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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Web site: http://www.rngr.net/publications/tpn

Email: diane.haase@usda.gov

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Spring/Fall 2019

Dear TPN Reader

This 2019 issue of TPN is a combined Spring/Fall issue. Because of the prolonged government shutdown early this year, I decided to postpone the Spring issue and include those papers with the Fall issue. With a whopping 18 articles packed with useful technical information, I'm sure you will find it worth the wait.

In addition to 5 technical articles and the annual report on seedling production in the United States, this issue includes 12 proceedings papers from the 2018 annual nursery meetings:

- Joint Annual Meeting of the Southern Forest Nursery Association and the Northeast Forest and Conservation Nursery Association (Pensacola, FL, July 17-19, 2018)
- Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25-26, 2018)

Note: proceedings papers from the annual nursery meetings have been published in TPN since 2014. All proceedings papers from the annual nursery meetings (1949 to now) are available online at: https://www.rngr.net/publications/proceedings/.

Keep up the good work! Our world is rife with human-caused environmental issues. It is my hope that *Tree Planters' Notes* contributes in some small way to counteract these issues by providing technical information used to support and improve reforestation, restoration, and conservation practices.

Best Regards ~

Diane L. Haase

Within your lifetime, the nation's need of trees will become serious. We of an older generation can get along with what we have, though with growing hardship; but in your full manhood and womanhood you will want what nature once so bountifully supplied and man so thoughtlessly destroyed; and because of that want you will reproach us, not for what we have used, but for what we have wasted.

~ **Theodore Roosevelt**, 1907 Arbor Day Message

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A Range-Wide Seed Collection to Support the Genetic Resource Conservation of Atlantic White-Cedar

Robert M. Jetton, W. Andrew Whittier, Barbara S. Crane, and Gary R. Hodge

Research Associate Professor, Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; Research Forester, Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Asheville, NC; Regional Geneticist, U.S. Department of Agriculture, Forest Service, Southern Region National Forest System, Atlanta, GA; Professor and Director, Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC

Abstract

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.) is a wetland tree species native to Atlantic and Gulf coastal regions of the United States and has undergone an 80-percent reduction in its natural distribution during the past 200 years. Reasons for this decline include harvesting, habitat conversion, and stress related to catastrophic wildfires and major hurricanes. Over the past 20 years, growing interest in the preservation and restoration of Atlantic white-cedar ecosystems and the need for genetically diverse planting stock led to the development of a cooperative genetic resource conservation effort for the species between Camcore (an international tree breeding and conservation program in the Department of Forestry and Environmental Resources at North Carolina State University) and the U.S. Department of Agriculture (USDA), Forest Service, Southern Region National Forest System and Forest Health Protection. The objective of this project was to target seed collections across the entire geographic range of the species from Maine south to Florida and west to Mississippi that incorporate genetic material representative of four seed zones defined for the species. Between 2012 and 2016, collections were made from 255 mother trees in 33 populations with a total yield of 1,049,648 seeds. Seeds were distributed to the USDA Agricultural Research Service–National Center for Genetic Resources Preservation for long-term storage, the USDA Forest Service Ashe Nursery Facility for seed orchard and restoration activities, and the Camcore Seed Bank for research and field plantings. Collectively, the seed stored at these three facilities represents the largest genetic resource for Atlantic white-cedar that is known to exist outside of remnant natural stands.

Introduction

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.; hereafter referred to as AWC), a member of the cypress family (Cupressaceae), is a tidal forested wetland tree species that grows in small, dense stands along the margins of freshwater swamps and bogs. The species distribution is a narrow, 80- to 210-km (50- to 130-mile) wide coastal belt that extends from southern Maine south to northern Florida and west along the Gulf Coast into southern Mississippi (figure 1). Given its coastal distribution, the species typically occurs at elevations from 0 to 43 m (0 to 140 ft) above sea level, although it is occasionally found in upland bogs at elevations as high as 457 m (1,500 ft), most notably at High Point State Park in New Jersey (figure 2). Soils where the species occurs are mucky peats in the Spodosols and Histosols orders and can be as deep as 12 m (40 ft). AWC grows characteristically in pure stands (figure 3), but can also be found growing

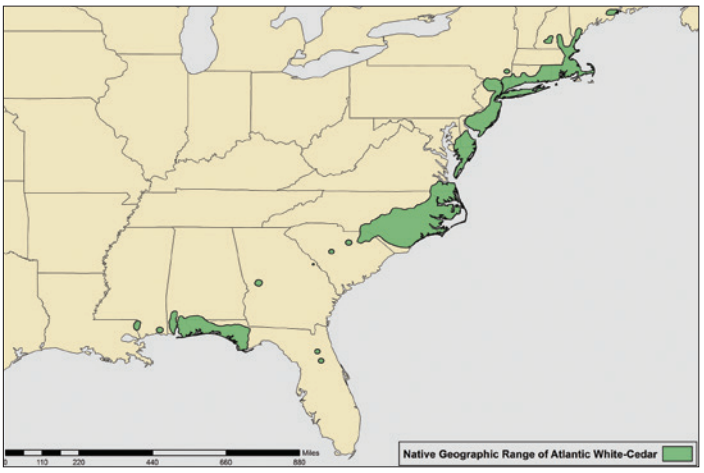

Figure 1. The geographic distribution of Atlantic white-cedar in the eastern United States.

Figure 2. Growing at an elevation above 457 m (1,500 ft), this mature Atlantic white-cedar stand in the Kuser Natural Area at High Point State Forest in New Jersey is the highest known elevation population of the species. (Photo courtesy of Camcore, North Carolina State University)

mixed with other tree species including pitch pine (*Pinus rigida* Mill.), slash pine (*P. elliottii* Engelm.), pond pine (*P. serotina* Michx.), eastern white pine (*P. strobus* L.), eastern hemlock (*Tsuga canadensis* [L.] Carr), baldcypress (*Taxodium distichum* [L.] Rich.), water tupelo (Nyssa aquatica L.), swamp tupelo/black gum (N. sylvatica Marsh.), red maple (*Acer rubrum* L.), and yellow birch (*Betula alleghaniensis* Britton) (Little and Garrett 1990).

AWC swamps are recognized as ecologically significant due to the ecosystem services they provide, particularly with respect to their role in hydrological processes (Kuser and Zimmermann 1995). AWC swamps stabilize stream flows, store flood waters, help to mitigate the effects of drought, and filter and purify water as it flows through them. They are also home to a great diversity of plant, mammal, amphibian, and bird species, many of them rare and/or threatened and some that are obligates to AWC habitats.

Prior to European settlement, there were an estimated 202,343 ha (500,000 ac) of AWC swamps and wetlands in the eastern United States, but today only about 40,469 ha (100,000 ac) remain (Kuser and Zimmermann 1995). The primary cause of this decline was over-harvesting. Across much of its distribution, AWC was historically of minor importance because the scarcity of suitable habitat made distribution of the species within its narrow range exceedingly patchy. It became an important commercial species during the 19th and early 20th centuries in areas where it was more widespread, such as eastern North Carolina, southeastern New Jersey, and the western Florida panhandle, where it was heavily harvested for its lightweight, decay-resistant wood (Laderman 1989). Annual harvests yielded up to 44,835 m^3 (19 million board feet) sold for a variety of uses including siding, fencing, decking, lawn furniture, boat planking, and small specialty products like roofing shingles and duck decoys (Ward 1989). Harvesting is not the only culprit behind the decline of AWC. Across most of its distribution, the draining of coastal wetlands for agriculture and the development of desirable coastal areas, catastrophic wildfires (figure 4), and shifting fire regimes that promote hardwood regeneration have all fragmented what remains of the species' natural habitat, contributing substantially to its continued decline (Kuser and Zimmermann 1995). Although listed as a species of least concern (LC) on the IUCN Red List of Threatened Species (Farjon 2013), AWC is considered rare in Georgia, Maryland, Mississippi, New Hampshire, and New York, of special concern in Maine, and extirpated in Pennsylvania (Nesom 2006).

With growing public awareness of the importance of these unique wetland ecosystems, efforts to protect, regenerate, or restore AWC swamps have increased during the past 20 years. Today, a relatively small number of protected and managed AWC wetlands survive within Federal, State, and private land holdings along the Atlantic and Gulf Coasts. The long-term outlook for these areas is mixed. In some areas, seed availability and moisture, light, temperature, and soil substrate conditions are favorable to natural regeneration following stand disturbance. In many instances, however, disturbance is so severe that stand conditions become suboptimal and, when combined with severe browsing from deer and other animals, natural regeneration failures are common. Over the past two decades, research and management activities have shifted to artificial regeneration of AWC ecosystems (Pickens 2009). These activities require that sufficient genetic resources be

Figure 3. This pure stand of Atlantic white-cedar at Appleton Bog Preserve in Maine is the northernmost known population of the species. (Photo courtesy of Camcore, North Carolina State University)

available in the form of seed stores or seed orchards to support nursery production of genetically diverse and broadly adaptable planting stock.

Given AWC's historic decline, its patchiness across its distribution, its exacting site requirements, and growing public awareness of its importance, the species was recognized by the U.S. Department of Agriculture (USDA), Forest Service as a good candidate for genetic resource conservation efforts to support ongoing ecosystem restoration and regeneration efforts. In 2012, a gene conservation effort was initiated as a collaborative effort between Camcore (an international tree breeding and conservation program housed in the Department of Forestry and Environmental Resources at North Carolina State University; NCSU) and the USDA Forest Service Southern Region National Forest System and Forest Health Protection Program. This article summarizes project objectives, seed collection strategy and protocols, and project results following 5 years of field work.

Figure 4. Severe wildfire damage to a population of Atlantic white-cedar at the Great Dismal Swamp National Wildlife Refuge. (Photo courtesy of Camcore, North Carolina State University)

Project Objectives

The overall goal of this project was to obtain a seed collection that is representative of the genetic and adaptive variation present across the range of AWC and to distribute that seed to facilities where it can be utilized for the conservation and restoration of the species. Specific project objectives were to: (1) collect seed from up to 40 populations and 400 mother trees (10 per population) distributed across four seed zones (10 populations and 100 mother trees per zone) that have been defined for the species; (2) place seeds into cold storage at the USDA Forest Service Ashe Nursery Facility in Brooklyn, MS, and the Camcore Seed Bank at NCSU in Raleigh, NC to support the establishment of seed orchards and subsequent restoration activities; and (3) submit 100 to 500 seeds per mother tree to the USDA National Center for Genetic Resources Preservation in Fort Collins, CO, for long-term preservation.

Seed Collection Strategy and Protocol

Locating AWC populations for seed collection was the first step in developing the seed collection strategy. This step was accomplished through survey of the available scientific and technical literature and analysis of species occurrence data available from Federal, State, local, and private land management agencies and conservancies. A total of 65 potential collection sites well distributed across the range of AWC were identified and visually assessed through field explorations. Of those assessed sites, 56 were found to contain intact AWC populations of varying size and became the focus of this gene conservation effort. Presence of trees could not be confirmed at the remaining nine locations.

The next step in the seed collection process was to determine how to distribute collections across the range of AWC to maximize the amount of genetic and adaptive variation captured in the seed collections. Mylecraine et al. (2004) assessed allozyme

variation across the AWC range. Their results showed that AWC has an overall moderate level of genetic diversity compared to other conifers, and a weak trend for increasing mean number of alleles per locus and percent polymorphic loci with decreasing latitude (r^2 0.29 and 0.23, respectively), indicating slightly higher levels of genetic diversity towards the southern portion of the species' range. All other diversity measures, however, showed no trends with latitude suggesting that overall genetic diversity in AWC is evenly distributed across the range and that seed collections should be similarly distributed to capture representative variation. Mylecraine et al. (2004) also showed significant genetic clustering within AWC with three distinct clusters (figure 5), one extending along the entire Atlantic coast from Maine to South Carolina, a second within peninsular Florida encompassing AWC occurrences on the Ocala National Forest, and a third that includes populations in Georgia and along the Gulf coasts of Florida, Alabama, and Mississippi. These genetic clusters in effect represent three distinct AWC gene pools that should also be considered when selecting seed collection locations.

Adaptive variation across the AWC geographic range was assessed by developing generalized seed zones using climate data. Seed zones were defined using the ecological niche model FloraMap™ (Jones and Gladkov 1999) and geographic coordinates and elevation for each of 34 AWC populations reported by Mylecraine et al. (2005). FloraMap™ was used to predict mean monthly minimum and maximum temperature and total precipitation for each population site by calculating average values from the five nearest meteo-

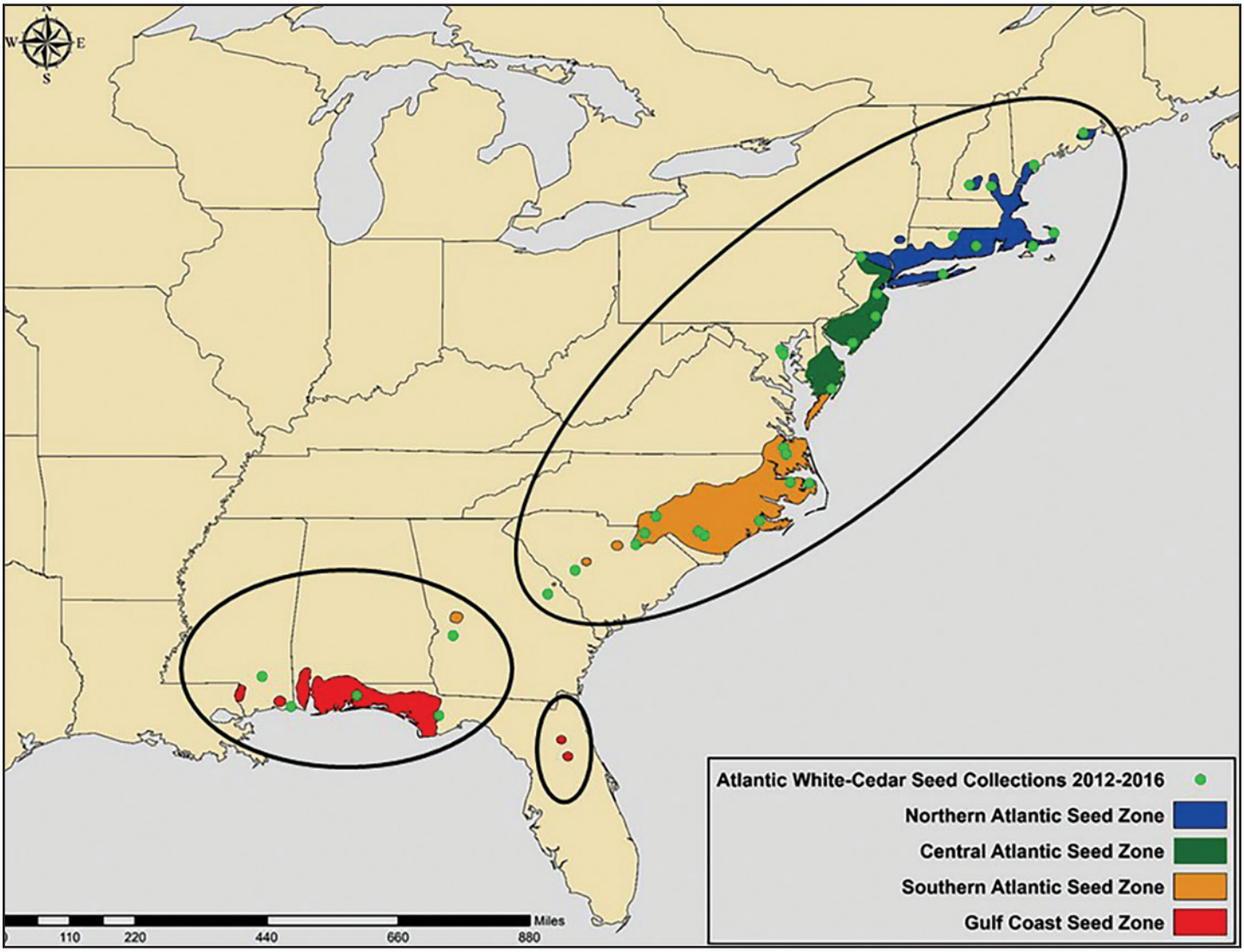

Figure 5. Atlantic white-cedar seed zones and locations of the 33 populations where seed collections were made. Black ovals represent the three genetic clusters identified by Mylecraine et al. (2004).

rological stations in its database, using a lapse rate correction to adjust temperature for elevation. In total, 36 climate variables (3 variables per month for 12 months) were derived. These variables were used to conduct a weighted paired group method (WPGMA) cluster analysis to group the 34 populations into clusters based on climate similarity. This analysis indicated that 85 percent of the variation in climate among the populations was explained by four clusters indicating four seed zones for AWC. These are: a Northern Atlantic zone extending from Maine south to New York; a Central Atlantic zone extending from New Jersey south to Maryland; a Southern Atlantic zone extending from Virginia south to Georgia; and a Gulf Coast zone extending from Florida west to Mississippi (figure 5). The seed zones are characterized by trends in increasing average annual minimum and maximum temperature moving from north to south across the geographic range of AWC (table 1).

Using these genetic parameters and seed zones, a seed collection protocol was developed. The collection target was to sample seeds from 40 populations and 400 mother trees (10 trees per population) distributed across the range of AWC. Collections were divided among the four seed zones with a target of 10 populations and 100 mother trees within each zone. These targets are actually higher than typically recommended for conifers of low to moderate levels of genetic diversity. Previous work by the Camcore program with tropical pine species demonstrated that 95 percent of genes occurring in a population at frequencies of 5 percent or higher can be captured with a seed sample from 6 to 10 populations and 10 to 20 trees per population distributed across the range of a species (Dvorak et al. 1999). Camcore has applied this approach successfully to genetic resource conservation efforts with eastern hemlock (*Tsuga canadensis* [L.] Carr.), Carolina hemlock (*T. caroliniana* Engelm.), and Table Mountain pine (*Pinus pungens* Lamb.) (Jetton et al. 2013, 2015). Details on how seed collections were carried out at a population level and the post-harvest handling of seeds are reported in Jetton et al. (2012) and summarized in figures 6 and 7.

Table 1. Climate attributes for four Atlantic white-cedar seed zones defined by FloraMap™.

Seed zone	Average annual minimum temperature (°C)	Average annual maximum temperature (°C)	Average annual total precipitation (mm)
Northern Atlantic	3.64	14.35	98.25
Central Atlantic	7.15	18.16	91.11
Southern Atlantic	10.22	22.90	102.11
Gulf Coast	13.90	25.62	126.54

$T(°F) = T(°C) \times 1.8 + 32$

Seed Collections and Distribution

The AWC gene conservation effort was conducted over five field seasons from 2012 to 2016. Collection efforts acquired seed from 255 mother trees and 33 populations well distributed across the entire geographic range of the species with multiple populations sampled within each of the four seed zones (table 2, figure 5). Best represented are the Northern and Southern Atlantic seed zones, with 78 mother trees in 9 populations and 108 mother trees in 13 populations sampled, respectively. Less represented are the Central Atlantic and Gulf Coast seed zones, where sampling was limited to 32 mother trees in 7 populations and 37 mother trees in 4 populations, respectively. Also represented in these

Figure 6. Andy Whittier of Camcore climbs an Atlantic white-cedar tree for seed collection at Jones Lake State Park in North Carolina. (Photo courtesy of Camcore, North Carolina State University)

Table 2. Population, tree, and seed attributes for 33 Atlantic white-cedar populations from which seed was collected for genetic resource conservation.

