shared in structuring Louisiana's forestry future (LDAF 2010).

Seedling Production for Reforesting Louisiana's Lands

Until the 1920s, artificial forest regeneration was considered largely experimental. Many questioned whether tree planting could sustain a viable industry. By the 1930s, however, the amount of barren land was beginning to be a problem for Louisiana, and State and industry officials recognized that without regeneration an entire industry could be lost. The Louisiana Constitution provides for the Louisiana Forestry Commission to protect, conserve, and replenish the natural resources of the State.

State Nurseries

Louisiana was a pioneer State in the South in establishing a nursery to produce seedlings for reforestation. The State's first nursery began operation in 1925 and was located at the Alexander State Forest, near Woodworth, LA. That year, the nursery produced and distributed more than 1 million seedlings throughout the State to landowners, schools, and organizations.

James Mixon (who later became the State forester), a graduate of Louisiana State University Forestry School, was assigned as State forest superintendent with responsibility for nursery operations in November 1940. Charles F. Delaney, who had directed the nursery operations since the program's inception, had died suddenly a few months earlier. By 1942, seedling production had increased to 10 million seedlings per year.

Thereafter, however, with the loss of CCC workers and the advent of World War II, seedling production declined. The nursery near Woodworth closed after the construction of two new State nurseries in 1947 and 1948 (Burns 1968).

By 1951, the two State nurseries, one in southwest Louisiana and one in northwest Louisiana, were producing 30 million seedlings annually. In 1953, the Southwest Nursery produced 34 million seedlings, leading the Nation in forest tree seedling production. To meet the demands created by the U.S. Department of Agriculture (USDA), Forest Service's Soil Bank Program, Louisiana constructed the Columbia Nursery in 1957, which helped boost production to a record high of 135 million seedlings in 1958. In 1959, the State built the Beauregard Nursery in the southwest corner of the State. As demand slowed, the Northwest Nursery closed in 1962. In 1965, the Southwest Nursery was put on standby and was later reopened in 1972 to produce hardwood seedlings (Louisiana Forestry Commission 1976). Closed again in 2002, the Southwest Nursery is now used as a scion bank for tree improvement.

Currently, the Louisiana Department of Agriculture and Forestry's Reforestation Division operates three seedling nurseries, which produce a combined average of 22 million advanced-generation seedlings each year. Most of these seedlings are bareroot loblolly, slash, longleaf, and spruce pine (figure 5). In addition, 500,000 containerized, improved longleaf pine are produced, an amount that is undergoing expansion in the upcoming season. In total, pine seedling production in Louisiana provides enough seedlings to replant approximately 33,000 ac (13,350 ha) of productive forest land each year. Annual hardwood production is about 4 million seedlings of 25 to 30 species (figure 6) and is enough to

Figure 5. The Louisiana Department of Agriculture and Forestry grows superior and advanced generation loblolly, slash, and longleaf pine. (Photo by Denise Barnette, Louisiana Department of Agriculture and Forestry, 2010)

Figure 6. Hardwood seedlings grown at the Louisiana Department of Agriculture and Forestry's nurseries are used in bottomland and upland plantings. (Photo by Doug Gillett, Louisiana Department of Agriculture and Forestry, 2008)

reforest approximately 15,000 ac (6,070 ha) per year. The 2 billionth tree grown at the State nurseries was planted at the Louisiana Tech School of Forestry in 1983. The 3 billionth tree will be grown in the 2013 through 2014 crop year.

Private Nurseries

In 1946, Continental Can established Louisiana's first privately owned forest seedling nursery northeast of Jonesboro to assure stock for company lands. Leonard W. Bosch became nursery superintendent in 1965, and by 1970, the nursery produced 6 million seedlings annually (Bosch 1970). Bosch nurtured the nursery and fledgling seed orchard through years of change from Continental Can to Continental Forest Industries, and in 1986, he negotiated an agreement to purchase the operation (Bosch 2012). He grew seedlings there until 2004 when, because of his declining health, the nursery was closed. At its peak, Bosch Nursery produced 31 million seedlings, with an average of 18 to 20 million seedlings during each year of its operation (Bosch 2012). From 1995 through 2005, several private nurseries were established to meet seedling demands of Federal cost-share assistance programs. The State currently has three private forest nurseries that each produce between 2 and 4 million seedlings annually, primarily for restoration programs.

Federal Nurseries

In 1921, the USDA Forest Service established the Southern and Appalachian Forest Experiment Stations at New Orleans, LA, and Asheville, NC, respectively. In 1924, the New Orleans station hired Phillip C. Wakely, a recent graduate of Cornell University (where he attended the first 4-year school of forestry in the United States). Over the years, Wakely made great strides in reforestation research. His research programs developed seed, seedling, and tree-planting technology still in use today (Willis 2005). In 1964, James Barnett took the reins from Phil Wakely. Barnett's work on improved seedling growth potentials and the growth of longleaf pine out of the grass stage, along with the production of containerized longleaf, helped pave the way in the effort to reestablish longleaf pine within its natural range (Willis 2005).

In August 1933, the USDA Forest Service selected an abandoned farm in an open stand of young longleaf pine as a site for the Catahoula Nursery, later known as Stuart Nursery. CCC workers from a nearby camp provided labor, except for building construction. In March 1934, the USDA Forest Service sowed 14.0 ac (5.7 ha) to longleaf, slash, and shortleaf pine. The Stuart Nursery produced 8,887,000 seedlings in 1934, which were planted on the Kisatchie National Forest, the DeSoto National Forest, and in Alabama, Arkansas, Florida, and Texas (USDA Forest Service 1935). The Stuart Nursery remained in operation until 1962 when it was converted into a seed orchard and designated as the Stuart Genetic Resource Management Area.

Tree Improvement

The predecessor of the Louisiana Department of Agriculture and Forestry, the Louisiana Office of Forestry, began tree improvement in 1963 and in 1969 became one of the charter members of the Western Gulf Forest Tree Improvement Program. Today, the Louisiana Department of Agriculture and Forestry maintains an extensive tree improvement program. This program, through selective tree breeding, produces seed native to Louisiana and the surrounding region with excellent disease resistance and superior growth aspects.

The main species included in the tree improvement program are loblolly, slash, and longleaf pines for the following reasons:

- Loblolly pine is the most widely grown tree in Louisiana.
- Slash pine is grown in the southern region of the State.
- Longleaf pine was part of the State's original virgin forests and is the focus in a new planting initiative.

In 2000, work began on a new hardwood orchard. The orchard currently consists of improved cherrybark oak, water oak, nuttall oak, sweetgum, green ash, willow oak, sycamore, and baldcypress. The baldcypress orchard includes salt-resistant cypress for the coastal region.

Regeneration and Conservation

More than 120 million pine and hardwood seedlings are planted each year in Louisiana. Industry is responsible for much of the planting, whether it is on their lands or private land under their management programs. The days of cut-and-run forest practices are gone; the timber industry is a major player in replenishing and managing a sustainable-yield forest. In addition, several State and Federal programs have resulted in significant tree planting during the past several decades.

State Programs

Louisiana initiated the Louisiana Forest Productivity Program in 1998, in response to concerns about possible future timber shortages. To be eligible for the program, landowners must own a minimum of 5 contiguous ac (2 ha) suitable for growing commercially valuable timber species. Landowners may receive 50 percent of the reforesting costs and timber stand improvement, up to $10,000 per year (LDAF 2012a). Since 1998, this program has been responsible for planting more than 35,000 ac (14,164 ha) (Aronstein 2012) leading to increased timber output (figure 7).

The Woodland Assistance Program provides technical support and planning for all facets of forest management on private land (LDAF 2012a).

The Forest Stewardship Program assists private forest landowners in more actively managing forest resources; maintaining forest productivity and health; and increasing social, economic, and environmental benefits of forest lands. This program encourages increased coordination on the part of Federal, State, and private land agencies to assist private, nonindustrial forest landowners (LDAF 2012a).

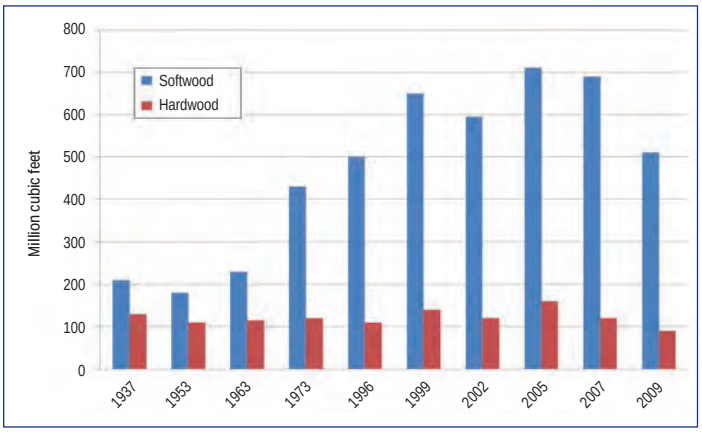

Figure 7. Hardwood and softwood timber product output over time in Louisiana. (Data source: Oswalt and Johnson, 2012)

In 1967, the Louisiana Department of Wildlife and Fisheries (LDWF) started restoring and establishing wildlife management areas (WMAs) across the State. To date, more than 22,000 ac (8,900 ha) of mostly wetland restoration has taken place (Dupuy 2012). LDWF owns the largest system of conservation lands in Louisiana, managing nearly 953,000 ac (38,445 ha) of fee title land within 61 WMAs and 5 refuges (The Nature Conservancy 2007). In addition, partners own 1.2 million ac (485,633 ha). Of those nearly 2.2 million ac (890,328 ha), 1.7 million ac (687,980 ha) are forested and under varying levels of forest management (The Nature Conservancy 2007).

The Louisiana Forestry Association facilitates the State's American Tree Farm System. The first tree farm under this program was approved for the Urania Lumber Company in 1951. It was fitting as the first location because Urania is considered the "Cradle of Reforestation" in the South because of Henry Hardtner's pioneering replanting program. Today, about 2,000 tree farms in the State total 1.5 million ac (607,041 ha) (Louisiana Forestry Association 2010a).

Federal Programs

A host of talented individuals who had their eyes on the future of the timber industry and the future of the country as a whole greatly influenced reforestation on Federal lands. In 1892, George W. Vanderbilt hired Gifford Pinchot as a forester for his Biltmore Estate near Asheville, NC, creating the first example of practical forest management on a large scale in the Nation (Pennsylvania Historical and Museum Commission 2012). In 1915, Louisiana's Henry Hardtner partnered with Samuel T. Dana of the USDA Forest Service. The two saw a need for forestry research and established large research plots on Hardtner's own reserve land in Urania, LA. In 1917, Yale University School of Forestry began sending graduating classes to Urania for 3 months of practical training on Hardtner's land (Willis 2005).

USDA Forest Service lands in Louisiana total 2,044,000 ac (827,195 ha). The Kisatchie National Forest is the State's only national forest and has played a pivotal role in the reforestation of Louisiana lands. When the largest sawmill west of the Mississippi River, the Gulf Lumber Company, closed in 1927, the USDA Forest Service was able to purchase some of its land, which later became part of the Kitsatchie National Forest. During its first 30 years, the Kisatchie National Forest was limited in its purchases because of the depressed economy and a tight Federal budget. In 1979 and 1980, however, it led all other national forests of the

South in revenue produced per acre (USDA Forest Service 2012). The Kisatchie National Forest is now home to some of the best natural longleaf pine habitat in the country.

Since 1998, the U.S. Fish and Wildlife Service (USFWS) has reforested or restored 41,000 ac (16,600 ha) of National Wildlife Refuge land in Louisiana in the lower Mississippi valley and Red River valley (Shelton and Meredith 2011). The USFWS owns and manages 24 refuges in Louisiana that encompass nearly 560,000 ac (226,600 ha), of which more than 50 percent is dominated by forest cover (The Nature Conservancy 2007). The USFWS, along with its partners, has done much work throughout this region in securing and replanting corridors and in connecting fractured timber tracts for wildlife and water quality.

The Forest Legacy Program of the USDA Forest Service, in partnership with States, supports efforts to protect environmentally sensitive forest lands. The program is voluntary and focuses on the acquisition of partial interest in privately owned forest lands (The Nature Conservancy 2007).

Federal cost-share assistance programs include the Forestry Incentive Program, the Conservation Reserve Program, the Wetlands Reserve Program, the Stewardship Incentive Program, the Environmental Quality Incentive Program, and the Wildlife Habitat Program. These programs have been responsible for the planting of hundreds of thousands of acres in Louisiana. Most of these plantings have taken place in the Red River and lower Mississippi River Alluvial Valley and have resulted in many acres of marginal croplands converted back to forest lands to the benefit of wildlife, recreation, water quality, and the environment. Louisiana's once-great bottomland hardwood ecosystem is slowly recovering because of these programs.

Challenges for Louisiana's Forests

Maintaining a healthy, vigorous forest requires hard work and dedication from public, private, and government entities to overcome the many challenges facing today's forests. In the following text, a few of the most significant challenges facing Louisiana's forests are addressed. There are many others such as insect, disease, and invasive species that must constantly be addressed as well.

Wildfire

From the early days of forest regeneration in Louisiana, the one most significant and constant challenge has been wildfire. The first State forester, R.D. Forbes, recognized the need for fire patrol, fire prevention, and public education. He proposed using spark arrestors on locomotive engines and advocated promoting public awareness of fire prevention using posters and lectures (LDAF 2010). In 1922 and 1923, two fire towers were constructed in Louisiana—the first on Great Southern Lumber Company land near Bogalusa and the second near Urania. By 1949, the State had 56 fire towers. By the late 1980s, the State replaced most of the fire towers with planes used for wildfire detection and support of ground crews. In 1925, newly appointed State Forester Billy Hine recognized the need for fire suppression personnel. Within 2 years, Hine hired 136 cooperative patrol staff members, 16 parish rangers, and 5 administrative staff members (LDAF 2010).

The acting State forester in 1942, Massey H. Anderson, stated, "Several large pulp and paper mills are now located in the State. Their raw products are entirely young, second growth timber. All of these wood-using industries are operating on the output of only partially productive forest land. If we can make our forest areas produce more wood products, through forest fire protection and wise management practices, more industry will be attracted to the State, giving permanency to our communities and increasingly larger payrolls" (Burns 1968). By the 1980s, the Office of Forestry employed 293 wildland firefighters, equaling approximately 129,246 ac (52,403 ha) of protection by a 2-person firefighting crew (LDAF 2010).

Education, along with mechanization and new forms of fire detection, has progressed generation to generation and resulted in major improvements in wildfire suppression. Even still, approximately 46,000 ac (18,615 ha) are destroyed in Louisiana annually by wildfire (LDAF 2010). Because of State budget constraints, the number of firefighters has been significantly reduced in the past 5 years, from 293 firefighters in the 1980s to 103 firefighters today (LDAF 2012b). This decrease in personnel, along with the extension of fire season because of recent drought situations within the State, could prove to be quite challenging in the future.

Urban Sprawl

Much of Louisiana's population is concentrated in the southern part of the State, which is also geographically the lowest elevation in the State. When populations shift from urban to rural in this area, they expand to higher, less flood-prone lands. This shift results in loss of timber lands and fragmentation of large timber blocks. It also increases the chance of wildfire and creates a much more difficult situation for fire suppression. Much of the forests' environmental, economic, and recreational benefits are lost as these large tracts are fragmented into smaller suburban and urban homesteads. In many cases, drainage and natural flow of waterways are

changed to accommodate urban sprawl, adding to the problem of wetland loss within a very sensitive ecosystem.

Wetlands Loss

Louisiana has a long history of levee building from early settlers and local governments to the USACE. These systems were built to protect people and property from floods but have proven over time to create new challenges. Diversion of the natural flow and flushing of river systems has disrupted much of the natural hydrology of Louisiana's wetlands and swamps. The building of flood control structures, forced drainage projects, canals, and navigation channels—along with naturally occurring forces—have added to this disruption. Tremendous acreage that once had seasonal wet and dry spells is now permanently flooded with fresh or salt water. Coastal land is sinking while gulf waters are rising (Torbett 2010). Much of the land that was once a healthy, thriving wetland forest is dead or dying because of stagnated swamps and salt-water intrusion. The harnessing of the Mississippi River and the digging of a checkerboard of canals have allowed more and more salt-water intrusion, devastating the cypress tupelo swamps of south Louisiana (figure 8).

Figure 8. Baldcypress, which grows throughout Louisiana, has been greatly affected by rising waters and salinity. This species is important in restoration and regeneration efforts. (Photo by Denise Barnette, Louisiana Department of Agriculture and Forestry, 2013)

Louisiana coastal regions are being lost at an alarming rate of 25 to 35 mi^2 (65 to 91 km^2) per year. This loss represents 80 percent of the coastal wetland loss in the entire continental United States (Louisiana Coastal Wetlands Conservation and Restoration Task Force and the Wetlands Conservation and Restoration Authority 1998). Mitigating this wetland loss must be a priority not only for Louisiana but also for the Nation. Families and businesses are being displaced and valuable infrastructure is being lost. The Breaux Act in 1990 and the Coastal 2050 initiative in 1997 paved the way for a solution to this problem. Since then, many individuals, local communities, and government agencies have done much work to help alleviate wetlands loss. Several things caused this problem, therefore, several things must be done to alleviate it. One important step is to aggressively reforest as much area as possible. If wetlands loss is not addressed quickly, one of the great wetland and bottomland hardwood ecosystems in the world will be lost, along with an entire culture and, for many, a way of life.

Baldcypress

Although less than 2 percent of the trees harvested in Louisiana are baldcypress (figure 9), a discussion of tree planting in the State would not be complete without including this important species. Baldcypress grows from one end of Louisiana to the other. Its wood quality characteristics have made it popular for more than 200 years for use in construction, boat building, and cabinet and furniture making. It is an important tree to the wood industry and for restoration efforts across the State.

Reforestation within the coastal region is difficult because of the many human-made canals throughout the landscape that have brought saltwater further inland, eroding the viability of standing timber, and preventing natural regeneration

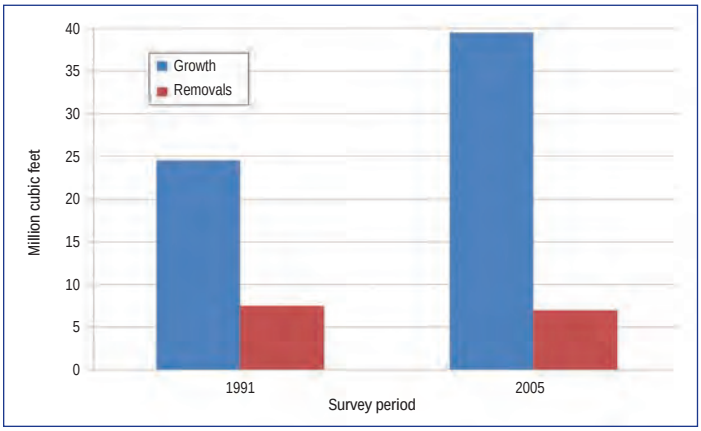

Figure 9. Cypress net growth compared with removals in Louisiana. (Data source: Louisiana Forestry Association 2010b; data from USDA Forest Service)

(Tompkins 2007). Sea level rise also results in gradual increases in flooding and salinity in coastal forested wetlands. Studies have shown that baldcypress is one of the most tolerant species of long flood durations and relatively deep flooding. Recent studies have also shown baldcypress to be tolerant of flooding with low-salinity water (Coastal Wetland Forest Conservation and Use Science Working Group 2005). Increases in the severity and length of flooding in coastal areas have reduced the productivity of the cypress-tupelo swamps. Baldcypress, however, is still a very important timber tree, and it is important that work continues in the regeneration and management of this tree. If baldcypress is limited only to restoration and not utilized for its economic value, there will be less planting of it, and, accordingly, less environmental gain. Appropriately managed, this tree can be good for the environment as well as the economy of the State.

Future Outlook

Louisiana's forests provide a sustainable yield of wood products, along with recreation, wildlife habitat, environmental benefits, and water quality. The future of the timber industry and forest restoration efforts in Louisiana is bright. An increased awareness of proper land use practices and partnerships among private landowners, State agencies, Federal agencies, and commercial entities has evolved into a dynamic forest cover throughout the State.

Tree planting in Louisiana includes not only planting in intensely managed yield forests but also planting to benefit wildlife habitat, restore wetlands, improve urban settings, and enhance recreation. Planting of more native species is increasing, and biodiversity within plantings is becoming the norm in restoration projects.

The combination of dedicated State, Federal, private, and commercial entities working together for the economic and environmental well-being of the State has restored Louisiana from its devastated landscape of the 1940s. Louisiana is again green and growing, although much work remains. Forests are a renewable resource and, when managed properly, have a strong, positive economic and environmental impact in Louisiana.

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Guidelines for Seed Collection and Stratification of Common Juniper (*Juniperus communis* L.)

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Abstract

Common juniper (*Juniperus communis* L.) is the most widely distributed conifer in the Northern Hemisphere. In the United Kingdom, juniper is one of only three native conifer species. Juniper populations are declining, however, particularly in southern England. In some cases, nursery production is seen as a means of boosting these populations. This article, which provides practical guidelines for collecting and processing berries and for stratifying the seeds, is intended for nursery managers, conservation practitioners, and related professionals who are concerned with propagating, restoring, and managing juniper ecosystems.

Introduction

Common juniper (*Juniperus communis* L.) is the most widely distributed conifer in the Northern Hemisphere, occurring in North America, Europe, Asia, and parts of North Africa (Thomas and others 2007). It is sometimes split into several subspecies (Eckenwalder 2009) or varieties (Farjon 1998). In the United Kingdom, three subspecies exist, viz. *J. communis* L. subsp. *nana* (J. & C. Presl) Nyman, *J. communis* L. subsp. *communis*, and *J. communis* subsp. *hemisphaerica* (J. & C. Presl) Nyman (BSBI 2012). This article refers to *J. communis* L. subsp. *communis*, which is widely distributed in the United Kingdom, although the populations are declining, particularly in southern England (Dearnley and Duckett 1999, Long and Williams 2007, Thomas and others 2007, Ward 1973). The decline is largely due to the lack of natural regeneration, which is attributed to poor seed quality, seed predation, and a shortage of suitable habitat conditions for germination and seedling establishment (Verheyen and others 2009, Ward 2010). Therefore, juniper is a United Kingdom Biodiversity Action Plan priority species (UK Biodiversity Reporting and Information Group 2007). Because of this priority, juniper is the focus of several *in situ* and *ex situ* conservation efforts. In some cases, nursery production is seen as a means of sustaining struggling juniper populations. Propagation from seeds, however, is neither simple nor straightforward. Like many other trees, juniper produces a large proportion of empty seeds. Empty seed production has a negative effect on the cost and efficiency of nursery production. Therefore, the overall aim of this article is to provide practical guidelines for propagating common juniper (*Juniperus communis* L. subsp. *communis*) from seeds.

Berry and Seed Quality Before Berry Collection

Testing berry and seed quality is essential for determining whether to collect berries and, also, how many to collect. The cut test is a crude, destructive, but quick means for determining berry and seed quality.

1. Check the berries for signs of seed predation. Parasitized berries usually can be readily distinguished from healthy berries. In the United Kingdom, the two main seed predators are eriophyid mites (*Trisetacus quadrisetus*) and juniper seed chalcids (*Megastigmus bipunctata*), the presence of which results in characteristic exit holes in the berries (figures 1a and 1b). In addition, eriophyid mites (figure 1c) cause fluted seed coats, which are visible when the berries are cut along the equator (figure 1d).
2. Select 10 plump purple berries per bush (figure 2a). Avoid green, wrinkly purple, or brown berries (figures 2b, 2c, and 2d). Remember that you can improve the accuracy of the cut test by increasing the sample size.
3. Cut the berries (at the equator) in half using a sharp penknife (preferably against a firm surface such as a notebook).
4. Count the seeds and assign them to either the filled or empty category based on a visual assessment of the cut seeds with regards to color, texture, and degree of embryo development in the following descriptions:
    a. Filled seeds contain a well-developed, firm, off-white (sometimes brownish) embryo and megagametophyte and, therefore, are scored as probably viable (figures 3a, 3b, and 3c).
    b. Empty seeds are entirely empty, contain shrivelled contents, or are embryo-less and, therefore, are scored as nonviable (figures 3a and 3d).

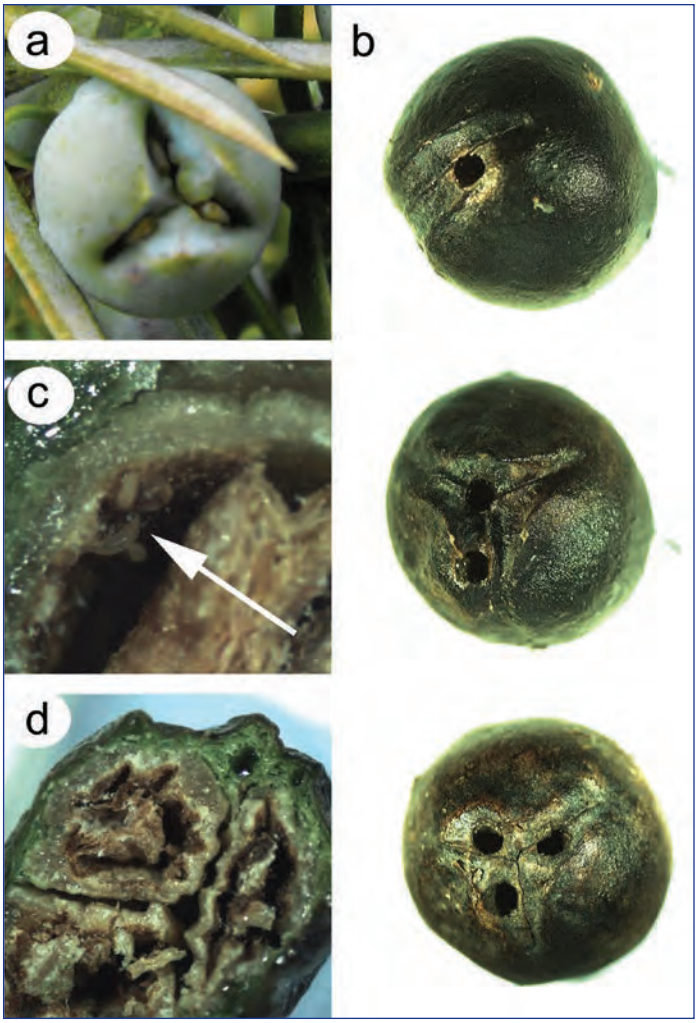

Figure 1. When collecting juniper berries, avoid parasitized berries with characteristic exit holes caused by (a) eriophyid mites or (b) juniper seed chalcids. The (c) eriophyid mites also cause (d) the seed coats to become fluted, which is visible when the berries are cut along the equator. (Photos by Shelagh McCartan, Forest Research, Forestry Commission, 2009)

A hand lens is sometimes useful. If less than 20 to 30 percent are filled seeds, then sample another bush. The age of the bushes also affects seed quality. In a small trial, we found that older bushes had only 4-percent filled seed compared with 70-percent filled seed in younger bushes (table 1). Using younger bushes may be particularly important for restoration management of juniper populations.