Population Name	County	State	Lat.	Long.	Elev. (m)	Trees (#)	Height (m)	DBH (cm)	Seeds (#)	Seeds (g)	Seed Year
Northern Atlantic Seed Zone											
Appleton Bog (TNC)	Knox	ME	44.33	-69.26	90	10	8.70 (± 4.42)	28.94 (± 9.38)	32,579	26.93	2016
Bolton Lakes	Tolland	CT	41.82	-72.41	215	10	14.30 (± 7.78)	25.71 (± 12.68)	133,235	110.11	2016
Cranberry Bog Preserve	Suffolk	NY	40.90	-72.67	5	8	6.81 (± 3.03)	10.36 (± 4.91)	24,163	19.97	2015
Lovernens Mill (TNC)	Hillsborough	NH	43.07	-72.02	348	10	6.11 (± 3.55)	11.18 (± 9.64)	32,740	27.06	2016
Manchester Cedar Swamp (TNC)	Hillsborough	NH	43.04	-71.49	125	6	6.60 (± 6.05)	14.36 (± 13.48)	12,112	10.1	2015
Marconi	Barnstable	MA	41.91	- 9.97	10	10	8.80 (± 5.47)	19.47 (± 8.58)	70,000	57.85	2016
Mashpee	Barnstable	MA	41.59	-70.48	12	10	7.20 (± 2.34)	15.59 (± 5.48)	30,671	25.35	2016
Pachaug State Forest	Voluntown	CT	41.59	-71.86	90	4	9.52 (± 4.55)	23.70 (± 12.31)	7,441	6.15	2015
Saco Heath Preserve (TNC)	York	ME	43.55	-70.46	51	10	7.31 (± 3.19)	21.63 (± 6.75)	41,648	34.42	2015
Central Atlantic Seed Zone											
Arlington Echo 1	Anne Arundel	MD	39.07	-76.6	0	8	3.78 (± 1.69)	6.81 (± 3.54)	24,576	20.31	2014
Arlington Echo 2	Anne Arundel	MD	38.96	- 6.54	5	10	4.75 (± 1.53)	11.52 (± 4.01)	12,922	10.68	2014
Belleplain State Forest	Cape May	NJ	39.24	-74.85	24	1	11.58	35.00	87	0.07	2014
Brendan Byrne State Forest	Burlington	NJ	39.89	-74.3	33	2	9.44 (± 2.15)	29.15 (± 14.63)	6,545	5.41	2014
Cheesequake State Park	Middlesex	NJ	40.43	-74.26	5	3	13.71 (± 4.03)	29.15 (± 9.94)	14,293	11.81	2014
High Point State Park	Sussex	NJ	41.33	-74.65	453	1	N/A	N/A	24	0.02	2014
Ponders Tract (TNC)	Sussex	DE	38.13	-75.37	13	7	8.92 (± 4.06)	29.15 (± 3.68)	1,996	1.65	2015
Southern Atlantic Seed Zone											
Alligator River NWR	Dare	NC	35.83	-75.91	1	10	8.22 (± 0.78)	15.62 (± 6.37)	18,276	13.77	2012
Catfish Lake (Croatan NF)	Craven	NC	34.94	-77.11	12	10	8.56 (± 2.82)	30.96 (± 12.37)	51,708	44.38	2012
Cheraw State Park	Chesterfield	SC	34.64	-79.89	41	10	10.72 (± 4.77)	19.51 (± 8.63)	10,920	9.21	2012
Fort Gordon	Richmond	GA	33.16	-82.24	98	13	7.69 (± 6.99)	11.10 (± 11.72)	35,903	29.67	2015
Fort Perry	Marion	GA	32.15	-84.54	150	12	7.54 (6.54)	13.13 (± 16.31)	59,786	49.41	2015
Gravatt Center	Aiken	SC	33.73	-81.58	117	10	15.30 (± 9.92)	25.76 (± 13.21)	25,393	20.59	2012
Great Dismal Swamp NWR NC	Camden	NC	36.54	-76.46	5	5	14.93 (± 7.72)	25.90 (± 14.89)	17,169	16.13	2012
Great Dismal Swamp NWR VA	Suffolk	VA	36.70	-76.52	7	6	10.56 (± 6.82)	22.41 (± 8.71)	13,668	11.48	2012
Jones Lake State Park	Bladen	NC	34.68	-78.59	25	8	12.26 (± 3.77)	28.80 (± 14.27)	9,972	6.2	2012
Kalmia Gardens	Darlington	SC	34.36	-80.11	56	1	18.00	41.10	1,272	1.05	2012
Pettigrew State Park	Tyrrell	NC	35.86	-76.37	11	5	10.51 (± 1.13)	15.95 (± 3.18)	5,286	3.5	2012
Sandhills Gameland	Richmond	NC	35.05	-79.62	109	10	10.88 (± 3.71)	23.48 (± 12.04)	6,960	7.04	2012
Singletary Lake State Park	Bladen	NC	34.58	-78.44	10	8	13.25 (± 5.40)	28.71 (± 10.77)	21,793	18.06	2012
Gulf Coast Seed Zone											
Apalachicola NF	Liberty	FL	30.21	-84.89	25	12	14.98 (± 5.31)	28.35 (± 14.32)	170,741	193.4	2013
Blackwater River State Park	Santa Rosa	FL	30.70	-86.87	3	10	11.88 (± 5.31)	30.85 (± 14.51)	74,698	77.2	2013
CaBlackwater River State Parkmp Shelby	Forest	MS	31.16	-89.17	59	5	17.67 (± 5.55)	23.06 (± 10.56)	13,821	18.2	2013
Escatawpa River NWR	Jackson	MS	30.43	-88.47	0	10	7.04 (± 2.56)	16.19 (± 7.14)	67,247	64.1	2013

DBH = diameter at breast height; 1 m = 3.28 ft; 1 cm = 0.393 in.

collections are two of the three AWC gene pools identified by Mylecraine et al. (2004). Missing are populations from the peninsular Florida genetic cluster in the Juniper Springs area of the Ocala National Forest. Two separate explorations of that area by the authors during the 2013 field season failed to locate populations for seed collection. Following those explorations, the authors inquired with local foresters about these stands and were told that they were likely extirpated by severe wildfires that heavily impacted the area in 2006 and 2009.

Seed collections were from as few as one mother tree per population (3 populations) to as many as 12 (2 populations) and 13 (1 population). The majority of populations are represented by 5 to 10 mother trees (24 populations). Average harvest per mother tree was 1,309 cones with an average of 4.47 seeds per cone. This is lower than the expected 8 seeds per cone reported for the species (Bonner 2008). Overall, an average 4,116 seeds per mother tree and 1,049,648 total seeds were collected to support the genetic resource conservation of AWC.

Germination tests have not been completed for all seedlots, but trials for the 2012 and 2013 collections resulted in 7 percent seed germination. These tests also included an evaluation of seed coat sterilization and photoperiod treatments for improving AWC seed germination (see Jetton and Whittier in this issue for details). The USDA Forest Service National Seed Laboratory (Dry Branch, GA) has completed x-ray tests on seed samples of 120 mother trees from 15 populations collected during the 2012 and 2013 field seasons. These tests indicated an average of 13.25 percent filled seeds per seedlot, ranging from a low of 1 percent to a high of 59 percent filled seed. Although this may seem low, previous reports indicate less than one-third of AWC seeds are expected to be filled (Bonner 2008) with a viability (based on actual seed germination) range of 3 to 25 percent (Little and Garrett 1990).

Of the 1,049,648 seeds collected, 278,938 representing 236 mother trees and 33 populations are stored at the USDA Forest Service Ashe Nursery Facility for future use in seed orchard and restoration activities. An additional 85,872 seeds representing the same 236 mother trees and 33 populations reside at the USDA Agricultural Research Service

Figure 7. Mature seed cones of Atlantic white-cedar were (a) collected and returned to the Camcore lab in Raleigh, NC where they were (b) dried and allowed to open. Seeds were (c) separated from foliage and other chaff prior to (d) packaging for placement in cold storage. (Photos courtesy of Camcore, North Carolina State University)

National Center for Genetic Resources Preservation for long-term preservation. Information on seed longevity specific to AWC is lacking, but seeds of Port-Orford-cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.) have survived storage at -15 °C (5 °F) for more than 11 years with no loss in germination capacity (Zobel 1990). The Camcore Seed Bank at NCSU retained 683,588 seeds representing all 255 mother trees and 33 populations as a backup collection for conservation, restoration, and associated research. An additional 1,250 seed were used for seed testing.

Summary and Conclusions

The 5-year cooperative effort between Camcore and the USDA Forest Service to conserve the genetic resources of AWC captured more than 1 million seeds for conservation. Seeds were acquired from 255 mother trees and 33 populations distributed across the full geographic extent of the species, representing the largest genetic base for the species known to exist outside of remnant natural stands. This material has been distributed to three seed repositories where it is maintained for long-term preservation and eventual use in research and restoration activities.

Although the number of populations and mother trees sampled fell short of the collection targets defined for this project, this project should be considered successful

given recent disturbances that have had significant impacts on AWC populations across much of the species' range during the past 15 years. Already mentioned are the wildfire impacts on the Ocala National Forest. Additionally, Hurricane Isabel in 2003 and subsequent wildfires in 2008 (South One Fire) and 2011 (Lateral West Fire) destroyed or severely damaged 90 percent of AWC stems in the Great Dismal Swamp (Belcher and Poovey 2009, Mitchell 2013). Populations along portions of the Gulf Coast were also damaged by Hurricane Katrina in 2005. Most notable was the region's largest population on the Grand Bay National Wildlife Refuge where winds snapped or uprooted 32 percent of trees in mature, reproductive age classes (McCoy and Keeland 2009). Similarly, the majority of AWC stands in New Jersey suffered significant mortality and stress from wind and seawater inundation associated with Hurricane Sandy in 2012 (New Jersey Department of Environmental Protection 2015). These disturbance events certainly impacted the number and quality of mother trees sampled during this project and highlight the importance of gene conservation efforts. The availability of genetically diverse and broadly adaptable seed resources for breeding and restoration is important for the resilience of tree species such as AWC whose already reduced abundance is further threatened by environmental stress (e.g., storms, drought, wildfire) and an uncertain climate future when sea level rise and saltwater inundation are expected to impact the health and sustainability of tidal forested wetlands in the eastern United States (Day et al. 2007).

Address correspondence to—

Robert M. Jetton, Research Associate Professor of Forest Health and Conservation, Camcore, Department of Forestry and Environmental Resources, Campus Box 8008, North Carolina State University, Raleigh, NC, 27695; email: rmjetton@ncsu.edu; phone: 919-515-6425..

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Effect of Seed Coat Sterilization and Photoperiod Treatments on the Germination of Atlantic White-Cedar Seeds

Robert M. Jetton and W. Andrew Whittier

Research Associate Professor, Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; Research Forester, Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Asheville, NC

Abstract

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.) (AWC) is an endangered, wetland tree species native to the coastal regions of the Eastern United States. Since 2012, AWC has been the target of *ex situ* gene conservation efforts by the Camcore Program at North Carolina State University and the U.S. Department of Agriculture, Forest Service. The gene conservation effort includes annual post-collection seed germination tests to evaluate seed quality and conservation value. Early germination trials were confounded by significant fungal growth that may have reduced overall seed germination. This study evaluated the effects of seed coat sterilization (no sterilization, bleach, hydrogen peroxide, and ethanol) and photoperiod (0:24, 8:16, 12:12, 16:8, and 24:0, light:dark) treatments on the germination of AWC seeds in the laboratory at 22 °C (71.6 °F) following cold-moist stratification at 4 °C (39.2 °F) for 30 days. Fungal growth in this study was minor and did not differ substantially between unsterilized and sterilized seeds. Treatment with hydrogen peroxide nearly doubled seed germination over the other sterilization treatments. There were no differences in seed germination among photoperiod treatments.

Introduction

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.; hereafter referred to as AWC) is a wetland tree species that occurs on the margins of freshwater swamps and bogs in the coastal regions of the eastern United States (Little and Garrett 1990). AWC occupies a narrow distribution that extends from Maine south to northern Florida and west along the Gulf Coast to southeastern Mississippi (figure 1). These wetlands have important functions for coastal hydrology, including stream flow stabilization and water filtration and purification (Kuser and Zimmermann 1995). As a timber species (figure 2), AWC has long been prized for its decay-resistant wood that is harvested and sold for a variety of purposes, including siding, roofing shingles, fencing, decking, lawn furniture, boat planking, and duck decoys (Ward 1989). There were an estimated 202,343 ha (500,000 ac) of AWC-dominated swamps and bogs prior to European settlement, but due to subsequent harvesting, draining of coastal wetlands for agriculture and development, and catastrophic hurricanes and wildfires, today only about 40,469 ha (100,000 ac) remain. Because of this decline, AWC was recognized by the U.S. Department of Agriculture (USDA) Forest Service as a good candidate for genetic resource conservation efforts to support ongoing ecosystem restoration programs. In response, Camcore (an international tree breeding and conservation program housed in the Department of Forestry and Environmental Resources at North Carolina State University) and the USDA Forest Service Southern Region National Forest System and Forest Health Protection, in 2012, initiated an *ex situ* gene conservation project for the species. Details on the objectives, protocols, and results of this project can be found in Jetton et al. 2019 (previous article in this issue).

The success of *ex situ* gene conservation depends on the collection of genetic material that is of high conservation value. This means that collections should capture not only representative genetic and adaptive variation of the species, but also sufficient amounts of viable seed (figure 3) to meet conservation objectives (Shaw and Hird 2014). The latter is challenging for tree species in the genus *Chamaecyparis*, where seed viability is variable and usually very low due to low

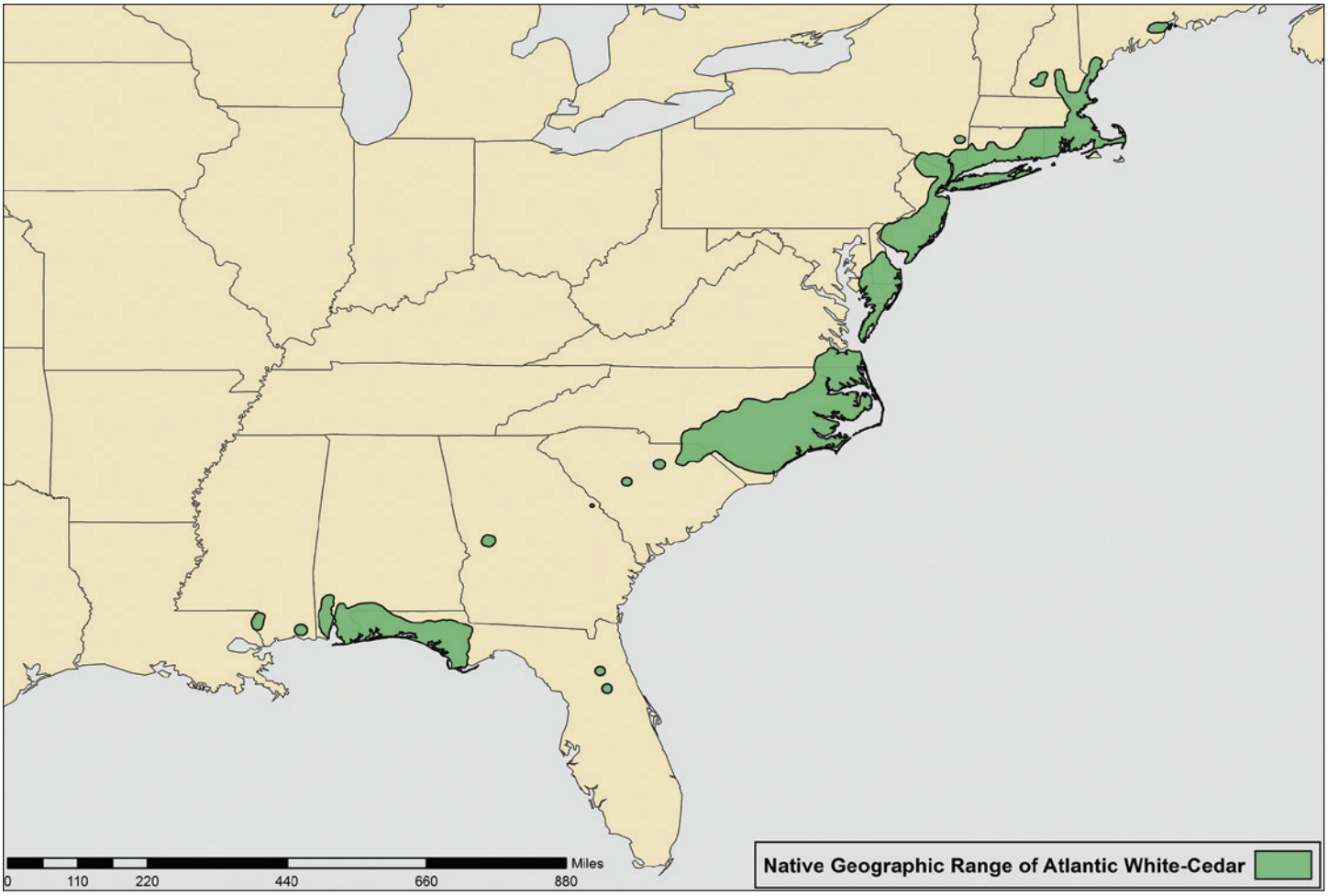

Figure 1. The geographic distribution of Atlantic white-cedar in the Eastern United States (Map courtesy of Camcore, North Carolina State University).

percentages of filled seeds (Bonner 2008a). This is particularly true for AWC, which produces on average 8 seeds per cone (figure 4) with one-third of the seeds expected to be filled, resulting in 3- to 25-percent germination under field conditions (figure 5), depending on location (Bonner 2008a, Little and Garrett 1990).

The potential for collecting large amounts of non-viable seeds requires regular post-collection germination testing of seeds to determine if conservation objectives are being met. Through the first two AWC seed-collection seasons (2012 and 2013), seed was collected from 15 populations and 120 mother trees distributed across the southern Atlantic and Gulf Coast seed zones (Jetton et al. 2019). Following the 2012 collections, Petri dish assays were conducted in Camcore's seed laboratory to test provenance-level germination (Jetton and Whittier, unpublished data). Seeds were first cold-moist stratified at 4 °C (39.2 °F) for 30 days, then germination assays were carried out in an environmental chamber at alternating temperature (30 °C:20 °C [86 °F:68 °F], day:night) and photoperiod regimes (8:16 light:dark). Although some seedlots had 20- to 28-percent germination, overall germination was low at only 7 percent. Significant fungal growth was noted in most of the Petri dishes that showed little or no germination and may have interfered with germination

Figure 2. A mature stand of Atlantic white-cedar at the Great Dismal Swamp along the North Carolina-Virginia border. The species is prized for its decay-resistant wood that is used for a number of wood products (Photo courtesy of Camcore, North Carolina State University).

Figure 3. Seeds of Atlantic white-cedar collected by Camcore for genetic resource conservation (Photo courtesy of Camcore, North Carolina State University).

of those seedlots. Seed coat sterilization with fungicides, bleach, hydrogen peroxide, and other sterilants to reduce infections of saprophytic and pathogenic fungi has been shown to improve seed germination in a number of conifer species (Barnett 1976, Barnett and Varela 2004, Wenny and Dumroese 1987). The objective of the study reported here was to test seed coat sterilization and photoperiod treatments on the germination of AWC seeds to optimize the laboratory seed germination protocol and reduce fungal growth.

Methods

In March 2014, a total of fifty, 25-seed bulks (1,250 seeds total) were prepared from the November 2012 (16 months post-collection) and November 2013 (4 months post-collection) AWC seed stocks stored at 4 °C in the Camcore seed bank. The individual seed bulks were prepared from a large, 2,000 seed bulk containing seeds from all 15 populations and 120 families collected during those years (Jetton et al. 2019). The seed bulks were cold-moist stratified on filter paper in Petri dishes at 4 °C (39.2 °F) for 30 days in a walk-in cooler following the protocol of Jetton et al. (2014). Following stratification, the 50 seed bulks were randomly assigned to one of 25 treatment combinations (5 seed-coat sterilizations by 5 photoperiods). The five sterilization treatments were: (1) unsterilized seeds on germination paper; (2) unsterilized seeds sown in growing medium (Fafard® Germination Mix, Sungrow Horticulture, Agawam, MA); (3) seeds sterilized by soaking in a 10-percent bleach solution for 10 minutes; (4) seeds sterilized by soaking in a 3-percent hydrogen peroxide solution for 1 hour; and (5) seeds sterilized by soaking in 10-percent ethanol for 10 minutes. The five photoperiods (light:dark) were: 0:24, 8:16, 12:12, 16:8, and 24:0. For those sown into growing medium, seeds were sown 1 cm deep in small garden pots after stratification. For all other seed treatments, seeds were placed into Petri dishes on moist germination paper.

Figure 4. Cones of Atlantic white-cedar at the Croatan National Forest in eastern North Carolina. Pictured in early summer, these cones will ripen by the fall and yield an average of eight seeds each (Photo courtesy of Camcore, North Carolina State University).

Two Petri dishes/garden pots (reps) per seed treatment were placed into each of the five photoperiod chambers. The germination experiment was conducted at 22 °C (71.6 °F) for 30 days. Each Petri dish or pot was checked daily for newly germinated seeds and for fungal growth on seed coats. Germination paper and garden pots were remoistened as needed with filtered, deionized water.

The probability of seed germination after 30 days was determined using a logistic regression model assuming a binomial distribution and logit link function in the GLIMMIX procedure of SAS 9.4 (SAS Institute 2013). The response variable was total percent germination, defined in the model statement by the events/trials syntax or the number of germinated seeds per Petri dish (or pot)/total seeds per Petri dish (or pot). The model tested the main effects of rep, sterilization treatment, photoperiod, and the sterilization treatment by photoperiod interaction. Where significant differences were found, means were compared using the Tukey-Kramer Multiple Comparison Test at $\alpha = 0.05$. All means reported are least square means, and all variances reported are standard errors.

Figure 5. Germinating seedling of Atlantic white-cedar at the Great Dismal Swamp along the North Carolina-Virginia border (Photo courtesy of Camcore, North Carolina State University).

Results

Overall, mean seed germination was 10 percent, or 125 of the 1,250 seeds. The hydrogen peroxide sterilization treatment, however, had about twice as much germination as all other seed treatments, regardless of photoperiod (table 1, figure 6). Total germination varied little among the photoperiod treatments and was lowest for seeds in the 24:0 photoperiod.

The overall number of seeds with fungal growth was 14, or approximately 1 percent of all seeds in the experiment. Among the sterilization treatments, the highest number of moldy seeds occurred in the control (6), followed by bleach (4), hydrogen peroxide (3), and ethanol (1). In the photoperiod treatments, the moldiest seeds occurred in 0 hours light (8), followed by 8:16 (2), 12:12 (2), 16:8 (1), and 24:0 (1).

Discussion

The bleach, ethanol, and hydrogen peroxide treatments reduced fungal growth relative to the unsterilized control, but this difference was minor and the overall occurrence of fungal growth was very low. This growth was much lower than that in the 2012 seed germination test, where more than 50 percent of seed coats had fungal growth (Jetton and Whittier, unpublished data). The 2012 result is likely related to the fact that tap water was used to maintain germination paper moisture, whereas filtered, deionized water was used in the current study.

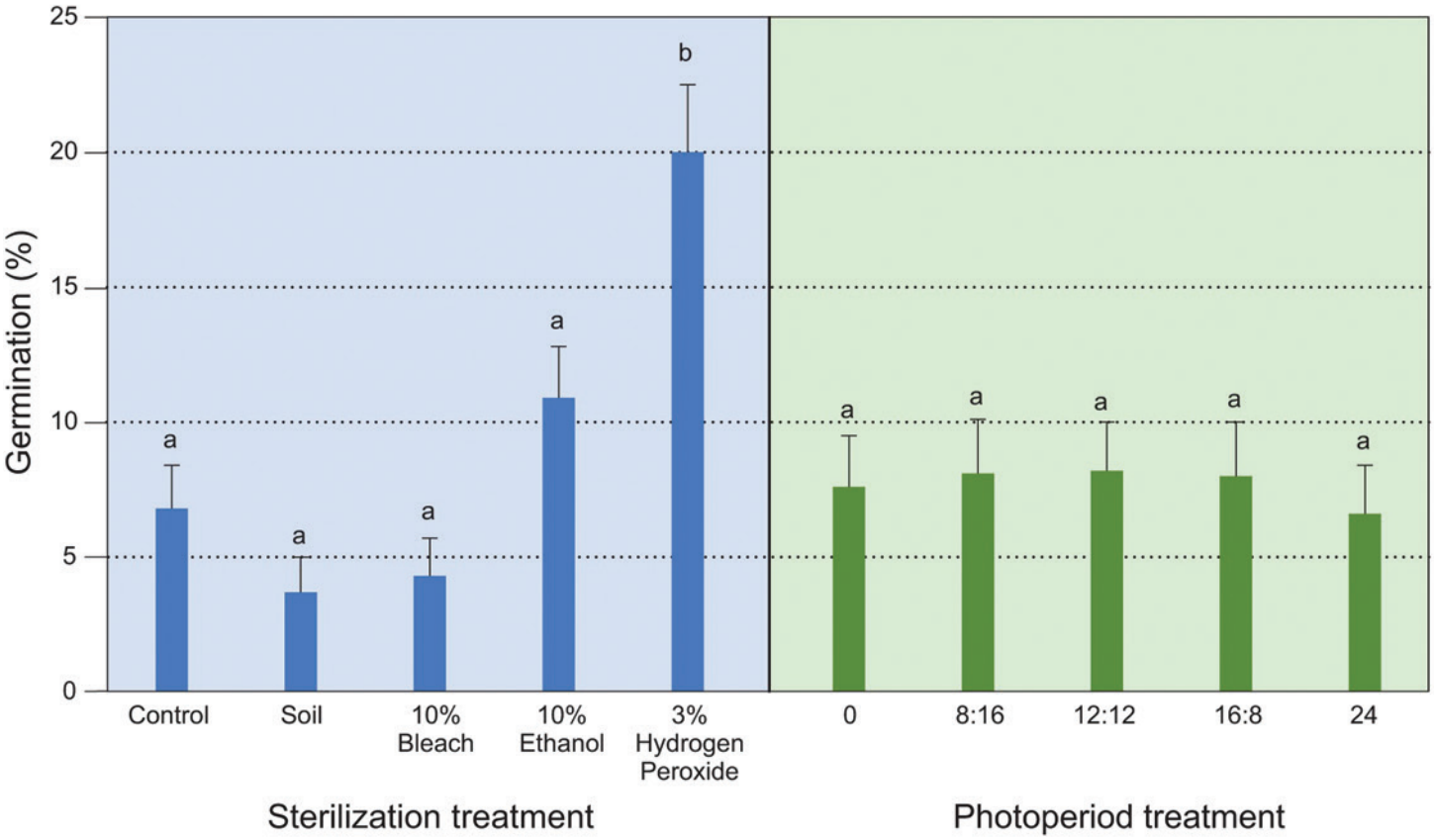

Figure 6. Least square mean (±SE) total germination of Atlantic white-cedar seed in response to sterilization treatments and photoperiod. There were no significant interactions. Within treatments, bars with the same letter did not differ signifcantly.

Table 1. Type III tests of fixed main effects in the logistic regression model for probability of seed germination after 30 days.

Effect	*DF*	*F*	*P*
Rep	1	1.16	0.2922
Sterilization treatment	4	10.58	<0.0001
Photoperiod	4	0.12	0.9748
Sterilization*Photoperiod	16	0.68	0.7893

The bleach and ethanol treatments did not significantly increase germination of AWC seeds relative to the control and soil treatments, but, not surprisingly, germination of seeds soaked in hydrogen peroxide was significantly higher than the other four sterilization treatments. Soaking seeds in hydrogen peroxide is known to be effective for sterilizing seed coats infected with saprophytic and pathogenic fungi (Barnett 1976, Barnett and Varela 2004) and can stimulate germination in seeds with scarified, nicked, cracked, or intact seed coats (Bonner 2008b). While not common in commercial nursery practice, this method is commonly used to initiate tissue culture in a number of conifer species (Amerson et al. 1985).

The lack of photoperiod effect on seed germination was surprising, given that AWC has demonstrated an obligate light requirement in previous research, although the duration of the light period that provides the best and worst germination varies among studies. Jull and Blazich (2000) reported 8-percent germination under zero light, but 48 percent and 55 percent under 1- and 24-hour photoperiods, respectively. Boyle and Kuser (1994) found that AWC seeds germinated at a higher rate under a 16-hour photoperiod (31.9 percent) compared with a 10-hour photoperiod (0.7 percent). Testing under an 8-hour photoperiod, Bianchetti et al. (1994) found that seed germination varied if temperature conditions were set to a constant or variable thermo-period.

Further research is needed to improve and optimize the laboratory seed germination protocols for AWC. Specific topics we plan to address are: (1) improving the seed cleaning process to remove more empty seeds and increase the number of filled seeds in each seedlot; (2) determine if 30 days at 4 °C (39.2 °F) or other combinations of duration and temperature are best for cold-moist stratification; and (3) further investigate the effect of alternating thermo-periods on AWC seed germination. In the meantime, based on the results of this study, the following protocol is recommended for germination testing of AWC seeds at Camcore. Following collection, extraction, cleaning, and storage, seeds should be cold-moist stratified at 4 °C (39.2 °F) for 30 days prior to testing. Following stratification, seeds should be surface sterilized by soaking in a 3-percent hydrogen peroxide solution for 1 hour, then sown on moist germination paper in Petri dishes. Germination should proceed under a 12:12 photoperiod at 22 °C (71.6 °F) for 30 days. Moistening of the germination paper should be done with filtered, deionized water to limit fungal growth on seed coats.

Address correspondence to:

Robert M. Jetton, Research Associate Professor of Forest Health and Conservation, Camcore, Department of Forestry and Environmental Resources, Campus Box 8008, North Carolina State University, Raleigh, NC 27695; email: rmjetton@ncsu.edu; phone: 919–515–6425.

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Forest Nursery Seedling Production in the United States—Fiscal Year 2018

Diane L. Haase, Carolyn Pike, Scott Enebak, Lori Mackey, Zhao Ma, and Meagan Rathjen

Western Nursery Specialist, U.S. Department of Agriculture (USDA), Forest Service, Portland, OR; Northeastern Area Regeneration Specialist, USDA Forest Service, West Lafayette, IN; Director, Southern Forest Nursery Management Cooperative, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL; Special Projects Coordinator, Center for Forest Nursery and Seedling Research, Department of Forest, Rangeland, and Fire Sciences, University of Idaho, Moscow, ID; Professor, Purdue University, Department of Forestry and Natural Resources, West Lafayette, IN; Graduate Student, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN

Abstract

Forest nursery production for the 2018 planting season was nearly 1.2 billion forest tree seedlings with more than 2.2 million ac (1 million ha) of trees planted. Similar to previous years, most production and planting occurred in the southern States, and approximately 75 percent of outplanted trees are bareroot stock.

Background

This annual report summarizes forest nursery seedling production in the United States. The number of seedlings reported is used to estimate the number of acres of forest planting per year. Prepared by the U.S. Department of Agriculture, Forest Service, Forest Inventory and Analysis (FIA), and State and Private Forestry, this report includes State-by-State breakdowns, regional totals, and an analysis of data trends. Universities in the Southern, Northeastern, and Western Regions of the United States made an effort to collect data from all the major producers of forest and conservation seedlings in the 50 States. Forest and conservation nursery managers provided the information presented in this report. As far as we know, it is the most complete compilation of such data in the country. Because all data are provided voluntarily by outside sources and some data are estimated, caution must be used in drawing inferences.

Methodology

State and Private Forestry, in collaboration with Auburn University, the University of Idaho, and Purdue University, produced the data for this report. These universities collected forest tree seedling production data directly from the forest and conservation nurseries that grow forest tree seedlings in their region of the United States (Auburn University collected from 13 States in the Southeast, the University of Idaho collected from 17 States in the West, and Purdue University collected from 21 States in the Northeast and Midwest). The approximation of planted acres for each State is derived from FIA estimates of tree planting area based on ground-plot data that States collected during 5-, 7-, or 10-year periods and compiled as an average annual estimate for the associated period. FIA estimates of acres of trees planted by State may not correlate with nursery production surveys because nurseries do not report shipments across State lines. Total acres by region, however, provide a reasonable comparison between the two methods. Data collected are reported by hardwood and conifer seedlings produced and acreage planted of each (table 1) and by bareroot and container seedlings produced (table 2). A complete list of the assumptions used in compiling this report appear in *Forest Nursery Seedling Production in the United States*—Fiscal Year 2013 (Harper et al. 2014).

Table 1. Hardwood and conifer tree seedling production and acres planted for each State and each region during the 2017-2018 planting year.

State	Hardwood seedlings produced	Hardwood acres planted[1]	Conifer seedlings produced	Canadian conifer imports	Conifer acres planted[1]	Total seedlings produced	Total acres planted[1]	FIA data acres planted[10]
Southeast								
Florida[2]	2,551,679	4,639	57,632,500	—	104,786	60,184,179	109,426	152,359
Georgia[2]	3,278,636	5,961	327,819,270	—	596,035	331,097,906	601,996	239,619
North Carolina[2]	500,000	909	67,758,200	—	123,197	68,258,200	124,106	99,215
South Carolina[2]	55,480	101	142,091,539	—	258,348	142,147,019	258,449	76,808
Virginia[2]	960,000	1,745	30,180,400	—	54,873	31,140,400	56,619	74,872
Regional Totals	**7,345,795**	**13,356**	**625,481,909**	**0**	**1,137,240**	**632,827,704**	**1,150,596**	**642,873**
South Central								
Alabama[2]	2,384,089	4,335	90,938,447	—	165,343	93,322,536	169,677	223,021
Arkansas[2]	9,092,233	16,531	95,442,900	—	173,533	104,535,133	190,064	117,744
Kentucky[3]	986,900	2,269	117,500	—	270.11	1,104,400	2,539	1,155
Lousiana[2]	—	—	46,599,000	—	84,725	46,599,000	84,725	160,801
Mississippi[2]	1,155,400	2,101	88,022,000	—	160,040	89,177,400	162,141	178,998
Oklahoma[2]	385,088	700	3,462,755	—	6,296	3,847,843	6,996	21,521
Tennessee[2]	2,309,000	4,198	3,142,000	—	5,713	5,451,000	9,911	28,005
Texas[2]	33,800	61	87,463,038	—	159,024	87,496,838	159,085	262,584
Regional Totals	**16,346,510**	**30,195**	**415,187,640**	**0**	**754,943**	**431,534,150**	**785,138**	**993,829**
Northeast								
Connecticut[3]	2,000	5	500	—	1	2,500	6	—
Delaware[2]	25,000	45	43,500	—	79	68,500	125	647
Maine[5]	3,800	6	19,000	3,180,000	5,332	3,202,800	5,338	8,168
Maryland[2]	1,845,900	3,356	1,571,075	—	2,858	3,417,770	6,214	1,445
Massachusetts[3]	7,373	17	1,853	—	4	9,226	21	—
New Hampshire[3]	23,160	53	184,795	—	425	207,955	478	—
New Jersey[3]	139,580	321	111,000	—	255	250,580	576	—
New York[5]	89,675	149	529,200	—	—	618,875	149	—
Pennsylvania[3]	1,045,812	2,404	1,928,062	—	4,432	2,973,874	6,836	2,680
Rhode Island	—	—	—	—	—	—	—	—
Vermont[3]	40,800	94	3,100	—	—	43,900	946	—
West Virginia[3]	188,647	434	117,184	—	269	305,831	703	870
Regional Totals	**3,411,747**	**6,885**	**4,510,064**	**3,180,000**	**13,656**	**11,101,811**	**20,541**	**13,810**
North Central								
Illinois[3]	610,440	1,403	104,750	—	241	715,190	1,644	2,498
Indiana[4]	1,726,813	2,657	623,000	—	958	2,349,813	3,615	1,753
Iowa[5]	568,710	948	151,075	—	252	719,785	1,200	621
Michigan[2,9]	3,328,118	6,051	14,399,441	1,665,000	26,181	19,392,559	32,232	9,467
Minnesota[2,9]	504,645	918	3,105,200	3,018,000	11,133	6,627,845	12,051	17,470
Missouri[3]	1,227,035	2,821	629,640	—	1,447	1,856,675	4,268	—
Ohio[3]	870	2	4,600	—	11	5,470	13	3,018
Wisconsin[6,9]	514,636	643	1,996,482	1,417,00	4,229	3,898,118	4,873	10,459
Regional Totals	**8,481,267**	**15,443**	**20,984,188**	**6,100,00**	**44,452**	**35,565,455**	**59,895**	**45,286**

State	Hardwood seedlings produced	Hardwood acres planted[1]	Conifer seedlings produced	Canadian conifer imports	Conifer acres planted[1]	Total seedlings produced	Total acres planted[1]	FIA data acres planted[10]
Great Plains								
Kansas[2]	8,825	16	48,400	—	88	57,225	104	—
Nebraska[2]	649,700	1,181	1,270,925	—	2,311	1,920,625	3,492	1,182
North Dakota[2]	41,236	75	836,490	—	1,521	877,726	1,596	—
South Dakota[2]	—	—	—	—	—	—	—	—
Regional Totals	**699,761**	**1,272**	**2,155,815**	**—**	**3,920**	**2,855,576**	**5,192**	**1,182**
Intermountain								
Arizona[2]	480	1	1,260	—	2	1,740	3	597
Colorado[2]	182,000	331	146,000	—	265	328,000	596	—
Idaho[2]	118,613	216	8,919,843	1,672,000	19,258	10,710,456	19,474	7,108
Montana[2]	19,325	35	807,550	—	1,468	826,875	1,503	8,082
Nevada[2]	2,451	4	527	—	1	2,978	5	—
New Mexico[2]	9,222	17	27,212	—	49	36,434	66	872
Utah[2]	135,000	245	57,000	—	104	192,000	349	—
Wyoming	—	—	—	—	—	—	—	997
Regional Totals	**467,091**	**849**	**9,959,392**	**1,672,000**	**21,148**	**12,098,483**	**21,997**	**17,656**
Alaska								
Alaska[2]	8,000	15	30,000	265,000	536	303,000	551	—
Pacific Northwest								
Oregon[7,9]	1,892,190	5,406	20,769,069	72,000	59,546	22,733,259	64,952	133,374
Washington[7,9]	420,402	1,201	23,606,224	172,000	67,938	24,198,626	69,139	97,872
Regional Totals	**2,312,592**	**6,607**	**44,375,293**	**244,000**	**127,484**	**46,931,885**	**134,091**	**231,246**
Pacific Southwest								
California[8]	105,020	233	13,917,812	—	30,928	14,022,832	31,162	33,657
Hawaii[8]	40,000	89	2,000	—	4	42,000	93	—
Regional Totals	**145,020**	**322**	**13,919,812**	**0**	**30,933**	**14,064,832**	**31,255**	**33,657**
Totals	**39,217,783**	**74,944**	**1,136,604,113**	**11,461,000**	**2,134,312**	**1,187,282,896**	**2,209,256**	**1,979,539**

[1] Acres planted were estimated assuming:

[2] 550 stems/acre

[3] 435 stems/acre

[4] 650 stems/acre

[5] 600 stems/acre

[6] 800 stems/acre

[7] 350 stems/acre

[8] 450 stems/acre

[9] Totals include an estimate of container conifers produced in Canada for distribution to neighboring States; bareroot imports for Maine and containers for other States.

[10] FIA = Forest Inventory and Analysis; average annual acreage planted estimated for all States (2017) on 5-year cycles, except for Alabama, Louisiana, Mississippi, and North Carolina, which are on 7-year cycles, and for Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, and Washington, which are on 10-year cycles. Data generated by Andy Hartsell, USDA Forest Service.

Table 2. Bareroot and container tree seedling production for each State and each region during the 2017-2018 planting year.

State	Bareroot	Container[1]	Total Seedlings Produced
Southeast			
Florida	54,800,613	5,383,566	60,184,179
Georgia	203,043,197	128,054,709	331,097,906
North Carolina	53,561,200	14,697,000	68,258,200
South Carolina	141,135,755	1,011,264	142,147,019
Virginia	31,140,400	—	31,140,400
Regional Totals	**483,681,165**	**149,146,539**	**632,827,704**
South Central			
Alabama	83,299,933	10,022,603	93,322,536
Arkansas	104,535,133	—	104,535,133
Kentucky	1,104,400	—	1,104,400
Louisiana	—	46,599,000	46,599,000
Mississippi	79,019,000	10,158,400	89,177,400
Oklahoma	3,691,621	156,222	3,847,843
Tennessee	5,451,000	—	5,451,000
Texas	87,496,838	—	87,496,838
Regional Totals	**364,597,925**	**66,936,225**	**431,534,150**
Northeast			
Connecticut	—	2,500	2,500
Delaware	9,500	59,000	68,500
Maine	—	3,202,800	3,202,800
Maryland	2,760,670	657,100	3,417,770
Massachusetts	—	9,226	9,226
New Hampshire	207,855	100	207,95
New Jersey	248,580	2,000	250,580
New York	581,325	37,550	618,875
Pennsylvania	2,972,674	1,200	2,973,874
Rhode Island	—	—	—
Vermont	42,200	1,700	43,900
West Virginia	305,831	—	305,831
Regional Totals	**7,128,635**	**3,973,176**	**11,101,811**
North Central			
Illinois	696,500	18,690	715,190
Indiana	2,341,413	8,400	2,349,813
Iowa	698,285	21,500	719,785
Michigan	17,278,239	2,114,320	19,392,559
Minnesota	3,493,945	3,133,900	6,627,845
Missouri	1,856,675	—	1,856,675
Ohio	4,500	970	5,470
Wisconsin	2,380,118	1,518,000	3,898,118
Regional Totals	**28,749,675**	**6,815,780**	**35,565,455**
Great Plains			
Kansas	—	57,225	57,225
Nebraska	1,106,625	814,000	1,920,625
North Dakota	791,000	86,726	877,726
South Dakota	—	—	—
Regional Totals	**1,897,625**	**957,951**	**2,855,576**
Intermountain			
Arizona	—	1,740	1,740
Colarado	121,000	207,000	328,000
Idaho	2,173,650	8,536,806	10,710,456
Montana	128,600	698,275	826,875
New Mexico	—	2,978	2,978
Nevada	—	36,434	36,434
Utah	—	192,000	192,000
Wyoming	—	—	—
Regional Totals	**2,423,250**	**9,675,233**	**12,098,483**
Alaska			
Alaska	0	303,000	303,000
Pacific Northwest			
Oregon	8,254,914	14,478,345	22,733,259
Washington	17,479,513	6,719,113	24,198,626
Regional Totals	**25,734,427**	**21,197,458**	**46,931,885**
Pacific Southwest			
California	—	14,022,832	14,022,832
Hawaii	—	42,000	42,000
Regional Totals	**0**	**14,064,832**	**14,064,832**
Totals	**914,212,702**	**273,070,194**	**1,187,282,896**

[1] Alaska, Idaho, Maine, Michigan, Minnesota, Oregon, Washington, and Wisconsin received container seedlings produced in Canada.

Data Trends

Nearly 1.2 billion forest tree seedlings were shipped from forest and conservation nurseries in the United States in fiscal year (FY) 2018. This production level is a decrease of 97.5 million seedlings compared with seedling production reported for FY 2017 (Hernández et al. 2018). The decrease is attributed to significantly lower reported numbers in the West and East (table 3). These lower numbers are due, in part, to a few nursery closures and production declines in some nurseries, but is likely an underestimate due to inconsistent participation from nurseries during data collection. Based on the total number of seedlings shipped and the average number of seedlings planted per acre in each State, more than 2.2 million ac (890,000 ha) of trees were planted during the fall 2017 through spring 2018 planting season.