In addition, seed quality can vary significantly among populations and within the same population among years. In

Figure 2. When collecting, pick (a) plump purple berries and avoid (b) green, (c) wrinkly purple, and (d) brown berries, because using plump purple berries reduces the amount of processing and improves the seed-lot quality. (Photo by George Gate, Forest Research, Forestry Commission, 2009).

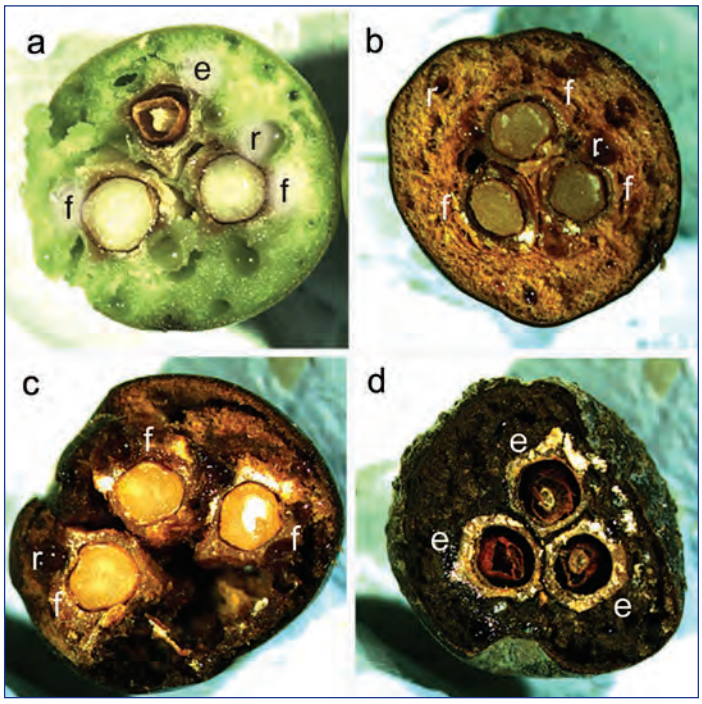

Figure 3. When determining seed quality, cut the berries along the equator and assess whether seeds are filled (see f in a, b, and c) or empty (see e in a and d). Also note the resin vesicles (r), which appear (a) green in immature berries and (b and c) amber in mature berries. (Photos by Shelagh McCartan, Forest Research, Forestry Commission, 2009 and 2012)

Table 1. Cut test results for plump purple berries collected from two populations in Aston Rowant, England. The young bushes were originally cuttings taken from the old bushes on an adjacent site. (Data source: OS grid reference SU7299, collected 2011)

Population	Total number of seeds*	Number of empty seeds	Number of filled seeds
Young (~10 years)	116	35	81
Old (~75 or more years)	106	102	4

OS = Ordnance Survey.
*Total number of seeds extracted from 40 berries.

2008, we found that berries from Harkerside Moor had only a 4-percent filled seed compared with 100 percent in Moughton Scarr, although both populations are found in the Yorkshire Dales National Park (table 2). Yet, in 2009, berries from Harkerside Moor had a 63-percent filled seed. Therefore, if seed quality is poor in a particular population, try again the following year.

Berry Collection

Collecting juniper berries is a labor-intensive process. The key to successful berry collection is timing; too early and the berries are not ripe, but too late and the birds will have eaten them. The optimum time in the United Kingdom is usually between late September and late October.

1. Get permission from the landowner.
2. Get outfitted properly using the following protective gear.
   a. Stout walking or Wellington boots appropriate for slippery or uneven terrain.
   b. Waterproof or thick trousers for going through overgrown bramble (*Rubus fruticosus* L.), bracken (*Pteridium aquilinum* [L.] Kuhn), and sloe (*Prunus spinosa* L.).
   c. Snug-fitting gloves (for instance, disposable latex gloves) to protect your hands from the prickly foliage.
3. Do a cut-test (described previously) on a small sample of berries to determine whether to collect the berries. If less than 20 to 30 percent are filled seeds or have signs of seed predation, then try another bush. Do not waste time collecting berries if the bushes show signs of heavy seed predation.
4. Collect plump purple berries (figure 2a) in heavy-duty polythene bags (to prevent rips from the prickly foliage). Juniper populations have 2- and 3-year reproductive cycles; berries appear to mature more rapidly in warmer climates than cooler ones (Ward 2010). Therefore, female cones and berries of two different generations usually occur simultaneously on a bush. We found that different color berries had different proportions of filled and empty seeds, ranging from 4-percent filled seed in brown berries to 77-percent in plump purple berries (table 3). So avoid green berries (figure 2b), which are still immature. Also avoid wrinkly purple (figure 2c) or brown berries (figure 2d), which often contain empty seeds. This strategy reduces the amount of processing and improves the overall quality of the seed lot.
5. Store berries in a loosely tied polythene bag (to allow for ventilation) in a refrigerator until required for processing. Remember to label the bag with collection details (collector's name, site location, and date).

One person can harvest about 7 oz (200 g) of berries in 1 hour from a good crop, possibly even more, depending on access to the bushes. It can take two people between 3 and 4 hours to collect a similar amount from a poor crop. In general, collect berries from at least 20 bushes (and also different growth forms) to maintain the genetic diversity of the population (Broome 2003). Also remember to leave some berries on the bushes for the birds.

Table 2. Cut test results for plump purple berries collected from three populations within the Yorkshire Dales National Park, England. (Data source: OS grid references SE01199818, SD79317135, and SD89299893, collected 2008)

Population (OS grid reference)	Total number of seeds*	Number of empty seeds	Number of filled seeds
Harkerside Moor (SE01199818)	28	27	1
Moughton Scarr (SD79317135)	21	0	21
Thwaitestones (SD89299893)	30	7	23

OS = Ordnance Survey.
*Total number of seeds extracted from 10 berries except for Moughton Scarr, where only 9 berries were used.

Table 3. Cut test results for different color berries collected from a population at Thwaitestones, England. The berries were collected on the same day from several bushes. (Data source: OS grid reference SD89299893, collected 2008)

Berry color	Total number of seeds*	Number of empty seeds	Number of filled seeds
Green (figure 2b)	29	17	12
Plump purple (figure 2a)	30	7	23
Wrinkly purple (figure 2c)	29	24	5
Brown (figure 2d)	28	28	1

OS = Ordnance Survey.
*Total number of seeds extracted from 10 berries.

Processing Berries

Processing berries is a sticky but worthwhile process, which removes potential chemical inhibitors, and thereby improves germination (Broome 2003). The process described in the following seven steps is suitable for small batches of berries (about 7 oz or 200 g):

1. Soak berries in water for 2 or more hours (to soften the flesh).
2. Macerate berries in a domestic blender (figure 4a). Use one or two short pulses of about 5 seconds on the lowest setting. Ensure that berries are just covered with water.
3. Screen pulp through stackable sieves with decreasing mesh sizes (for instance, 0.25 in [6.30 mm], 0.13 in [3.35 mm], and 0.09 in [2.24 mm]) under running water (figure 4b). This step is important for removing small pieces of pulp and, therefore, reduces time spent on step 4. If a large number of intact berries remain, repeat steps 2 and 3. Do not put naked seeds in the blender, because the blades will damage them.
4. Float off remaining pulp and empty seeds under running water. Use a large beaker or bucket (about 5.3 qts or 5.0 L) to allow sufficient depth for the filled and empty seeds to separate (figure 4c). Adjusting the flow rate is an art, so it is recommended to trap waste in a hand-held sieve in case of a mishap. As an alternative method, fill the beaker with water, stir the pulp vigorously, wait a few seconds to allow the filled seeds to settle (or sink), and then scoop out the floating waste with a tea strainer.
5. Spread filled seeds thinly on a tray and air-dry overnight. If you have a sufficient number of seeds, do a cut test (described previously) on a small sample of the 'sinkers' to confirm that the seeds are filled. A small proportion of empty seeds have very thick seed coats, which makes separation impossible.

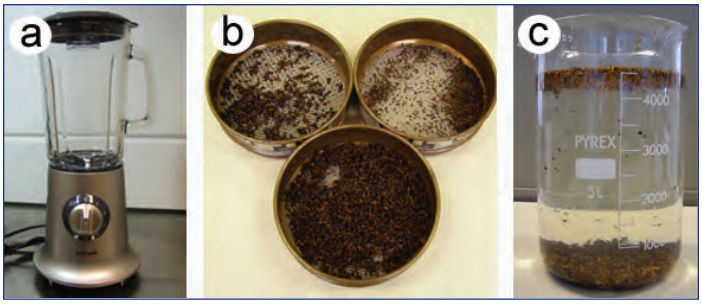

Figure 4. When processing, (a) macerate the berries in a domestic blender, (b) wash the pulp through a series of stackable sieves with decreasing mesh sizes, and then (c) float off the empty seeds in a large beaker. (Photos by Shelagh McCartan, Forest Research, Forestry Commission, 2008 and 2009)

6. Store the seeds in a loosely tied polythene bag (to allow for ventilation) in a refrigerator until required for propagation (up to 4 weeks). Do not store seeds for too long because they may deteriorate at high moisture content. For long-term storage, air-dry the seeds to low moisture content (about 10 to 15 percent fresh weight basis) and then store in an airtight, 500-gauge polythene bag in a refrigerator (39.2 °F [4.0 °C]) until needed.
7. Clean sticky hands, glassware, and sieves with methylated spirits.

This process is very efficient at separating filled and empty seeds. However, you can modify the steps to fit your needs. Remember that a trade-off exists between efficiency (time and effort of processing) and gain (proportion of empty seeds removed from seed lot). For production purposes, note that seed size varies among populations, which influences the number of filled seeds per gram. We found that this seed count ranged from about 89 seeds per gram for Dalcataig to 124 for Harting Down (table 4).

Seed Stratification and Germination

Juniper seeds are dormant and require stratification to germinate (Johnsen and Alexander 1974). Stratification of deeply dormant seeds such as juniper often requires alternating warm and cold periods as described in the following steps.

1. Sow seeds in trays containing moistened potting medium (peat: grit [1:1 v/v]) and lightly cover with the same mixture. Do not sow seeds too densely because it can make pricking out the seedlings difficult.
2. Place trays in loosely tied polythene bags (to reduce water loss but allow for ventilation).
3. Transfer the trays to an incubator set at an alternating 50/59 °F (10/15 °C) (12/12 hr) or leave in a room with a similar temperature range for 2 weeks or more. We found that extending this warm phase enables a few more seeds to germinate but can delay seedling emergence by an equivalent length of time (figure 5).

Table 4. Average number of filled seeds per gram (0.04 oz)* for four populations in the United Kingdom. (Data source: OS grid references SU803185, NT229649, SE01199818, and NH367143, collected 2009)

Population (OS grid reference)	Average number of seeds per gram (0.04 oz)
Harting Down, England (SU803185)	124 ± 4
Pentlands, Scotland (NT229649)	106 ± 3
Harkerside Moor, England (SE01199818)	96 ± 3
Dalcataig, Scotland (NH367143)	89 ± 4

OS = Ordnance Survey.
*This calculation was based on the weight of 100 seeds (N = 8 for each population).

4. Transfer trays to a refrigerator (39.2 °F [4.0 °C]). After 18 to 20 weeks in the cold phase, the seedlings start emerging readily at temperatures between 39.2 and 59.0 °F (4.0 and 15.0 °C). Some seeds will not germinate even when they are filled.

If germination is good, then few benefits remain to repeating steps 3 and 4 because hardly any additional seeds will germinate in the next cycle. Significant differences may exist between populations, largely because of genotype and environmental factors (Tylkowski 2009). In some cases, seeds may require two or more growing seasons to break dormancy, particularly when they are grown under nursery or field conditions (Broome 2003, Tylkowski 2009). If germination is poor, then it may be quicker and more efficient to simply collect and process more berries the following year.

Pricking Out Seedlings

Even after stratification, seedlings emerge slowly and erratically over several weeks. Therefore, it is critical to check progress regularly.

1. When the cotyledons start unfolding, carefully lift the seedlings using a table fork or similar implement. Then, using a dibber, transplant them into plug-trays containing potting mixture (peat: grit [1:1 v/v]). Transplant sooner rather than later to minimize damage to the roots.
2. Place plug-trays in a mist-bed for a few weeks. Gradually reduce the humidity as the seedlings harden off.

For further information on reintroducing juniper seedlings to the natural habitat, see Wilkins and Duckworth (2011).

Conclusions

Common juniper has a long and complicated reproductive life cycle spanning 2 or 3 years. Usually female cones and berries of two or more generations occur simultaneously on a bush. Therefore, it is critical to pick only mature (plump purple) berries and to avoid immature (green) or unproductive (wrinkly purple or brown) berries. Cautious berry picking improves the overall quality of the seed lot and reduces the time and effort spent on processing. Unlike propagating many other conifers, processing junipers is complicated; extracting juniper seeds involves macerating, sieving, and floating berry pulp to separate filled and empty seeds. This extraction is a sticky, time-consuming but worthwhile process. Processing offers the following benefits:

- A higher proportion of filled seeds due to the removal of empty seeds.
- Faster germination of seeds due to the removal of chemical inhibitors in the berries.

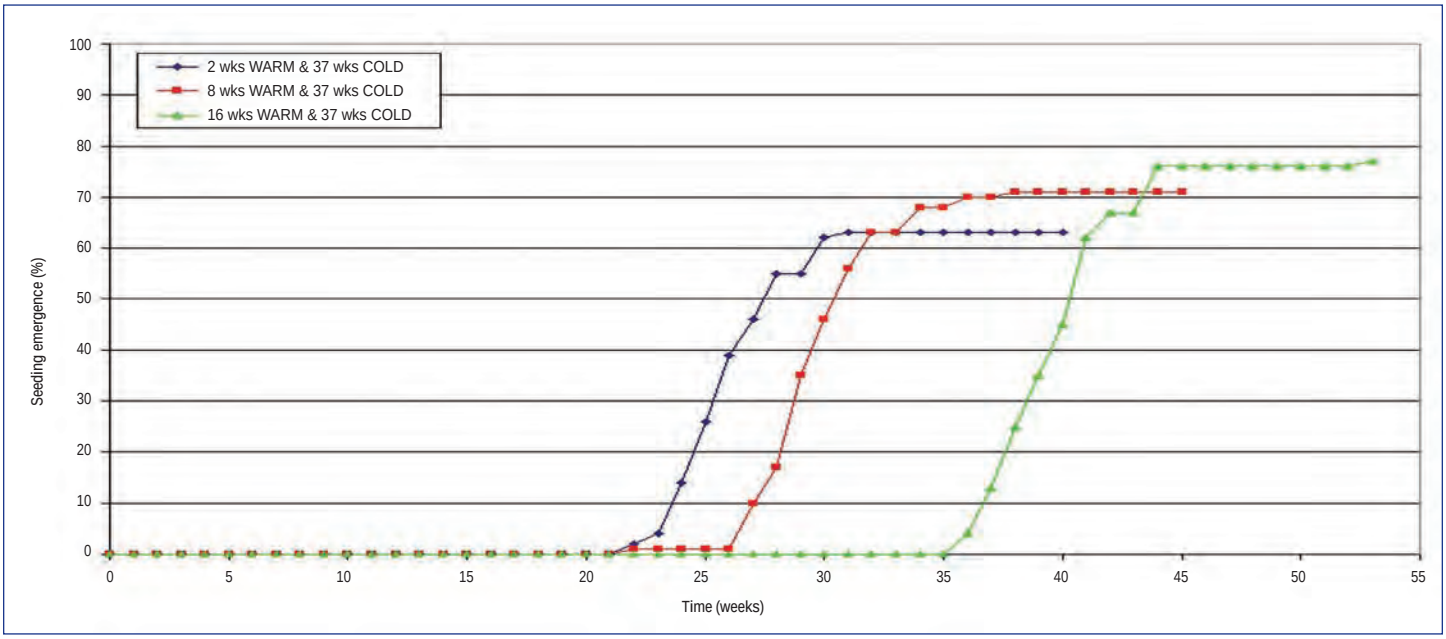

Figure 5. Seedling emergence of juniper after various lengths of warm (alternating 50/59 °F or 10/15 °C [12:12 hr]): cold (39.2 °F or 4.0 °C) stratification (seeds collected from Thwaitestones, England). Note: The seedlings readily emerged at 39.2 °F (4.0 °C). (Data source: SD89299893, collected 2008)

In addition, juniper seeds require stratification to break dormancy. Stratifying the seeds serves two purposes: (1) the warm phase allows the embryo to mature and/or inhibitors to leach out of the seed coat, and (2) the cold phase breaks dormancy (Johnsen and Alexander 1974, Pack 1921, Thomas and others 2007). Stratifying seeds offers the following benefits:

- A higher germination percentage.
- Faster and more uniform germination of seeds due to dormancy breakage.

We hope these guidelines are a significant step toward cost-effective propagation and *ex situ* conservation of common juniper.

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Comparing Seven Planting Tools for Container-Grown Longleaf Pine Seedlings

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Abstract

We compared seven tools for planting container-grown longleaf pine seedlings in fine sandy loam in Louisiana and in fine sand in Alabama. The tools were (1) JIM-GEM® KBC dibble bar, (2) JIM-GEM® OST Dibble Bar, (3) Terra Tech Styro 8 Dibble Stick, (4) container seedling tube dibble, (5) hoedad, (6) auger, and (7) shovel. Significant differences in variances between the two sites 15 months after planting negated comparing tools between sites. When tools were compared at individual sites, significant root collar diameter and shoot dry weight differences were reported in Louisiana and root distribution differences were reported in Alabama. Root mass, root/shoot ratio, and number of first-order lateral roots egressed from the root plug did not differ significantly among planting tools at either site.

Introduction

Interest in restoring longleaf pine (*Pinus palustris* Mill.) across its native range in the Southeastern United States has partly focused on increasing its acreage from 1.4 to 3.2 million ha (3.4 to 8.0 million ac) by 2024 (America's Longleaf 2009). The Longleaf Partnership Council estimated that in 2012 1.7 million ha (4.2 million ac) of forest were dominated by longleaf pine (Gaines 2012). The States within the longleaf range have projected that there will be 2.4 million ha (6.0 million ac) of longleaf pine range wide by 2027 (Gaines 2012). To achieve either of these outcomes will require forest, pasture, and croplands to be reforested or converted to longleaf pine, primarily via planting seedlings.

Up to 69 million longleaf pine seedlings are produced annually, of which 70 to 90 percent are grown in containers (Barnard and Mayfield 2009, McNabb and Enebak 2008, South and others 2005). With the preference for container stock, research continues across the longleaf pine range to examine the effects of size and type of container on longleaf pine seedling quality, both in the nursery and after outplanting (e.g., Barnett and McGilvray 2002; Haywood and others 2012; South and others 2005; Sword Sayer and others 2009, 2011). One emphasis of this research has been to improve the distribution of fibrous roots within the container cavity and thereby the outplanted seedling's root architecture for years to come (Barnett and McGilvray 2002; Sword Sayer and others 2009, 2011). How important is the planting tool, however, in determining shoot and root development of planted seedlings?

Several kinds of planting tools have been used in container studies. South and others (2005) used augers to plant seedlings while Sword Sayer and others (2009) used solid, round dibbles or punches although, in both cases, the research focused on container type and size. Jones and Alm (1989) and Johnson and others (1998) evaluated planting tools, but their emphasis was on planting errors, seedling survival, and height growth rather than on root-system development. Leduc and others (2011) found root structure differences when comparing two tools on a single site. Bolstered by these results, we expanded to comparisons of seven planting tools used at two distinct locations—a fine sandy loam in Louisiana and a fine sand in Alabama. The objectives were to determine if planting tool affects subsequent shoot and root development of longleaf pine seedlings.

Methods

The U.S. Department of Agriculture (USDA), Forest Service Southern Region, Atlanta, GA, supplied the longleaf pine seeds that came from a Florida source. Seeds were sown in mid-May 2009 in Copperblock™ Styroblocks (Beaver Plastics model number 112/105, 3.6 cm [1.4 in.] diameter with 14.8 cm [5.8 in.] depth). Using protocols adapted from Barnett and McGilvray (1997, 2000), USDA Forest Service personnel at the Alexandria Forestry Center, Pineville, LA, grew the seedlings for 28 weeks. Briefly, the growing medium was a 1:2 (volume:volume) mixture of peat moss and vermiculite amended with Scott's Osmocote® 14-14-14 slow-release fertilizer at a rate of 3.6 kg/m^3 (6.1 lb/yd^3). Between mid-July and late August 2009, personnel applied a 0.05 percent (weight/volume) solution of Peter's Professional® 20-20-20

water-soluble fertilizer three times to root plug saturation. In mid-August, personnel drenched seedlings with a 0.12 percent solution of Scott's Banrot® broad-spectrum fungicide at 2.50 L/m^2 (0.06 gal/ft^2), followed by 1.25 L/m^2 (0.03 gal/ft^2) water to rinse chemical residue off the needles. Seedlings were grown for 4 weeks under ambient light in a greenhouse before being moved outdoors and grown until outplanting.

Seedlings were outplanted in December 2009 on the Palustris Experimental Forest (31.162° N., 92.668° W.) in southwest Rapides Parish, LA, and the Escambia Experimental Forest (31.027° N., 87.041° W.) in southwest Escambia County, AL. At the Palustris site, the soil is a Malbis fine sandy loam (fine-loamy, siliceous, subactive, thermic Plinthic Paleudults), and the soil at the Escambia site is a Troup fine sand (loamy, kaolinitic, thermic Grossarenic Kandiudults).

Before seedlings were planted, a 15- by 20-m (49- by 66-ft) area was rotary mowed. Single tree plots were established in a completely randomized experimental design laid out as 10 rows of 14 trees each at 1- by 1-m (3.3- by 3.3-ft) spacing. Twenty container seedlings were replicates for each treatment that were randomly planted with each of the seven tools for a total of 140 seedlings. The seven planting tools were (1) JIM-GEM® KBC Dibble Bar, (2) JIM-GEM® OST Dibble Bar, (3) Terra Tech Styro 8 Dibble Stick (dibble stick), (4) container seedling tube dibble (tube dibble), (5) hoedad, (6) auger with a 4.45-cm (1.75-in.) inside-bit diameter, and (7) shovel (figure 1). The shovel was used to carefully plant each seedling as one would for landscaping purposes and was meant to be the good-as-planting-can-be check treatment (figure 2). The hoedad was considered the most difficult tool to use and required the most time to plant seedlings on these relatively flat sites, and the auger and shovel required more time than the KBC dibble bar, OST dibble bar, dibble stick, and tube dibble to plant seedlings. The KBC dibble bar, OST dibble bar, dibble stick, and tube dibble were similarly easy to use and required about the same amount of time to plant seedlings. Cost of tools varied among the following tools: KBC dibble bar ($35.96), OST dibble bar ($35.50), dibble stick ($81.50), tube dibble ($62.65), hoedad ($92.40), auger ($158.00), and shovel ($61.50).

On the Palustris site, growing season (March through November, 2010) precipitation totaled 74.3 cm (29.3 in), which was 34.0 cm (13.4 in) less than the 50-year average (National Climatic Data Center 2011). Average daily temperature was 23.0 °C (73.4 °F) for the growing season, which was great-er than the monthly 50-year average from April through November. Similarly, on the Escambia site, growing season precipitation totaled 82.3 cm (32.4 in), which was 26.3 cm (10.4 in) less than the 50-year average. Average daily temp-erature was 21.8 °C (71.3 °F) for the growing season, which was greater than the monthly 50-year average from April through November. Based on monthly Palmer Drought Severity Index values, the Palustris site was in mild-to-severe drought conditions April through November and the Escambia site was in mild-to-moderate drought conditions June through November.

In March 2011, 15 months after planting, all longleaf pine seedlings were excavated at a 15-cm (6-in) radius from the stem base and effort was made to extract the roots to their deepest point. Excavated seedlings were washed before measurements were taken. Root-collar diameter (RCD) was measured with calipers. Seedlings were separated into

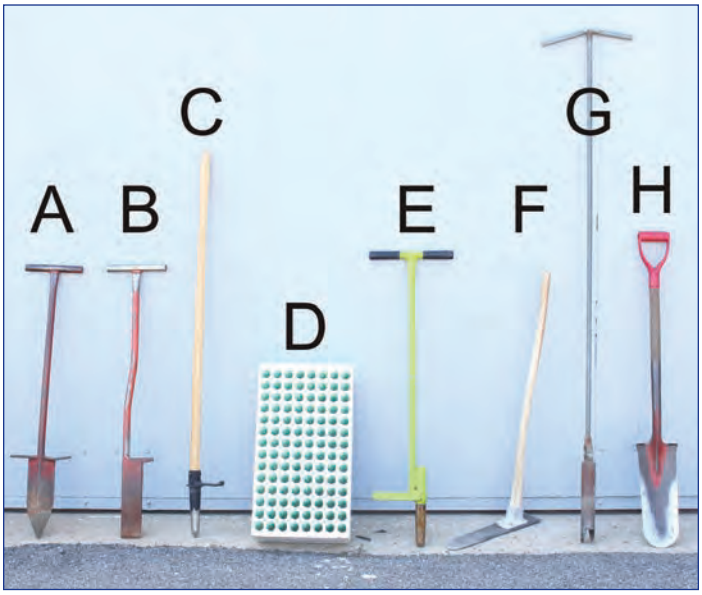

Figure 1. The seven planting tools and type of Styroblock used in this study were (A) JIM-GEM® KBC dibble bar, (B) JIM-GEM® OST dibble bar, (C) Terra Tech Styro 8 dibble stick, (D) Copperblock™ Styroblock, (E) container seedling tube dibble, (F) hoedad, (G) auger, and (H) shovel. (Photo by Daniel J. Leduc, USDA Forest Service, Southern Research Station, Alexandria Forestry Center, 2012)

Figure 2. Photograph illustrating the careful planting of a seedling in the good-as-planting-can-be check treatment. (Photo by Daniel J. Leduc, USDA Forest Service, Southern Research Station, Alexandria Forestry Center, 2009)

above- and below-ground portions using the root collar as the dividing point. After drying to equilibrium at 70 °C (158 °F) in a forced-air oven, dry weights of the above- and below-ground portions were determined. The root/shoot ratio of each seedling was calculated.

To determine root-system architecture, the number of first-order lateral roots (FOLRs) that had egressed from the root plug was counted. FOLRs are the primary lateral roots with diameters greater than 1.00 mm (0.04 in.) at 5.00 mm (0.20 in.) from the taproot. To do the counting, each seedling's root system was placed on a diagram divided into quadrants with a solid black central circle that delineated the outside wall of the root plug before outplanting (Leduc and others 2011), and each egressed FOLR was counted. In addition, quadrants with at least one end of an egressed FOLR were counted as described by Leduc and others (2011).