Table 3. Annual forest nursery seedling production in each region for FY 2012 to FY 2018.

Year	Total seedling production	West (17 States)	East (20 States)	South (13 States)
FY 2018	1,187,282,896	76,253,776	46,667,266	1,064,361,854
FY 2017	1,284,824,689	151,321,764	67,595,266	1,065,907,659
FY 2016	1,260,216,076	152,785,327	72,314,630	1,035,094,369
FY 2015	1,302,237,795	175,464,446	95,417,986	1,031,355,363
FY 2014	1,217,607,888	115,620,820	85,684,417	1,015,564,370
FY 2013	1,181,554,535	96,344,063	102,066,671	983,143,801
FY 2012	1,190,552,819	170,975,830	81,672,547	936,918,542

FY = fiscal year.
Sources: This report, Harper et al. (2013, 2014), Hernández et al. (2015, 2016, 2017, 2018)

Address correspondence to—

Diane L. Haase, Western Nursery Specialist, U.S. Department of Agriculture, Forest Service, P.O. Box 3623, Portland, OR 97208; email: diane.haase@usda.gov; phone: 503–808–2349.

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Effects of Site Treatments During 26 Years for Ponderosa Pine (*Pinus ponderosa* Lawson and C. Lawson [Pinaceae]) Plantings in Colorado's Northern Front Range

Douglas J. Stevenson, Pradip Saud, Carl D. Miller, Cynthia Stevenson, Trisa J. Ward, and Bryan D. Murray

Senior Research Specialist, Oklahoma State University, Department of Natural Resources and Environmental Management, Stillwater, OK; Postdoctoral Researcher, Department of Extension Animal Sciences and Natural Resources, New Mexico State University, Las Cruces, NM; CEO, Miller Engineering, Escanaba, MI; Vice-President (retired), Stevenson Forests, Stillwater, OK; Tree Planting Specialist, Colorado State Forest Service, Boulder, CO (formerly), Cecil County, Perryville, MD (currently); Assistant Professor, Oklahoma State University, Department of Natural Resources and Environmental Management, Stillwater, OK.

Abstract

Getting tree seedlings to grow on dry, grass-covered sites in the Colorado Front Range and piedmont is a long-standing problem. We tested various planting treatments by growing ponderosa pine (*Pinus ponderosa* Lawson and C. Lawson [*Pinaceae*]) for 25 and 26 years on a mountain site and a piedmont site in Colorado's Front Range. Weed barrier, black plastic, scalping, and polyacrylamide gel applied alone or in combination proved effective at promoting seedling growth and survival compared with the untreated control treatment. Results suggest that controlling grass competition may be more important than water in regulating growth and survival of seedlings on sites where annual rainfall averages 40 cm (15 in) and summers are dry.

Introduction

Successfully planting trees in dry grassy areas has been a problem since at least 1902, when the Bureau of Forestry (later the U.S. Department of Agriculture [USDA] Forest Service) foresters established a nursery later named for Charles E. Bessey in the Nebraska Sand Hills. The Nebraska National Forest began planting in 1902 (Gardner 2009, Pool 1953). Much has been written on tree planting in the Great Plains (e.g., Baer 1989, Engle et al. 2008, Read 1964), and there are some research projects from the Colorado Rockies (Droze 1977). Less is known, however, about tree planting in the piedmont area between the mountains and the plains west of the South Platte River, particularly with scalping. This area has soils derived from mountain outwash and is often quite rocky. Down-canyon winds pile fine particles into small dunes or layers of wind-blown sand, creating extremely variable planting conditions.

Grass competition, especially from early season grasses such as smooth brome (*Bromus inermis* Leyss.) (Bond 2008, Davis et al. 1998, Goldberg and Barton 1991, Rietveld 1975), is a challenge to establishing new stands of trees in the Front Range and Great Plains. Scalping (Graham et al. 1989), plastic mulch (Green et al. 2003), wood chip mulch (Mashayekhan and Hojjati 2013), polypropylene fabric weed barrier (Geyer et al. 2006), and even carpet mulches have been tried in an effort to improve reforestation success with various species, producing variable results. Fallowing a site with plastic mulch or herbicide a year before planting tripled growth of Rocky Mountain juniper (*Juniperus scopulorum* Sarg. [*Cupressaceae*]) (Nickerson 2002). Nickerson used woven plastic weed barrier strips up to 2.3 m (7.5 ft) wide in his windbreak and living snow fence projects, covering an area of 5.57 m^2 (60 ft^2) per seedling.

The intent of mulch treatments is to form a physical barrier that prevents evaporation (thereby increasing water availability) and to starve competing plants of light (Chang-Hung 1999). Flint and Childs (1987) showed significant improvement in diameter growth of Douglas-fir (*Psuedotsuga menziesii* [Mirb.] Franco) seedlings using herbicide, scalp, and mulch treatments. Rietveld and Heidman (1974) used 45.7 cm (18-in) square plastic sheets to mulch around ponderosa pine (*Pinus ponderosa* Lawson and C. Lawson) seedlings

in Arizona and remeasured seedlings annually for 3 years, terminating their experiment in the third season when seedling survival was down to 19 percent and mulch had deteriorated significantly. In the third year, seedlings mulched with polyethylene were significantly taller than those without mulch.

Water-absorbing polymers are believed to work by increasing soil water-holding capacity, increasing the size and number of pores, and mitigating soil compaction (Orzolek 1993). Callaghan et al. (1989) found that polymer treatment resulted in 57 percent survival for eucalyptus seedlings, compared with 0 percent for controls when seedlings were irrigated at 6-week intervals. Johnson and Leah (1990) found that polyacrylamide application increased mean shoot fresh weight for three species of grains up to seven times that of controls and Pryor (1988) found a 30-percent increase in tomato fruit production when polymers were applied. Polymers lose their effectiveness with time (Al-Humaid and Moftah 2006).

The objective of our study was to compare effects of a variety of planting treatments at planting time on subsequent height, diameter, and survival of southwestern ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) in the Northern Front Range and piedmont. To achieve our objective, we planted two sites with southwestern ponderosa pine seedlings and tracked growth and survival annually for 6 years with later measurements at 20 and 26 years. We hypothesized that there were significant differences in height and diameter growth and survival as a result of applying a variety of treatments.

Materials and Methods

Sites

The Flagstaff site is located west of Boulder, CO, at 39°58'N, 105°20'W (figure 1) at an elevation of 2,350 m (7,710 ft) on Ferncliffe stony sandy loam (Moreland and Moreland 1975). The underlying material consists of landslide debris. Bedrock generally occurs at depths exceeding 150 cm (60 in). The planting site slopes from 2 to 16 percent southeastward. Average annual precipitation was 54 cm (21 in) between 1988 and 2009, and mainly occurred in April and May (Prism Climate Group 2016). Existing vegetation at the time of establishment consisted of southwest ponderosa pine

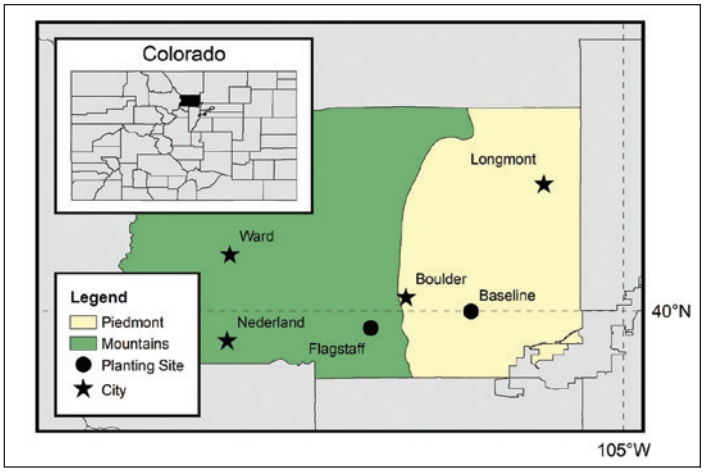


Figure 1. Experimental planting sites in Boulder County, CO. "Mountains" roughly corresponds to the "Southern Rockies Ecoregion," while "Piedmont" roughly corresponds to the "High Plains Ecoregion." Data from U.S. Environmental Protection Agency (2012).

and Douglas-fir at 4 to 8 m^2/ha (45 to 85 ft^2/acre) basal area and broadleaved plants with some short, annual, late-season (C_4) and perennial, early-season (C_3) grasses.

The Baseline site is east of Boulder, CO, at 40°00'N, 105°11'W (figure 1) at an elevation of 1,615 m (5,300 ft) on Nunn silt loam (Moreland and Moreland 1975). Bedrock is at an unknown depth greater than 150 cm (60 in). The planting site is located on a stream terrace about 4 m (15 ft) above Dry Creek in valley-fill material. Average annual precipitation is about 40 cm (16 in) (Prism Climate Group 2016). Planting site slopes about 2 percent northward. Existing vegetation at the time of planting was a heavy sod of early season (C_3) perennial grasses.

Seedlings

We used 2-year old (2+0) ponderosa pine seedlings from the Colorado State Forest Service Nursery (Fort Collins, CO). Seeds were collected west of Fort Collins, CO, and the seedlings grown in 5 by 5 by 15 cm (2 by 2 by 6 in) tarpaper pots. Seedlings were hand-planted by stripping off tarpaper and placing the seedling in a hole 17 cm (7 in) deep by 17 cm (7 in) wide. The Flagstaff site was planted in April 1990 and the Baseline site was planted in April 1991. One-month seedling viability was 97 percent or better at both sites.

Treatments and Experimental Design

A variety of treatments using polymer gel, black plastic, weed barrier, and scalping were applied with

the goal of increasing soil moisture availability and decreasing competition.

The polyacrylamide gel (polymer) (Hydrogel, Plant-Best, Inc., Markham, Ontario) was mixed at the rate of one part polymer crystals to 50 parts tap water and allowed to stand overnight. In the morning, surplus water was discarded. Seedlings treated with polymer gel received either 237 ml (8 oz) or 474 ml (16 oz) of fully hydrated polymer mixed 1:1 with back-fill soil and placed around the seedling's roots to match the original soil line.

Two mulch treatments were used. The first treatment was a black plastic mulch consisting of 1.83 m (6 ft) squares of 6-ml black polyethylene plastic (Visqueen, British Polythene Ltd., Greenock, UK) with an "X" cut in the center to lessen the risk from sharp edges vibrating in the wind and cutting through the seedling. The added carbon black increases polyethylene resistance to ultraviolet light, making it last longer in sunlight. The other mulch treatment was 1.83 m (6 ft) squares of woven black plastic weed barrier (DeWitt Sunbelt, The DeWitt Company, Sikeston, MO), also with an "X" cut in the center. Weed barrier is heavier, porous, and longer lasting than black plastic. All mulch sheets were anchored to the ground with rocks, slash, or iron sod staples.

The following treatments were applied at planting time:

(1) Control (Con). Seedlings were planted without scalping, polymer, plastic, or weed barrier.

(2) Scalping (Sca). About 2.5 cm (1 in) of sod and other plant material was removed from a 1 m (3 ft) radius circle. The seedlings were planted in the center of the circle.

(3) Plastic (Pla). A black plastic square was anchored around each seedling as described previously.

(4) Polymer (Poly). Fully hydrated polymer gel (237 ml; [8 oz]) was applied as described previously.

(5) Scalping/plastic (ScaPla). Scalping and plastic treatments were combined.

(6) Scalping/polymer (ScaPoly). Scalping and polymer treatments were combined.

(7) Plastic/polymer (PlaPoly). Plastic and polymer treatments were combined.

(8) Scalping/plastic/polymer (SPP). Scalping, plastic, and polymer treatments were combined.

(9) PolymerX2 (PolyX2). Polymer gel (474 ml [16 oz]) was applied as described previously.

(10) Weed barrier (Bar). A square of landscape fabric was anchored around each seedling as described previously.

All 10 treatments were installed at the Baseline site, while only the control, plastic, and weed barrier treatments were installed at the Flagstaff site. At Baseline, 200 seedlings were planted in a randomized complete block design consisting of two blocks, with 10 seedlings assigned to each treatment per block. At Flagstaff, 90 seedlings were planted, with 30 seedlings assigned to each treatment. Many plastic sheets were blown away in a windstorm 3 days after planting. The landowner found and replaced most, but eight could not be found. These seedlings were reassigned to the control group, leaving 38 control, 22 plastic, and 30 weed barrier seedlings total.

Seedlings were measured each October from 1990 to 1997, and then measured again in 2009 and 2016. From 1990 to 1997, measurements consisted of stem diameter at 2.54 cm (1 in) above the ground, the height above the small node on the stem at the original soil line to the tip of the terminal bud, and survival. Browse by mule deer (*Odocoileus hemionus* Rafinesque) and damage to leaders by southwestern pine tip moth (*Rhyacionia neomexicana* Dyar [Lepidoptera: Olethreutidae]) were noted. In 2009 and 2016, multiple heights were measured on each tree using a clinometer and tape, starting from the top and moving down to each whorl to estimate annual heights until there were too many branches to be able to see the whorl.

Site Index Model Development

A site index equation was developed by modifying Barrett's (1978) site index model:

(1)

$$SI_i = b_1 - \left(b_2 Age_i + \frac{b_3}{Age_i}\right) b_4 \left[1 - exp\left(b_5 Age_i\right)\right]^{b_6} + \left(b_7 Age_i + \frac{b_3}{Age_i}\right)(Ht_i - Ht_M) + Ht_M$$

where:

SI_i = Site index,

Age_i = Age in years (with age-at-planting = 1),

Ht_i = Measured tree height (model is applied tree-by-tree),

i = seedling index number,

Ht_M = Measurement height

b_1, b_2, b_3, b_4, b_5 and b_6 are coefficients to be estimated using PROC NLIN (SAS Institute 1985).

When equation 1 is solved for height, the result is:

(2)

$$Ht_i = \frac{(SI_i - H_M - b_1)}{\left(b_2 Age_i + \frac{b_3}{Age_i}\right)} + b_4\left[1 - exp\left(b_5 Age_i\right)\right]^{b_6} + H_M$$

To determine metric values of b_1, b_2, b_3, b_4, b_5, and b_6 applicable to southwest ponderosa pine, we converted data from Minor (1964) to metric (84 observations), combined it with our data (2,420 observations), and fit equation 2. Minor's data was for sawlog-sized trees and not applicable to seedlings. We combined our dataset with Minor's so our model would be continuous with Minor's data. This produced the following values:

b_1 = 3.5713

b_2 = 0.000857

b_3 = 91.7196

b_4 = 3.6808

b_5 = -0.0763

b_6 = 3.8552

We tested these coefficients with a ten-tree sample of existing mature trees from the Flagstaff site. Using Barrett's model (equation 2), average 100-year site index was 22.6 m (74.2 ft), which agrees well with an average site index of 23.7 m (78.2 ft) obtained using the tallest 20 percent of surviving experimental seedlings at age 26.

In applying equation 2 to our data, we substituted Trt_i for SI_i where Trt_i was defined as:

(3)

$$Trt_i = b_1 Var_1 + b_2 Var_2 + \ldots + b_n Var_n$$

where:

Var_n is a dummy variable identifying a specific treatment

b_1, b_2 … bn are coefficients to be estimated.

The trajectory that the seedling follows is estimated by the values fitted to coefficients in equation 3. The value of Trt_i can be estimated for individual trees or for an entire treatment class ($p < 0.0001$, $s = 0.620$):

	Mountain:	Piedmont:
Control:	b_1 = 9.7110a	b_1 = 8.6111a
Scalp:	No data	b_2 = 10.0393ab
Plastic:	b_3 = 9.5612a	b_3 = 9.6676abc
Polymer:	No data	b_4 = 5.0050abcd
ScaPla:	No data	b_5 = 11.3921bce
ScaPoly:	No data	b_6 = 5.9321df
PlaPoly:	No data	b_7 = 10.4092beg
SPP:	No data	b_8 = 11.5255begh
PolyX2:	No data	b_9 = 6.7869abdf
Barrier:	b_{10} = 11.2333	b_{10} = 12.7536ch

Values followed by the same lowercase characters indicate identical statistical values.

We found a simple straight line equation using seedling height worked well to predict diameter:

(4)

$$Dia_i = b_0 + (b_1 Var_1 + b_2 Var_2 + \ldots + b_n Var_n) Ht_i$$

where:

Dia_i is diameter at 2.54 cm (1 in) above groundline

Ht_i is seedling height

Var_i is a dummy variable identifying a specific treatment

b_1, b_2, … , bn are coefficients to be estimated, similar to Trt_i above:

	Mountain:	Piedmont:
Constant:	Not significant	b_0 = -0.0854
Control:	b_1 = 3.3184	b_1 = 3.4704a
Scalp:	No data	b_2 = 4.8217acd
Plastic:	b_3 = 3.9484	b_3 = 4.5606ab
Polymer:	No data	b_4 = 4.5086abc
ScaPla:	No data	b_5 = 4.9236acd
ScaPoly:	No data	b_6 = 6.9273
PlaPoly:	No data	b_7 = 4.9167acd
SPP:	No data	b_8 = 4.7269acde
PolyX2:	No data	b_9 = 4.6951abcde
Barrier:	b_{10} = 3.2535	b_{10} = 4.1638c

Values followed by the same lowercase characters indicate identical statistical values.

Survival was modelled using a logarithmic decay curve:

(5)

$$S = b_0 b_1^t$$

where:

S is the proportion of seedlings still alive in Year t.

t is the planting age in years (seedlings were 2 years old when planted),

b_1, are coefficients to be estimated:

	Mountain:	Piedmont:
Control:	$b_0 = 0.927$; $b_1 = 0.979$a	$b_0 = 0.982$a; $b_1 = 0.952$a
Scalp:	No data	$b_0 = 0.976$b; $b_1 = 0.986$b
Plastic:	$b_0 = 0.975$; $b_1 = 0.988$ab	$b_0 = 0.976$b; $b_1 = 0.999$b
Polymer:	No data	$b_0 = 0.992$a; $b_1 = 0.959$a
ScaPla:	No data	$b_0 = 0.941$c; $b_1 = 0.992$b
ScaPoly:	No data	$b_0 = 0.927$; $b_1 = 0.986$b
PlaPoly:	No data	$b_0 = 0.976$b; $b_1 = 0.989$b
SPP:	No data	$b_0 = 0.943$c; $b_1 = 0.995$b
PolyX2:	No data	$b_0 = 0.893$; $b_1 = 0.959$a
Barrier:	$b_0 = 0.920$; $b_1 = 0.993$	$b_0 = 0.974$b; $b_1 = 0.996$b

Values followed by the same lowercase characters indicate identical statistical values.

Tables of statistical significance for each coefficient in all three models are available from the author.

To show combined effects of growth and mortality on plantings, we prepared illustrations of surviving volume over time. The equation used a cone of seedling height minus 2.54 cm (1 in) averaged over the treatment.

(6)

$$Vol/hec = \pi\left(\frac{Dia}{100}\right)^2\left(\frac{h}{2}\right)(Sur)(Stock) = \pi\left(\frac{Dia}{20000}^2\right)h(Sur)(Stock)$$

where:

Vol is average volume of surviving seedlings for the treatment

Dia is average seedling diameter at 2.54 cm (1 in) above groundline

h is average height for the treatment,

Sur is survival, and

Stock is the initial stocking rate in seedlings per hectare.

Equation 6 is derived from a physical model and contains no coefficients needing estimation.

Results

When we applied Barrett's (1978) site index model with both sites, every block, treatment, and interaction term, three among-sites differences, and most treatments were significant at $\alpha = 0.05$. Analysis of variance produced $F_{(10,3162)} = 1897.29$, FIT = 0.857 and standard error of 0.62 m (2.03 ft) (equation 2). Treatment coefficients are proportional to height, diameter, and survival. We used height to predict stem diameter at 0.0254 m (1 in) equation 4). R^2 was 0.893 with a standard deviation of 1.854 cm (4.71 in). Except for Scalp/Polymer, treatment coefficients were similar. To model survival probability we fit proportions of surviving seedlings determined from stem counts made each October, to equation 5 (tables 1 and 2). All FIT values were significant and similar (lowest FIT = 0.990).

Mule deer damage occurred at the Flagstaff site during the first and third winters. Seedlings at both sites were damaged by southwestern pine tip moth each year measurements were taken. Deer damage affected both growth and survival, while tip moths affected only height growth. When treatments were added to the model, deer and tip moth damage became insignificant ($\alpha = 0.950$).

By 1997, 6 and 7 years after seedlings had been planted at the Baseline and Flagstaff sites, respectively, the black plastic was reduced to fragments. Broad-leaved plants were re-invading space formerly covered by plastic. Nevertheless, seedlings in treatments that included plastic mulch were growing well (figures 2 and 3). By 2009, black plastic on both sites was completely gone and the weed barrier was so brittle it could not be moved without tearing. Weeds were coming up through the weed barrier, but by this time, seedlings were mostly suppressing weeds on their own. Weed barrier produced the highest surviving volumes of any treatment at both sites (figures 2 and 3).

We observed significant differences in grass suppression among treatments. Weed barrier treatment had the greatest suppression compared with control and polymer treatments (figures 2 and 3; table 1). Polymer treatment did not increase seedling performance

Table 1. Measured ponderosa pine seedling height and diameter at the Flagstaff site over time. At ages 2 to 6 means are not statistically different at $\alpha = 0.05$. At age 7 and older, means followed by the same letter are not statistically different from each other.

Age	Height (m)			Diameter (cm)			Survival (%)		
	Control	Plastic	Barrier	Control	Plastic	Barrier	Control	Plastic	Barrier
2	0.22	0.21	0.25	0.75	0.71	0.88	100	100	100
3	0.27	0.26	0.28	0.85	0.82	0.93	84	100	93
4	0.27	0.29	0.34	0.87	0.88	1.00	82	82	73
5	0.31	0.37	0.46	1.10	1.14	1.25	71	77	73
6	0.40	0.47	0.60	1.40	1.65	1.87	63	77	70
7	0.50a	0.61a	0.74b	1.86a	2.21a	2.48b	61a	73a	70a
20	2.92a	2.90a	3.38b	11.94a	12.16a	13.45b	39a	65b	47b
26	4.34a	4.11a	4.80b	13.40a	16.67a	14.75b	39a	65b	47b

relative to the control but appeared to have a negative effect, with some polymer treatments having lower morphological values and survival over time compared with the control (table 2, figure 3).

Discussion

Our findings are consistent with previous research (Maguire et al. 2009, Rose et al. 1999, Rose et al. 2008) in that the area around a seedling influencing its growth and survival is much larger than previously thought, and that early treatments have lasting effects. Maguire et al. (2009) used chemical site treatments on 5.57 m^2 (60 ft^2) plots with varying application frequencies over 5 years, and found that plots that were treated all 5 years had significantly greater height growth. Rose et al. (2008) showed that vegetation control around individual trees had a profound effect on stem volume of Douglas-fir seedlings (*Pseudotsuga menziesii* Mirb. Franco) 12 years after planting.

Our comprehensive literature search did not return any field studies that evaluated long-term effects of polymers, landscape fabric (weed barrier), large (greater than 91.4 cm [3 ft] on an edge) sheets of plastic, or combinations of these. In addition, we found few long-term studies of planting treatments dealing with ponderosa pine in Colorado, or of tree planting in the Colorado piedmont area, with the exception of Shepperd et al. (2006) who reported average ponderosa pine heights of 1.4 m (4.5 ft) 23 years after planting on a scarified site.

Table 2. Height, DBH, basal diameter, and survival of ponderosa pine seedlings at two sites in the Northern Front Range (Colorado).

Treatment	Height (m)	Basal diameter (cm)	DBH (cm)	Survival (%)
Flagstaff (age 28)				
Control	4.3	13.5	8.1	29
Plastic	4.1	16.8	9.1	59
Barrier	4.8	14.7	8.9	27
Baseline (age 27)				
Control	2.6	12.2	6.9	25
Scalp	4.7	23.6	13.2	30
Plastic	4.4	24.6	13.7	55
Polymer	2.2	12.4	7.1	10
SPla	4.7	25.4	14.2	65
SPoly	2.4	12.3	11.7	30
PPoly	4.5	24.4	13.7	80
SPP	4.8	25.4	14.2	65
PolyX2	3.4	18.3	10.2	15
Barrier	5.5	27.2	15.2	75

Treatment abbreviations are: SPla=Scalp&Plastic, SPoly=Scalp&Polymer, PPoly=Plastic&Polymer, SPP=Scalp, Polymer&Plastic, PolyX2=Double Polymer and weed barrier. DBH is diameter at breast height (54 in or 1.37 m). Basal diameter is at 2.54 cm (1 in) above groundline.

Unlike studies that have examined seedling field performance from a reforestation point of view (Rietveld and Heidman 1974, Rose et al. 1999, 2008; Shepperd et al. 2006), our study was based on treatments to improve windbreaks and shelter plantings. Under these

Figure 2. Regression models of height, basal diameter, survival, and volume over time at the Flagstaff (mountain) planting site. Models assume an initial stocking of 1500 seedlings/ha.

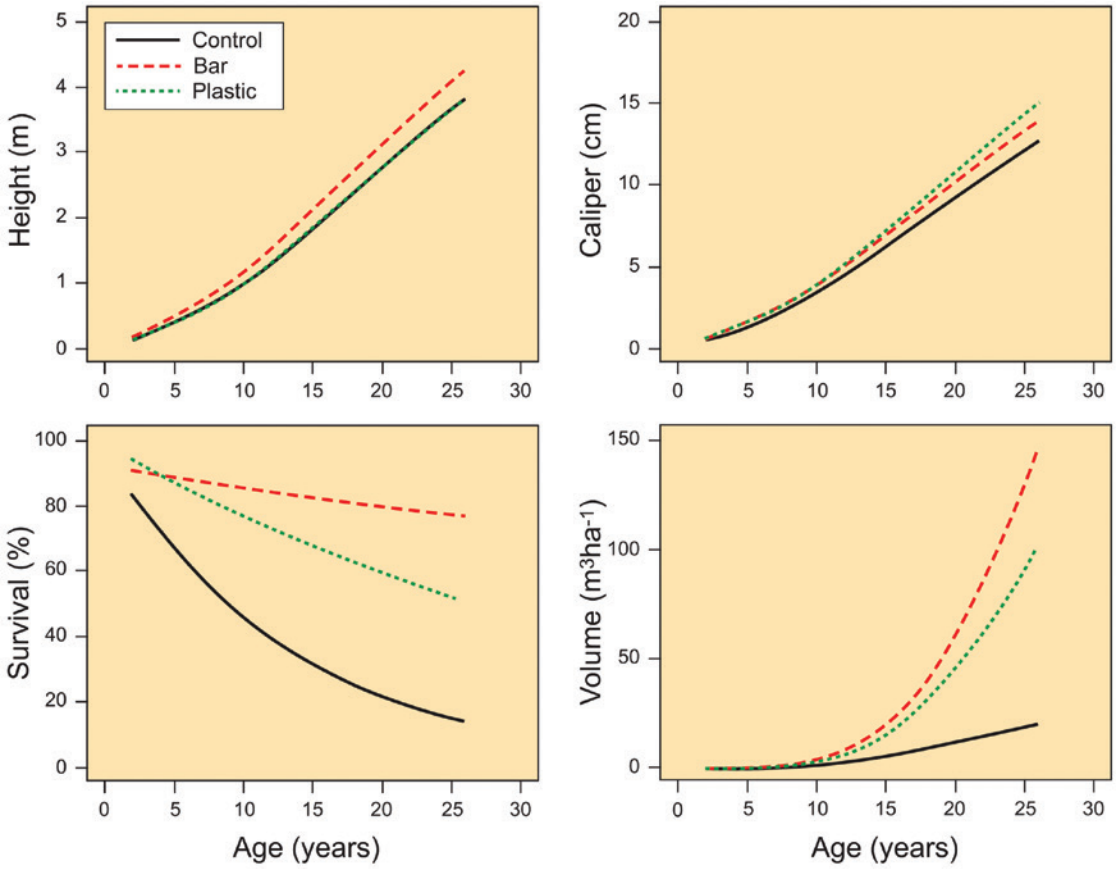
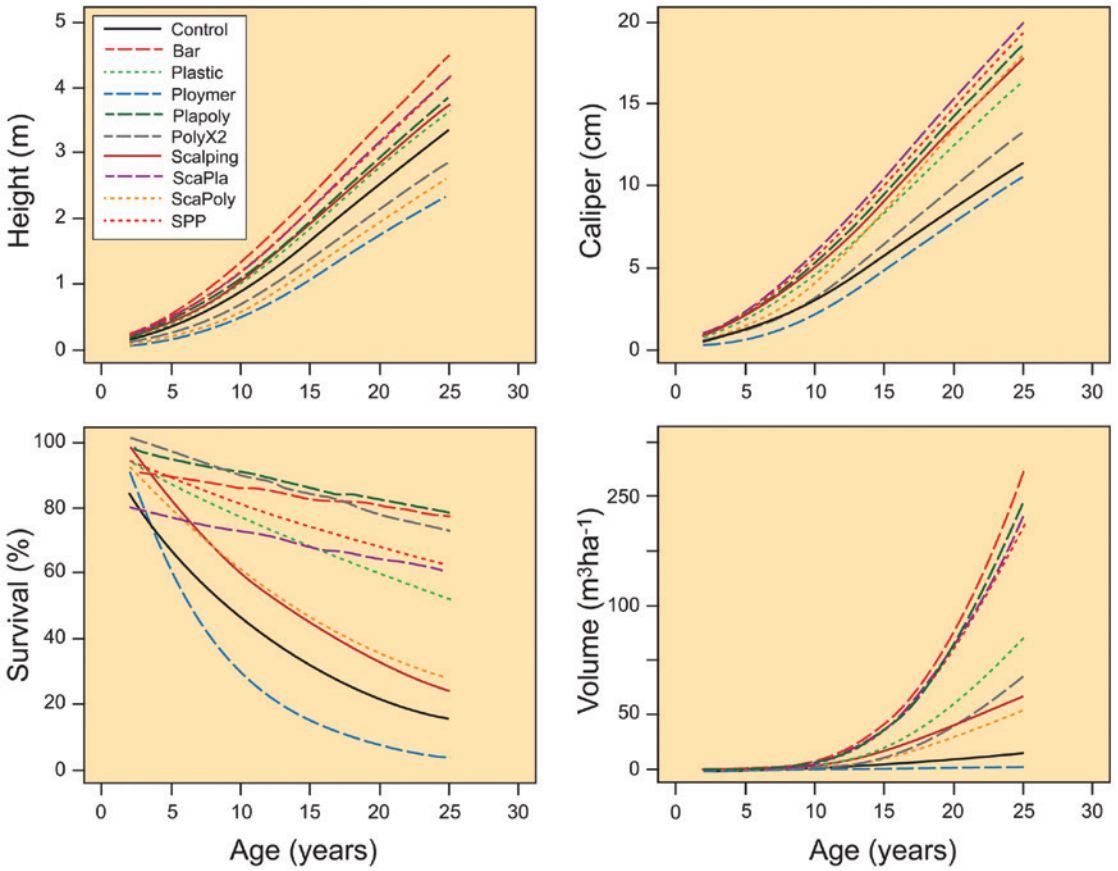


Figure 3. Regression models of height, basal diameter, survival, and volume over time at the Baseline (piedmont) planting site. Trees treated wtih Weed barrier (Bar), Plastic/ Polymer (PlaPoly) and Saclp/ Plastic/ Polymer (SPP) treatments did well in each metric. Models assume an initial stocking of 1500 seedlings/ha.



circumstances, high-cost treatments with high success rates might be a better investment than low-cost treatments with low success rates. Walker and McLaughlin (1989) used plastic mulch sheets around loblolly pine (*Pinus taeda* L.) and yellow-poplar (*Liriodendron tulipifera* L.) and found improved growth and survival compared with controls. Similarly, Lowenstein and Pitkin (1970) successfully prevented weed encroachment using black plastic mulch around pine seedlings and found significant increases in height growth after 5 growing seasons. Rietveld and Heidman (1974) reported no significant difference in survival between black polyethylene mulched trees and controls; while height growth was slightly improved using black polyethylene; they speculated that a "larger mulched area" would produce improved survival and growth. Rose et al. (1999) found that maximum growth response occurred between 5 and 6 m^2 (54 to 64 ft^2) of chemical control.

We noted on both sites that early season grasses (C_3), like smooth brome, were much more competitive than late-season (C_4) grasses. We suspect that grass allelotropes may be involved (Bonner 1950, Chung and Miller 1995, Myers and Anderson 1942). We found that treatments that reduce grass cover improve seedling growth and survival and are essential to planting success in the Northern Front Range and piedmont.

Polymer did not improve long-term seedling performance in our study. Al-Humaid and Moftah (2006), working with buttonwood (*Canocarpus erectus* L.), found that a polymer (Stocksorb) concentration of 0.4 to 0.6 percent resulted in twice as much soil water retention compared with unamended control soil. Callaghan et al. (1989) found that a 0.5 percent mixture of polymer to soil combined with watering at 3-day intervals increased survival of coolibah (*Eucalyptus microtheca* Blakely) from 0 to 100 percent over a 56-day trial. Orzolek (1993) reported 2.8 percent weight loss of polyacrylamide after 6 weeks in the ground and a 30 percent increase in tomato production. The Al-Humaid and Moftah (2006), Callaghan et al. (1989), and Orzolek (1993) studies, however, all used supplemental irrigation, suggesting that polymers must be re-wetted more frequently than the two or three precipitation events provided each season by naturally occurring summer storms. Although these researchers found notable short-term effects on growth and survival, our long-term study indicated that polymers may have no effect or a negative effect. Efficacy of hydrogel products can vary and have been known to increase mortality when seedlings are subjected to moisture stress following outplanting (Starkey et al. 2012). The ability to hold water in the soil has been well documented, but research results are mixed regarding hydrogel influences on water availability and plant uptake, and can vary by product and environmental conditions (Landis and Haase 2012).

Conclusions

Research on treatments at the time of planting is usually monitored for only a few years. Results after 1 to 3 years, however, may be more reflective of seedling treatment in the nursery and early seedling establishment than site treatments. Longer term studies (i.e., 10 or more years) can provide a more comprehensive evaluation of treatment effects (figure 4).

Mulch treatments need to be large enough to keep grass and weeds from reaching water that would otherwise be accessible to the seedling. Spot treatments should extend outward from the seedling for at least 0.91 m (3 ft), preferably more. Treatments that suppress grass, like scalping, plastic mulching, and particularly weed barrier, are the most effective at promoting seedling growth and survival. Although black plastic is not as effective as woven weed barrier, it is less expensive. Polymer treatments may not be effective unless supplemental watering is included. Further experimentation to determine the best polymer products and rates (if any) is needed.

Grass allelotropes may affect seedling growth and survival. This observation warrants further research. Our study shows that mulching treatments can result in successful ponderosa pine plantings on Ferncliffe and Nunn soils in the northern Front Range and piedmont. Similar studies are needed on other soil types.

Address correspondence to—

Douglas J. Stevenson, Senior Research Specialist, Oklahoma State University, Department of Natural Resources and Environmental Management, 008F Agriculture Hall, Stillwater, OK 74078; email: doug.stevenson@okstate.edu.

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Figure 4. (a) Baseline site 30 years since planting. (b) The author at the Baseline site; the tree in the foreground was treated with weed barrier. (Photos by Cynthia Stevenson, 2019)

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Field Establishment Techniques for Guindo Santo, an Endemic Species from Central Chile

Carolina Alvarez, Manuel Acevedo, Marta González, R. Kasten Dumroese, Eduardo Cartes, and Iván Quiroz

Researcher, Instituto Forestal, Sede Biobío, San Pedro de la Paz, Chile; Researcher, Instituto Forestal, Sede Biobío, San Pedro de la Paz, Chile; Researcher, Instituto Forestal, Sede Biobío, San Pedro de la Paz, Chile; Research Plant Physiologist, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Moscow, ID; Researcher, Instituto Forestal, Sede Biobío, San Pedro de la Paz, Chile; Researcher, Instituto Forestal, Sede Biobío, San Pedro de la Paz, Chile.

Abstract

First-year outplanting performance was measured in guindo santo (*Eucryphia glutinosa* (Poepp. & Endl.) Baill.), a Chilean endemic tree species in the Mediterranean climate zone, which is catalogued as a near-threatened species. The effects on survival of initial plant size, fertilization at establishment, and shade (with or without nurse trees) were evaluated during the first growth season. Planting guindo santo under nurse trees was the most important treatment to increase survival, compared with trees planted in full sun. We believe that the positive effects of nurse trees on survival are linked to a decrease in plant drought stress during summer, in response to higher levels of soil water content and a decrease in incident irradiation. We strongly suggest the use of shade, like nurse trees or tree shelters, during guindo santo establishment in a Mediterranean climate.

Introduction

The success of many restoration programs relies on the field establishment, survival, and growth of nursery-produced plants. Transplant shock often impairs plant performance during the first growing season (Close et al. 2005), especially under adverse site conditions such as poor soil fertility or severe climatic conditions such as those present in Mediterranean-type climates (Valladares et al. 2004). Thus, several post-outplanting treatments such as fertilization (Fox et al. 2006, Li et al. 1999), shading (Bellot et al. 2002, Puértolas et al. 2010), and weed control (Fleming et al. 1998, Navarro-Cerrillo et al. 2005, Nilsson and Orlander 1999) have been shown to enhance survival and growth of plants during the first few seasons after outplanting.

First-year fertilization of outplanted seedlings has the main objective of increasing survival and short-term productivity (Fox et al. 2006). In Chile, the effects of early plantation fertilization treatments have been mainly focused on exotic species such as Monterey pine (*Pinus radiata* D. Don) and *Eucalyptus* spp. (Rubilar et al. 2008, Schönau and Herbert 1989), which are subjected to intensive forest management programs. We know of only one field fertilization experiment with native species under restoration programs, namely coigüe (*Nothofagus domeyi* Mirb.) (Donoso et al. 2009). Although fertilization can improve early performance of several species (Barros et al. 1992, Drechsel and Schmall 1990, Li et al. 1999, Mhando et al. 1993), benefits of this treatment depend on several factors, such as soil characteristics and species-specific nutrient requirements (Rodríguez 1993), for which this information is nonexistent for native species under restoration programs in Chile.

In Chile, evidence is mounting that manipulating micro-environmental conditions on the planting site can increase field survival and performance (Soto et al. 2017, Valenzuela et al. 2016). The use of shelters, mesh guards, or outplanting under nurse trees are the most-used treatments to decrease environmental stresses on seedlings (Jiménez et al. 2005, Navarro-Cerrillo et al. 2005, Puértolas et al. 2010). These treatments improve micro-site conditions and facilitate seedling establishment, especially in open, deforested areas with stressful environmental conditions (Oliet et al. 2015, Padilla and Pugnaire 2006). Specifically, the use of adult nurse plants can ameliorate extreme environmental stresses by reducing soil water evaporation, lowering air and soil temperature, and decreasing the amount of radiation reaching the plants (Padilla and Pugnaire 2006). In Chilean native species, mesh guards and shelters have

been successfully used for quillay (*Quillaja saponaria* Molina), Roble (*Nothofagus obliqua* Birb.), laurel (*Laureliopsis phillipiana* Looser.), and olivillo (*Aextoxicon punctatum* Ruiz & Pavon.) (INFOR, unpublished data). Likewise, the native conifer ciprés de las guaitecas (*Pilgerodendron uviferum* D. Don) had a 200-percent growth increase when outplanted under a nurse canopy (Bannister 2015).

Fertilization and outplanting under nurse trees are silvicultural treatments that can be applied during restoration programs on Mediterranean sites of Central Chile, which has been declared one of the world's most threatened habitats (Dinerstein et al. 1995). Despite its vulnerability, little is known about applying cultural treatments during outplanting restoration species. Such is the case of guindo santo (*Eucryphia glutinosa* (Poepp. & Endl.) Baill.), a Chilean endemic species whose habitat extends from Linares province (-36°05´S, -71°10´W) to Malleco province (-38°14´S, -71°45´W) at elevations between 200 and 1,400 m (650 to 4,600 ft) and is only found near rivers and streams west of the Andes (Muñoz 1966). Guindo santo is a small, deciduous tree that reaches only 5 m (16 ft) tall. The tree has white flowers up to 6 cm wide with red stamens and foliage that turns orange or purple during autumn; such characteristics confer high ornamental value and importance for honey production (Hechenleitner et al. 2005) (figure 1). Guindo occurs in relatively separated subpopulations of native forest (Echeverría and Rodríguez 2014), indicating high levels of fragmentation. The International Union for Conservation of Nature (IUCN) has categorized guindo santo as a near-threatened species due to excessive clearing to establish exotic species plantations and construct hydroelectric plants.

The objective of our study was to evaluate effects of fertilization and outplanting under nurse trees on the survival and growth of guindo santo during the first growing season in a Mesomediterranean area of Central Chile.