Differences among tools, sites, and their interaction were evaluated using PROC GLM (SAS Institute Inc. 1985); and the residuals were then tested for departures from normality using PROC UNIVARIATE. If the distribution of the residuals was found to be significantly different from normal by the Kolmogorov-Smirnov test, then differences in tools were tested using PROC NPAR1WAY. After the first series of tests, it was determined that significant differences in growth magnitude existed between the two sites (figure 3) as well as unequal variance among treatment groups. Therefore, response variables from each location were analyzed separately with the exception of mortality, which had similar variances for both sites and was therefore analyzed across both sites to maintain adequate degrees of freedom.

Results and Discussion

Of the 280 longleaf pine seedlings planted for both locations, 37 died—likely because of the drier-than-normal and warmer-than-normal growing season in 2010. No significant differences were noted in survival between locations (data not shown).

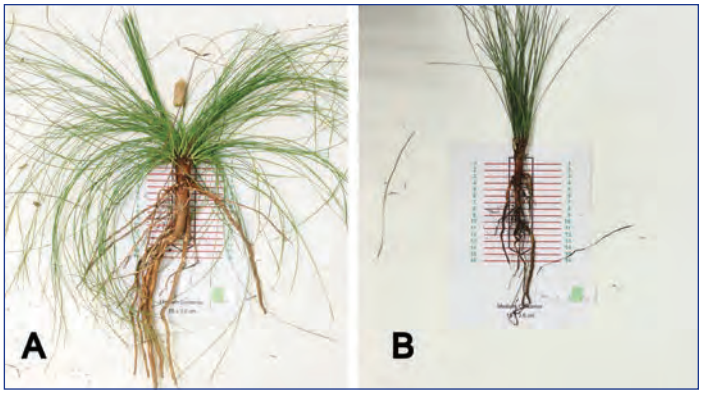

Figure 3. Two average-size longleaf pine seedlings on the Escambia (A) and Palustris (B) sites; seedlings were grown in Copperblock™ Styroblocks and planted using an OST dibble bar. (Photo by Daniel J. Leduc, USDA Forest Service, Southern Research Station, Alexandria Forestry Center, 2011)

The largest differences in seedling development were not among tools but between locations. The seedlings planted on the Escambia site grew much larger than those planted on the Palustris site (figure 3). Some of the size differences might be attributed to less severe drought conditions on the Escambia, but more likely, it was due to better site conditions than the Palustris site based on soil type and level of vegetation competition.

The planting tools had few significant differences in seedling growth. On the Palustris site, only RCD and shoot dry weight differed significantly among tools (table 1). For both variables, seedlings planted using the OST dibble bar were largest although not significantly larger than those planted using the dibble stick, hoedad, or shovel.

On the Escambia site, only the distribution of roots into quadrants differed significantly among tools (table 2). Seedlings planted using the dibble stick, tube dibble, and shovel had the best root distribution, while those planted using the KBC dibble bar, OST dibble bar, and auger had the poorest root distribution. In addition, although not significant, the root/shoot ratio was greater for seedlings planted using the KBC and OST dibble bars than for those planted using the dibble stick.

Table 1. Mean growth variables for longleaf pine seedlings planted using seven different tools on the Palustris Experimental Forest (Malbis fine sandy loam). For each variable, columnar means followed by the same letter are not significantly different based on Duncan's Multiple Range Test.

Tools	RCD (mm)	Shoot dry weight (g)	Root dry weight (g)	Root/shoot ratio	Number of quadrants with egressed root ends	Number of egressed FOLR
KBC dibble bar	14.7bc	8.7ab	8.8a	0.93a	1.2a	7.9a
OST dibble bar	16.6a	10.1a	9.4a	0.94a	1.5a	8.1a
Dibble stick	15.6 ab	8.3ab	9.0a	1.09a	1.4a	8.4a
Tube dibble	13.7c	6.5b	7.0a	1.07a	1.2a	6.9a
Hoedad	14.9abc	8.3ab	8.7a	1.06a	1.8a	7.7a
Auger	14.0bc	7.3b	7.2a	0.97a	1.4a	7.2a
Shovel	15.5ab	10.1a	9.5a	0.95a	1.4a	8.3a

FOLR = first-order lateral roots. RCD = root collar diameter.

Table 2. Mean growth variables for longleaf pine seedlings planted using seven different tools on the Escambia Experimental Forest (Troup fine sand). For each variable, columnar means followed by the same letter are not significantly different based on Duncan's Multiple Range Test.

Tools	RCD (mm)	Shoot dry weight (g)	Root dry weight (g)	Root/shoot ratio	Number of quadrants with egressed root ends	Number of egressed FOLR
KBC dibble bar	20.9a	28.0a	33.2a	1.19a	1.6c	7.8a
OST dibble bar	19.4a	23.9a	29.7a	1.20a	1.6c	7.7a
Dibble stick	21.5a	33.9a	34.4a	1.00a	2.0abc	10.0a
Tube dibble	21.0a	30.9a	32.8a	1.04a	2.7a	9.8a
Hoedad	20.4a	33.8a	37.9a	1.13a	1.8bc	9.1a
Auger	19.7a	26.9a	28.9a	1.06a	1.6c	8.4a
Shovel	20.6a	30.2a	32.4a	1.06a	2.6ab	9.7a

FOLR = first-order lateral roots. RCD = root collar diameter.

Contradictory outcomes between the two sites occurred for several other variables. For example, planting using an OST dibble bar or shovel on the Palustris site resulted in the greatest shoot dry weight, while on the Escambia site, no significant shoot dry weight differences existed among planting tools and seedlings planted using an OST dibble bar were ranked last for shoot dry weight (tables 1 and 2). On the Palustris site, planting seedlings using a tube dibble resulted in significantly smaller RCD than planting using several other tools and the seedlings were ranked last in RCD, shoot dry weight, and root dry weight. On the Escambia site, RCD, shoot, and root dry weights of seedlings planted using a tube dibble were not significantly different compared with the other planting tools.

Barnett (1978) found that loblolly pine (*P. taeda* L.) seedlings survived better in a heavy silt loam when the holes were cored rather than punched. He suggested that tools such as the dibble stick compact the soil and possibly reduce the ability of the root system to penetrate the sides of the hole. In contrast, he reported better survival for seedlings planted in punched holes rather than cored holes on a sandy loam soil. Similarly, survival and height growth of lodgepole pine (*P. contorta* Douglas ex Louden) in compacted clay loam was best when a soil core was removed before planting using a tool similar to the tube dibble (Bohning 1981). Seedling survival in noncompacted soils (bulk density < 1.6 g/cm^3 [100.0 lb/ft^3]), however, was as good when planting in punched holes compared with cored holes. Based on USDA Natural Resources Conservation Service (2012) soil surveys, the bulk density at one-third bar-soil moisture for the Escambia site was 1.54 g/cm^3 (96.00 lb/ft^3) and for the Palustris site was 1.51g/cm^3 (94.00 lb/ft^3). These low bulk densities at both sites help to explain why planting tools had little influence on survival or other parameters in this study.

Leduc and others (2011) determined that a solid round dibble (similar to the dibble stick) was superior to a tube dibble in terms of the number of FOLRs and number of quadrants with roots. In our current study, however, the statistical differences in root architecture among the dibble stick, tube dibble, and auger were not sufficient on either site to conclude that planting seedlings using one of these three tools would result in better root-system architecture than the other tools (tables 1 and 2).

Conclusions

In Sword Sayer and others (2009), the development of FOLRs and root-system architecture were considered important in predicting seedling access to surface-soil resources, growth, and the future stability of saplings and trees in high, sustained winds. For practical purposes, the type of planting tool in the current study did not affect root-system architecture on either site, at least for the first 15 months after planting. We concluded that none of the planting tools in general were superior to the others and that, as concluded by Adams and Patterson (2004), how well seedlings are handled and the care taken to plant them may be more important than the tool used. In addition, cost differences and the expected useful life of the tools might help determine which tool to use.

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Root Growth, Plug Cohesion, Mineral Nutrition, and Carbohydrate Content of 1+0 *Picea mariana* Seedlings in Response to a Short-Day Treatment

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Abstract

A short-day (SD) treatment was applied to containerized 1+0 black spruce (*Picea mariana* [Mill.] B.S.P.) with the objective of increasing root mass and root-plug cohesion. The SD treatment resulted in the induction of bud formation, cessation of height growth, and significant increases in carbohydrate content (sucrose, pinitol, and starch), root nutrient contents, and root dry mass. Allometric models showed that given the same shoot mass, the average seedling grown under the SD treatment had 25 percent more root mass than those in the control treatment, which led to a significant improvement in root-plug cohesion. Seedling quality evaluation before delivery to the planting site showed that 91 percent of 1+0 black spruce seedlings subjected to SD treatments conformed to quality standards compared with 71 percent for those subjected to the control treatment. These results indicate that the use of an SD treatment may improve the profitability of forest nurseries by increasing the quality and quantity of shippable seedlings.

Introduction

More than 150 million forest seedlings are grown annually in Québec, Canada (Lamhamedi and others 2007). Nearly 2,500 different stock types are produced in 24 forest nurseries (18 private and 6 government) using different production scenarios. Most (90 percent) of the seedlings are container grown and the remainder produced are bareroot seedlings. Nursery managers must work within environmental constraints beyond their control, which can reduce the morphophysiological quality of the seedlings. The most notable constraints include climatic extremes and interannual variability as well as the very short growing season in northern forest nurseries.

Before being planted, seedlings grown in Québec forest nurseries are subjected to a morphophysiological evaluation for 25 norms (Veilleux and others 2008). These norms are specific to each stock type and production scenario. Evaluation of 1+0 and 2+0 containerized seedlings is conducted both in the autumn and spring preceding delivery to the planting site. Foliar nitrogen (N) concentration and rooting sufficiency are among the 25 quality norms that are evaluated. During the evaluation, a seedling is deemed to have insufficient root development if the root-plug breaks, either partially or completely, is incomplete after extraction from the container cavity, or exhibits two or more discontinuous sections of undamaged roots separated by more than 5.0 mm (0.2 in). The seedling is also rejected if more than 33 percent of the roots on the periphery of the root plug are dead or decaying (Veilleux and others 2008). An assessment of data from the 24 Québec forest nurseries between 2003 and 2006 indicated that large portions of seedlings were rejected because of failure to meet nine principal norms. Among these norms, the proportion of black spruce seedlings grown in 67-50 containers (67 cavities, 50 cm^3 [3 in^3]/cavity) had insufficient root development that reached an average of 54.3 percent during the autumn before delivery (Lamhamedi and others 2007). This level of insufficient root development varies significantly among nursery growers.

Several cultural techniques have been modified, at an operational scale, to improve seedling root growth and plug cohesion (Landis and others 1989, 1990). These techniques include container design, cavity volume and arrangement (Landis and others 1990), the use of peat and compost-based growing media with the desired physicochemical characteristics (Bernier and others 1995, Heiskanen 1993, Veijalainen and others 2008), and the optimization of irrigation and fertilization regimes (Lamhamedi and others 2006, Landis and others 1989).

In addition, short-day (SD) treatments may be applied early in the growing season to improve root dry mass and plug cohesion. In general, this treatment is used at an operational scale for several coniferous species produced in North American and Scandinavian forest nurseries to induce bud

formation, control height growth, and improve frost tolerance (Bigras and others 2001, Colombo 1996, Colombo and others 2001, Hawkins and Shewan 2000, Kohmann and Johnsen 2007, Krasowski and others 1993, MacDonald and Owens 2006, Rostad and others 2006, Turner and Mitchell 2003). The treatment is also used to improve seedling performance under xeric conditions (Luoranen and others 2007) and to combat other stresses on the plantation site (Tan 2007).

Our hypothesis was that an SD treatment, applied for a limited time during the active growing phase, increases root-plug cohesion and root mass by inducing a greater allocation of biomass to root growth and development. This shift in allocation toward the root system has generally been inferred from instantaneous shoot/root ratio assessments (Hawkins and Draper 1988, Krasowski and Owens 1991), but to our knowledge has not been quantified continuously with allometric models in response to early SD treatments applied during rapid shoot elongation. A need also exists to increase our knowledge about the effect of SD treatments on tissue carbohydrate and nutrient contents. The general objectives of the present study were to (1) evaluate the effects of SD treatments during the active growing season on shoot and root growth, plug cohesion, mineral nutrition, carbohydrate content, and bud formation of 1+0 black spruce seedlings produced under tunnel conditions in a forest nursery; and (2) quantify the effects of SD treatments on dry-matter allocation between shoots and roots through the use of allometric models.

Materials and Methods

Plant Material and Experimental Design

Black spruce seeds (seedlot: EPN-V1-LEV-2-2; production code GI05EPN05-C06) were sown at the end of March 2006 into 67-50 containers (IPL 67-50, Saint-Damien, Québec, Canada; 67 cavities, 50 cm^3 [3 in^3]/cavity; cavity depth: 8.7 cm [3.4 in]; cavity diameter: 3.2 cm [1.3 in]; 864 seedlings/m^2 [80 seedlings/ft^2]). The cavities were filled with a moist, peat-based substrate adjusted to a bulk density of 0.09 g/cm^3 (0.0002 oz/in^3). The percentage of large (10-mesh sieve), medium (20-mesh sieve), short (40- and 100-mesh sieves), and fine particles (200-mesh sieve) represented 12, 29, 60, and 11 percent of the growing medium, respectively. The containers were installed in a standard production tunnel (figure 1) at Serres et Pépinière Girardville, a private forest nursery located in the Saguenay-Lac St. Jean region of Québec, Canada (latitude 49° 01' 06"; longitude 72° 30' 42"). The tunnel, with an average capacity of 250,000 seedlings, was covered with milk-white polyethylene, 100 mm thick (4 mil = 400 gauge = 0.004 inches), which transmitted 50 to 55 percent of incident light (multi-layer greenhouse cover film, type UVA/White 45 percent, (Ginegar Plastic Products Ltd, Kibbutz, Israel). The cover could be retracted along both sides to facilitate aeration and modify the air temperature inside the tunnel. After seed germination was complete (early May), the germinants were thinned to one per cavity. No germinants were transplanted to empty cavities.

The SD treatment was applied during the most rapid period of shoot elongation as a cultural technique to increase root growth and improve plug cohesion. It was applied between June 30 and July 18, 2006, and consisted of modifying the photoperiod to light/dark: 8hr/16hr. This treatment differs from the typical SD treatment in Québec, which is applied in forest nurseries toward the end of the growing season (mid-August) to improve hardening processes and frost tolerance. A black polyethylene cover, positioned approximately 40 cm (16 in) above the shoot tips, was manually installed above the seedlings and removed each day to create the dark period. The containers of seedlings subjected to the control treatment were grown under natural light conditions where the day length varied between 15.3 and 16 hr per day (Saguenay: 48° 25' 00" N., 71° 04' 00" W., Québec, Canada). The two treatments (SD and control) were installed along the length of a standard production tunnel in five randomized complete blocks (figure 1). In each block, the group of 6 containers subjected to the control treatment was isolated from the 11 containers subjected to SD treatment by a wall of white polystyrene foam to prevent incident light from reaching the neighboring containers during the dark periods implicit to the SD treatment (figure 1). In each block, 5 of the 6 control containers and 10 of the 11 SD containers were sampled for growth and mineral nutrition and the remaining container in each was used to assess bud formation.

Growth and Environmental Conditions

Seedlings in both treatments were grown using standard nursery cultural techniques for the production of 1+0 black spruce in Québec. The irrigation regime optimized substrate water contents during the different growth phases (Bergeron and others 2004; Lamhamedi and others 2003). The seedlings were irrigated with sprinklers arranged in a square pattern (7.3 by 7.3 m, [24.0 by 24.0 ft]) (Rain-Jet, model 66U, Harnois, Québec, Canada). Substrate water content was monitored by gravimetry. Covering the range of the sprinkler distribution and both treatments, 12 containers were randomly selected and weighed two or three times per week. Average substrate water content ranged between 20 and 50 percent (v/v) during

Figure 1. (a) Production of black spruce seedlings in a standard production tunnel at Serres et Pépinière Girardville (Québec, Canada). (b) Short-day (SD) and control treatments were installed along the length of a standard production tunnel in five randomized complete blocks. (c) Control containers in each block were grouped and were not covered with the black plastic during SD treatment; they were isolated from the containers subjected to SD treatment by a wall of white polystyrene foam to prevent incident light from reaching the neighboring containers during the periods of darkness implicit to the SD treatment. (Photos by: Mohammed S. Lamhamedi, 2006)

the growing season. The control of substrate water content was identical for both treatments and at no time were any of the seedlings subjected to water stress (Bergeron and others 2004).

Seedlings in both treatments were fertilized twice weekly using the approach developed for Québec forest nursery production and *PLANTEC* software (Girard and others 2001). This approach is adapted to the seedlings' demand for nutrients as well as the established growing standards specific to black spruce seedlings (Girard and others 2001, Langlois and Gagnon 1993). The quantities of N, phosphorous (P), and potassium (K) applied per seedling from May 5 to September 26, 2006, were 18 mg (0.0006 oz), 15 mg (0.0005 oz), and 18 mg (0.0006 oz), respectively. During each fertilization session, seedlings also received micronutrient elements. As the growing season progressed, N concentration in the substrate progressively decreased from 143 to 8 ppm and the electrical conductivity (EC) decreased from 188 to 70 µS/cm.

Temperatures in the growing medium surrounding the roots and at the substrate surface were continuously monitored (soil temperature probe model 107B, Campbell Scientific, Edmonton, Alberta, Canada) under both the SD and control treatments. Air temperature and relative air humidity inside the tunnel at 2.0 m (6.5 ft) above the ground surface were measured with a Vaisala RH and Temperature Probe (model HMP35C, Campbell Scientific, Edmonton, Alberta, Canada). A data acquisition system (model CR10X, Campbell Scientific, Edmonton, Alberta, Canada) was used to record the data.

Growth, Mineral Nutrition, and Bud Formation Measurements

Seedling growth, substrate fertility, and tissue mineral content were evaluated from June through early November. Seedlings and substrate subjected to the SD treatment were sampled 10 times and those in the control treatment were sampled 5 times based on previous study methodologies (Lamhamedi and others 2003). Three of the samples for the control treatment were taken after completion of the SD treatment. On the first sampling date, a container was randomly chosen from the first block. On subsequent sampling dates, a container was systematically sampled from the same position in the other four blocks. In each of these containers, 50 of the 67 seedlings were randomly selected and gently extracted, for a total of 250 seedlings/treatment/date. All sampled seedlings were healthy without any visible damage.

The height and root-collar diameter (50 seedlings/block/treatment), root and shoot dry mass (5 composite samples of 10 seedlings/block/treatment), and tissue mineral content

(1 composite sample of 50 seedlings/block/treatment) were measured. Root and shoot tissues were oven-dried for 48 hr at 60 °C (140 °F) before determination of dry mass. After grinding and acid digestion, each composite sample was analyzed for N using the Kjeldahl method and for P, K, calcium (Ca), and magnesium (Mg) by inductively coupled argon plasma analysis (Parkinson and Allen 1975, Walinga and others 1995). The mineral nutrient composition is expressed as content (concentration x dry mass) per seedling (or tissue type) for each element to accurately reflect seedling mineral nutrient uptake and accumulation (Timmer and Miller 1991). Given that seedling tissue was pooled for analyses, nutrient composition was based on the average seedling (or tissue) mass. Substrate fertility ($N\text{-}NO_3$, $N\text{-}NH_4$, $N_{mineral}$ P, K, Ca, and Mg), pH (H_2O), and EC were determined on one composite sample from each treatment (50 root plugs/composite sample/block) on each sampling date.

Between June 29 and August 10, 2006, the 9 seedlings in the center row of a randomly selected container in each of five blocks within each treatment (total of 45 seedlings/treatment) were monitored for bud formation. The same seedlings were evaluated three times weekly until a white terminal bud was visible on all of the monitored seedlings (stage II development, per Lesser and Parker 2004). On May 11, 2007, before delivery to the planting site, bud-break status of seedlings subjected to both treatments was evaluated. Bud break was considered to have occurred when green needles were visible under the bud scale cap (Wilkinson 1977). Before being dispatched to the planting site (May 14 and 15, 2007), 120 seedlings were randomly selected from five blocks of each treatment (24 seedlings/block/treatment) and subjected to an assessment of the 25 quality criteria (including insufficient root development, height, diameter, foliar N concentration, forks, and height/diameter) established by the ministère des Ressources naturelles MRN du Québec (Veilleux and others 2008). This assessment of seedling quality, carried out by a team of evaluators accredited by the MRNF, not only determines the level of uniformity of each seedling lot, but it is also used to calculate the compensation the nursery receives for producing the seedlings.

Carbohydrate Analyses

From each treatment/block, 10 seedlings were randomly selected before (June 27, 2006) and after (July 18, 2006) application of the SD treatment. Seedling roots were washed to remove the substrate, then separated from the shoots and stored at 4 °C (39 °F). For each composite sample (roots and shoots; 10 seedlings), the following carbohydrates were extracted with 80 percent hot ethanol and quantified by high-performance liquid chromatography (model 2414, Waters, Milford, MA, United States): glucose, fructose, sucrose, raffinose, and the sugar alcohols, pinitol and inositol. Individual sugars were identified based on retention times relative to known standards. Starch concentrations in roots and shoots were measured on a spectrophotometer (model Spectronic 20, Bausch & Lomb Incorporated, Aliso Viejo, CA, United States).

Statistical Analyses and Dry-Matter Allocation Between Roots and Shoots

Statistical analyses of morphophysiological variables were conducted with the MIXED procedure of SAS (SAS Institute, Cary, NC, United States). The assumption of normality of the error terms was respected for all of the variables and the assumptions of normality and homogeneity of variance were verified. Independence between the sampling dates was presumed and growth variables were measured on different seedlings on each date. The treatment effect was considered significant at 10 percent to account for spatial variability of resources (Lamhamedi and others 2006) in each cavity and an inherent variability in seedling growth resulting from a bulk collection of open-pollinated seeds.

To quantify the effect of each treatment on biomass partitioning between roots and shoots, an allometric equation was developed from individual seedling data pooled over all sampling dates after the application of SD treatments using natural logarithmic transformation:

$$\ln(y_{ij}) = b_{00} + b_{01}trt + b_{10}\ln(x_{ij}) + b_{11}\ln(x_{ij}) * trt + u_i + \varepsilon_{ij}$$

Where:

- y_{ij} : shoot dry mass measured for seedling j, from block i,
- x_{ij} : root dry mass measured for seedling j, from block i,
- trt : 1 if seedling was subjected to « short day » and 0 if not,
- b_{00} : intercept for control seedlings,
- b_{01} : addition component for treatment intercept ($b_{00}+b_{01}$ = intercept for short-day seedlings),
- b_{10} : slope for control seedlings,
- b_{11} : addition component for treatment slope ($b_{10}+ b_{11}$ = slope for short-day seedlings),
- u_j : random effect of block j ~ $N(0,\sigma_u^2)$,
- e_{ij} : residual error, ~ $N(0,\sigma^2)$.

Parameters b_{10} and b_{11} describe the partitioning of biomass between shoots and roots and are a measure of the ratio of their relative growth rates during the exponential growth phase (Ledig and others 1970).

Results

Environment Variables

During the germination and active growing periods (mid-April to late July 2006), mean air temperature ranged from 13 °C (55 °F) to 29 °C (84 °F) (figure 2). During the period of bud formation and natural hardening (early August to late September 2006), the average daily temperature inside the tunnel decreased progressively, varying from 23 °C (73 °F) to 6 °C (43 °F). Relative humidity fluctuated between 65 and 85 percent (figure 2). Average maximum temperatures, 2.0 m (6.5 ft) above the ground and at the substrate surface were similar for the two treatments. Night temperatures at the substrate surface were always higher under SD treatment, however, than under the control treatment (figure 3). In general, substrate temperatures in the rooting zone under the SD treatment were also warmer than those of the control treatment (figure 3).

Seedling Growth, Bud Formation, Bud Break, and Uniformity

With the exception of total seedling dry mass ($p = 0.3127$), all growth variables had a significant date by treatment interaction ($p < 0.0001$). SD treatment resulted in the cessation of height growth ($p = 0.0031$) within 2 weeks of

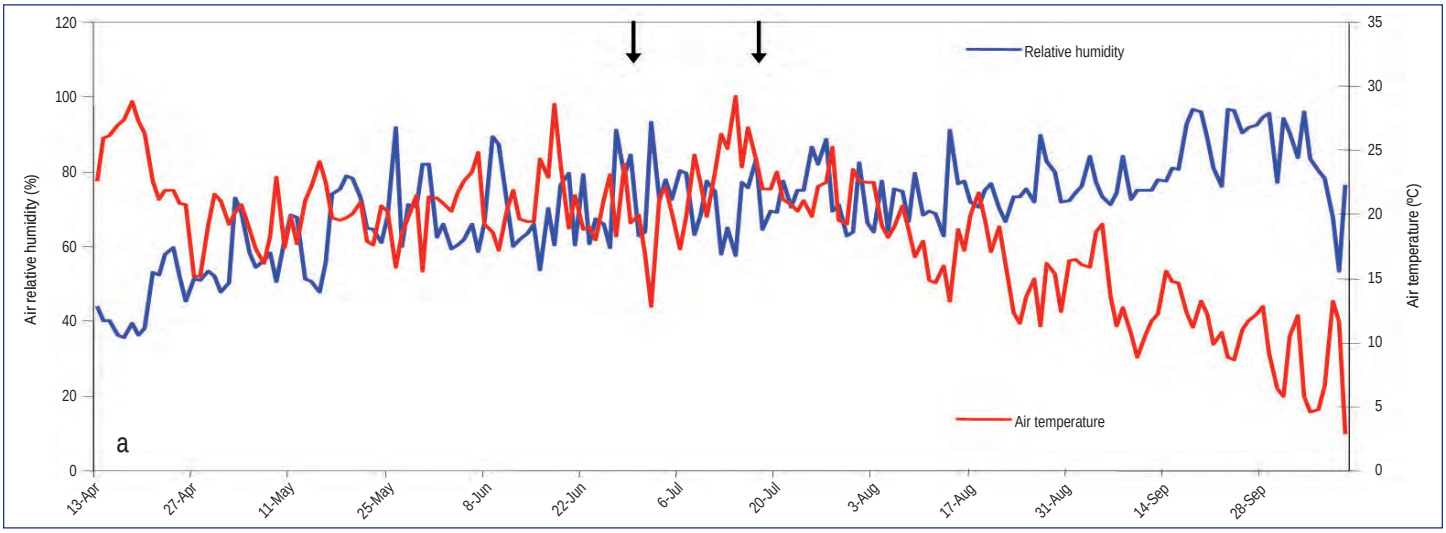

Figure 2. Variations of daily mean air temperature and relative humidity during the growth of 1+0 black spruce seedlings produced under tunnel conditions in a forest nursery. Arrows indicate the beginning and end of the short-day treatment.

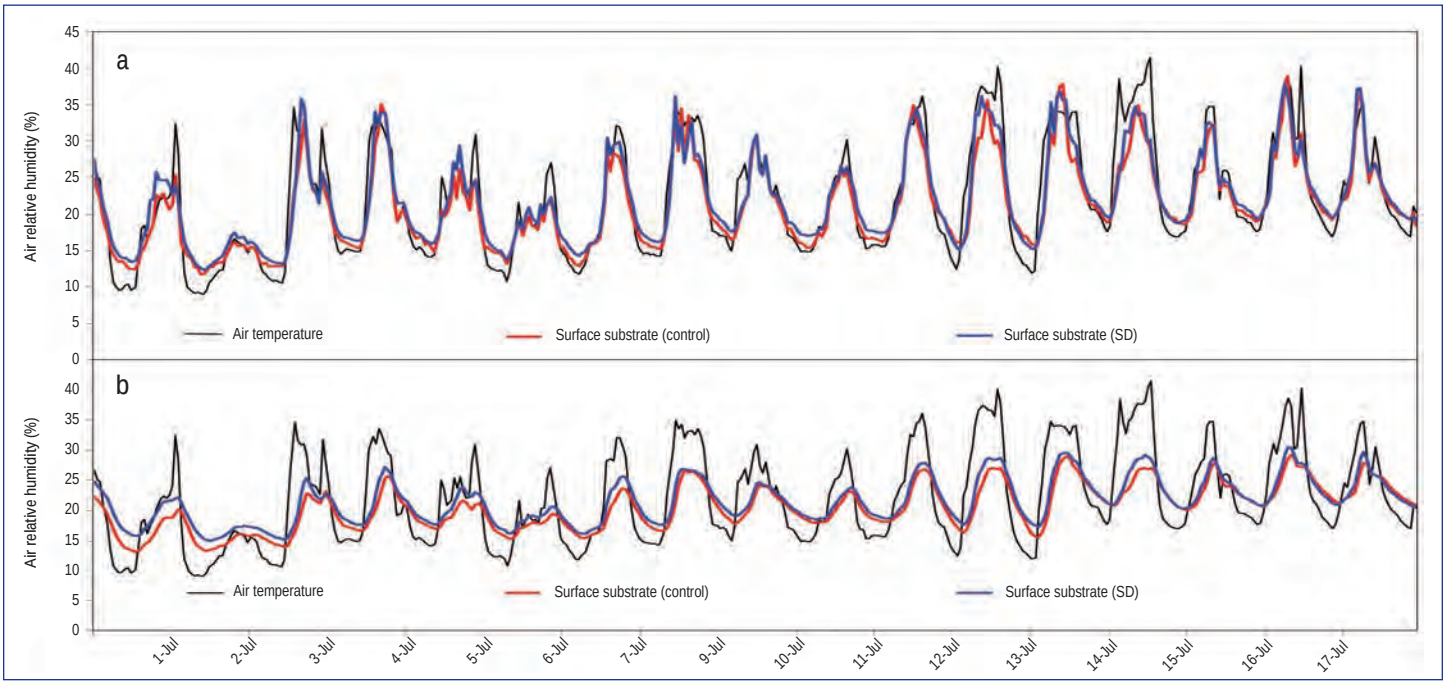

Figure 3. (a) Hourly air temperatures 2 m above and at the substrate surface. (b) Hourly air (2 m) and substrate temperatures for 1+0 black spruce containerized seedlings subjected to control and short-day (SD) treatments.

application, an increase in root mass with time ($p < 0.0001$), and a reduction ($p = 0.0026$) in shoot and total mass of the 1+0 black spruce seedlings (figure 4). Unlike height, the effect of SD treatment on root-collar diameter was not evident after 2 weeks (figure 4) despite slight differences between the two treatments in mid-September ($p = 0.0569$).

All seedlings in the SD treatment formed visible white buds after 2 weeks of treatment, whereas bud formation of seedlings in the control treatment occurred during a prolonged period (mid-July to mid-August) as a function of natural environmental conditions (figure 5). Few seedlings (< 1 percent) broke bud after bud formation despite the favorable environmental conditions for shoot growth during summer and autumn of 2006. After spending the winter outside under the snow, seedlings that had been subjected to the SD treatment broke bud sooner than those grown under the control treatment (figure 5). In addition, the percentage of 1+0 black spruce seedlings that conformed to the 25 MRN norms was higher under the SD treatment (91 percent) than under the control treatment (71 percent).

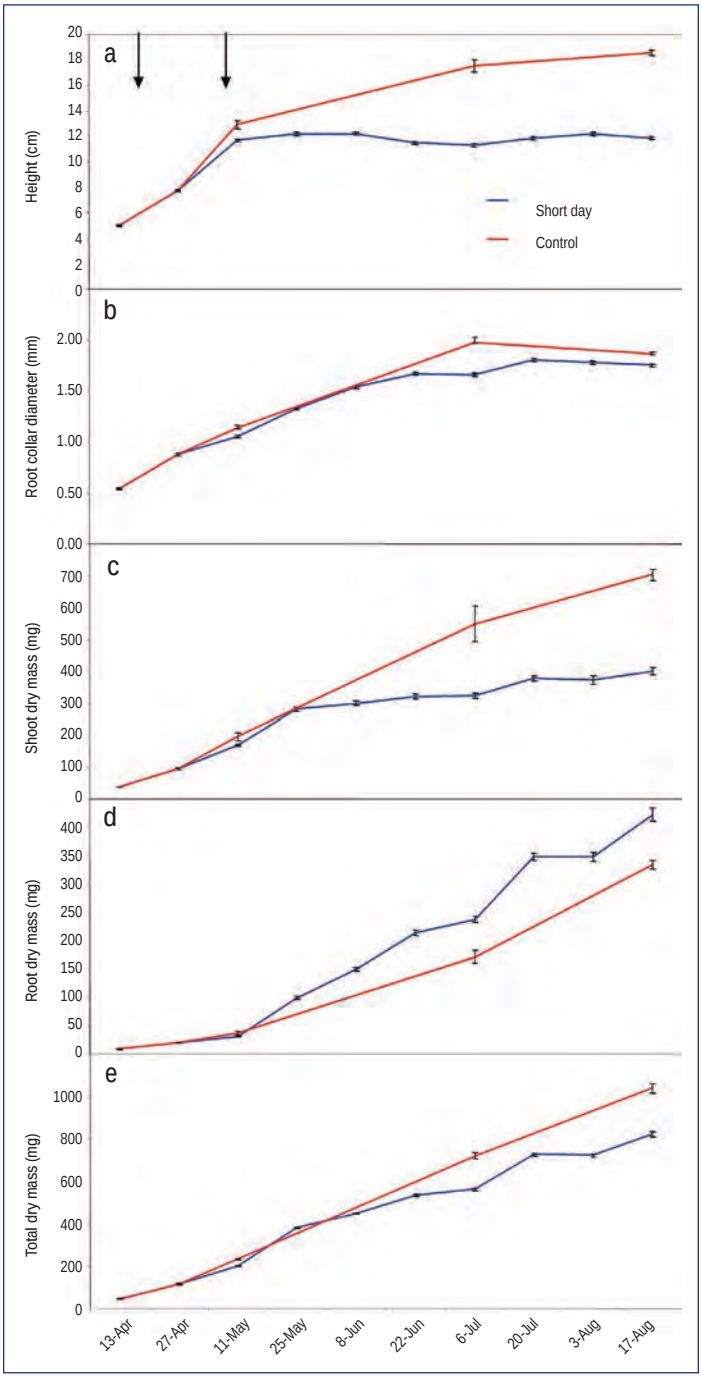

Figure 4. (a) Seasonal changes in height, (b) root-collar diameter, (c) shoot dry mass, (d) root dry mass, and (e) total seedling dry mass of 1+0 black spruce seedlings subjected to the control and short-day (SD) treatments (n = 250 ± SE). Arrows indicate the beginning and end of the SD treatment.

Allocation of Dry Matter Between Shoots and Roots

The allometric models showed that significantly more dry matter was allocated to root growth under the SD treatment than under the control treatment ($p < 0.0001$). Seedlings grown under the SD treatment had a 25-percent increase in root mass compared with seedlings with similar shoot mass that were grown under the control treatment (figures 6 and 7).

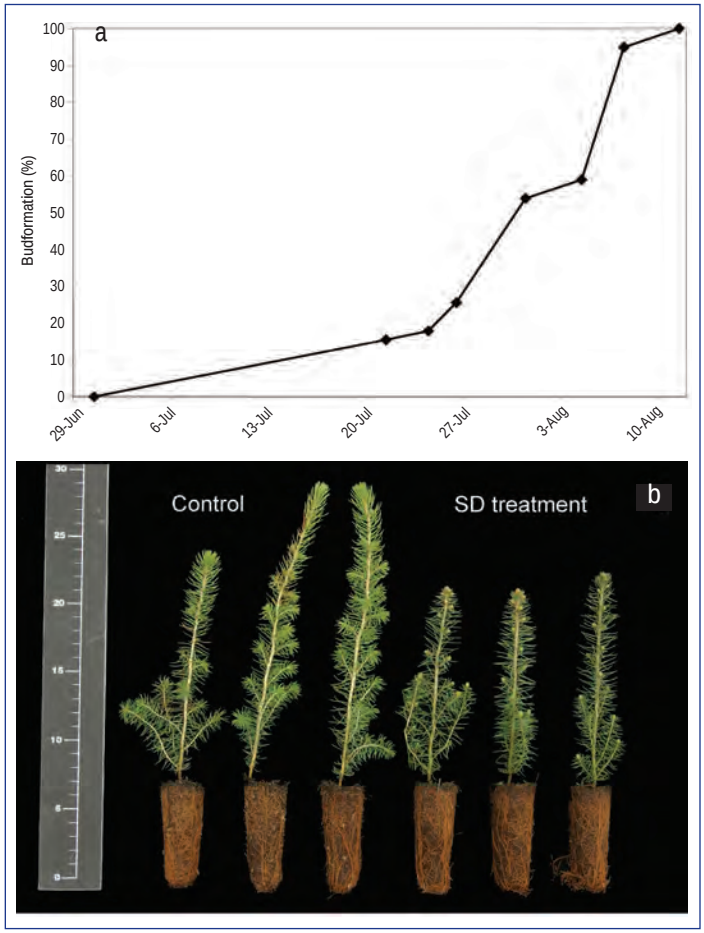

Figure 5. (a) The percentage of bud formation over time in the control seedlings. The seedlings subjected to a short-day (SD) treatment had all formed buds by the end of the SD treatment (July 18, 2006). (b) After spending the winter outside under the snow, seedlings subjected to the SD treatment broke bud sooner than those grown under the control treatment (May 14, 2007).

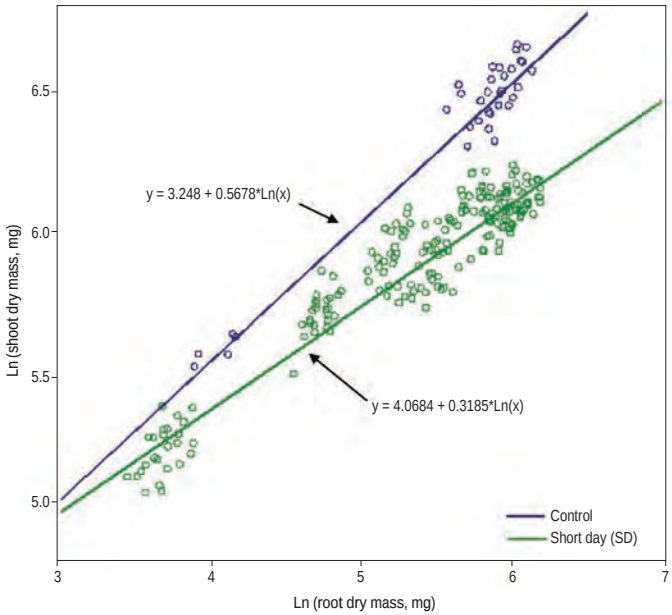

Figure 6. Allometric models of dry-matter allocation between shoots and roots of seedlings subjected to the control and short-day treatments.

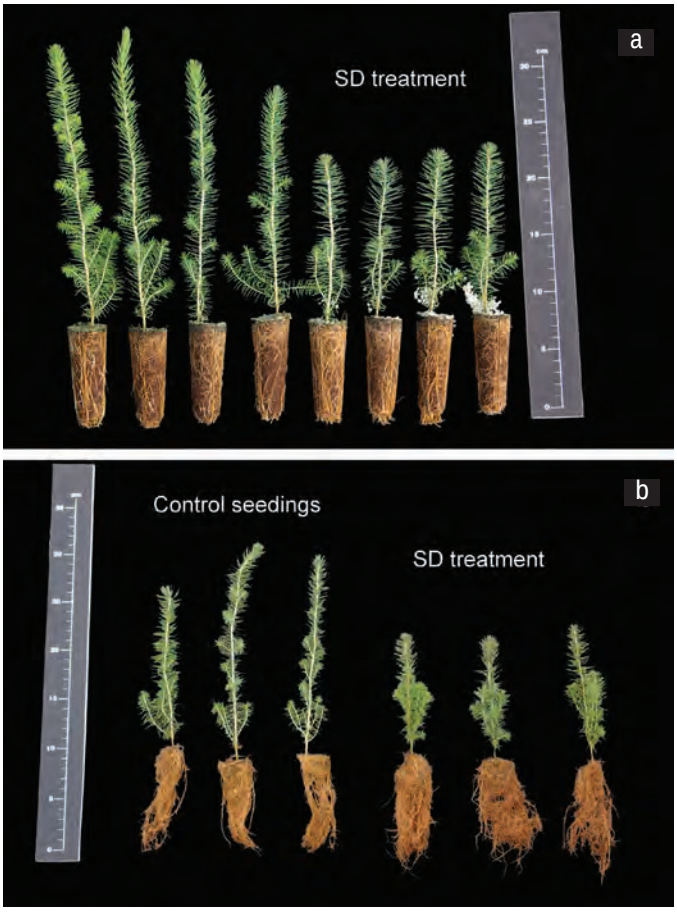

Figure 7. (a) Growth of shoots and roots of black spruce seedlings grown under short-day (SD) and control treatments (September 11, 2006). (b) Growth of roots under the SD treatment was greater than that under the control treatment.

Mineral Nutrition

A significant date by treatment interaction occurred for root and shoot mineral nutrient contents. Root N ($p = 0.0463$), P ($p = 0.0321$), K ($p = 0.0815$), and Mg ($p = 0.0089$) contents were increasingly greater over time for seedlings grown under the SD treatment compared with those in the control treatment (figure 8). Conversely, shoot N ($p = 0.0750$), P ($p = 0.0266$), and K ($p = 0.0023$) contents were increasingly lower over time under SD than under the control treatment (figure 8). With regard to mineral nutrient concentrations, the average root-tissue concentrations, before (N: 2.33 percent, P: 0.85 percent, and K: 2.27 percent) and after the SD treatment (SD, N: 1.34 percent, P: 0.40 percent, and K: 0.88 percent; Control, N: 1.33 percent, P: 0.39 percent, and K: 0.86 percent) were similar for the two treatments. On the final sampling date, average shoot N concentrations were slightly lower in the control plants (SD, N: 1.77 percent; Control, N: 1.34 percent).

Carbohydrates

Fructose content of the shoot tissue did not differ between treatments ($p = 0.2654$), whereas raffinose ($p < 0.0001$), sucrose ($p = 0.0027$), glucose ($p = 0.0138$), pinitol ($p < 0.0001$), and starch ($p = 0.0005$) contents all differed significantly between the two treatments. The SD treatment resulted in a significant increase ($p < 0.001$) in sucrose (4.17 ± 0.44 mg/seedling, [0.00015 oz]), pinitol (4.08 ± 0.21 mg/seedling, [0.00015 oz]), and starch (5.78 ± 0.43 mg/seedling, [0.00020 oz]) contents of the shoot tissue with respect to the carbohydrate contents before application of the SD treatment (sucrose: 2.09 ± 0.50 mg/seedling [0.00007 oz]; pinitol: 1.75 ± 0.30 mg/seedling [0.00006 oz], starch: 4.75 ± 0.46 mg/seedling [0.00017 oz]).

In root tissue, contents of sucrose ($p = 0.6739$), glucose ($p = 0.2247$) or starch ($p = 0.8070$) in the root tissue were unaffected by treatment, whereas raffinose content was significantly higher ($p = 0.0134$) for SD seedlings compared with control seedlings. In contrast, control seedlings showed significantly higher pinitol ($p = 0.0061$) and inositol ($p = 0.0012$) contents compared with SD seedlings.

Discussion

Our results showed an average increase of 25 percent in dry-matter allocation to roots of black spruce seedlings in response to SD treatment under operational conditions (figures 4 and 6) resulting in a 20-percent increase in the proportion of shippable plants with sufficient root development. This cultural technique could potentially

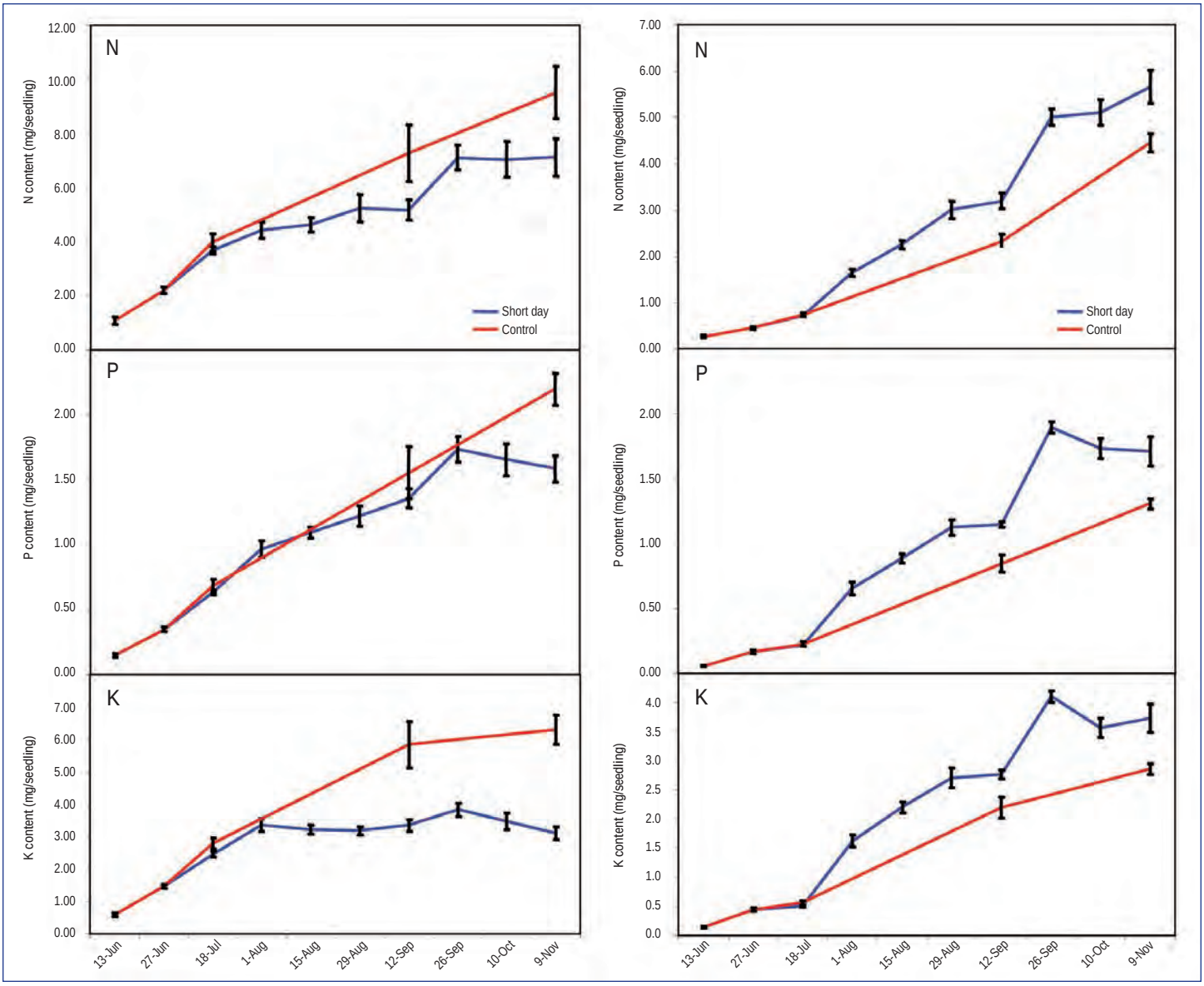

Figure 8. Mineral nutrient content of the shoot and root tissue of seedlings subjected to the control and short-day treatments (n = 5 composites samples ± SE).

improve nursery profitability. Plug cohesion and root development, particularly after the cessation of height growth, are closely linked to current net photosynthesis (Lippu 1998, Ritchie 2003, van den Driessche 1987) and the carbon source: sink dynamics within the plant (Kozlowski 1992). Root growth may become a stronger sink for photosynthates after the initiation of bud formation. In a study by Hawkins and others (1994), an SD treatment increased net photosynthesis in interior spruce (*Picea glauca* [Moench] Voss x *Picea engelmannii* Parry) seedlings and possibly reduced respiration, thus providing a surplus of photosnythates directed toward root growth after bud formation and cessation in height growth. In accordance with other studies (Colombo and others 2001), our results showed that SD could be used as a cultural technique during the most rapid period of shoot elongation to increase root-plug cohesion and root dry mass by inducing a greater allocation of dry matter to root-system development.

The SD treatment in this study caused a significant increase in the tissue contents of certain carbohydrates (sucrose and pinitol) similar to results observed in Norway spruce (Rostad and others 2006), which may have a positive effect on root growth. Krueger and Trappe (1967) showed that the percentage of active root tips and root growth of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings were inversely correlated with decreasing carbohydrate content. Similar results were observed for *Pinus taeda* L cuttings where dry mass and root surface area were positively correlated with certain carbohydrates (myo-inositol, glucose, fructose, sucrose and raffinose) and foliar N concentration (Rowe and others 2002). In our study, substrate N concentration was high (143 ± 10 ppm) at the beginning of the active growing period to enable seedlings to achieve target heights before application of the SD treatment. After the treatment, environmental

conditions were still favorable to physiological activity; thus, N fertilization was significantly reduced (from 40 to 8 ppm) for both treatments to control height growth, avert reflushing, and induce dormancy and hardiness. Mengel and Kirkby (1987) reported that carbohydrates usually accumulate when N fertility is reduced. The biweekly evaluation of tissue and substrate mineral concentrations throughout the growing season indicated that the nutritional status, however, notably N concentrations, were above thresholds deemed critical for growth and gas exchange in black spruce seedlings (Lamhamedi and Bernier 1994, Munson and Bernier 1993). We did not observe any symptoms of N deficiency in either treatment, because black spruce seedlings can grow under conditions of low nutrient availability (Lafond 1966). From our results, it appears that the increase in carbohydrates for seedlings in the SD treatment was caused by the treatment and was unaffected by factors related to mineral nutrition. The increase in shoot N and P content and root K content of seedlings subjected to the SD treatment over those of the control treatment is likely a result of the increased root growth that occurred in response to the SD treatment; the increase in dense, fine white roots increases the absorptive surface area, thereby enabling seedlings to exploit most of the cavity volume and the air spaces between the substrate aggregates.

In addition to increased root growth, this study showed that an SD treatment applied to black spruce seedlings for 2 weeks in late June through early July caused height growth cessation and bud initiation sooner than the control treatment, thereby ensuring crop uniformity. Similar results have been reported for black spruce (Calmé and others 1993; Colombo and others 1981, 2001; D'Aoust 1981) and other coniferous species (Eastham 1990, Hawkins and Draper 1988). Despite the fact that the SD treatment was applied early (June 30 through July 18) and that the growing conditions were favorable during the entire growing season, we only observed reflushing in a few seedlings in the intervening period between the end of the SD treatment and the onset of autumn. In Québec, SD is generally applied in forest nurseries at the end of the growing season (mid-August) to induce hardening and frost tolerance, rather than to increase root-plug cohesion and root dry mass, which is the same objective of our study. The absence of reflushing may be explained by a strict control of substrate water content and fertility throughout the growing season and from the judicious choice of a seed provenance that responded very well to the SD treatment. In a greenhouse study with a seedlot originating from another seed orchard, Lamhamedi and others (2007) observed late-summer apical and lateral bud break in more than one-half of the 1+0 black spruce seedlings after SD treatment. Kohmann and Johnsen (2007) also observed that reflushing after SD treatment was dependent on the genetic origin of *Picea abies* seeds as well as the geographic location of the nursery. These different findings indicate that the application of an SD treatment during the active growing phase (early July) does not guarantee a definitive cessation of growth. To avoid reflushing after an SD treatment, Kohmann and Johnsen (2007) suggest prolonging the length of the SD treatment to a total of 3 weeks, and increasing dark period (> 14 hr). After outplanting, the relatively rapid budburst of seedlings subjected to the SD treatment compared with those under the control treatment may impart an early growth advantage but could also result in susceptibility to late spring frost injury, especially in ecological regions where the probability of spring frosts is relatively high.

Conclusion

All of the 1+0 black spruce seedlings subjected to the 2-week SD treatment ceased height growth and set buds, thus enhancing height uniformity. In addition, the SD treatment significantly increased mineral nutrition and carbohydrate contents, and it increased dry-matter allocation to roots, thus improving root-plug cohesion and the presence of roots on the periphery of the root plug. The percentage of seedlings subjected to the SD treatment that met the quality standards was 20 percent higher than those under the control treatment indicating that the use of an SD treatment may improve the profitability of forest nurseries. Good plug cohesion is essential to maintaining root-system integrity during lifting, shipping, and planting. Fine roots on the periphery of the root plug improve contact at the root-soil interface, thus increasing seedling survival and growth rates after planting.

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Storage Conditions Influence Cultural Detection of the Shoot Blight Pathogen *Diplodia pinea* From Asymptomatic Red Pine Nursery Seedlings

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Abstract

The pine shoot blight, canker, and collar rot pathogen *Diplodia pinea* can persist on or in asymptomatic red pine nursery seedlings, and it can proliferate after outplanting to cause disease and mortality. After lifting from nursery beds, seedlings are routinely kept in cold storage at nurseries. During and after shipment to customers, however, seedlings may be stored without refrigeration. In each of 2 years, we assayed seedlings from a bareroot nursery before and after storage for presence of this pathogen. Each trial included a storage treatment in which seedlings were kept at room temperature for 1 week after cold storage. Results demonstrated the effectiveness of nursery cultural practices and protective fungicide applications, as well as cold storage, to reduce the frequency of association of the pathogen with asymptomatic seedlings. We recommend that seedlings be kept in cold storage, even at moderately cool temperatures, before, and especially after, delivery to customers.

Introduction

Red pine (*Pinus resinosa* Aiton) is the most planted tree in the North-Central region of the United States (Gilmore and Palik 2006), and most contemporary red pine stands are plantations of this single species (USDA Forest Service 2002). Seedlings are planted after clearcut harvests of mature plantations. Most of these seedlings are 2- or 3-year-old bareroot seedlings produced in State and Federal nurseries in Michigan, Wisconsin, and Minnesota.