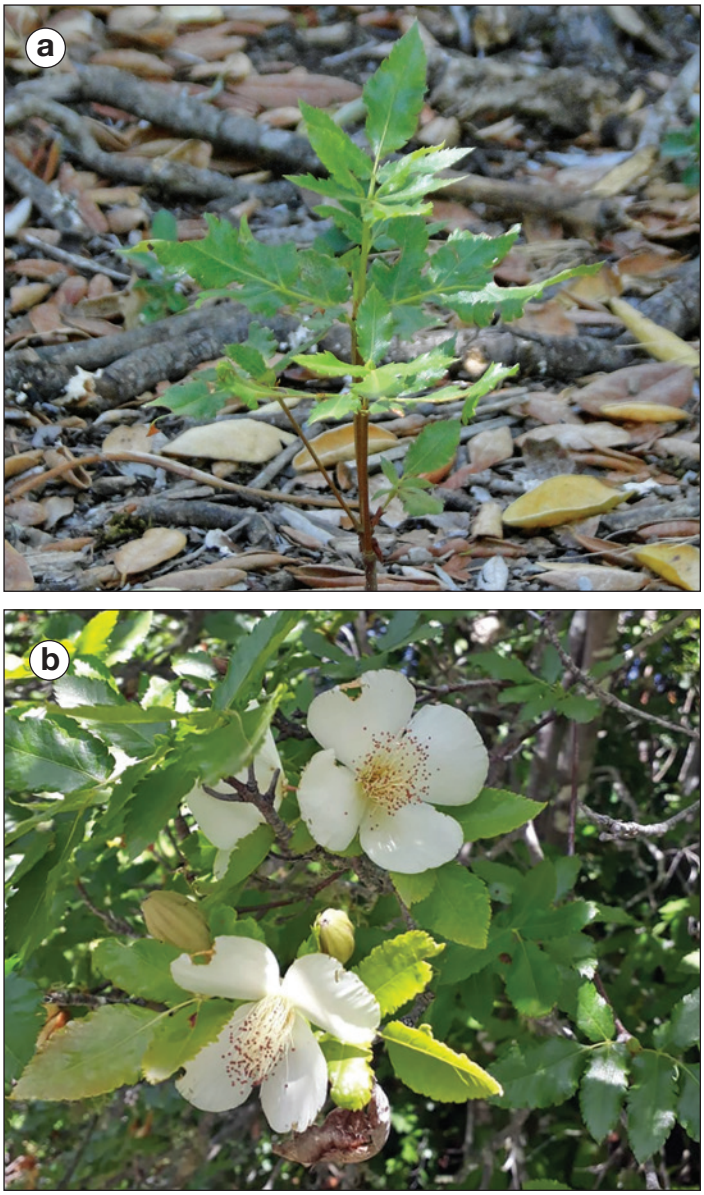

Figure 1. (A) Guindo santo seedling and (B) a close-up of the flowers known for their high value as an ornamental for honey production. (Photos by Hernán Soto, Instituto Forestal, 2012).

Methods

Nursery Production Stage

We collected guindo santo seeds in April 2011 in San Fabián de Alico commune, Biobío region (-36°35´S, -71°28´W), which is within the species' natural range. Seeds were stored in zipper plastic bags and refrigerated at 4 °C until October 2011, when they were soaked in water 24 hours prior to sowing. Seeds were sown into 20 expanded polystyrene containers, each having 84 cavities (130 ml cavity^{-1} [4.39 fl oz cavity^{-1}]) and 336 cavities m^{-2}), and germination occurred under greenhouse conditions. Containers were filled with composted Monterey pine bark having total, aeration, and water retention porosity of 49, 25, and 24 percent, respectively. The germination period lasted 61 days after sowing (DAS) from October through November 2011, during which we irrigated 5 min per day. Once germination was complete and until the end of the growing season, irrigation was applied daily to return the amount of water in the medium to container capacity.

After the germination phase, we fertilized seedlings following a three-stage scheme. Initially, between 61 DAS until 123 DAS, fertilization was applied using Ultrasol Inicial fertilizer 15-30-15 (N-P-K) (SQM, Chile) applied at 2 g L^{-1} (0.265 oz gal^{-1}) or 300 mg L^{-1} N (0.037 oz gal^{-1}) with sprinklers once a week to return the water volume to container capacity. The second fertilization stage lasted from 123 DAS until 242 DAS using Ultrasol Crecimiento 25-10-10 (N-P-K) (SQM, Chile) alternating with Ultrasol Desarrollo (18-6-18) (SQM, Chile), both in application rates of 3 g L^{-1} (0.397 oz gal^{-1}) of fertilizer (500 and 180 mg L^{-1} N, respectively [0.064 oz gal^{-1} and 0.023 oz gal^{-1}]), in the same manner as the previous stage. The third stage of fertilization lasted between 242 DAS until 303 DAS. During this stage, fertilization was applied using Ultrasol Producción (13-6-40) (N-P-K) (SQM, Chile) twice a week, at a rate of 2 g L^{-1} (0.265 oz gal^{-1}) of fertilizer (260 mg L^{-1} N [0.034 oz gal^{-1}]). Additionally, calcium nitrate was also applied twice a week during this stage in doses of 2 g L^{-1} (0.265 oz gal^{-1}) and Coldkiller® (AQM, Chile) was applied once a week in a dose of 2 ml L^{-1} (0.462 in3 gal^{-1}) to prevent frost damage.

Plant height varied widely at the end of the nursery stage. Thus, seedlings were sorted into two height categories before outplanting: 10 to 20 cm (approximately 4 to 8 in; H1) and 25 to 35 cm (approximately 10 to 14 in; H2).

Field Stage

The field study was installed at the Bullileo sector (-36°35´S, -71°28´W), San Fabián de Alico commune in Biobío region, Chile. This location is the southern limit of the Mediterranean climate and is in the Andean foothills of south-central Chile. The climate is Mesomediterranean with perhumid conditions, mild winters, and dry summers (Donoso 1996, Amigo and Ramírez 1998). Almost 80 percent of the annual precipitation occurs between May and September. During the first growing season after outplanting (from November to April), the accumulated precipitation was 1,041 mm (41 in), the mean temperature was 19.2 °C (66.56 °F), and the maximum air temperature in summer was 30.9 °C (87.62 °F). The soils are shallow with a predominance of volcanic material, including andesitic and basaltic materials (Donoso 1996).

In August 2012, we established the seedlings under two contrasting sun exposure conditions corresponding to two neighboring fields: one at full sun exposure (figure 2A) and the other with boldo (*Peumus boldus* Molina.) trees (4 to 6 m tall [13 to 20 ft]) as nurse trees (shade condition) (figure 2B). Herbaceous plants were removed from both fields before outplanting holes (30 by 30 by 30 cm [approximately 12 by 12 by 12 in]) were dug with a planting shovel at a 1 by 1 m (approximately 3 by 3 ft) spacing equivalent to 10,000 plants ha^{-1} (approximately 4,000 plants ac^{-1}). Two field fertilization treatments were applied: a control treatment with no fertilizer application and a fertilization treatment with a single application of 115g (4.05 oz) of Vitra 8-20-7 (N-P-K) plus 1 percent B (sodium borate). Fertilizer was applied around the plant in a groove of 15-cm (5.9-in) diameter and 5-cm (approximately 2-in) depth (figure 3).

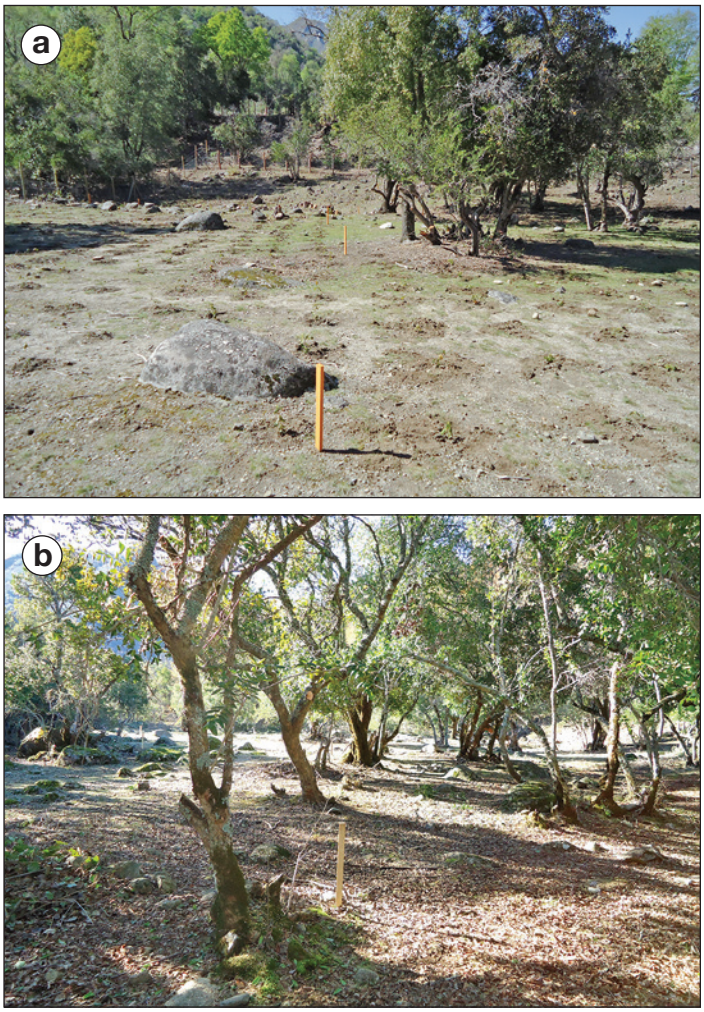

Figure 2. Guindo santo seedlings were outplanted near Bullileo with either (A) full sun or (B) shade provided by boldo nurse trees. (Photos by Manuel Acevedo, Instituto Forestal, 2012)

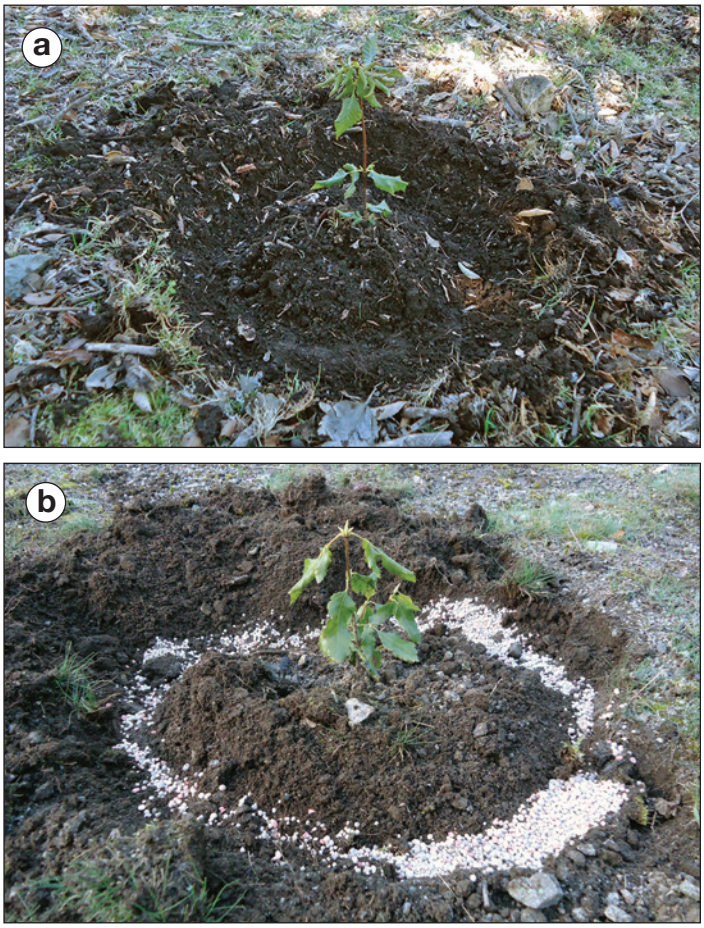

Figure 3. At outplanting, control seedlings of guindo santo were either (A) not fertilized or (B) had a ring of fertilizer applied in a 15-cm-diameter circle. (Photos by Manuel Acevedo, Instituto Forestal, 2012)

Survival and Growth Increment Measurements

Survival was evaluated monthly during the first growing season (October 2012 through April 2013). Each seedling was measured for stem diameter at ground line and height at plant establishment (October 2012) and at the end of the first growing season (April 2013). Stem diameter and height growth increments were calculated by subtracting the first measurement from the last measurement.

Pre-Dawn Water Potential Measurements

Pre-dawn water potential was measured monthly in the upper third leaves of 72 seedlings (3 plants per treatment combination replicate). During the evening before each measurement, leaves were wrapped in aluminum foil until pre-dawn at which time leaves were collected and kept on ice until the determination of leaf water potential using a Scholander pressure chamber (PMS Instruments, Albany, OR).

Gravimetric and Volumetric Soil Water Content

Gravimetric soil water content was determined monthly. Three soil samples from each treatment combination replicate (72 samples total) were collected with a shovel from the top 20 cm (7.9 in) of the soil profile, then weighed, dried in a forced ventilation oven for 48 hours at 65 °C (149 °F), and reweighed. Soil water content was calculated according to the following formula:

$$\%\ soil\ water = \frac{(weight\ of\ wet\ soil(g) - weight\ of\ dry\ soil\ (g))}{weight\ of\ dry\ soil\ (g)}\ x\ 100$$

Also, for field characterization purposes, continuous volumetric water content was determined using soil moisture sensors (EC-5, METER Group, Pullman, WA) installed 20 cm (7.87 in) below the soil surface and recorded hourly (Em-50, METER Group) from January 2013 through April 2013.

Photosynthetic Photon Flux Measurements

Monthly determinations of photosynthetic active radiation (PAR) were performed with a quantum sensor (LI-190, LI-COR, Lincoln, NE) attached to a light meter (LI-250A, LI-COR). Five measurement points were distributed randomly in each experimental unit. These measurements were performed at midday on clear days at plant level.

Experimental Design and Data Analyses

Our field experiment was laid out in a split-plot design consisting of two sun exposure conditions (whole plots) by two outplanting fertilization levels by two seedling height categories with three replications, each having 49 seedlings per treatment combination for a total of 1,176 plants. Variance analysis of repeated measurements was performed by modelling the structure of the variance and co-variance. Multiple comparisons were performed using the Tukey-Kramer test with a 95-percent confidence to test the effects of light condition, fertilization, plant size, and time on plant survival. Average stem diameter and height growth increments were obtained for each experimental unit and used for variance analysis. All statistical analyses were performed with Infostat software (V.2011p) and R extension (V.2.15.0).

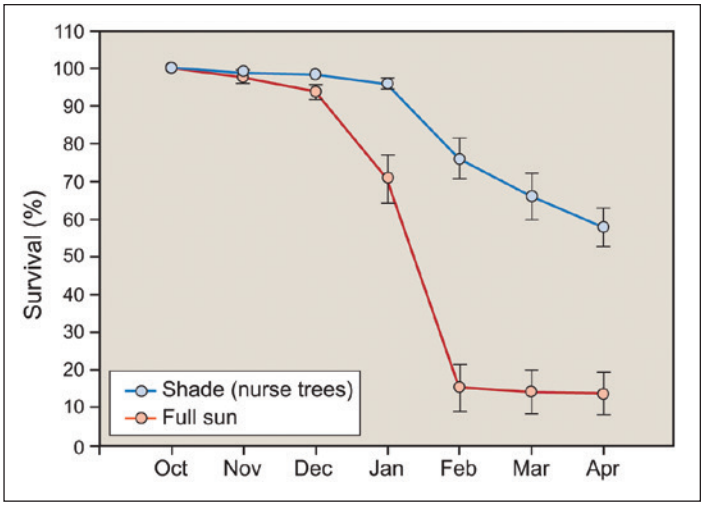

Figure 4. Survival (± standard deviation) of guindo santo seedlings during the first growing season (October 2012 through April 2013) differed over time between those grown under full sun exposure and those that were shaded by nurse trees.

Results

The interaction of time and level of sun exposure significantly affected survival at the end of the first growing season. Survival decreased for seedlings grown in full sun as well as in shade, but the magnitude of the decrease was greater for seedlings grown in full sun (figure 4). Both exposure treatments had the steepest declines in survival during the warmest summer temperatures in January (figure 4).

No significant interactions were observed for height growth, but the main effects of sun exposure, initial plant height, and field fertilization were significant (table 1). Exposure to full sun reduced seedling height growth compared with those that were planted under nurse trees. Short seedlings (10 to 20 cm [approximately 4 to 8 in]) had less height increment than did tall seedlings (25 to 35 cm [approximately 10 to 14 in]). Fertilization increased the height increment.

Table 1. Effect of initial seedling height, shade exposure condition, and fertilization on guindo santo height growth and root collar diameter growth during the first growth season. For each variable, means followed by different letters indicate significant differences between treatment according to Tukey at $p<0.05$.

Treatment		Mean height growth (cm	Root collar diameter (mm)
Initial seedling height	10 – 20 cm	9.26 b	2.04 a
	25 – 35 cm	15.18 a	2.20 a
Sun exposure	Full sun	5.34 b	2.06 a
	Nurse plants	14.14 a	2.14 a
Fertilization	No	10.25 b	2.04 a
	Yes	14.51 a	2.23 a

Stem diameter increment was unaffected by treatment and averaged 2.12 mm (0.083 in) for all treatments ($p=0.338$).

Pre-dawn water potential was significantly affected by the interaction between time of measurement and exposure condition. Similar to survival results, pre-dawn water potential was maintained until January 2013 (figure 5) and then decreased significantly during February 2013, although seedlings under full sun exposure had a significantly lower pre-dawn water potential (higher stress) than seedlings grown under shade. During the next month (March 2013), an increase in water potential values was observed in both treatments, although plants never reached the values observed during field establishment.

Gravimetric soil water content in both sun exposure conditions decreased from the time of outplanting until April 2013 and was significantly higher in the shaded plots than in those under full-sun exposure (figure 6A). While the gravimetric water content indicated a steady decrease in soil water during the first growing season, the continuous monitoring of the volumetric water content using soil water sensors indicated an increase in this parameter on February 17, 2013, due to a precipitation event (figure 6B), especially in the soil under full sun exposure. After this event, volumetric soil water content steadily decreased in both exposure conditions, reaching values in April 2013 found previous to the precipitation event.

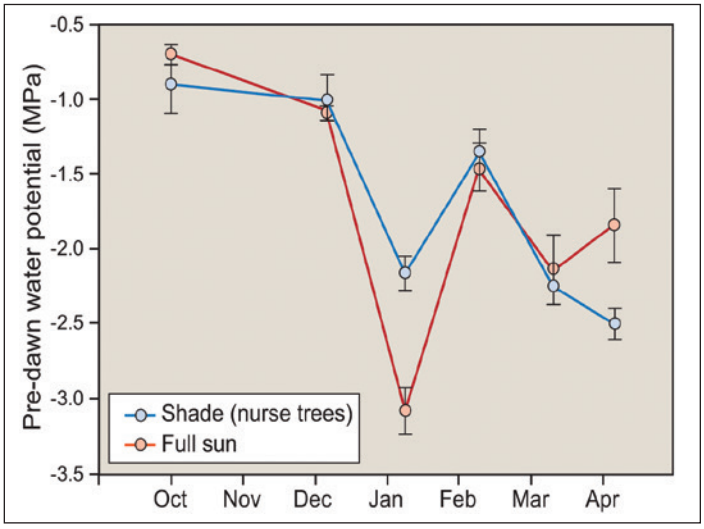

Figure 5. Pre-dawn water potential (MPa) of guindo santo seedlings varied during the first growing season but tended to be higher for seedlings grown under shade condition compared with those grown under full sun exposure.

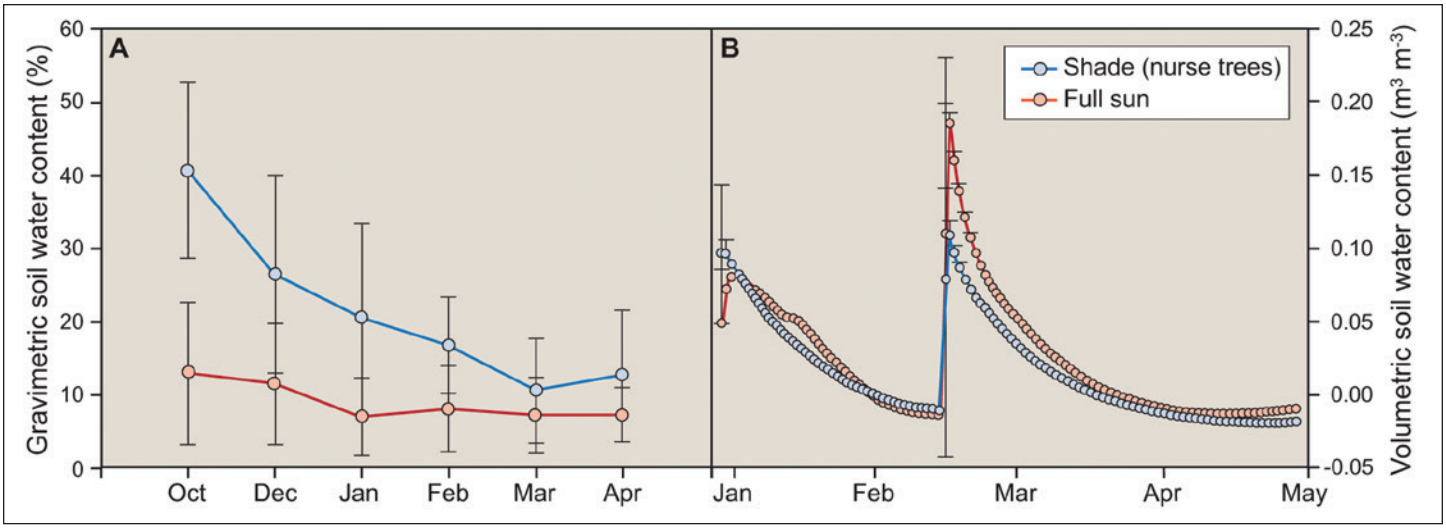

Figure 6. (A) Gravimetric and (B) volumetric soil water content during the first growing season under nurse tree shading and under full sun exposure.