Shoot blight, canker, and collar rot caused by *Diplodia pinea* (syn. *Sphaeropsis sapinea*) frequently damages red pine nursery seedlings. For example, Palmer and others (1986) reported a 42-percent disease incidence in 2-0 seedlings. In plots located in proximity to red pine windbreaks, which are a source of inoculum, the frequency of shoot blight can be even greater (Stanosz and others 2005). *D. pinea* survives in dead colonized needles, stems, and cones on which it bears asexual fruiting bodies (pycnidia) (figure 1) that release spores (conidia). Spores are disseminated by rain splash and are abundant during spring and early summer (Palmer and others 1988), when young shoots are most susceptible. The pathogen infects through stomata, directly through the surface of young stems, or through fresh wounds (Brookhouser and Peterson 1971, Chou 1976). Pycnidia with conidia can develop within a few weeks after infection on dead seedlings, killed organs of living seedlings, and shoots excised from top-pruned seedlings (Munck and Stanosz 2008, Palmer and others 1988), so that multiple cycles of disease within a single growing season are possible. The similar fungus *D. scrobiculata* also can damage red pines, but it has been less often associated with red pine nursery seedlings (Stanosz and others 2005).

Red pine seedlings of all age classes may be rendered unmerchantable because of Diplodia shoot blight, canker, and collar rot, all of which lead to deformity or death (Palmer and Nicholls 1985). Infection of young seedlings during the first season of growth can result in rapid mortality, with retention of reddish to brown dead needles (figure 2). Colonization of elongating shoots on older seedlings can lead to shoot

Figure 1. Pycnidia of *Diplodia pinea* emerging from the base of a red pine needle. (Photo by Glen R. Stanosz)

death before full needle elongation and result in curling or crooking of the stem (figure 3). Needles of diseased shoots often turn yellow, then red to brown, or gray. Cankers on seedling stems begin as discrete, purplish, resinous lesions that result from direct infection or pathogen growth into stems from diseased needles. Collar rot symptoms include relatively rapid desiccation of needles and seedling death (figure 4), with blackening of the lower stem and root collar inner bark, and with dark staining of the underlying wood (figure 5). Although obviously symptomatic seedlings can be discarded during sorting and grading before packing, shipments of bulk-lifted seedlings (those that are packed immediately after lifting without sorting or grading) may include blighted or dead seedlings that bear the pathogen.

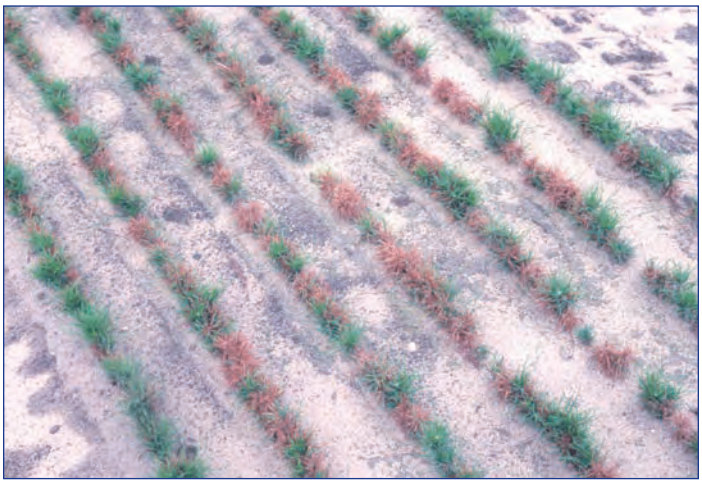

Figure 2. Dead red pine seedlings killed by *Diplodia pinea* in the first season of growth. (Photo by Glen R. Stanosz)

Figure 3. Distorted red pine shoot killed by *Diplodia pinea* during elongation. (Photo by Glen R. Stanosz)

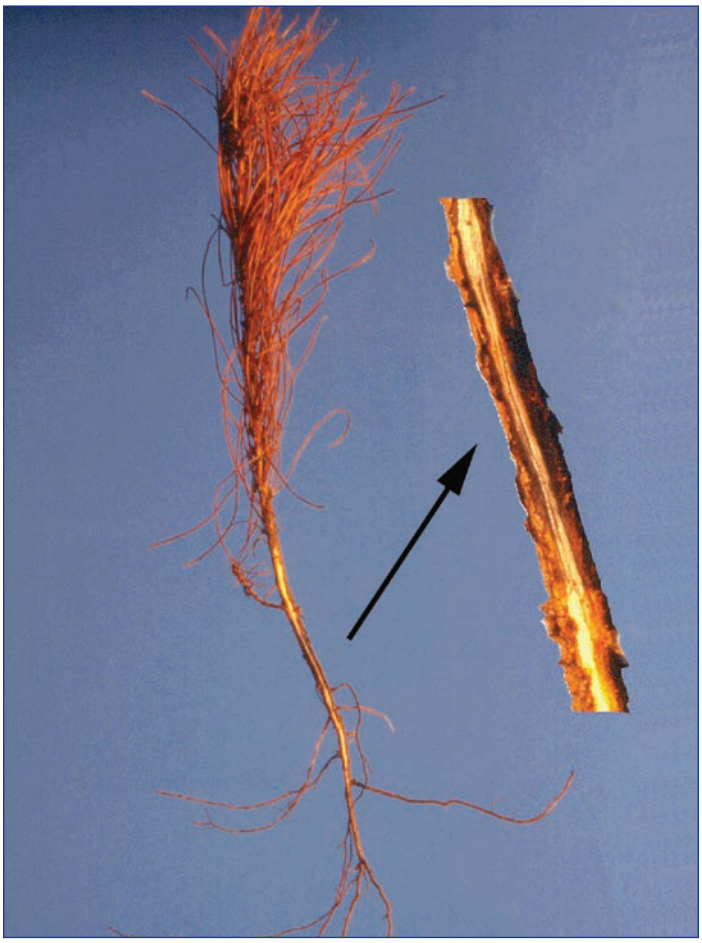

Figure 4. Red pine seedling that was rapidly killed by Diplodia collar rot (inset) shortly after outplanting. (Photo by Glen R. Stanosz)

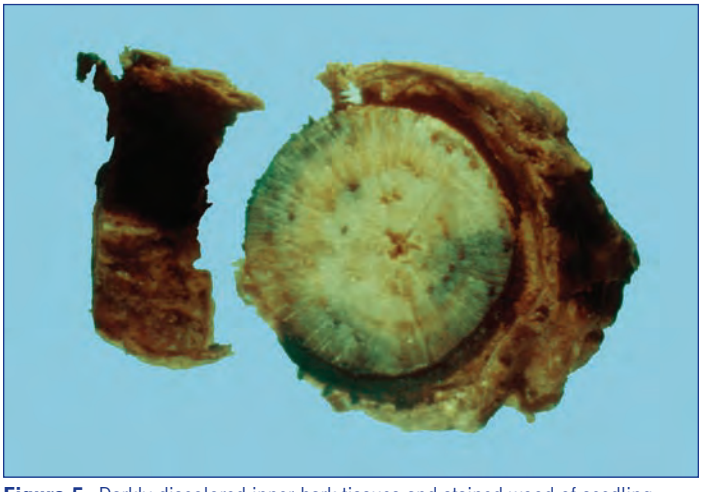

Figure 5. Darkly discolored inner bark tissues and stained wood of seedling killed by Diplodia collar rot. (Photo by Glen R. Stanosz)

Application of protectant chemicals to reduce losses caused by *D. pinea* has produced mixed results. Palmer and others (1986) reported that only 2.7 percent of 2-0 red pine seedlings were diseased when treated with benomyl during both growing seasons. In one nursery, however, Stanosz and others (2005) found that, in spite of benomyl application, the average disease incidence, based on visible symptoms, was 43 percent in plots of 2-0 seedlings in close proximity to a windbreak inoculum source. In addition, fungicide applications may not prevent persistence of *D. pinea* on or in seedlings in the absence of disease development. Stanosz and others (2005) culturally assayed surface-disinfested lower stem segments from healthy-appearing seedlings. The pathogen was detected on 63 percent (Wilson State Nursery, Wisconsin) and 88 percent (Badoura State Nursery, Minnesota) of asymptomatic seedlings in beds that were in close proximity to a windbreak inoculum source and in which symptomatic seedlings also were common. In addition, *D. pinea* can subsequently proliferate and kill previously asymptomatic seedlings under conditions that induce host stress (Stanosz and others 1997, Stanosz and others 2001). This ability of *D. pinea* to act as a latent pathogen may explain the frequent mortality associated with collar rot of recently outplanted red pine seedlings (Stanosz and Cummings Carlson 1996).

After dormant bareroot red pine seedlings are lifted and packed in early spring, they usually are stored until delivery to customers. For example, at the Minnesota Department of Natural Resources General Andrew Nursery, seedlings are placed in plastic bags and then into shipping cartons and maintained in a cold room at 3.3 to 4.4 °C (38 to 40 °F) for as long as 3 weeks. After seedlings are transferred to customers, however, conditions during transport and storage for days or even weeks until seedlings are planted are highly variable and often do not include cold storage.

Nurseries in which Diplodia shoot blight, canker, and collar rot have caused serious losses have implemented practices intended to reduce both the incidence of these diseases in nursery beds and the persistence of *D. pinea* on healthy-appearing seedlings. The influence of storage conditions on the activity of *D. pinea* on or in the asymptomatic seedlings, however, has not been explored. The objectives of this study were to (1) quantify the effectiveness of disease management practices on the persistence of *D. pinea* on or in asymptomatic red pine nursery seedlings and (2) determine the influence of storage, including a period of nonrefrigerated storage, on asymptomatic persistence of the pathogen on red pine nursery seedlings. Studies were conducted in each of 2 years, using cultural methods to detect the pathogen and molecular methods to confirm pathogen identity.

Methods

Experiments With Noninoculated Seedlings

Experiment 1 was designed to compare the frequency of cultural detection of *D. pinea* among seedlings assayed (1) upon receipt from the nursery (without extended storage), (2) after storage for 3 weeks in a cold room, or (3) after storage for 3 weeks in a cold room and then 1 additional week at a room temperature. The third treatment was intended to simulate proper cold storage of seedlings after lifting, followed by storage at a warmer temperature during delivery or after receipt by a customer.

Asymptomatic, dormant red pine seedlings were lifted from two nursery beds in late April 2009 and 2010 from the Minnesota Department of Natural Resources General Andrews State Nursery, Willow River, MN (46.32° N., 92.84° W.). Seedlings from each nursery bed were packaged 10 per plastic bag (a replicate), with these bags placed within a larger plastic bag and corrugated cardboard box normally used for seedling shipment. The two boxes were shipped overnight to the laboratory at the University of Wisconsin-Madison, where five replicate bags of seedlings from each nursery bed were randomly assigned to each of the three treatments, and then replaced in the larger plastic bag in the shipping boxes.

Experiment 2 was conducted similarly in 2010 with five replicate bags of seedlings from each nursery bed assigned randomly to (1) storage for 4 weeks in a cold room or (2) storage for 3 weeks in a cold room, followed by 1 week at room temperature. Storage temperatures during each experiment were recorded hourly using Hobo data loggers (Onset Computer Corporation, Bourne, MA) placed among the bags of seedlings.

After storage, seedlings were culturally assayed using procedures similar to those previously developed to evaluate asymptomatic persistence of the pathogen on or in red pine seedlings (Stanosz and others 2005). A segment approximately 5 cm (2 in) long was cut from the lower stem/root collar of each seedling, needles were removed, and then surface-disinfested by 30 sec immersion in a 95-percent ethanol solution followed by two immersions for 2 min each in a solution of 1.05 percent NaClO plus two drops of Tween-80 per liter (8 drops per gallon) deionized water. Each segment was then placed on one side in an 84-mm-diameter (3.3-in-diameter) Petri dish containing tannic acid agar medium (Blodgett and others 2003) and twice-autoclaved red pine needles were placed on the other side (figure 6). The dishes were incubated 30 cm (12 in) beneath one cool white

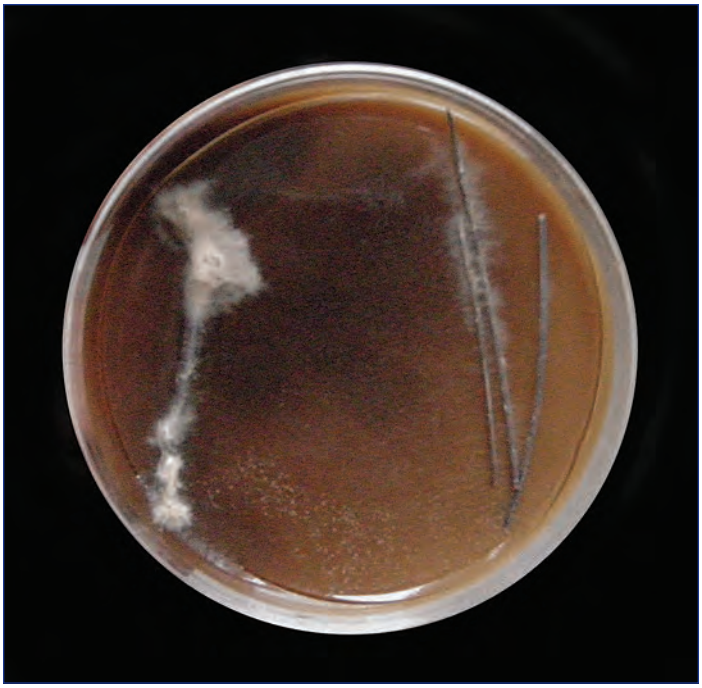

Figure 6. *Diplodia pinea* mycelium that has grown from a surface-disinfested red pine stem segment (left) to red pine needles (right) in a Petri dish containing tannic acid agar medium. (Photo by Glen R. Stanosz)

fluorescent light tube and one ultraviolet light tube for up to 6 weeks at approximately 24 °C (75 °F). Conidia from pycnidia produced on the needles were examined for characteristics consistent with those of *D. pinea* (Punithalingam and Waterston 1970).

To confirm the pathogen species, pycnidia from the Petri dishes were transferred to potato dextrose broth and incubated for approximately 1 week. After incubation, DNA from these subcultures was extracted using the procedures of Smith and Stanosz (1995). The fungus was then identified using specific mt SSU rDNA PCR primers that allow differentiation of *D. pinea* from the similar conifer pathogen *D. scrobiculata* and other related fungi (Smith and Stanosz 2006).

Experiments With Inoculated Seedlings

Because nursery disease management practices likely reduced *Diplodia* frequency on red pine seedlings, additional experiments were conducted to further evaluate storage effects on the pathogen's persistence and disease. Dormant seedlings from the same nursery were lifted from two nursery beds in late April 2009 (experiment 3) and 2010 (experiment 4), packaged 10 per plastic bag, and shipped to the laboratory as described previously for experiments 1 and 2. Conidial inoculum of *D. pinea* was applied to seedlings after receipt, however, to ensure presence of the pathogen with seedlings during storage treatments. Experiment 3 was conducted in 2009 with 10 bags (replicates) from each nursery bed assigned randomly to each of two treatments: (1) storage for 3 weeks in a cold room or (2) storage for 3 weeks in a cold room, followed by 1 additional week at room temperature. Experiment 4 was conducted in 2010 with 10 bags from each nursery bed assigned randomly to (1) storage for 4 weeks in a cold room or (2) storage for 3 weeks in a cold room, then 1 additional week at room temperature.

Conidial inoculum was obtained from twice-autoclaved red pine needles incubated for several weeks on colonies of *D. pinea* on water agar medium. Needles bearing pycnidia were crushed in sterile, deionized water. The resulting suspension was filtered through two layers of cheesecloth, and more water was added to adjust the concentration of conidia to 5 by 10^4 spores per millimeter. An atomizer was used to apply 1 ml of con-idial suspension to seedlings in each replicate bag and then the bag was resealed. Germination of conidia in the inoculum suspensions was assessed by examination of 50 conidia per trial of experiments 3 and 4 after 4 hours incubation on water agar medium at 24 °C (75 °F) in the dark. For both exper-iments, germination exceeded 80 percent. After storage, seedlings were culturally assayed using procedures described above.

Experimental Design and Data Analysis

Because results were similar, data for seedlings from the two nursery beds were pooled into a single, completely randomized design for each experiment. Means of temp-eratures recorded hourly for each experiment were calculated, and maximum and minimum temperatures were determined. For each experiment, mean percentages of seedlings from which the pathogen was detected were calculated. Because the data lack normality, analyses were performed using a nonparametic method. Differences among storage treatments in each experiment were determined using the Kruskal-Wallis test of equality of medians using Minitab for Windows version 14 (Minitab Inc., State College, PA).

Results and Discussion

Experiments With Noninoculated Seedlings

Use of molecular methods confirmed *D. pinea* as the pathogen cultured from noninoculated seedlings in every case except one, when the similar pathogen *D. scrobiculata* was detected. The detection of *D. pinea* in this study is consistent with prevalence of this pathogen with asymptomatic nursery seedlings at other nurseries, but contrasts with previous results for the General Andrews State Nursery. When surveyed in 2002, 7 of

10 seedlings from the General Andrews State Nursery for which molecular methods were used to confirm pathogen identity yielded *D. scrobiculata* (Stanosz and others 2005). Whether the current result indicates a shift in pathogen population in, or in the vicinity of, this nursery is unknown. These findings, however, underscore the importance of employing methods that allow for unambiguous identification of fungal pathogens.

Noninoculated seedlings were infrequently (0 to 7 percent) culturally positive with or without extended storage in both 2009 and 2010 (table 1). Detection of a *Diplodia* pathogen was rare in these 2 years compared with 2002, when seedlings from this nursery were similarly assayed. At that time, averages of 20 and 26 percent of asymptomatic seedlings from the two locations sampled tested positive for either pathogen, with as many as 40 percent of seedlings positive in one plot (Stanosz and others 2005). At other nurseries sampled that year in Minnesota and Wisconsin, as many as 88 percent of asymptomatic seedlings in proximity to windbreaks bore *D. pinea* or *D. scrobiculata*. The much lower frequency of detection in the current study can be attributed to efficacy of current disease management practices at the General Andrews State Nursery and the other affected nurseries. Removing red pine windbreaks, rouging affected seedlings, avoiding top pruning, and adopting a 2-year production cycle (instead of a 3-year cycle) reduce the exposure of seedlings to inoculum. Coupled with judicious application of fungicidal sprays, these measures have drastically reduced association of the pathogens with seedlings (Minnesota Department of Natural Resources 2009, Wisconsin Department of Natural Resources 2011).

Experiments With Inoculated Seedlings

Results differed significantly between storage treatments for seedlings to which inoculum had been added in 2009 ($p < 0.01$). The frequency of culturally positive inoculated seedlings was 6 percent when seedlings were cold stored (approximately 3.5 °C) for 3 weeks compared with 33 percent for seedlings that were stored for 1 additional week at room temperature (table 1). This difference demonstrates the potential for pathogen proliferation after removal of seedlings from cold storage. Detection, even after rigorous surface disinfestation, suggests that a pathogen is not merely persisting superficially, but that infection has occurred.

In 2010, temperature in cold storage was not as low as desired, averaging nearly 8 °C (14.4 °F) (table 1). The frequency of cultural detection was 12 percent for seedlings that were cold stored for 4 weeks and 21 percent for seedlings that were removed from the cold room and stored for a 4th week at room temperature ($p = 0.12$). Even though cold storage temperatures were higher than planned, a tendency still existed for more frequent pathogen detection after exposure to a warmer temperature for the final week.

Implications for Nurseries and Customers

Similar to the current study, previous research to examine the effect of temperature on growth of *D. pinea* and *D. scrobiculata* found that temperatures of 20 °C (68 °F), 25 °C (77 °F), and 30 °C (86 °F) were conducive to colony growth after 3 days on potato dextrose agar, whereas no discernable growth was observed for cultures at 5 °C (41 °F) or 10 °C

Table 1. Percentages of asymptomatic red pine seedlings from which cultural detection of *Diplodia pinea* or *D. scrobiculata* occurred.

Treatment	Noninoculated seedlings (%)[a]		Inoculated seedlings (%)[b]	
	2009 (experiment 1)	2010 (experiment 2)	2009 (experiment 3)	2010 (experiment 4)
No storage	3	7	—	—
Stored 3 weeks at 3.5 ± 1.1 °C (38.3 ± 2.0 °F)	1	—	6	—
Stored 3 weeks at 3.5 ± 1.1 °C (38.3 ± 2.0 °F), followed by 1 week at 23.0 ± 1.1 °C (73.4 ± 2.0 °F)	1	—	33	—
Stored 4 weeks at 7.8 ± 1.0 °C (46.0 ± 1.8 °F)	—	0	—	12
Stored 3 weeks at 7.9 ± 0.5 °C (46.2 ± 0.9 °F), followed by 1 week at 24.8 ± 1.6 °C (76.6 ± 2.8 °F)	—	4	—	21
	$p = 0.40$[c]	$p = 0.10$	$p < 0.01$	$p = 0.12$

[a] Experiments 1 and 2: n = 10; 5 replicates from each of two nursery beds.
[b] Experiments 3 and 4, n = 20; 10 replicates from each of two nursery beds.
[c] Values of p for treatment differences using Kruskal-Wallis test of equality of medians.

(50 °F) (Palmer and others 1987). Temperatures of 0 °C to 2 °C (32 °F to 36 °F) are recommended as ideal cold storage temperatures for seedlings for up to 2 months (Landis and others 2010). Many nurseries now have facilities for storage of seedlings at these temperatures, although customers may not. Results of this study and others support the likely benefit of preplanting storage by customers at even moderately cool temperatures (≤ 10 °C [≤ 50 °F]).

In addition to the direct influence of temperature on fungal growth, lengthy cold storage durations or storage without refrigeration could affect seedling physiological condition (Landis and others 2010) and render seedlings susceptible to infection or disease development. For example, a controlled experiment with potted red pine seedlings demonstrated that moisture stress induces more severe Diplodia shoot blight symptoms (Blodgett and others 1997). As mentioned previously, stress can stimulate proliferation of *D. pinea* to kill previously asymptomatic seedlings (Stanosz and others 2001). Storage in sealed plastic bags lessens drying in storage and no visible indications of drying were apparent in the current study.

Conclusions

We infrequently cultured *Diplodia pinea* and *D. scrobiculata* from asymptomatic red pine seedlings grown in a nursery where practices included removal of inoculum sources, chemical protection, and other measures to reduce or eliminate presence of these pathogens. When we inoculated the seedlings with *D. pinea* immediately before storage, however, a period of storage without refrigeration led to more frequent cultural detection of this pathogen. Storage of seedlings at even moderately cool temperatures before, and especially after, delivery to customers is recommended.

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Developing Reforestation Technology for Southern Pines: A Historical Perspective

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Abstract

Early in the 20th century, the forests of the South were decimated by aggressive harvesting, resulting in millions of acres of forest land in need of reforestation. Foresighted individuals committed efforts to restore this harvested land to a productive condition. The effort required dedication, cooperation, and leadership. The efforts of this small cadre of individuals resulted in successful restoration of the South's forests and these new forests became the basis of the South's economy.

The Need for Reforestation

As late as the 1870s, millions of acres of virgin longleaf pine (*Pinus palustris* Mill.) forests covered the South's Coastal Plain from the Carolinas to east Texas. During the late 1800s and early 1900s, however, massive timber harvest on this forest land occurred. The dire economic conditions after the Civil War allowed for procurement of vast areas of timber land with low investments. The development of steam-powered logging and milling equipment resulted in the establishment of the huge lumbering industry. The area of clear-cut forests in the southern Coastal Plain equaled the combined areas of Alabama, Mississippi, and Louisiana—about 92 million ac (37 million ha) (Heyward 1958).

The mass timber harvest has been described as the golden age of lumbering and did much to provide for the economic recovery of the South, but, after the timber was removed, a spirit of desolation and bleakness returned (figure 1). Few individuals could envision how forested conditions could be restored within a timeframe that could be economically practical, because the harvested pine stands had been 150 years old or older. Even if reforestation had been practical, Wakeley (1930) estimated that, based on the then-rate of planting, it would take up to 1,000 years to reforest the Nation's denuded forest land.

Recognizing Reforestation's Economic Potential

In the 1910s, Henry E. Hardtner of the Urania Lumber Company (Urania, LA) believed that an economic opportunity existed in developing second-growth forests and worked to convince others of the economic potential of reforestation (Burns 1978). Hardtner invited Professor H.H. Chapman of the Yale University School of Forestry to bring his forestry students to Urania for the school's annual 3-month spring camp. Beginning in 1917, and continuing for several decades, Chapman led his students at Urania in developing novel concepts for determining growth possibilities, evaluating the role of fire in longleaf pine establishment, and using periodic controlled burning as a means of suppressing hardwood competition (Barnett 2011).

Austin Cary, a Forest Service employee from the Washington, DC, office, traveled the South in the early 1900s and did much through his pithy ways to convince lumbermen of the value of second-growth stands (White 1961). He was known to charge into a lumber company president's office and take him to the woods. With his ever-present axe, he would cut trees to show the rapid growth rate of young pine stands.

Hardtner, Chapman, Cary, and other pioneers demonstrated that reforestation was economically viable. But, tree nurseries and technology to reforest these massive areas of depleted forest land were needed to successfully achieve reforestation goals.

Figure 1. This cut-over forest land was typical of millions of acres of land across the South in the early 20th century. (Photo from USDA Forest Service files)

Early Development of Nursery and Reforestation Technology

In 1908, the Great Southern Lumber Company began operation at Bogalusa, LA, and established the world's largest sawmill, with four 8-foot band saws producing 1 million board feet of lumber every 24 hours (Heyward 1963). W.H. Sullivan, general manager of the company, visited with Hardtner at Urania and decided to begin a reforestation program. In 1919, Sullivan assigned J.T. Johnson as forester in charge of reforestation. Johnson had no formal forestry training, but he "contributed an immeasurable quality of skill, labor and ingenuity to building the South's great pine forests" (Wakeley 1976). Johnson established a one-half acre pine seedling nursery during 1921–22 across from the Bogalusa City Hall—believed to be the first pine seedling nursery in the South (Wakeley and Barnett 2011). Larger nurseries soon followed.

Johnson was fortunate to have F.O. "Red" Bateman as his assistant. Bateman was the company's head ranger (figure 2). With only a 9th-grade education, Bateman became the prime mover in developing nursery and planting principles and techniques for the southern pines. By the time Philip C. Wakeley (figure 3) was hired in 1924 by the recently established USDA Forest Service Southern Forest Experiment Station and assigned to Bogalusa to begin a reforestation cooperative program, Bateman had worked out general principles still employed today, such as slit planting of bareroot seedlings grown at moderate seedbed densities in the nursery without shade (Wakeley 1976). He also developed a planting dibble that is still in use today and established a 6-ft by 8-ft outplanting spacing that became the nearly universal planting density standard used throughout the South for decades.

When Philip Wakeley began his research, the Southern Forest Experiment Station had been in existence for only 3 years. At the time, fewer than 20 professionally trained foresters were working across the entire South (Wakeley and Barnett 2011). Wakeley's intensive collaborative effort to understand and develop southern pine seed collection and processing, seedling nursery culture, and planting technology was applied to the entire southern Coastal Plain from east Texas to the Carolinas (figure 3).

Before the Great Depression caused the Great Southern Lumber Company to go into receivership in the early 1930s, Bateman had planted 12,700 ac (5,140 ha) of southern pines. With the exception of the Biltmore Estate near Asheville, NC, no other successful pine plantations in the South had more than 100 ac (40 ha) (Wakeley and Barnett 2011). White pine (*Pinus strobus* L.) seedlings grown in Europe had been imported to establish the Biltmore plantations (Schenck 2011).

An example of Red Bateman's ingenuity was the development of a nursery seeding tool for longleaf pine seeds. Wakeley expressed his frustration one morning at the inability to drill sow longleaf pine seeds because of their persistent seed coat

Figure 2. F.O. (Red) Bateman of the Great Southern Lumber Company developed many southern nursery practices still in use today. (Photo by C.W. Goodyear, 1929)

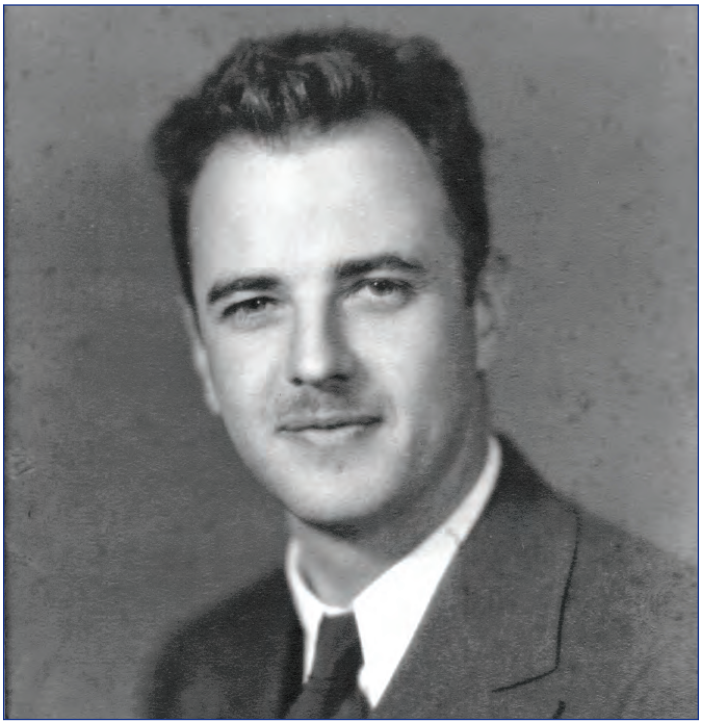

Figure 3. The research of Philip C. Wakeley of the Southern Forest Experiment Station established basic nursery and planting technology for southern pines. (Photo from Philip Wakeley family, 1935)

wing. Before noon, Bateman came by Wakeley's worksite and asked him to stop by the nursery. When he arrived, Bateman demonstrated a seeder for sowing longleaf pine seeds that he had developed that morning—a wooden trough, 5.0 ft (1.5 m) long to fit across nursery beds and hinged at the bottom to drop seeds on the bed. A pair of tall, curved handles at each end permitted it to be opened without stooping or kneeling, which made the device easy to use (figure 4). The seeder resulted in marked improvement in the uniformity and quality of longleaf pine nursery stock.

Refining Nursery and Reforestation Technology

The results of Wakeley's cooperative nursery research with the Great Southern Lumber Company were applied by other organizations interested in reforestation. Several forestry companies established small nurseries to evaluate the economic potential of reforestation. In 1929, Wakeley wrote a bulletin about the results of the cooperative seed, nursery, and planting research. He then surveyed six nurseries: Louisiana State University School of Forestry at Baton Rouge, LA; Louisiana Division of Forestry at Woodworth, LA; Industrial Lumber Company at Elizabeth, LA; Long Bell Lumber Company at DeRidder, LA; and the Texas Forest Service nurseries at Kirbyville and Conroe, TX. Wakeley found the nursery managers to be "observant, ingenious, and uninhibited men" (Wakeley and Barnett 2011: 82).

Charles Delaney and his brother Luther were managers of the Louisiana Department of Conservation, Division of Forestry nursery located on the Alexander State Forest near Woodworth, LA (figure 5), and frequently interacted with Wakeley to develop the South's first State tree-seedling nursery (Barnett and Burns 2011, 2012). The Texas Forest Service State nurseries followed soon thereafter.

Wakeley's collaboration with Johnson and Bateman ended in the early 1930s with the advent of the Great Depression and the demise of the Great Southern Lumber Company. During Wakeley's association with Great Southern, he developed information for seed collecting, processing, and treating and for seedling stock specifications and a variety of nursery cultural treatments. Thereafter, Wakeley's reforestation research program was moved to the Forest Service's new Stuart Nursery in central Louisiana.

In 1933, the Stuart Nursery was established by the Kisatchie National Forest (KNF) in central Louisiana in conjunction with the creation of the Civilian Conservation Corps (CCC). Although KNF employees managed the nursery, a nearby CCC camp of 200 young men provided labor for its operation (figure 6). Nursery production was about 25 million seedlings annually, with most of the seedlings shipped to CCC projects that had reforestation emphases. Wakeley's research, now located at the nursery, took advantage of the CCC crews to apply a variety of nursery cultural practices and to establish

Figure 4. This seeder, which F.O. (Red) Bateman developed for winged longleaf pine seeds, exemplifies his innovative skill. (Photo from USDA Forest Service files)

Figure 5. The Louisiana Department of Conservation, Division of Forestry nursery at the Alexander State Forest near Woodworth, LA, was the first State tree-seedling nursery in the South. Charles (left) and Luther Delaney (bent over) managed the nursery. (Photo from Louisiana Department of Agriculture and Forestry files)

Figure 6. The Kisatchie National Forest's Stuart Nursery in central Louisiana used Civilian Conservation Corps crews to operate the nursery. These crews grew and planted 670,000 seedlings in research studies. (Photo from USDA Forest Service files)

outplanting studies. Over the duration of the CCC involvement and support, nearly 750,000 tree seedlings were planted in research studies on the Palustris Experimental Forest (Barnett, Haywood, and Pearson 2011). The resources available at the Stuart Nursery facilitated the development of Wakeley's southern pine seedling grade specifications and other cultural guidelines that are still in use today throughout the South (Wakeley 1954).

By the end of the 1930s, Wakeley and his colleagues published guidelines for southern pine seed (Wakeley 1938a), seedling production (Huberman 1938, Wakeley 1938b), and planting technology (Wakeley 1935). Early versions of these publications were used by the organizations using CCC crews to grow seedlings for reforestation projects. Most of these CCC-related projects ended with the closure of the CCC program and the beginning of World War II (WWII). The availability of the CCC program provided an opportunity to field test seed, seedling, and planting research results, however, and pioneer reforestation guidelines for southern pines.

Modern Nurseries Across the South

After WWII, a concentrated effort was made to continue developing and applying reforestation technology. In 1954, Wakeley published *Planting the Southern Pines*, which incorporated results of the research programs with both the Great Southern Lumber Company and Stuart Nursery (Wakeley 1954). This single publication provided the modern foundation for southern pine nursery development and plantation establishment (figure 7).

Since Wakeley's publications, all Southern State forestry organizations and most major forestry companies have established nurseries. Few nurseries established before WWII remain in operation, however. The Stuart Nursery and W.W. Ashe Nursery in southern Mississippi continued operation for many years, but now are closed. The Soil Bank Program in the early 1960s did much to increase the demand for planting stock and expansion of nursery production. In the late 1970s and early 1980s, reforestation programs and nursery production of forest industries expanded even further so that large portions of nursery production shifted from Federal- and State-operated nurseries to commercially operated forest industry nurseries.

Although many refinements and improvements have been made in nursery technology during the past 75 years, the basic guidelines that Wakeley and his colleagues developed in the early 20th century remain as the foundation for today's southern nursery and reforestation programs (figure 8).

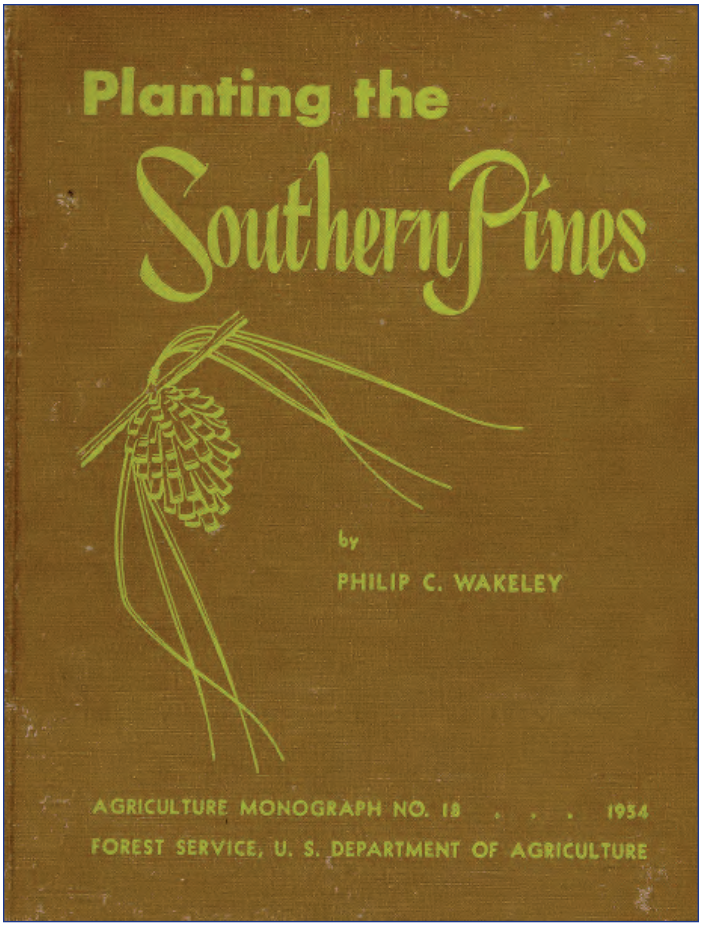

Figure 7. Wakeley's 1954 book, *Planting the Southern Pines*, provided the knowledge and technology for operating nurseries and establishing pine plantations across the South. (Photo by James Barnett, 2010)

Figure 8. This longleaf pine plantation was established as a research study by Wakeley in the winter of 1934 to 1935. (Photo by James Barnett, 2012)

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Coastal Douglas-Fir Controlled-Crossing Guidelines

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Abstract

Controlled pollination is an essential element in forest tree breeding. Although the concept is simple, the process requires multiple steps, which must be done correctly to obtain successful crosses. As tree-breeding cooperatives of the Pacific Northwest begin a third cycle of breeding and testing, it is timely to describe this process for the major forest conifer species, Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco).

Stimulation by stem girdling and injection of gibberellic acids 4 and 7 is crucial for early flower induction; it can often begin 2 years after grafting into a seed orchard or breeding orchard. Other stimulation techniques (root pruning, calcium nitrate fertilization) can also be effective. Estimates of 10 seeds per female cone and 2 pollen buds to pollinate a female flower can be used for planning. Pollen dried to 6 to 8 percent can be used immediately or stored for future seasons. Important ingredients of successful crossing include attention to detail, constant vigilance, and exact timing, especially when bagging, harvesting pollen buds, and applying pollen to receptive female flowers.

Introduction

Cooperative tree improvement of Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) has proceeded fully through two cycles[1] and now stands on the cusp of the third cycle. A tree-improvement cycle includes selection of superior trees to use as parents, controlled breeding to produce fully pedigreed families, replicated progeny tests to determine the genetic worth of the parents, and reselection of elite parents and superior progeny to begin the next cycle (Howe and others 2006). As we move into the third cycle, we build on the considerable efforts of past tree breeders. These early forest geneticists often had to make crosses in the tops of selected plus trees in natural stands (figure 1; Ching 1960, Orr-Ewing 1956). Today, all crossing is completed in either established seed orchards or breeding archives (figure 2).

In recent years, much institutional knowledge has been lost as experienced tree breeders have retired or moved on to other pursuits and as important tree-breeding centers have been downsized or closed. Most applied publications date back decades (for example, Ching 1960, Orr-Ewing 1956, Wilson 1969) and, although quite informative, do not include many recent advances or adequate details on a number of steps. Although timber-growing organizations now employ a fraction of the skilled staff their predecessors once did, timelines are aggressive, and the need for efficiency is higher than ever before. At the same time, mating designs call for making between 200 and 400 full-sib crosses within each cooperative third-cycle breeding zone, adding up to several

Figure 1. How it once was—crossing on a first-generation parent tree in 1976. (Photo by Marc Vomocil, Starker Forests, 1976)

Figure 2. Breeding orchard specifically designed for third-cycle crossing. (Photo by Keith Jayawickrama, Oregon State University, 2012)

[1] First cycle: 1966–2001; second cycle: 1984–present; third cycle: 2008–present.

thousand Douglas-fir crosses across the Pacific Northwest (PNW). A critical need exists for scientists to document successful controlled-crossing techniques for Douglas-fir so that the next generation of tree breeders has the tools necessary to efficiently and cost-effectively complete mating designs.

This article describes the necessary information to conduct a Douglas-fir crossing program. Where more than one way exists to achieve a given goal, each is discussed in turn. Much of this information is gathered from practitioners in the field rather than from published literature, which is limited. A short section on information specific to western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), the secondary conifer species in cooperative PNW tree-improvement programs, is also included.

Controlled Crossing

Controlled crossing is an important part of tree-improvement programs (White and others 2007). Conditions in a controlled-breeding environment differ from natural stand conditions in at least six key ways:

1. For maximum cost efficiency, breeding cycles are accelerated so that trees are stimulated to flower and produce seed at much earlier ages.
2. Instead of allowing female flowers to be open-pollinated by a mix of abundant natural pollen, pedigrees are controlled to a single male parent, producing full-sib families or mixtures of pollen from known male parents for polymix crosses.
3. Instead of waiting for natural seed production to occur every 7 to 10 years, breeding trees are encouraged to flower every 2 years.
4. Instead of remote, steep, and sometimes high-elevation conditions, trees are established in farm-like, low-elevation, and fairly flat conditions.
5. Grafting is used to establish seed orchards and breeding orchards (Jayawickrama and others 2009). Instead of reproduction by a single-genotype tree developing naturally from seed, a composite tree is built via grafting, using a root system from compatible rootstock, with the reproduction occurring on selected donor tissue known as the scion.
6. Controlled breeding is conducted on trees that ideally can be reached from the ground or from short ladders.

Although the term is taxonomically imprecise, tree breeders commonly use "flower" when referring to floral structures in trees. Before flowering, the term "female bud" or "cone bud" is used for the structure that produces female flowers, and the term "male bud" or "pollen bud" is used for the structure that produces male flowers. Female flowers produce seed cones, and male flowers produce pollen cones. A conelet is a female flower that has been pollinated but is not yet fully mature. We use this accepted terminology throughout the article.

Although Douglas-fir is not the most difficult tree species to breed successfully, it is certainly not the easiest to breed. The reluctance to flower and the abortion of conelets because of frost and other factors are the main obstacles. The time period from pollination to mature seed is relatively short, however; crosses made in April yield mature seed about 5 months later. With diligence, effort, and attention to detail, PNW tree breeders have successfully completed an estimated 25,000 controlled crosses of Douglas-fir during the past 50 years (compiled from estimates by Jeff DeBell, Washington Department of Natural Resources; Michael Stoehr, British Columbia Ministry of Forests; Jim Reno, Weyerhaeuser Company; the Northwest Tree Improvement Cooperative; and the authors' personal experience).

Flower Induction

To quickly complete a given mating design, flower induction is required. In most PNW seed orchards, using a flower induction treatment, also referred to as stimulation, has been standard practice for many years, resulting in consistently reliable seed crops. Flower induction treatments applied in the spring take effect that summer, and the resulting pollen buds are observable by fall. Cone buds are less obvious but are usually quite evident by February of year 2. A healthy tree can be stimulated every other year for several years. Stimulating a given tree too often or too aggressively can result in lower vigor, poor seed quality, and, ultimately, death of the tree. In breeding orchards with many ramets (individual trees) per clone, however, it is acceptable to lose some ramets to complete the crossing program faster.

Gibberellic Acids

Until the early 1990s, tree breeders believed that Douglas-fir orchard trees would not produce appreciable quantities of pollen and seed cones until 10 to 15 years after grafting into a seed orchard. Promising results were obtained by stimulation techniques such as drought stress and root pruning, but such treatments were (and still are) difficult to apply in large orchards. Today, with the ability to apply certain growth hormones, specifically combinations of gibberellic acid (GA), it is relatively simple to get much younger ramets to flower

(Ross and Bower 1989). The type of GA (designated by number), timing, application method, and dosage influence the outcome. Although GA is a powerful tool, it requires careful use to be safe and effective.

GA4/7

GA4 combined with GA7 (GA4/7) is very effective in stimulating flower production in members of the Pinaceae family, including Douglas-fir, and is available in either crystalline or liquid, ready-to-use formulations. Crystalline GA must be dissolved in ethyl alcohol before use. The liquid form is known by the trade name ProCone™ (Valent Corporation) and is considered the industry standard. As when using all chemicals, the user should consult the label for safe and proper use.

Best results are usually obtained with freshly purchased ProCone. The manufacturer can provide data on how long an individual batch can be used without losing strength. ProCone needs to be stored in a standard refrigerator to prolong its shelf life.

GA Application

GA application to ramets can usually start 2 to 3 years after grafting (Cherry and others 2007). Tree breeders have different opinions about the best timing for GA application: about the time of vegetative bud break (Cherry and others 2007); when 50 percent of the trees have flushed (Ross and Bower 1991); when most of the trees have flushed (Ross and Bower 1989); and when 50 to 90 percent of the year's vegetative growth has occurred (ProCone label). Anecdotal evidence suggests that younger trees in breeding orchards need to be treated after vegetative bud flush, but older trees in seed orchards respond better to application directly before or at bud break. The ProCone label indicates that, based on application timing, the chemical can stimulate either pollen-bud or cone-bud development.

Although guidelines exist for calculating proper GA dosages, determining the best dosage is as much an art as it is a science. Some clones respond well to lower GA dose amounts, others require higher doses, and yet others can have a phytotoxic response, resulting in excessive needle drop, dieback, and even mortality.

The application rate is based on the cross-sectional area at the point of injection, with the number of holes increasing as tree diameter increases. It is useful to create a spreadsheet ahead of time, measure the diameter of each tree at the point of intended injection and calculate both the amount of GA needed and the number of holes required for that tree. Tree breeders have used a variety of injectors over the years (figure 3). One example is a repeater pipette, which repeatedly delivers the same volume up to 10 times. An injector with the capacity to quickly adjust the dose rate is ideal. In a breeding orchard organized in this fashion, it is possible to treat two trees per minute, using two people. After drilling the downward angled holes into each tree, the product is injected into each hole.

Stem Girdling

In older orchards, GA may not be needed. Instead, stem girdling is a safer, less expensive, and more predictable stimulation method. In some low-elevation orchards, this technique alone is sufficient to stimulate consistent and reliable flower crops. Stem girdling can be applied when the ramets are at least 5 cm (2 in) in diameter at ground level. Tree breeders have varying recommendations regarding optimal dates for stem girdling: 2 weeks before vegetative bud break, at the time of vegetative bud swell (Ross and Bower 1989, Ross and Bower 1991), 6 weeks before vegetative bud break (Clemo, personal communication 2012), or 1 to 3 weeks before vegetative bud flush (Woods 1989).

Figure 3. Examples of gibberellic acid injectors. (Photos by Keith Jayawickrama, Oregon State University, 2010)

Targeted trees receive overlapping half-circumferential girdles. These trees are cut through the bark and cambium until the saw teeth just reach the xylem (wood), but no deeper (figure 4). Cutting any deeper into the tree results in no benefit and can potentially weaken the stem. Girdling is usually done with a small hand-held pruning saw. Small trees (with a diameter-at-breast-height of 5 to 8 cm [2 to 3 in]) are more safely treated with a knife or a hacksaw. Larger trees are often girdled with a battery-operated, thin-kerf chainsaw.

The first cut needs to be placed at a comfortable working height, with the second cut placed about 1.5 times the stem diameter above or below the first cut, for example, 15 cm (6 in) apart on a 10-cm (4-in) diameter tree. For large trees, space the cuts no more than 30 cm (12 in) apart. Girdles must overlap each other vertically, and the kerf must be left clean of sawdust. New girdles should not be located within an existing scar.

Calcium Nitrate

Calcium nitrate fertilizer can stimulate flowering in Douglas-fir (Ebell 1972). When applied near the end of March, application rates of 205 to 413 kg nitrogen per ha (200 to 400 lb/ac) have been shown to stimulate increased flowering. If an orchard gets a lot of rain, this fertilizer needs to be applied later in the spring to prevent rapid leaching; in drier orchards without irrigation, it needs to be applied earlier to enable rainfall to dissolve the material and move it down into the soil by the time of active root growth.

Figure 4. Stem girdling on a small ramet in a breeding orchard. (Photo by Larry Miller, Oregon Department of Forestry, 2012)

Other Stimulation Methods

Girdling, calcium nitrate, and GA may be applied singly or in combination to increase effectiveness. Root pruning (using a tree spade or a single ripper preceded by a rolling coulter) is another option. This process is usually implemented around the time of pollen-bud swell and is most effective when used in conjunction with GA. This treatment is very effective in potted orchards, which may be one reason to establish some ramets in pots. This technique may be too severe to be used every other year.

Initial Planning Before Crossing Season

Although third-cycle breeding programs need to begin with a clear crossing plan, considerable variation in fecundity and phenology is to be expected, which may necessitate changes in plans both within and between years. Douglas-fir is monoecious, so a clone may be used as a male parent, a female parent, or both. Because most third-cycle material consists of recently grafted ramets—often from juvenile forward selections from second-cycle tests—pollen buds may be limited. A simple database helps to organize the data needed for each clone. Information to be tracked includes the following items:

- Clone number.
- Number of ramets.
- Orchard row-column coordinates.
- Tree size.
- Vigor rating (excellent, fair, or poor).
- Female flower rating (5 = heavy, 0 = none).
- Male flower rating (5 = heavy, 0 = none).
- Female flower maturity (1 = dormant, 5 = post receptive).
- Pollen flower maturity (1 = totally closed, 5 = shed).
- Phenology (early, mid-, or late season).
- Comments.
- Pollen available from previous years.
- Estimated number of existing seeds for a given cross.

Collecting and tracking such data ensures that early trees are bagged or collected first, and late trees are delayed to the latter part of the breeding season.

A good pollen tree has plenty of pollen buds, matures early, is in excellent health, and can be easily worked, either from the ground, a small orchard ladder, or the bed of a pickup truck. An optimal tree for crossing has similar traits but produces more female flowers than male flowers. Considering the relatively short breeding season, ready accessibility of reproductive buds is important.

Pollen

All stages of pollen collection, processing, storage, and viability testing are comprehensively described in Webber and Painter (1996). Only the highlights, and local experience in Oregon and Washington, will be presented here.

Pollen Planning

Pollen is rarely limiting in older, first- and second-cycle seed orchards. A good orchard-sized tree can produce more than 100,000 pollen buds per year, enough for several liters of pollen. The challenges are to find such good pollen trees, collect and process the pollen in time for use on bagged female flowers, and keep this pollen viable.

In third-cycle crossing programs, however, pollen is likely to be limited. Experience shows that 1 ml of fresh pollen can treat 10 flowers once. If pollen is limited, however, up to 30 flowers may be lightly treated with this same 1 ml of pollen. Rare pollen lots can also be diluted with either dead pollen or talc, using a maximum ratio (volume-to-volume) of 1:1. Because seed yields may be reduced, pollen "stretching" needs to be reserved for only extreme situations. Pollen lots need to be labeled as "use sparingly" or "use liberally."

Another way to calculate pollen needs is to assume two pollen buds per female flower. A target of 200 seed for a cross would therefore require 10 cones and at least 40 pollen buds.

Breeding plans and crop survey data need to be compared to determine how much pollen is needed from which clones. When in doubt, err on the side of collecting too much pollen. In some trees, the amount of time required to pick and extract 400 male buds can be nearly the same as for 40 buds. Breeding is considerably faster and seed yields much higher if extra pollen is available. Extra pollen can be stored for use in subsequent years.

When starting a new crossing program, it is advisable to build a stock of pollen from a number of parents before putting much effort into bagging and crossing. Without experienced workers, it is challenging to collect and process pollen in time to pollinate with it during the same year. Flowering involves a 2-year cycle, so a missed opportunity today will not be available again on the same trees for 2 more years.

Pollen Collection and Forcing

Especially in young breeding orchards, pollen availability is often limiting. Thus, it is advisable to build up pollen inventories for 1 to 2 years before the onset of controlled crossing. The first step is to carefully track the maturation of pollen buds, using a 1 through 5 rating system (Webber and Painter 1996):

1. Dormant.
2. Buds beginning to swell but still closed tightly.
3. Pollen cone breaks through bud scales.
4. Pollen cone elongated, with visible spaces between microsporangia.
5. Pollen cones completely shed.

Using this rating system ensures that pollen cones of most lots will be picked at the optimum time, giving good yields of high viability pollen. The best time to harvest pollen is when pollen cones are at Stage 4, elongated, and resembling a clump of grapes (figure 5). Squeezing a mature pollen cone

Figure 5. Mature pollen cone of coastal Douglas-fir that is ready for picking, directly before pollen shed. (Photo by Bill Marshall, Cascade Timber Consulting, 2011)

yields very little liquid; what liquid exists is expected to be yellowish and thick. Those that release clear liquid are not ready to pick. Differences in pollen cone color are normal and bear no relationship to maturation—mature flowers may be yellow, pink, purple, or red.

Early pollen collection combined with forcing (see the description that follows) also is possible, but this collection usually reduces yield and viability. Conversely, if picked too late, pollen buds will be partially shed, also reducing yields.

When days are warm, sunny, and dry, pollen cones can develop from Stage 3 to Stage 5 in a few hours. Even if pollen cones have started to shed, usable amounts of pollen can be acquired by increasing the number of cones collected. Another option is to assess other ramets of the same clone, which may mature later if located in cooler and shadier parts of the orchard. Because pollen cones mature at different rates depending on exposure, it is also important to examine bud development all around the tree. If the pollen crop is large, collecting beyond the optimal window will still give an adequate yield. When the pollen crop is small, however, timing is critical and repeated visits to the same tree to pick at the optimum maturation stage will maximize pollen yields.

Pollen can be transferred from tree to tree on hands, clothing, and tools, which can cause cross contamination of different pollen lots and, thus, loss of pedigree control. Fortunately, sanitation is a simple process. Using rubbing alcohol, spray down skin and tools before beginning work on any tree. If hand cream does not adequately counteract the drying effect of the alcohol, surgical gloves can help protect sensitive skin.