PAR was significantly higher in the field under full sun exposure, compared to the field shaded with boldo nurse trees. This difference was maintained through all the first growing season (figure 7).

Discussion

One of the main constraints for restoration programs in Mediterranean climates is seedling survival during the first growth season, which is often hampered by highly stressful conditions such as water scarcity during summer (Becerra et al. 2011). Thus, research efforts should focus on treatments with potential to reduce environmental pressure on seedlings and thereby increase survival. Such factors or treatments include the use of nurse plants and fertilization during establishment.

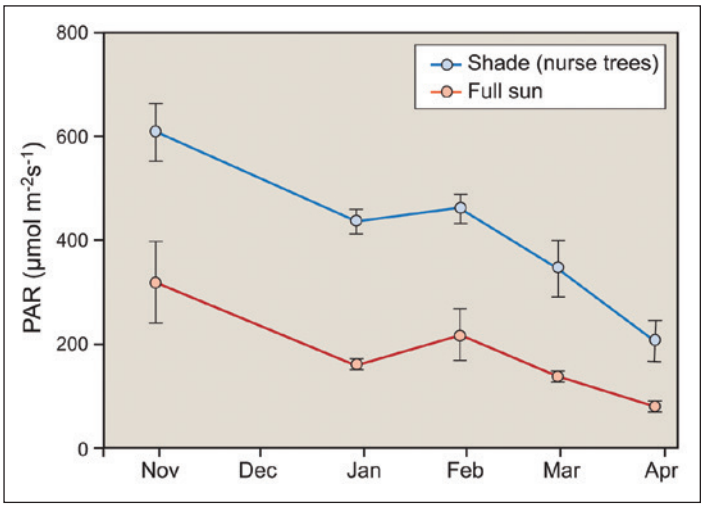

Figure 7. Photosynthetic active radiation (PAR) for guindo santo plants during the first growing season under nurse trees and under full sun exposure.

Our results show that the use of nurse plants for establishment of guindo santo modifies aboveground and belowground environments to improve seedling survival. These beneficial effects are consistent with other reports with Mediterranean species, such as longleaf pine (*Pinus palustris* Mill.) (Knapp et al. 2013, Rodriguez-Trejo et al. 2003), black pine (*Pinus nigra* Arnold) and scots pine (*Pinus sylvestris* L.) (Castro et al. 2002), and English yew (*Taxus baccata* L.) (Peragón et al. 2015). In our study, the boldo nurse trees resulted in gravimetric soil water content and decreased solar radiation at plant level, common shade effects known to be beneficial to plant growth and survival (Valladares et al. 2008). Indeed, in our study, outplanted seedlings under the nurse trees had consistently higher pre-dawn water potential compared with seedlings grown in full sun, suggesting that shaded plants were less water-stressed during summer (February 2013).

The use of nurse trees or shrubs has not always been favorable to plant survival and growth, however, especially under high-density situations when the nurse plants have intercepted rainfall (Valladares and Pearcy 2002), leading to lower water availability and increased drought stress. In our study, volumetric water content increased equally with or without nurse trees, suggesting that the boldo canopy density was ideal for decreasing irradiation but not impairing precipitation from recharging the soil. Shade can also have adverse, species-specific effects on plant growth, especially under drought conditions (Valladares and Pearcy 2002).

Although soil organic matter (OM) content was not measured, research regarding the use of nurse trees for restoration in Mediterranean species found higher OM content under nurse trees, such as kermes oak (*Quercus coccifera* L.) and mastic tree (*Pistacia lentiscus* L.) (Arévalo et al. 1993, De la Torre and Alías 1996), and grasses (Maestre et al. 2001). Soil OM increases water retention and positively affects soil structure, nutrient cycling, and nutrient availability (Sánchez et al. 2006) and increases cation exchange capacity (Page-Dumroese et al. 2000). Removal of OM from the soil surface can have a negative impact on seedling growth as observed in other species such as western white pine (*Pinus monticola* Douglas ex D. Don) and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Page-Dumroese et al. 1997).

Field fertilization improved growth but not survival in our study, which agrees with studies published in Mediterranean species. In ponderosa pine (*Pinus ponderosa* Douglas ex Lawson), Fan and Moore (2002) indicated that fertilized seedlings had greater height and stem diameter at the time of outplanting compared with unfertilized control plants, although higher fertilizer rates increased mortality in this species. Sloan and Jacobs (2013) found that neither controlled-release fertilizers nor immediately available fertilizers significantly affected field survival of white spruce (*Picea glauca* (Moench) Voss) and aspen (*Populus tremuloides* Michx.), though growth of white spruce was positively affected by both types of fertilization. Sutton (1975) also found a growth response to fertilizer in white spruce. Seedling responses to field fertilization depend on field factors such as competing vegetation (Brockley 1988), soil nutrient availability (Nilsson and Allen 2003), and soil moisture (Everett et al. 2007, Rubilar et al. 2008), but, in general, many studies with conifers (Brockley 1988, Fan and Moore 2002, Fan et al. 2004, Haase et al. 2006) and hardwoods (Jacobs et al. 2005) have shown an increase in height and root-collar diameter growth when planted with fertilizers.

Although it has been reported that larger nursery plants perform better under field conditions (Villar-Salvador et al. 2012) and are preferred for reforestation in Mediterranean climates, we found that nursery plant size had no effect on field survival during the first growth season. For silvicultural purposes, seedling uniformity is a desired trait (Basey et al. 2015), but for ecological restoration, non-uniformity and high variability in morphological and growth traits indicate genetic diversity (Smith et al. 2007). Thus, using nursery stock of varied sizes may be suitable for restoration programs, especially in species such as guindo santo, in which plant height variability has no negative effect on field survival.

Conclusions

Our results show that growth and survival of guindo santo plants in a Mediterranean climate, regardless of their initial size, was enhanced by the use of boldo nurse trees, which increased soil gravimetric water content and decreased incident irradiation, and which, in turn, decreased plant stress as noted by higher predawn water potential values during the summer. Fertilization also increased plant growth but had no effect on survival. Similar results may be obtained with other nurse tree species or with tree shelters; more research is required to discern the best approach.

Address Correspondence To—

Carolina Alvarez, Instituto Forestal, Sede Biobío, Camino a Coronel km 7.5, San Pedro de la Paz, Chile and Instituto de Ciencias Agronómicas y Veterinarias, Universidad de O´Higgins, Campus Colchagua, San Fernando, Chile; email: carolina.alvarez@uoh.cl; phone: 56 41 2853263.

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Studies to Evaluate Hydroponic Culture of Teak Seedlings in a Temperate Greenhouse

W. Andrew Whittier, Gary R. Hodge, Juan L. Lopez, Carole Saravitz

Research Associate, Camcore, Department of Forestry and Environmental Resources, North Carolina State University (NCSU), Asheville, NC; Director, Camcore, Department of Forestry and Environmental Resources, NCSU, Raleigh, NC; Associate Director, Camcore, Department of Forestry and Environmental Resources, NCSU, Raleigh, NC; Phytotron Director and Research Associate Professor, NCSU, Raleigh, NC

Abstract

Teak (*Tectona grandis* L.f.) is one of the premier timber species globally. High demand, combined with harvesting restrictions across its natural range, has resulted in extensive plantation establishment. Plantations, in turn, depend on the production of healthy seedlings for successful establishment. As a lead up to assist growers in diagnosing seedling nutrient issues, we conducted a series of studies to test the feasibility of growing teak seedlings hydroponically in temperate greenhouses. Teak seedling studies were conducted in both sand and liquid culture hydroponic systems. Within each system, different strength nutrient solutions, solution pH levels, and pH buffers were tested to determine optimal conditions for growing seedlings. These studies indicated that teak seedlings could be successfully grown hydroponically in temperate greenhouses and responded best to a full-strength nutrient solution with a pH of 5.8 and a sodium hydroxide buffer. These results will be useful for conducting future studies to evaluate nutrient disorders in teak seedlings.

Introduction

Teak (*Tectona grandis* L.f.) is one of three species in the *Tectona* genus (others include *T. hamiltoniana* and *T. phillipensis*) within the Verbanaceae family. Of the three, *T. grandis* is the most highly valued and is considered to be one of the premier timber species in the world. High levels of resistance to insect damage and water-related decay coupled with a combination of durability, strength, workability, and aesthetically pleasing color result in this valuation.

Within India, high demand for teakwood has resulted in prices ranging from US$225 to $900 per m^3 for plantation grown logs (ITTO 2017, Thulasidas 2013) while in the United States, values as high as US$4,000 per m^3 have been reported for quality logs (Ladrach 2009).

Appetite for teak lumber, coupled with restrictions on harvesting from natural stands in its native southeastern Asia, has resulted in numerous plantations being established throughout the tropics. In the Americas, the first reported plantation was in Trinidad in 1913 (Keogh 1979). In Puerto Rico and the U.S. Virgin Island of St. Croix, approximately 130 ha have been established (Weaver 1993). As of 2010, teak plantations were reported to have been planted in 65 countries, making up an estimated 75 percent of the world's high-quality, tropical hardwood plantations (Koskela et al. 2014).

While there is a considerable body of literature on teak under natural and plantation conditions, the amount of information pertaining to the production of seedlings is modest (Swaminathan and Srinivasan 2004). Furthermore, even less research has been done on the nutrient requirements of teak seedlings. To date, no single study has examined the 12 essential micro- and macronutrients and how they each impact the growth of teak seedlings. One of the best ways to study plant nutrients is with hydroponic culture.

Crop production in soilless culture systems requires an adequate supply of all the elements essential for plant growth in the nutrient solution (Kilnic et al. 2007). A nutrient solution for hydroponic systems is an aqueous solution containing mainly inorganic ions from soluble salts of essential elements for higher plants (Trejo-Téllez and Gómez-Merino 2012). Most modern hydroponic nutrient solutions are based on the work of Hoagland and Arnon (1950) and have been adapted to

numerous crops (Whipker 1988). Plants have marked powers of adaptation to different nutrient conditions (Hoagland and Arnon 1950). Nonetheless, it is important to determine suitable nutrient solutions for each plant species (Kilnic et al. 2007).

In addition to determining the nutrient solution composition, one needs to consider the solution strength. Several studies have found that reducing solution strength had no significant impact on fruit production. Kane et al. (2005) found that biomass production of onion was as great in half-strength Hoagland's as in the more concentrated solution. Siddiqui (1998) found a 25-percent strength solution did not decrease tomato fruit yield.

As plants grow, they absorb minerals, which alters nutrient levels, and oftentimes pH, in the solution. Periodic replacement of all or a portion of the nutrient solution helps to replace lost nutrients and maintain consistency in nutrient concentrations. In general, the recommended pH for hydroponic culture is 5.5 to 5.8 to optimize overall nutrient availability (Bugbee 2004). Suitable teak soils are sandy and slightly clayey, fertile, deep, and well-drained, with a neutral or slightly acid pH (DeCamino et al. 2002). In hydroponic systems, pH is constantly changing as plants grow and take up nutrients (Berry and Knight 1997). Once a species-specific pH level has been targeted for nutrient solutions, maintaining this pH can be achieved by adding acids or bases to lower or raise the pH, respectively. In choosing a buffer, care must be taken to utilize one that does not alter the nutrient solution composition. Two commonly used pH buffers are calcium hydroxide and sodium hydroxide. The use of calcium hydroxide for growing teak is attractive, as teak has a noted calcium demand. Unfortunately, however, calcium hydroxide tends to precipitate out of solution and can clog the fine tubing used in automated delivery systems (Saravitz 2013).

A series of studies was conducted to investigate the feasibility of growing teak hydroponically in a temperate greenhouse. Specifically, these studies addressed the following questions: (1) How do teak seedlings respond to both sand and liquid culture hydroponic systems? (2) What nutrient solution concentration is optimum for growing teak seedlings? (3) What is the associated pH of the optimum nutrient solution? And, (4) what is the recommended pH buffer for use in the liquid culture hydroponic system? The results from this study will be useful for future studies of teak seedling nutrition.

Nutrient Solution Strength Study

Materials and Methods

During the summer of 2013, teak seedlings were grown in a sand culture hydroponic system with three nutrient solution treatments in a greenhouse located in Raleigh, NC (35.8° N, 78.7°W). Greenhouse conditions during the study were night/day temperatures of 21 ºC/18 ºC with ambient light and natural photoperiod.

Following a 24-hour room temperature water stratification, seeds were sown directly into 72-cell germination trays (4.0 by 4.0 by 5.8 cm cell dimensions) filled with a sterile peat and perlite medium (figure 1). After 34 days, seedlings were transplanted into 14-cm deep plastic pots filled with acid-washed silica sand. In transplanting, efforts were made to retain the entire root system. Eighteen seedlings were transplanted into three different nutrient concentration treatments for a total of six seedlings per treatment. Seedling pots were placed into three separate lengths of polyvinyl chloride (PVC) irrigation pipe (1.8 m long and 10.2 cm diameter) fitted with six PVC funnels placed into openings in the pipe (figure 2). Each pot had two drip irrigators placed on opposite sides of the stem with flow oriented toward the plant (figure 3). Daily irrigation occurred once every 3 hours from 0600 to 1800. Irrigation was automated and pumped through the system using sump pumps placed in 19-L buckets located below the seedlings. The nutrient solution used in irrigation drained from the bottom of each pot into the sloped PVC pipe. Used solution was captured and recirculated throughout the system. Nutrient solutions were changed weekly to replace nutrients taken up by the plants. Plants were monitored daily for

Figure 1. Recently germinated teak seedlings. (Photo by Andrew Whittier, 2013)

Figure 2. Sand culture hydroponic system. (Photo by Andrew Whittier, 2013)

Figure 4. Ten percent strength nutrient solution seedling at 30 days. (Photo by Andrew Whittier, 2013)

Figure 3. Recently transplanted teak germinant in sand culture hydroponic system. (Photo by Andrew Whittier, 2013)

nutrient solution response and measured for height and basal diameter weekly for 5 weeks.

Treatments began immediately after transplanting into pots and consisted of 10-, 50-, or 100-percent concentration of a complete modified Hoagland's all-nitrate nutrient solution (Hoagland and Arnon 1950). The full-strength stock solution was mixed with deionized (DI) water to a total volume of 100 L (table 1). The 100-percent solution consisted of only premixed solution, the 50-percent solution was a 1:1 mix of the full-strength solution and DI water, and the 10-percent solution was 1:9 ratio of full-strength solution to DI water. Growth means were analyzed using PROC ANOVA of the Statistical Analysis System software package (SAS 1988).

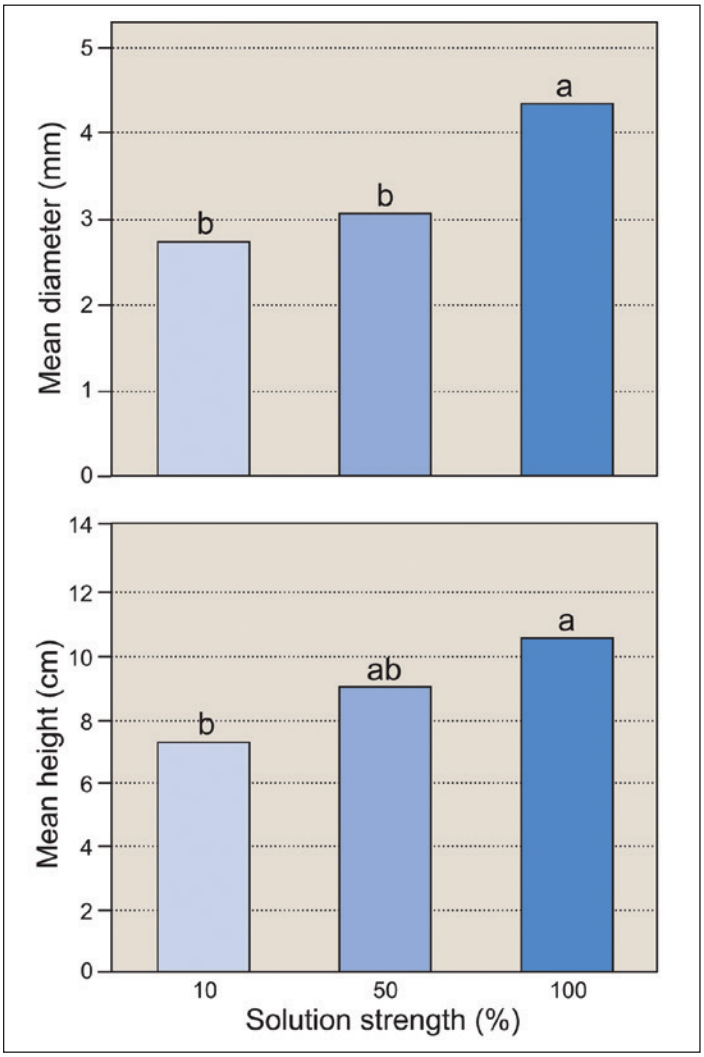

Figure 5. Teak seedling basal diameter and height at week five by solution strength in sand culture. Bars with the same letter are not significantly different at the $P \leq 0.05$ level.

Results

After 5 weeks, basal diameter and height of teak seedlings grown in the 100-percent solution were significantly larger than those grown in the lower-strength solutions (figures 4 and 5). None of the 18 seedlings in any of the three solution strengths died over the 5-week period. This study indicates that teak seedlings will

Table 1. Salts and bases used to formulate nutrient solutions based on Hoaglund and Arnon (1950). Salts and bases were added (mL/100L solution) to deionized water to make 100L of each solution.

Fertilizer salt and base	Stock solution molarity	100% solution	50% solution	10% solution
Potassium nitrate (KNO_3)	1M	500	250	50
Calcium nitrate tetrahydrate [$Ca(NO_3)_2 \bullet 4H_2O$]	1M	500	250	50
Potassium phosphate monobasic (KH_2PO_4)	1M	100	50	10
Magnesium sulfate heptahydrate ($MgSO_4 \bullet 7H_2O$)	1M	200	100	200
Iron diethylenetriam-epentaacetic acid (FeDTPA)	1M	100	50	10
Manganese chloride tetrahydrate ($MnCl_2 \bullet 4H_2O$)	20 mM	90	45	9
Zinc chloride ($ZnCl_2$)	20 mM	15	7.5	1.5
Cupric chloride dihydrate ($CuCl_2 \bullet 2H_2O$)	20 mM	15	7.5	1.5
Boric acid (H_3BO_3)	100 mM	45	22.5	4.5
Sodium molybdate dihydrate ($Na_2MoO_4 \bullet 2H_2O$)	1 mM	10	5	1
Sodium hydroxide (NaOH)	1 M	40	20	4

grow suitably in a sand culture hydroponic system under varying concentrations of a complete modified Hoagland's all-nitrate solution. Based on these findings, the full-strength solution was deemed optimum for subsequent teak nutrient experiments.

Hydroponics Solution pH Study

Materials and Methods

To fine-tune hydroponic conditions for growing teak seedlings, three nutrient solution pH levels were examined in a liquid culture hydroponic system at North Carolina State University Phytotron (Raleigh, NC). Seeds were soaked in water for 24 hours, then sown into 164 ml3 Ray Leach Cone-tainers (Stuewe and Sons, Inc., Tangent, OR) filled with sterilized river sand. Containers were placed in a greenhouse with ambient light with night/day temperatures of 30 ºC/26 ºC. All seed was hand-watered twice daily. After 33 days, 36 healthy seedlings were chosen and carefully removed from the container and sand was washed from their roots through repeated submersion in tap water combined with gentle agitation. Once all sand was removed, seedlings were placed in glass beakers filled with tap water, then placed in the liquid culture hydroponic system.

The liquid culture hydroponic system consisted of three individual hydroponic units installed in a controlled environment room (figure 6). Each individual hydroponic unit consisted of one 100-L PVC tank placed on a rolling metal frame with another 100-L PVC tank below for a total of 200-L of solution per unit. Seedlings were all grown in a 100-percent strength nutrient solution throughout the study. Nutrient solutions were circulated between tanks by enclosed pumps at a rate of 16 L/min. Aeration was supplied as solution from the upper tank fell back into the lower tank. A check valve located between the two tanks allowed for the isolation of tanks, which facilitated the replacement 100 L of nutrient solution weekly. Seedlings were grown with a 12:12 daily photoperiod and temperatures of 30 ºC/26 ºC.

The upper tank of each hydroponic unit was separated into three compartments with PVC walls. Each of the three divisions were further divided into four sections to isolate roots from each other while maintaining a uniform solution. While plants and roots were kept

Figure 6. Liquid culture hydroponic system. (Photo by Andrew Whittier, 2013)

Figure 7. Healthy teak seedling grown in full strength nutrient solution 6.0 pH. (Photo by Andrew Whittier, 2013)

separate, the nutrient solution was able to flow freely throughout the entire system. The upper tank was fitted with a PVC cover that held PVC discs over the three compartments. In each of these discs were openings that held foam plugs that were suspended directly over the hydroponic solution. Seedlings were placed in slits cut into the foam plugs with roots submerged into the nutrient solution.