Mature pollen cones need to be collected in lightweight, paper "lunch sacks" labeled with the clone number and date. Alternatively, branch tips with pollen cones may be clipped and placed into larger sacks. Pack each bag loosely, fold over the top, and staple to close. Collected pollen cones need to be stored in the shade or in a cooler during a warm day until transferred to the processing facility. Although undesirable, pollen cones may be harvested when working in the rain. This harvest needs to be done only when the pollen will be processed in a forced-air system to avoid molding. After pollen has been collected from a ramet, the ramet needs to be checked off the field sheet.

Orr-Ewing (1956) and Ching (1960) reported on a system to force pollen indoors, which is currently used in one Douglas-fir breeding program in Germany to acquire pollen for use within the same year. They place the end of each clone's freshly cut branch in a glass container with AKN-solution (Heisel 1983) and keep the room at a fairly uniform temperature not exceeding 27 °C (81 °F) during the day, and slightly cooler at night. If branches are cut early and pollen has yet to ripen, the room needs to be maintained at 80- to 100-percent relative humidity (RH); if branches are cut directly before shedding, then the room needs to be kept at 60- to 70-percent RH. Shortly before shedding, the area below the branches needs to be cleaned and lined with newspaper. Pollen sheds over a 2- to 4-day period, and can be collected each day. Pollen shed can be forced by slight tapping of the branches.

Pollen Processing

Pollen must be dried to a moisture content (MC) of 6 to 8 percent to be used efficiently and stored properly (short- or long-term). Thus, having a warm (at least 27 °C [81 °F]), dry room enhances pollen cone handling and processing results. Because it is frequently rainy during the pollen collection season, a clothesline needs to be installed on which bags of freshly collected pollen cones may be hung for initial drying. The best system for pollen processing is a forced-air apparatus that uses screened funnels with attached containers to collect shedding pollen. Examples of good pollen-processing facilities are described in Franklin (1981) and Webber and Painter (1996) references and shown in figure 6. Pollen can be processed under less optimal conditions, but will require more time and return more variable results.

Using a forced-air system, pollen cones collected at Stage 4 will shed most of their pollen in 24 to 36 hours. Tapping the funnels occasionally helps pollen fall into the collection vial; this pollen will be clean and free of debris after passing through the screen. By 48 hours, forced-air-dried pollen will reach the target MC and can either be used immediately or stored in a freezer. The MCs of a few early collections need to be tested before freezing to ensure that the target MC has been reached. Pollen dried to the proper MC will, when swirled in the vial, flow like water.

Without a forced-air system, good supplies of high quality pollen can still be collected, but the process will take longer, the MC will be more variable and therefore the pollen will not be reliable after long-term storage. Under this scenario, pollen needs to be sieved after collection to remove pieces of bud scales, insect parts, and other nonpollen debris, and then bottled for immediate use or refrigerated for short-term storage.

Best results are obtained when collected pollen cones are processed immediately. If the cones cannot be processed immediately, the paper bag containing buds can be placed in a refrigerator at about 3 °C (38 °F) for up to 10 days (Webber and Painter 1996).

Figure 6. Examples of pollen extraction systems. (Photos by Keith Jayawickrama, Oregon State University, 2012)

Pollen Storage

Unused pollen collections from the same clone can be combined at the end of the season and placed in storage. Pollen needs to be resieved as needed and checked for MC. Before long-term freezer storage, pollen lots must be dried to 6 to 8 percent MC to avoid damage. Depending on the measuring device used, varying amounts of pollen are needed to accurately determine MC. Contact the authors for examples of equipment used to measure pollen MC.

Regardless of the type of storage container (glass, plastic, or aluminum-foil pouches), it is very important to seal the container well. Sealing the lids with Parafilm™ provides an extra layer of protection against air infiltration. Label all pollen containers and lids clearly. Pollen can be stored in graduated bottles, which makes it easier to estimate the volume. Storage containers need to be filled completely to remove as much air as possible before storage.

Storage in vacuum-sealed containers or those filled with an inert gas such as nitrogen, maintains pollen viability longer than does storage in containers containing ambient air. Another approach is to use an array of container sizes so that most will be completely filled. Under such conditions and when frozen at -18 °C (0 °F) in a standard household freezer where no temperature fluctuations exists, pollen viability will usually remain high for 2 to 3 years. Storage for longer periods of time requires more careful preparation and much colder storage temperatures.

Pollen Inventories

Existing pollen lots need to be tested by February of the year of intended use. Germination testing procedures for Douglas-fir, as described in Webber and Painter (1996), are relatively straightforward and have been widely used in the PNW. Pollen lots with at least a 50-percent germination rate are suitable for use (Webber and Bonnet-Masimbert 1993). Pollen with lower viability may be used, but reduced seed set is likely.

Controlled Pollination

Female Flower Development

Webber and Painter (1996) identify five developmental stages of Douglas-fir female buds and cones:

1. Dormant.
2. Beginning to elongate.
3. Approaching bud burst.
4. Receptive flower with 30 to 40 percent of the bracts exposed.
5. Post-receptive conelet.

It is very important to follow flower development closely so that pollination bags are installed at the proper time for successful controlled crossing.

Maturation of female flowers depends on weather and crown position, with as much as 4- to 8-days difference in optimum receptivity in different parts of the tree (Webber and Painter 1996). Flower maturity varies even within the same pollination bag. Until the tree breeder becomes experienced, it is necessary to assess the receptivity of each bag on numerous occasions throughout the spring. As with pollen, flower color is not an indicator of its receptivity.

Douglas-fir cones usually have 35 scales, each with two ovules, for a maximum of 70 viable seeds per cone. Ovules

at the basal and at the distal portions of each cone usually are not fertile, so the middle two-thirds of each cone produce most of the viable seed. For predicting breeding workloads, assume a recovery of 10 filled seeds per pollinated cone. Individual flowers stay receptive for 6 to 8 days after bud burst, with the optimal time to pollinate being days 2 through 8 (Webber and Painter 1996).

Flower Inventories

In most of the PNW, male and female buds develop sufficiently by late February to be readily identifiable (figure 7). At this point, we can determine which clones will mostly be used for pollen production, for females, or for both based on the relative abundance of male and female buds. The sooner trees are identified for use as females (such as the tree in figure 8), the more efficient breeding will be for that year because of the time required to install pollination bags.

Figure 7. Well-developed male (top) and female (bottom) buds. (Photos by Larry Miller and Lisa Clemo, Oregon Department of Forestry, 2012 and 2013)

Pollination Bags

Pollination bags are used to isolate female flowers from nontarget pollen to ensure complete control of the pedigree. Ideal pollination bags:

- Exclude 100 percent of nontarget pollen.
- Are strong enough to handle wind gusts, abrasion from adjacent branches, rain, snow load, and sun.
- Reduce heat transfer.
- Permit exchange of air and water vapor.
- Include a window to allow cone development to be checked without removing the bag.

Bags with all the above characteristics are relatively expensive; however, the temptation to cut costs on bag quality needs to be resisted. It is very frustrating and costly to have most of the bags torn after a windstorm or heavy rain. Such

Figure 8. Prolific flowering on a very small ramet. A major challenge facing the breeding program is making such flowering the norm rather than the exception. (Photo by Keith Jayawickrama, Oregon State University, 2012)

a setback can delay the crossing program by 1 year or more. It is more cost-effective to buy a roll of bagging material and cut the bags to fit different-sized branches. Sources of pollination bags are somewhat limited; contact the authors for known suppliers.

Timing and Placement of Pollination Bags

The best time to begin installing pollination bags depends on tree phenology and the size and scope of the breeding program for a given year. In most years, bagging is typically done in late March, before bud burst and pollen flight. When a large breeding effort is planned, early bagging may be warranted, although this approach can subject the bags to longer periods of potential damage from wet, windy weather. Waiting too long to install bags increases the risk that female flowers will be contaminated by unknown pollen (by the time the female flowers are receptive as in figure 9, it is too late).

To account for variations in fertility and losses from frost, we often use a factor of 10 for Douglas-fir. For example, to achieve a target of 200 seeds, 20 female flowers need to be bagged. Barring a catastrophe, applying this factor is usually sufficient. Crossing results are notoriously variable among clones, however. After we know how specific clones produce, we can adjust the factor accordingly in subsequent years.

Third-cycle crossing will involve multiple bags on small ramets (figure 10). From the standpoint of risk management, hanging all bags on one tree is not a good practice—a total loss could be incurred because of localized frost, a ramet could snap off in a windstorm or when loaded by snow, as examples. Where practical, it is best to distribute pollination bags across at least two ramets of a clone. Maintaining correct identity of crosses is the top priority, and any strategy for using the same tree for multiple crosses must be balanced against the risk of confusing cross identity.

Figure 9. Female flower of coastal Douglas-fir at time of peak receptivity. (Photo by Larry Miller, Oregon Department of Forestry, 2012)

Bags need to be placed in the easiest portion of the crown in which to work. An optimal area will be easily accessible and will have many females and few males. The target number of flowers to be bagged on a given tree can be spread across several bags, each enclosing 6 to 8 flowers. This allows for some flower abortion due to injury from cold and from rubbing against the bag. Experience shows that fewer than 4 flowers per bag significantly increases labor costs and more than 10 flowers per bag causes crowding, which often leads to cone abortion unless large bags are used. Bags need to be placed in the same general area, yet not so close together that they may rub against each other in the wind. Placing the bags in a similar orientation promotes even flower development, thereby reducing the number of pollination visits.

Installation of Pollination Bags

A sturdy wire hook attached to a pole is helpful for pulling branches to within working distance. Lifts and ladders may be needed when crossing tall trees. Ladder use has a surprisingly high accident rate, so safety training is strongly advised, with careful attention to applicable regulations.

Pollen buds inside the bag or directly outside it's opening must be removed before female flowers are bagged. Watch for

Figure 10. Multiple pollination bags on a small ramet. Ramets of this size will be the norm as cooperative third-cycle crossing gets under way. (Photo by Keith Jayawickrama, Oregon State University, 2012)

small hidden pollen buds, especially between and underneath branches. Vegetative buds need to be removed from branch tips, otherwise the expanding shoots will elongate inside the bag, causing overcrowding. Do not leave sharp twigs in or near the bag because they can tear holes in the bag. Trim off excessive foliage inside the bag to reduce crowding. Because pollen is often applied by opening the top of the bag, place bags so the enclosed flowers are easily visible and reachable from the top of the bag.

Pollination bags act like parachutes in the wind, so small branches may break if not adequately reinforced; splints made from twigs or bamboo work well for this purpose. Internal supports made from aluminum wire can be formed into "halo" or spiral shapes and then tied to the flowering branch to help keep the bag "inflated." Do not let flowers touch the inside of the bags—this can cause a high rate of flower loss. Bags placed on lateral branches can be supported by tying the branches to the main stem. Additional support is generally required on smaller ramets or on those with small diameter branches.

Dacron batting works well as a pollen gasket applied around the branch where the pollination bag will be attached. This gasket needs to be located close enough to the branch ends to allow about 5.0 cm (2.0 in) of bag extending below the Dacron for an adequate seal and about 5.0 to 7.5 cm (2.0 to 3.0 in) of extra room between the branch tips and the top of the bag so the bag can be opened and closed several times.

Write the number of flowers contained in the pollination bag on its outside with a permanent marker. Next, open the bag, fully extending all the gussets, and slip it over the branch. To hold the bag in place, apply a zip-tie, over the area padded with Dacron batting. Turn the bag's window away from the sun, and tighten the zip-tie. Finally, trim away external foliage that might damage the bag.

Pollination bags cause a greenhouse effect, thereby accelerating maturation of the enclosed flowers. If labor is short and work must begin relatively early in the season, flag and prepare the intended branches as described above, but leave the bags off. Prepared branches can then be quickly bagged directly before the breeding season.

Check each bag periodically, especially after a heavy windstorm. If a bag was intact before the storm and is assessed for damage immediately afterward, it is reasonable to assume minimal pollen contamination under wet/windy conditions. Minor pin-holes can be repaired by stapling a crease over the damaged portion of the bag or sealing with duct tape. If bags must be replaced, transfer all labeling and notes to the replacement bag. If the exclusion of foreign pollen cannot be assured, remove the bag and delete the cross, updating the record accordingly. If rainwater accumulates in a bag, open it and let the water out to prevent molding. To minimize this problem, avoid bagging limbs that are oriented horizontally or downward.

Pollen Application

Perfect control pollination conditions are dry foliage, calm winds, and minimum condensation inside the pollination bag. Phenology assessments, pollen preparation, and other tasks are therefore best done in the morning, with pollination occurring from mid-day into the evening. If female flowers and pollen are scarce, complete as many crosses as possible in a given year, and finish the rest in a later year. If pollen is insufficient to complete even one cross, it is better to wait until a later year when more pollen is available.

Each day, pollen lots intended for use need to be transferred into syringes or bottles in the lab using funnels. Pollen needs to be taken to the field in coolers. When tree breeders are in the field, any pollen that is not in use needs to be stored in a cooler, keeping the cooler in the shade as much as possible. Many methods are used to apply pollen to female flowers, including using spray bottles, syringes, and brushes, and those tools are described in the following section.

A highlighted orchard map is useful for planning and tracking each day's workload. Before pollinating the intended tree, verify that it is the correct clone, and sanitize your hands and forearms with rubbing alcohol. Next, carefully inspect each bag. If any pin-holes or abrasions exist, repair, replace, or remove as appropriate. If any unshed pollen flowers exist, remove them. If missed pollen cones are at or near pollen shed, remove the bag and delete the cross.

For all completed crosses, use a ball-point pen to label a write-on aluminum tag for each bag. Such tags need to be marked with a sequential bag number (circled), and the pedigree (Female # x Male #) of the cross. Securely fasten each tag to the appropriate branch directly below the base of the corresponding pollination bag. Before leaving for the next tree, update the field notes and double check to ensure that all bags have been checked or pollinated, all bags have been resealed and are free of damage, all records are complete and legible, and all pollens have been returned to the cooler.

Spray Bottle and Syringe Pollination

If pollen is abundant, it can be poured from a test tube or squeezed from a plastic bottle onto bagged flowers. Plastic nose-spray bottles can be used as a low-tech, inexpensive option for blowing the pollen onto bagged flowers.

When pollen supply is short, syringe pollination is recommended. Adding a little talc (less than 10 percent) to each pollen lot helps prevent needle clogging. Using large-diameter needles, called "blunts," and by avoiding pollinating when bags are wet can also minimize clogging. Take care to apply pollen directly onto all bracts of each flower. Syringe pollination requires only small needle-holes in the pollination bag, taking care to seal with duct tape when finished.

When using either a syringe or spray bottle, poke a hole in the bag, and blow the pollen onto the flowers (figure 11). Lightly tap the bag to keep the pollen suspended for a few extra seconds. This step will improve the chances of the pollen grains falling onto a receptive flower, thereby improving seed set. A nail tapped into a block of wood may be used to poke a hole in the bag. Seal the hole using duct tape before moving on to the next bag.

Brush Pollination

Brush pollination is another good method (figure 12). Camel or horse-hair brushes work well, as do those made from sable (expensive) or squirrel (preferred). Some breeders opt to not use brushes made of synthetic materials, because the pollen tends to cling to them via static electricity. Others use very inexpensive brushes and discard after one use to minimize the risk of contamination.

If using brushes, have two pollen vials for each male parent clone: one partially filled vial prepared with a brush and one as a back-up vial of extra pollen. If the brush vial is dropped or the pollen becomes damp, transfer the brush to the back-up vial.

For brush pollination, take the pollen from the cooler and carefully open the end of the pollination bag. Remove the brush from the pollen vial and apply pollen to each flower enclosed in the bag. Most tree breeders prefer not to touch the flowers with the brush and try not to allow flowers to rub against the side of the bag. It is good practice for the breeder to place his or her thumb over the top of the vial whenever the brush/cap is off. This practice helps avoid inadvertent pollen spillage, keeps the wind from sucking out the pollen via a Venturi effect, and minimizes the risk of contamination by foreign pollen.

When pollen is in very short supply, the breeder should wait to pick the pollen buds until they have started to shed pollen directly into a small poly bag or vial. Apply this naturally shed pollen from the bag or vial to the female flowers with a small brush. Maturation of the pollen and cone buds must be monitored very carefully to take full advantage of such small pollen crops.

Seal each pollination bag immediately after brush pollination by double-folding the opened end and stapling it shut (three staples are usually adequate).

Figure 11. Pollination of Douglas-fir flowers with a squeeze bottle. (Photo by Keith Jayawickrama, Oregon State University, 2012)

Figure 12. Brush pollination of Douglas-fir flowers. (Photo by Dan Cress, Regenetics Forest Genetics Consulting, 2006)

Second Pollinations

If pollen is not limited, it needs to be applied generously to each flower to increase seed set. Another option is to pollinate each flower twice. The first pollination needs to occur when roughly 25 percent of the bracts have extended from the bud scales—about a week after the bract tips first emerge. The second pollination needs to occur when about 75 percent of the bracts have emerged.

Before any second pollinations, review the phenology data and breeding records to locate bags that may be ready for repollinating. Check that each bag is still free of abrasions and pin-holes. Repair or replace bags as needed, or delete crosses as appropriate. When bags are replaced, transfer all notes from the old bag to the new. Inspect the enclosed flowers and take detailed notes on phenology. If three-fourths of the bracts are exposed, the flowers can be pollinated again. This repollination often occurs about 3 days after the first pollination, sooner in warm weather, or later in cold, wet conditions.

For bags ready for a second pollination, check that the information on the aluminum tag matches all other records. Use a staple puller to reopen the bag, and carefully repollinate all parts of each flower. Seal each bag when finished.

In years with a long, cold spring, three pollinations may be needed. Careful field notes will identify any bags that still contain immature females at the second pollination.

Protection and Maintenance After Controlled Pollination

Cone and seed insects occur commonly in seed and breeding orchards. Most damage results from one or more of the following:

- Douglas-fir cone gall midge *(Contarinia oregonensis* Foote).
- Western conifer seed bug (*Leptoglossus occidentalis* Heidemann).
- Douglas-fir cone moth (*Barbara colfaxiana* Kearfott).
- Fir cone worm (*Dioryctria abietivorella* Groté).
- Douglas-fir seed chalcid (*Megastigmus spermotrophus* Wachtl).

Insecticides are available for treating these pests; however, they must be used carefully. On rare occasions, some insecticides can cause pollinated female flowers to abort. Consult the pesticide label for proper application to control cone and seed insects.

After the breeding season, three options exist regarding the pollination bags. Some choose to replace pollination bags with mesh insect bags, such as fiberglass window screening sown into a simple flat bag. Using this type of bag reduces the risk of the cones overheating in the summer sun (compared with the pollination bags), increases the chance of reusing the pollination bags, and keeps insects out. Some breeders choose to leave the original pollination bag, taking care to have the window facing down. Either the mesh or pollination bags will provide a buffer against late cone harvesting because any seeds that shed will remain within the bag; they also make it obvious which cones are products of controlled pollination. The third option is to remove the pollination bag after risk of common cone and seed pests is minimal. If using this option, it is helpful to paint the branch below the pollinated cones or otherwise mark it for ease of future location.

Cone Harvesting

Harvesting the control-pollinated cones is one the simpler parts of the process, yet improper timing or poor record keeping can waste an entire year's breeding effort. Optimal timing to obtain fully ripened seed varies by orchard and year, but typically occurs in late August to early September. Seed wing color is a much better indicator of seed maturity than cone color. A mature cone has brown seed wings, is somewhat flexible in a lengthwise direction, and floats in water. The greenhouse effect of pollination bags left intact may accelerate cone ripening, so the condition of nonbagged cones on the same trees may not be a good indicator of bagged-cone ripeness. As cones ripen, they dry out and flare open. Flared cones begin to shed their seeds; therefore, unbagged cones need to be harvested directly before they flare since the riper the cones the better the resulting seed.

Some breeders prefer to collect cones from controlled crosses in advance of operational harvests to avoid possible damage or loss of bagged cones. Others breeders prefer to wait until after the operational harvest to assure ripeness, because bags are fairly easy to avoid. If a branch with a bag is broken off, the cones from that bag can be harvested earlier than planned.

After a tree is ready for harvesting, insect or pollination bags are removed if present, and the cones are carefully picked and placed into small sacks with a tight, breathable mesh, such as cloth rice sacks. If cone sacks are large enough and cone collection personnel are fully experienced, multiple bags of the identical cross may be combined. Capture any seeds that have shed into the bag, if bags are present. The cross identity is then transferred from the aluminum tag(s) to a paper tag,

the aluminum tag(s) are placed in the sack with the cones, and the paper tag is securely attached to the outside of the cone sack.

If the cones are inside pollination or mesh bags, a second option is to allow flaring to take place, and harvest the entire bag, limb, and cones. The seed and flared cones can then be processed indoors.

Cone sacks must be only one-half full or less; over-filling will interfere with proper after-ripening and cone opening. Cone sacks must be kept dry and off the ground, with good air circulation. For best results, spread sacks in a single layer on wooden racks erected in an open-air shed. Multiple bags of the same cross, and their reciprocals, need to be carefully combined before shipping to the seed plant. Avoid harvesting cones during rainy weather to prevent molding, reduced seed recovery, and reduced seed viability.

Germination tests have shown that seed yield from controlled cross bags can be similar to that of open-pollinated cones, but it is also recognized that germination rates of different crosses can be different. The importance of scrupulous record keeping cannot be over-emphasized.

Crossing of Western Hemlock

In general, it is easier and faster to reach a target quantity of control-pollinated seed for western hemlock than it is for Douglas-fir. Techniques and protocols for controlled crossing of western hemlock are similar to those used for Douglas-fir, but key differences exist (Webber 2000, authors' personal experience):

- Western hemlock cones are much smaller than Douglas-fir cones, so it is possible to fit dozens of female cones into a pollination bag.
- Female flowers rarely abort by coming into contact with the pollination bag.
- Hemlock cones yield about 15 filled seed per cone, so a single bag can produce a very large number of seed.
- Female flowers have a longer receptive period (up to 12 days).
- Removing pollen buds from pollination bags is time consuming because they are very small and far more numerous.
- Wire or wooden splints are often needed to strengthen the very flexible limbs, but wire internal bag supports are unnecessary.
- The optimum timing for flower induction in western hemlock in general is later than in Douglas-fir.
- Western hemlock cones mature later in the fall, from mid-September to early October.

Conclusion

The first substantial cooperative third-cycle crossing effort was in the spring of 2012, with about 100 crosses attempted. We expect 100 to 200 crosses to be made each year for the next decade. The plan is to test the resulting third-cycle seedlings and establish new third-cycle orchards starting around 2025. Those orchards are expected to affect the plantations growing through a substantial part of the 21st century: assuming that 40 million trees are planted per year over 15 years, the result could be 600 million trees or about 1.5 million ac derived from third-cycle crossing. It is our hope that these guidelines help make that crossing effort as successful as it can be.

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Increasing Seed Germination of *Bursera graveolens*, a Promising Tree for the Restoration of Tropical Dry Forests

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Abstract

This article describes a method to increase the germination rate of *Bursera graveolens*, a socially, commercially, and ecologically valuable tree species in southwest Ecuador and northwest Peru. The species suffers from (regional) unsustainable harvesting for its aromatic resin. Increased propagation success could lead to increased use of the tree in reforestation, restoration, and social forestry projects and on industrial plantations. Ecological observations of *B. graveolens* were made while the primary author managed the reforestation program and tree nursery in the Cerro Blanco Protected Forest in Guayaquil, Ecuador (2˚11' S. and 79˚53' W.), from 1995 to 2006.

Introduction: The Need for Restoration of Tropical Dry Forests

Worldwide, tropical dry forests and woodlands are characterized by annual precipitation between 10 and 40 in (250 and 2,000 mm) (Holdridge 1978), frost-free conditions year round with a mean annual temperature of 62.6 ˚F (17 °C), and an evaporation rate that exceeds precipitation (Holdridge 1978, Murphy and Lugo 1986). Holdridge (1978) defines tropical dry forests as having 40 to 80 in (1,000 to 2,000 mm) of annual precipitation, very dry tropical forests as having 20 to 40 in (500 to 1,000 mm) of annual precipitation, and tropical thorn scrubs (which is, in essence, degraded tropical dry forest woodland) as having 10 to 20 in (250 to 500 mm) of annual precipitation.

Subtropical and tropical dry forests of the world are quickly disappearing under human pressure. According to Janzen (1988), the tropical dry forest is the tropical lowland habitat most threatened with destruction—not the tropical rain forest. In Ecuador, less than 1 percent of the original dry forest currently exists (Neill and Nunez 1996, Sánchez and others 2006). Murphy and Lugo (1986) state various reasons for the destruction of tropical dry forests, one being that more dry forest just was, and is, available to destroy than was rain forest. Of the Earth's tropical landmass, 40 percent is dominated by forest, of which 42 percent is dry forest, 33 percent is moist forest, and only 25 percent is wet or rain forest.

Tropical dry forests worldwide have been severely impacted by human settlement for thousands of years. People prefer tropical dry forests because they are healthier places to reside compared with tropical humid forests, and they are easier to clear for agriculture because of shorter trees and a favorable climate for raising livestock. Dry forest soils tend to be more fertile than wet or rain forest soils due to less nutrient leaching by rain. In addition, many valuable hardwood species, such as teak (*Tectona grandis* L. F.) and caoba (*Swietenia macrophylla* King), grow in dry forests. In fact, Murphy and Lugo (1986) suggest that scientists may never know the former true extent of tropical and subtropical dry forests because many savannas, scrubs, or thorn woodlands are thought to be originally dry forests degraded beyond recognition.

Although severely reduced in extent, tropical dry forests in Ecuador and coastal Peru are biologically diverse. These forests are part of the Tumbesian ecoregion and cover approximately 70,625 mi^2 (113,000 km^2) along the Pacific coast of South America from northern coastal Ecuador to just north of Lima, Peru (Stattersfield 1998). The ecoregion is named for the Peruvian city of Tumbes, located on the Pacific coast close to the border of Ecuador and Peru. These Tumbesian tropical dry forests support many plant and animal species, including 313 woody species, 66 of which are endemic to the region. This high level of endemism is most likely due to their isolation from the tropical dry forests of the Pacific coast of Central America and the Brazilian Cerrado (Neill and Nuñéz 1996, Sánchez and others 2006).

Worldwide, there is increasing interest in restoring degraded lands back to the ecological community that existed on site before degradation. That ecological community ideally includes both plants and animals. Because reintroducing animal species to areas of their former range can be

difficult, restorationists tend to focus initial efforts on plant communities. After a suitable habitat is created, the hope is that native animals will recolonize the now-restored site (Hobbs and Harris 2001, Ruiz-Jaen and Aide 2005).

Description and Ecology of *Bursera graveolens*

Bursera graveolens (Kunth) Triana and Planch, known as palo santo, is a deciduous tree species native to the tropical dry forests of Ecuador and Peru. The wood has been traditionally burnt as incense and mosquito repellent because it possesses aromatic resins and oils (Soudkoup 1970, Valverde 1990). In recent years, these resins and oils are being extracted from the wood by the perfume industry (Yukawa and Iwabuchi 2004). *B. graveolens* is a relatively fast-growing species that colonizes rocky outcrops. Not only does the tree produce a commercially valuable product, it has potential for use in the ecological restoration of tropical dry forests and minelands.

B. graveolens is common in dry tropical forests from the Yucatan Peninsula of Mexico, south to Peru, and on the Galapagos Islands of Ecuador (Valverde 1998). *B. graveolens* grows from sea level near the equator up to elevations as high as 5,000 ft (1,500 m), particularly in the Andes of Southern Ecuador and northern Peru **(**Colter and Maas 1999, Sánchez et al. 2006). *B. graveolens* grows on rocky, arid, and nutrient poor soils (Clark and Clark 1981, Guerrero and López 1993). In the driest areas that support tropical thorn scrub or very degraded dry forest, *B. graveolens* is found on a wide variety of soils, and is generalized throughout the landscape. In somewhat moister landscapes, such as dry and very dry tropical forests, *B. graveolens* occurs on xeric sites such as rocky slopes, ridge tops, and abandoned quarries instead of growing through the landscape (figure 1).

Figure 1. Leafless trees of *Bursera graveolens* during the dry season. (Photo by Eduardo Jaime Arias)

B. graveolens grows to a mature height of 24 to 50 ft (8 to 15 m) and a diameter at breast height of 12 to 24 in (30 to 50 cm). The leaves are compound leaves and the bark is smooth and gray, streaked with white, where resin drips down from cuts or abrasions (figure 2). *B. graveolens* has tiny, white unisexual flowers (Valverde 1990) (figure 3). Many taxa of the Burseraceae family are dioecious (Daly 1993, Opler and

Figure 2. Trunk of *Bursera graveolens* with resin streaks. (Photo by Eduardo Jaime Arias)

Figure 3. Watercolor of leaves, fruit, and flowers of *Bursera graveolens* and photo inset of *B. graveolens* leaves and flowers. (Art and photo by Eduardo Jaime Arias)

Bawa 1978), though it is not clear whether *B. graveolens* is or not because there is no obvious visible difference in male or female plants other than the presence or absence of fruit. Recent communication with New York Botanical Garden taxonomists inclines toward a belief that the species is monecious (Cornejo, personal communication 2007; Daly, personal communication 2007).

The fruit of *B. graveolens* is an aril: a small black seed, covered by a red pulp, contained in a green capsule one-half in (1.2 cm) long by one-fourth in (0.6 cm) wide, attached to a stalk (figure 4). The two halves of the capsule fall off when the fruit is ripe. The aril is rich in lipids, which makes it attractive to ants, rodents, and birds (Daly 1993). Lone individuals, and particularly groups of trees, emit an odor similar to anise (Guerrero and López 1993, Valverde 1990).

Chazdon and others (1996) treat the congener *Bursera simaruba* (L) Sarg., as an early successional species**.** *B. graveolens* could also be considered an early successional species or long-lived pioneer that establishes in a forest opening and persists in the overstory for many years. In fact, growth rings of *B. graveolens* have been used for dendro-chronical studies in Peru to record changes in precipitation and occurrences of El Niño Southern Oscillation Event (ENSO) events over a 47-year period from 1954 to 2001 (Rodríguez and others 2005).

Flowering and pollination of *B. graveolens* occurs during the transitional period between the absolute drought of the dry season and the abundant downpours of the rainy season. Opler and others (1976) found that light rains in a Venezuelan tropical dry forest triggered *B. graveolens* flowering in anticipation of the heavier rains that will follow. Around the city of Guayaquil (2˚11' S. and 79˚53' W.), *B. graveolens* flowering occurs soon after the first light rains start around the middle of December (weather data from Instituto Nacional de Meteorología en Hidrología de Ecuador [INAMHI]), 1995 through 2006). Pollination is ambophilous (achieved by both insects and wind). Wind pollination is favored by the absence of rain, low relative humidity, and good air movement. These are common conditions in tropical dry forests (Bullock 1994). Flores (2002) reported that the congener *B. simaruba* is pollinated by wasps, which coincide with observations by the primary author of small wasps visiting the scentless, white flowers of *B. graveolens.*

Ripe fruit begin to appear in the last week of April and continue to ripen until the first week of June. The fruits do not all ripen at once but rather in ones and twos (Guerrero and López 1993, Valverde 1990). The seed capsules dehisce leaving the fruit attached to a stalk and hanging from the branch. The fruit either fall to the ground or are eaten by birds. These birds either consume the seeds or disperse them by defecation or regurgitation after digesting the red pulp that surrounds the seed.

In the Cerro Blanco protected forest and the Guayaquil area, there are 220 species of birds (Berg 1994, Pople and others 1997, Sheets 2004). Some are exclusively frugivorous; many more are partially frugivorous (or omnivorous) like the tyrant-flycatchers (of which 29 species are in Cerro Blanco) or the yellow-rumped Cacique (*Cacicus cela* L.) (figure 5). Some are granivores, totally or partially, like the finches, grosbeaks, and the aptly named seedeaters of the genus *Sporophila*. All these species could be seed dispersers, predators, or both. The primary author was unable to determine which bird species are consumers and which are dispersers of *B. graveolens* seed.

Figure 4. Closed and opened seed capsules of *Bursera graveolens.* Note the black seed surrounded by a red pulp. (Photo by Eduardo Jaime Arias)

Figure 5. Yellow-rumped Cacique (*Cacicus cela* L.), one of many bird species that disperse seeds of *Bursera graveolens.* (Photo by Eduardo Jaime Arias)

Research in the Galapagos Islands found dispersal and predation of *B. graveolens* seeds by the Galapagos dove (*Zenaida galapagoensis* Gould), the Galapagos mockingbird (*Nesomimus parvulus* Gould) (Clark and Clark 1981), and four species of Darwin's finches (*Geospizia* spp Gould) (Grant and Grant 1980). In Mexico, the white-eyed vireo (*Vireo griseus* Boddaert) and the grey catbird (*Dumetella carolinensis* C.T. Wood) ate the fruits and dispersed seeds of *Bursera fagaroides* (Kunth) Engl., while the white-tipped dove (*Leptotila verreauxii* Bonaparte) consumed the seeds (Ortiz-Pulido and Rico-Gray 2006). It is obvious that the Galapagos bird species are not present on the mainland of South America or the Cerro Blanco Protected Forest where the seeds were collected for the experiment described in this article, but their congeners are. Both the Pacific or West Peruvian dove (*Zenida meloda* Tschudi) and the long-tailed mockingbird (*Mimus longicaudatus* Tschudi) occur along the landward edge of mangroves, in areas of tropical thorn scrub that contain trees of *B. graveolens*, and in areas of very dry tropical forest that grade into tropical thorn scrub. Interestingly enough, these two species do not occur in the high hills or low mountains of the Cerro Blanco Protected Forest but rather in the nearby plains that extend to the Pacific Ocean. The hills are somewhat moister than the plain. Within the Cerro Blanco Protected Forest, the white-tipped dove (*Leptotila verreauxii*), which eats seeds of *B. fagara* in Mexico, is common. Also, another 11 species of dove or pigeon are present. Instead of the white-eyed vireo (*Vireo griseus*) that consumes *B. fagara* fruit in Mexico, there is the red-eyed vireo (*Vireo olivaceus* Linnaeus).

After the bird-dispersed or fallen seed is on the ground, it stays in the leaf litter for approximately 6 months until the rainy season begins again before germinating. During that period, some seed is subject to predation by ants and rodents as observed by the primary author and described by Daly (1993).

In addition to providing food and habitat for birds, *B. graveolens* also provides overstory conditions favorable for other forest tree species to colonize a site. Observations of forest changes over 18 years in the Cerro Blanco Protected Forest showed that plots along three ascending gradients of moisture, elevation, and successional status with an overstory of *B. graveolens* had saplings of *Simira ecuadorensis* (Standl.) Steyerm. and *Capparidastrum petiolare* (Kunth) Hutch. developing underneath the canopy, two species representative of the next phase of forest succession (Morgan, unpublished data 1995–2006).

Study Objectives

One obstacle to the wider adoption of *B. graveolens* for ecological restoration projects is its low germination rate and, by extension, availability as planting stock. For example, informal germination tests performed in the tree nursery of the Cerro Blanco Protected Forest found, at best, germination rates of 8 percent. The objectives of this study were to (1) determine the most effective treatment to increase seed germination and (2) determine if there is a required period of seed dormancy that can be met through seed storage. We hypothesized that a pretreatment that mimics the passage of a seed through the digestive tract of a bird will increase seed germination, and that a time period equal to the length of the coastal Ecuadorian dry season must pass before the seeds germinate.

Materials and Methods

This study has an interesting aspect to it because research and observations were performed over various years and in two locations. Field observations were made over the course of 11 years in Ecuador, as were some informal germination trials. However, the formal experiments were performed at the University of Florida.

Seed Collection

Seeds for this experiment were obtained from the Cerro Blanco Protected Forest, more commonly known by its Spanish name, Bosque Protector Cerro Blanco. It is located outside Guayaquil, Ecuador's largest city and port with close to 2.5 million inhabitants (Instituto Nacional de Estadística y Censo del Ecuador, 2010). Cerro Blanco, which means *white mountain* or *white hill*, is a private forest reserve of approximately 15,000 ac (6,000 ha) of very dry tropical forest. Elevations range from nearly sea level to 1,696 ft (514 m). It is administered by the Ecuadoran nongovernmental organization Fundación ProBosque, which employed the primary author for 11 years managing the tree nursery and reforestation program.

The climate is tropical with an average annual temperature of 77 °F (25 °C), ranging from minimums of 57 °F (14 °C) and maximums of 99 °F (37 °C). Average annual precipitation is 39.52 in (988 mm) and is concentrated in the wet season months of December through May. It is supplemented by fogs or "garuas" in the summer months of the dry season. Fogs are not an insignificant source of precipitation, although difficult to quantify (Bonifaz and Cornejo 2004). Ecuador's Pacific coast is periodically subject to the ENSO where the amount

of rainfall can be double, triple, or quadruple that of a normal year (weather data from INAMHI 1995 through 2006, BBC 2008).

Seed for this experiment was collected from a small stand of 30 *B. graveolens* trees on a rocky slope that was quarried approximately 40 years ago for limestone before the establishment by government decree of the Cerro Blanco Protected Forest in 1989. The stand is at an elevation of 240 to 330 ft (80 to 100 m) above sea level. The nursery staff of Fundación Pro-Bosque collected fruits every day or two from April 2006, when the fruits started to ripen, until June. The staff collected fruits on the lower branches by hand and those on higher branches with a pole-mounted pruning shear. Because fruits do not ripen all at once, but rather in ones and twos, it was necessary to return every day or two to collect seeds. Collected fruits were put on a table in a shed to dry for 1 week and the seeds were removed from the fruit capsules as they opened. In 2007, a second batch of seeds was obtained fortuitously from former co-workers of the primary author and was used for an additional trial, the fourth and final one.

Seeds were washed in a 10-percent household bleach solution (3 to 6 percent sodium hypochlorite, NaOCl) to disinfect pathogens and remove the pulp that surrounds the seeds. Seeds were then air-dried and stored in sealed jars in a dry, dark place for approximately 2 months. Seeds were transported to the University of Florida in Gainesville where germination trials were conducted.

Germination Trials

In the first trial (March 16 through May 7, 2007), 6-month-old seeds were subjected to four treatments: untreated control; physical scarification with sandpaper; 4-minute soak in 95 percent pure sulfuric acid (H_2SO_4) (figure 6); and a hot water treatment. The hot water treatment consisted of placing seeds in water heated to 122 °F (50 °C) and allowing them to soak for 24 hr as the water cooled. Seeds were sown into commercially available trays of pressed peat pellets (36 pellets per tray, 1.44 in [36 mm] diameter, Jiffy brand). One seed was sown per pellet and one tray comprised a single treatment replication; there were three treatment replications total. The trial was performed in a growth chamber illuminated for 12 hr daily and maintained at 77 °F (25 °C). Pellets were kept moist at all times.

Three subsequent germination trials were conducted in a greenhouse. The substrate used for those trials was a 1:1 mixture of sand and vermiculite in 4-in (100-mm) diameter petri dishes. As in the first trial, the substrate was kept moist at all times. Temperatures in the greenhouse ranged between 77 °F (25 °C) and 104 °F (40 °C).

Treatments applied to 1-year-old seeds in the second trial (June 8 through August 11, 2007) included four sulfuric acid treatments, three hot water treatments, and an untreated control. The acid treatments consisted of immersing the seed for 1, 2, 3, or 4 min in 95-percent pure H_2SO_4 (figure 6) and thoroughly rinsing the seeds with water upon removal from the acid bath. Seeds treated with hot water were soaked for 24 hr in water heated to 122 °F (50 °C), 140 °F (60 °C), or 158 °F (70 °C); thereafter, the water was allowed to cool to room temperature. Each treatment had four replications with 15 seeds in each replication.

The third trial (August 17 through October 10, 2007) consisted of immersing relatively fresh seeds into six hot water treatments: 122 °F (50 °C), 140 °F (60 °C), 158 °F (70 °C), 176°F (80 °C), 194 °F (90 °C), and 212 °F (100 °C). After immersion, the water was allowed to cool down and the seeds were soaked for 24 hr. In addition to using the hot water treatments, the third trial included a control treatment. The seeds were collected in 2007 from the same stand of trees as before and were approximately 2 months old at the time of the trial. Each treatment had four replications (petri dishes) with 15 seeds in each with the exception of the 212 °F (100 °C) treatment. Because it was expected that the 212 °F (100 °C) water treatment would destroy the seed embryo, that treatment had only one replication with 11 seeds.

Because of contradictory results of the third trial, a fourth trial was conducted November 1, 2008, through February 1, 2009, using 8 replications of 15 seeds. Seeds were subjected to the same gradient of water temperature treatments as used in the third trial (122 °F [50 °C] to 212 °F [100 °C]). In addition, some seeds were soaked in an acid bath for 1, 2, 3, 4, or 5 min.

Figure 6. Seeds were subjected to acid scarification treatments by placing them in this spoon-shaped container for loose tea leaves and soaking in 95 percent pure sulfuric acid for 1, 2, 3, 4, or 5 min. (Photo by Michael Morgan)

Percent seed germination was evaluated at the end of each trial. Data were normalized by calculating the square root of the proportion of germinated seeds and then multiplying by the arcsine. Transformed data were subjected to analysis of variance. Treatments were compared with Tukey Post-Hoc tests (Chen and Maun 1998, Longnecker and Ott 2004, Pereira de Souza and Válio 2001. Each trial was analyzed separately to compare differences among treatments. To see if storage time affected germination, the germination rates of the control seeds in the four trials were compared, because the seeds in each trial had been stored for different periods.

Results

Results from all four seed germination trials are shown in table 1.

In the first trial, physically scarified seeds had significantly higher germination than control seeds ($p = 0.019$) (figure 7). Physical scarification, however, was considered too labor-intensive for practical application in large nurseries.

The second trial showed no significant differences among the acid treatments and the control. Seeds treated with the 70 ºC hot water treatment had the highest average germination (53 percent, $p = 0.016$), suggesting that hot water treatments are effective at breaking the seed coat and promoting germination.

In the third trial, germination tended to increase as water temperature increased, then declined and ceased ($p = 0.00018$) as follows: started at 122 °F (50 °C), increased at 140 °F (60 °C), peaked at 158 °F (70 °C), dropped sharply at 176 °F (80 °C), and ceased at 194 °F (90 °C). It was unexpected however, to find that the control seeds had the highest germination (20 percent). If we exclude the results of seeds subjected to the 90 ºC treatment, in which no seeds germinated, the p value is 0.22, indicating no statistical difference among treatments. Because these results were unexpected, the fourth trial was conducted with identical hot-water treatments. It was surprising to find that the control seeds again had the highest mean germination (18 percent). As with the second trial, acid treatments did not increase average germination relative to the controls.

No significant differences existed in germination among the control seeds of the four trials, indicating that neither seed age nor storage duration was a factor in the germination of these seeds (figure 8). These results also indicate that no physiological seed dormancy exists for this species; after the seed coat is broken and the seed embryos absorb water, the seeds can germinate. Tropical seeds tend to have a short

Table 1. Percent germination of seed for each trial and treatment. Within each trial, means followed by the same letter do not differ significantly at $p \leq 0.05$.

TRIAL	TREATMENTS	MEAN	MIN	MAX	SE
1	Control	24.07 b	19.44	30.56	2.72
1	Acid 4 min	23.15 b	33.33	25.00	0.76
1	Sandpaper	34.26 a	33.33	36.11	0.76
1	Hot water 50 °C	28.70 ab	25.00	30.56	1.51
2	Control	23.25 b	13.00	33.00	4.33
2	Acid 1 min	23.33 b	12.50	33.33	4.45
2	Acid 2 min	20.00 b	6.25	31.25	5.63
2	Acid 3 min	11.67 b	6.67	18.75	2.94
2	Acid 4 min	21.67 ab	12.50	33.33	4.42
2	Hot water 50 °C	21.67 b	0.00	37.50	7.81
2	Hot water 60 °C	36.67 ab	20.00	56.25	8.07
2	Hot water 70 °C	53.33 a	33.33	75.00	8.85
3	Control	20.00 a	12.50	31.25	4.43
3	Hot water 50 °C	11.75 a	7.00	20.00	3.09
3	Hot water 60 °C	13.25 a	7.00	20.00	2.66
3	Hot water 70 °C	19.75 a	13.00	33.00	4.71
3	Hot water 80 °C	11.50 a	0.00	20.00	4.17
3	Hot water 90 °C	0.00 b	0.00	0.00	0.00
3	Hot water 100 °C	0.00 b	0.00	0.00	0.00
4	Control	18.00 a	6.67	20.00	3.5
4	Acid 1 min	5.00 b	0.00	13.33	2.7
4	Acid 2 min	14.00 a	0.00	26.67	3.4
4	Acid 3 min	6.6 a	0.00	20.00	2.1
4	Acid 4 min	10.00 a	6.67	20.00	1.7
4	Acid 5 min	10.00 a	6.67	13.33	3.3
4	Hot water 50 °C	17.50 a	6.67	33.33	3.7
4	Hot water 60 °C	12.50 a	0.00	33.33	4.6
4	Hot water 70 °C	16.20 a	0.00	33.33	9.4
4	Hot water 80 °C	5.00 b	0.00	13.33	1.6
4	Hot water 90 °C	0.00 c	0.00	0.00	0.00
4	Hot water 100 °C	0.00 c	0.00	0.00	0.00

Figure 7. Seeds of *Bursera graveolens* germinating. (Photo by Michael Morgan)

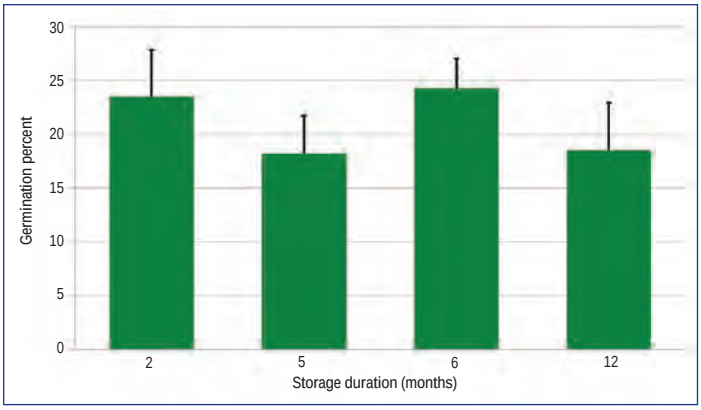

Figure 8. Effect of seed age and seed storage on germination (± SE); there was no difference in germination among untreated (control) seed in the four trials.

storage period, because the higher temperatures of the tropics result in faster chemical reactions, such as respiration and photosynthesis, than in cooler climates. Hence, refrigeration and cold rooms are essential for seed storage. These trials demonstrate that *B. graveolens* seeds can be stored for up to a year without affecting germination.

Discussion

The results of this experiment bring up some interesting points about frugivory, seed dormancy, and pregerminative treatments of seeds. In general, plants reproduce themselves by exchanging genetic material in the form of pollen with other plants of the same species. From the successful exchange of genetic material, seeds are formed. The seeds, once in the soil, start to germinate when there is sufficient light, air, and moisture.

Seeds, once fully formed, need to get from the branch to the soil. The most obvious way is to fall off the plant onto the ground below. This option, however, is not necessarily the best for the seed. Many seeds and seedlings will not germinate under the shade of its parent(s). The shady microsite underneath the parent might favor the development of pathogens that prey on seeds and seedlings. Or, a concentration of succulent seedlings will attract herbivorous predators. Therefore, many plants have evolved or devised ways for their offspring to be transported away from the parent and to a (hopefully) suitable site for germination, establishment, growth, and future reproduction. Dispersal distances can vary widely. Many trees produce light, windborne seeds that are carried away in the wind some distance. Two examples of trees with wind-dispersed seeds are the ashes (*Fraxinus* sp) in the temperate regions of the world and laurel (*Cordia alliodora* [Ruiz and Pav.] Oken), from the neotropics. On the other hand, red mangroves (*Rhizophora mangle* L.) and coconuts (*Cocos nucifera* L.) seeds float on water and use the ocean waves and currents to transport the seeds to a suitable site. Other species use animals to transport the seeds. Some seeds are sticky such as beggartick (*Bidens frondosa* L.) and attach themselves to animals' fur to carry them away. Other plants use edible fruits to attract dispersing animals. The seeds of algarrobo (*Prosopsis juliflora* [SW] DC) are contained within a sweet pod that ruminant animals, such as cows, eat. The seeds are either spit out while the animal chews its cud or defecated later. Walnuts (*Juglans* spp) produce big nuts that are collected and cached by squirrels (*Sciurus* sp) to eat later some distance away from the parent tree. Sometimes the animal does not return for its seeds and a seedling sprouts from the forgotten cache. Birds also disperse many seeds. They are attracted to the ripe fruits and either defecate or regurgitate the seeds. Some birds, such as parrots, and the appropriately named seedeaters consume or predate upon seeds if they are not poisonous. For this reason, seeds of many species are poisonous to avoid predation.

Some fresh seeds have physiological and/or physical dormancy and do not germinate when planted in conditions with appropriate light, moisture, temperature, and aeration. Physiological (or chemical) dormancy avoids having the seed germinate during a brief window of favorable conditions only to result in the tender seedling being killed when conditions revert to being too dry or too cold. The classic example of chemical dormancy is that of acorns from oak (*Quercus* sp). These seeds overwinter under the snow and/or leaf litter where it is cool and damp before they germinate in the spring. In fact, these seeds will not germinate in a nursery if planted immediately or soon after collection; they must first be stored some weeks in a refrigerator in a plastic bag full of wet leaves. In the case of *B. graveolens*, we would expect that if there is a seed dormancy period, the best seed germination would happen approximately 6 months after fruiting and seed fall, at the start of the rainy season. Results of this experiment, however, disproved the need to break chemical dormancy. Most tropical seeds can germinate readily after seed fall provided there is sufficient moisture available (Smith and others 2002).

Physical (or seed coat) dormancy requires that the seed coat be broken to allow the entrance of water, so that the seed embryo can imbibe water and start metabolizing. For those species that are animal dispersed, the seed coat protects the seed embryo, while the overlying fruit is being consumed, allowing for later dispersal. We know that *B. graveolens* produces a flesh-covered seed that is defecated by frugivorous birds. This implies that the seed coat needs to be broken before the seeds can germinate (Smith and others 2002). Not only do birds have stomach acid, they have rough gizzards,

sometimes filled with stony grit to help them grind up and digest their food (Gill 1990); the seed coat allows the plant embryo to survive passage through the gastro-intestinal tract.

Various scarification techniques have been used to break physical seed dormancy. One way is to use sandpaper on a seed until it loses its shine, because the oily lipids that seal the seed have been abraded away. Another is to crack the seed with a hammer (Smith 2002). Acid baths and hot water soaks are often used to imitate stomach acids (Smith 2002). These treatments also allow scarification of many seeds at once. Problems associated with this method are that the seed embryo can be damaged or killed by soaking too long (i.e., be cooked) and that handling hot water and acid is potentially dangerous to personnel. A safer method is to soak seeds in cool water for several hours or days (changing the water regularly to remove leachate and/or pathogens) so that chemicals that inhibit germination leach out and the seeds can then imbibe water. The drawback with this method is that seeds can rot if soaked too long. For example, Cascol (*Caesalpinia paipai* Ruiz Lopez and Pavon) is a hard-coated seed from a tropical dry forest tree. The seed is found in woody pods eaten by ruminants such as cattle or deer. One would think that soaking overnight would be an appropriate pretreatment. Soaking for more than 4 hr, however, leads to rotting seeds (Morgan, unpublished data 1995–2006). Some more unconventional seed pretreatments include feeding seeds to livestock, or even birds, and collecting the defecated or regurgitated seeds; setting fire to the seeds to burn off a thick pericarp; allowing ants to eat the pericarp; and treating seeds with fungal spores (Centro Agrícola Tropical de Investigación y Ensenañza, 2000).

In this study, *B. graveolens* responded to seed scarification (albeit inconsistently). In other seed germination research, *Bursera simaruba*, a congener of *B. graveolens*, is dispersed by both birds and monkeys and had germination between 80 and 100 percent without scarification (Navarette-Tindall 1990). Murray and others (1994) experimented with the bird dispersed tree species *Witheringia* spp.) and the black-faced solitaire (*Myadastestes melanops* Salvin) to determine if the fruit of *Witheringia* had a laxative effect, while increasing seed germination. They found that the longer the seed was in a bird's digestive tract, the less likely it was to germinate; however, 62 percent of the seeds passed through a bird's stomach germinated, as opposed to 51 percent of mature seeds just picked off the tree.

Perhaps in some cases, frugivory is more important as a means of seed dispersal than as a pregerminative treatment. Ortiz-Pulido and Rico-Gray (2006) found that 17 percent of *B. fagaroides* seeds germinated if eaten and defecated by *Dumetella carolinensis* and 0 percent germinated when eaten and defecated by *Vireo griseus*. These rates were actually lower than the germination rate observed for seeds without any treatment (20 percent).

Conclusions

Results suggest that mechanical scarification with sandpaper improved germination of *B. graveolens seeds*. It is unfortunate that this method is too laborious for the production of large quantities of seedlings. Further results suggest that immersing *B. graveolens* seeds in 158 ˚F (70 ˚C) hot water and allowing them to soak for 24 hr increase germination. The average ger-mination of three trials with this treatment was 30 percent. Exposure to hot water temperatures greater than 158 ˚F (70 ˚C) resulted in reduced, or no, germination. Although birds consume fruit from this species, it appears that this action serves primarily as a means of dispersal, because germination of control seeds averaged 21 percent across four trials and did not differ greatly from several of the scarification treatments.

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