Nutrient solutions were mixed with reagent grade chemicals and DI water and were based on a full-strength complete modified Hoagland's all-nitrate solution (Hoagland and Arnon 1950; table 1). Sulfuric acid was added to the solution following mixing in order to achieve an initial target pH. Using a pH meter (Model 5993-35, Cole-Parmer, Vernon Hills, IL) that displayed current levels on a pH monitor/controller, the three hydroponic units were set to three pH levels: 5.3, 5.8, and 6.3. Target pH was maintained through automated use of peristaltic pumps adding a calcium hydroxide (Ca(OH)2) base to the nutrient solution as needed. Only bases were added as the nutrient solution became gradually more acidic as seedlings took up nutrients. Weekly replacement of half of the solution in each unit was done to maintain a consistent nutrient solution throughout the study.

Height and basal diameter were measured weekly for 38 days, after which seedlings were removed and separated into leaf, stem, and root components. Fresh plant weights at the time of removal from hydroponics were recorded. Plant components were then dried in a forced-air oven at 60 ºC for 48 hours, then measured for dry weights. Height, basal diameter, wet plant weight, and dry plant weight means were analyzed using PROC ANOVA of the Statistical Analysis System software package (SAS 1988).

Results

After 38 days, none of the plants in the study had died. Growth was impressive regardless of pH levels (figure 7). Height of seedlings grown in pH 5.8 was significantly taller than those grown in pH 5.3, whereas basal diameter was unaffected by treatment (figure 8). There were no significant treatment differences in fresh or dry plant weights. This study indicates that teak grow well across a range of acidic pH values when adequate nutrients are supplied. Future studies looking at a more extreme pH range would help to more fully understand the upper and lower pH limits that hydroponically grown teak will tolerate.

Nutrient Solution pH Buffer Study

Materials and Methods

Following a 24-hour stratification in tap water, 220 teak seeds were sown 1 cm deep with micropyles down in germination flats filled with moist, sterilized river sand. Sown flats were placed in a greenhouse with ambient light and day/night temperatures of 30 ºC/26 ºC and were hand-watered twice daily. After 58 days, 20 germinants were randomly chosen and carefully removed from trays. Sand was washed from roots through repeated agitated dunking in tap water. Once the roots were thoroughly cleaned, 10 plants were installed into each of two hydroponic tanks. The hydroponic units utilized were the same as those described in the nutrient solution pH study. Each of the two tanks was filled with a 100-percent Hoagland nutrient solution and monitored for pH, as described in the nutrient solution pH study. The pH in both units was maintained at 6.0. In one tank, pH was maintained through the automated addition of a sodium hydroxide (NaOH) buffer through peristaltic pumps. In the other tank, the pH

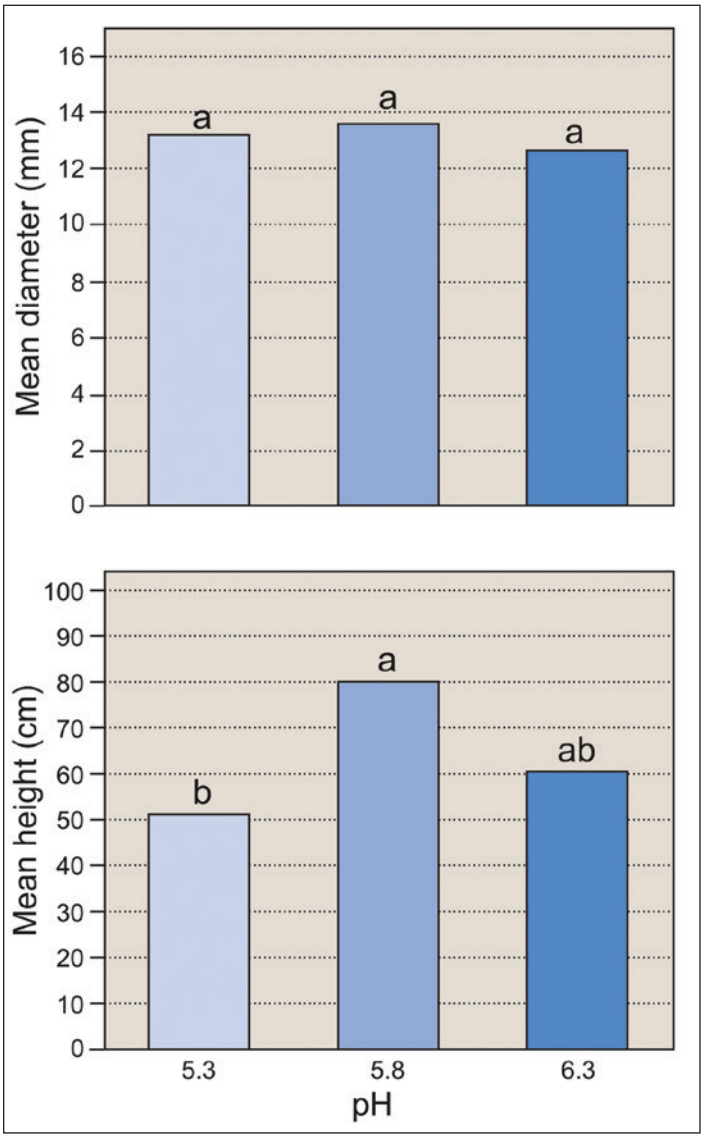

Figure 8. Teak seedling mean basal diameter and height after 5 weeks in three different pHs in liquid culture. Bars with the same letter are not significantly different at the P ≤ 0.05 level.

was maintained with a calcium hydroxide ($Ca(OH)_2$) buffer added through the same peristaltic pump system.

Seedling height and basal diameter were measured weekly for 8 weeks. Initial height of all 20 seedlings averaged 1.4 cm. Initial diameter was not recorded, as plants were too small and delicate to measure with calipers. At the completion of the study, plants were removed from the hydroponic solution and weighed for total fresh weight, as well as fresh weight of leaves, stems, and roots. Dry weight of plant parts was measured after 48 hours in a forced-air oven at 60 ºC for 48 hours. Height, basal diameter, wet plant weight, and dry plant weight means were analyzed using PROC ANOVA of the Statistical Analysis System software package (SAS 1988).

Results

After 56 days in the buffer study, one plant in each buffer treatment had died. Mean height and diameter between the two buffer treatments did not differ significantly at the P ≤ 0.05 level (figure 9). Wet and dry plant weights between the two buffer treatments were also not statistically different.

The lack of significantly different rates of growth was unexpected, as teak has a reported high calcium demand. The lack of positive response to additional calcium may indicate that seedling calcium demands were met with the calcium provided in the full-strength Hoagland nutrient solution. The use of sodium hydroxide as a pH buffer is preferable to avoid issues with precipitates when using calcium hydroxide.

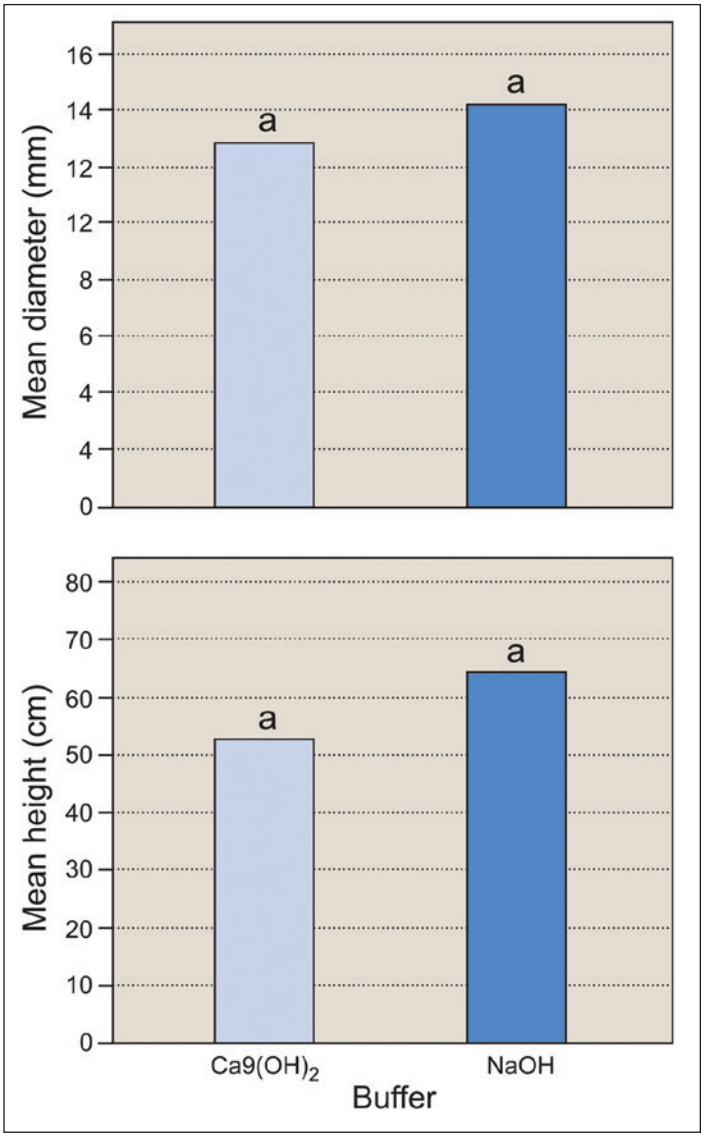

Figure 9. Teak seedling mean basal diameter and height at 8 weeks in two different buffers in liquid culture. Bars with the same letter are not significantly different at the P ≤ 0.05 level.

Recommendations

These preliminary studies helped to answer questions involving a suitable methodology for growing teak seedlings hydroponically in temperate greenhouses. Growth was adequate within greenhouses during the summer in a temperate climate. The use of a full-strength standard nutrient solution produced adequate growth in hydroponically grown teak seedlings.

Most hydroponic systems are designed to be slightly acidic (Bugbee 2004). Although the results in this study indicate that seedling growth was suitable at each of the tested pH levels, a target pH of 6.0 is recommended in future hydroponic studies due to the slightly improved height of seedlings in the 5.8 and 6.3 pH solutions.

To maintain a desired pH while plants take up nutrients from the hydroponic solution, buffers are commonly added to the nutrient solution (Bugbee 2004). We expected teak seedlings would respond well to a calcium hydroxide buffer because the species has a known high calcium demand (Weaver 1993), but there were no significant differences between buffer treatments. In future liquid culture hydroponic studies, we recommend sodium hydroxide as a buffer because of its ease of use with peristaltic pumps.

In summary, this research illustrated that teak seedlings would respond well to both sand and liquid culture hydroponic greenhouse setups. Based on these findings, we recommend that future hydroponic teak seedling studies use a full-strength standard Hoagland nutrient solution at a pH of 6.0 with a sodium hydroxide buffer.

Address correspondence to—

W. Andrew Whittier, Camcore, Department of Forestry and Environmental Resources, c/o USFS Southern Research Station, 200 WT Weaver Blvd, Asheville, NC 28804; email: wawhitti@ncsu.edu; phone: 828–257–4369 ext. 369.

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A Performance Comparison of Bareroot and Containerized *Pinus taeda* L. Seedlings as Affected by Ophiostomatoid Fungi

Pratima Devkota, Scott A. Enebak, and Lori G. Eckhardt

Post-doctoral Research Associate, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI; Professor, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL; Professor, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL

Abstract

The objective of this study was to evaluate responses of containerized and bareroot seedlings from the same *Pinus taeda* L. families to ophiostomatoid fungi, *Leptographium trerebrantis* and *Grosmannia huntii*. Seedlings from four families were artificially inoculated with *L. terebrantis* and *G. huntii*. After 8 weeks, tissue necrosis and occlusion caused by the fungi were measured. Seedlings from both *P. taeda* stockypes showed similar susceptibility to fungi, suggesting both seedling stocktypes can be used to screen the susceptibility of *P. taeda* families against tested ophiostomatoid fungi. This paper was presented at the Joint Annual Meeting of the Southern Forest Nursery Association and the Northeast Forest and Conservation Nursery Association (Pensacola, FL, July 17–19, 2018).

Introduction

Pinus taeda L. (loblolly pine) is one of the most commercially valuable timber species in the southern United States. *Pinus taeda* plantations contribute a considerable portion of the economy of this region by providing marketable forest products, habitat for wildlife, recreational areas, and others (Poudel et al. 2017, Schultz 1997). However, root-infecting fungi, including bark beetle-vectored ophiostomatoid fungi, are frequently associated with root infection and the decline of this species (Eckhardt et al. 2007).

Leptographium terebrantis and *Grosmannia huntii* are the most pathogenic ophiostomatoid fungi associated with *Pinus taeda* decline (Matusick et al. 2010; Singh et al. 2014). Root-feeding bark beetles act as vectors in introducing these fungi into the roots of pine trees during their feeding activity (Jankowiak and Bilański 2013). Inoculation of these fungi and subsequent host defense responses result in tissue necrosis and occlusions of xylem and phloem tissues, respectively (Devkota et al. 2018b). Thus, the fungal infection, together with activated host defense responses, disturbs plant water transport and results in tree decline.

Bareroot and containerized seedlings are the two major stocktypes used in forest restoration programs. Bareroot seedlings are grown in soil beds in an open field with the removal of soil during harvest. Containerized seedlings are grown in containers containing artificial media under a shelter or controlled greenhouse environment with root and growing medium maintained together from harvest to outplanting (Grossnickle and El-Kassaby 2016). The lifting of bareroot seedlings in nurseries in the southeastern United States involves a number of operational procedures that might affect the root viability (Starkey and Enebak 2013). In contrast, root damage is minimal in containerized seedling. The use of a greenhouse and artificial growing medium may, however, increase susceptibility of containerized seedlings to biotic diseases, compared with bareroot seedlings (Grossnickle and El-Kassaby 2016).

Few studies have been conducted to screen the susceptibility and resistance of different *Pinus taeda* families to *Leptographium* and *Grosmannia* spp. (Devkota et al. 2018a, Devkota and Eckhardt 2019). These studies examined either bareroot or containerized seedlings, but never compared the relative performance of both seedling stocktypes from the same family. For example, Singh et al. (2014) studied bareroot and container-grown seedlings from different families in the screening in the year 2011 and 2012, respectively. Variation in seedling stocktype used in

individual trials have made it difficult to compare family performance between trials.

Thus, there is a need to compare variations in susceptibility and resistance of containerized and bareroot seedlings from the same family of *Pinus taeda* to ophiostomatoid fungi. The objective of our study was to utilize the established method of fungal inoculation for evaluation of bareroot and containerized seedlings from the same four *P. taeda* families to *Leptographium terebrantis* and *Grosmannia huntii*.

Methodology

An artificial seedling inoculation study was conducted in 2014. This study was a subset of a larger experiment conducted to screen the tolerance of various *Pinus taeda* families to *Leptographium terebrantis* and *Grosmannia huntii*. Bareroot and containerized seedlings from four half-sib *Pinus taeda* families were studied. Each family was assigned a random name (i.e., L109, L81, L38, and L09). The genetic distinction between these families and the original names are not disclosed to maintain confidentiality.

Seeds from all families were collected and sown within a single forest company nursery (Elberta, AL) in March 2013. Bareroot seedlings from all families were grown in an operational nursery bed and containerized seedlings were grown in 600 cm^3 containers. Seedlings were lifted from nursery beds and containers in early January 2014 and transported to the research facility at Auburn University, Auburn AL.

To minimize individual seedling variation, seedlings with 30 ± 0.5 cm average height and 4.5 ± 0.1 mm root-collar diameter (RCD) were chosen for the inoculation experiment. A total of 128 seedlings were chosen from each seedling stocktype. Seedlings were transplanted into plastic pots (16.19-cm diameter and 18.41-cm height) with peat-based potting medium (ProMix BX®, Premier Tech, Quebec, Canada) and grown in an outdoor growing area. The study design was a randomized complete block with six blocks (figure 1). Soil water was regularly monitored and the pots were watered to meet the volumetric content of each pot (V/V: 0.28).

Two months after transplanting, seedling mortality, RCD, and height of seedlings were measured. Then, seven randomly selected seedlings from each family/stocktype combination per treatment in each block were inoculated with one of three treatments. Single isolates of *Leptographium terebrantis* and *Grosmannia huntii* were used as the two fungal treatments and sterile agar plugs were used as the control treatments. The

Figure 1. Randomized blocks with bareroot and containerized seedlings transplanted in an outdoor research facility of Auburn University at Auburn, AL. (Photo by Pratima Devkota, 2014)

fungal isolates were originally from the roots of declining *Pinus* stands from the southern United States, as described by Eckhardt et al. (2007). The fungal isolates were cultured in 2 percent malt extract agar (MEA) plates for 14 days prior to the inoculation experiment.

To perform the inoculation (figure 2), a 1-cm vertical flap of bark was cut with a sterile razor blade in the seedling stem 2 cm above the soil line (Devkota and Eckhardt 2018). Then, a 3-mm agar plug with actively growing fungi (fungus side down) was inoculated in the wound. To prevent the desiccation of the agar medium, the inoculation point was covered with a moist cotton ball and wrapped with Paraflim®.

Seedling mortality, RCD, and seedling height were evaluated 8 weeks after inoculation. Then the individual seedlings were clipped above the soil line and placed in a bucket filled with 0.25 g Fast-Green dye (FastGreen

Figure 2. Artificial inoculation of fungal mycelial plug in the stem wound. (Photo by Pratima Devkota, 2014)

Figure 3. Dark necrotic lesions in *Pinus taeda* seedling stems inoculated with *Leptographium terebrantis*. (Photo by Pratima Devkota, 2014)

FCF; Sigma Chemical Co., St. Louis, MO) mixed in 1 L of deionized distilled water. To allow the dye to translocate throughout the stem, seedlings were left upright in the stain mix for 48 hours. After 48 hours, the bark around the inoculation point was carefully scraped until the necrotic tissue was observed and lesion and occlusion length were measured with a digital caliper. The vertical length of the dark dead necrotic tissue (figure 3) and the occluded tissue not taking up the dye was regarded as lesion and occlusion length, respectively. Two 2-mm sections of the stem tissue around the lesion was plated on MEA containing 800 mg L^{-1} of cycloheximide and 200 mg L^{-1} of streptomycin sulfate medium to confirm re-isolation of the inoculated fungus.

Seedling height growth was calculated by subtracting seedling height before inoculation from height during harvest. Similarly, seedling diameter growth was calculated by subtracting seedling RCD before inoculation from RCD during harvest. Data were analyzed using a general linear model (GLM) in SAS statistical software (SAS Institute, 9.4 versions, Cary, NC). Assumptions of normality and equal variance were satisfied. Pair-wise comparisons between the stocktypes were performed using the Post Hoc Tukey's test at $\alpha = 0.05$.

Results

Seedling mortality 2 months after transplanting and prior to the fungal inoculation differed significantly between stocktypes (5 and 30 percent of bareroot and container seedlings, respectively). There was, however, no further seedling mortality 8 weeks after the fungal inoculation. Dark-brown, necrotic lesions and vascular occlusions were observed in the inoculated seedling stems. *Leptographium terebrantis* and *Grosmannia huntii* were re-isolated from 90 and 92 percent of the inoculated seedlings, respectively.

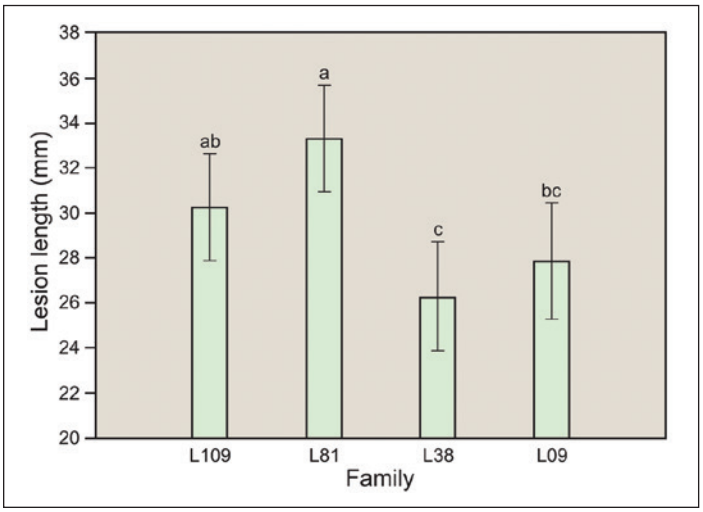

Figure 4. Lesion length caused by *Leptographium terebrantis* and *Grosmannia huntii* in seedlings from the four different *Pinus taeda* families. Different letters indicate Tukey's Honest Significant Differences among different families at $\alpha = 0.05$.

At first, the model was fitted to the data with three treatments (two fungi and one control). Tukey's multiple comparison test revealed that the lesion and occlusion length in the control seedlings did not occur beyond the inoculation point. Also, when compared to the fungal treatments, the lesion and occlusion length in seedlings receiving control treatments were significantly shorter. Thus, the model was fitted again with the two fungal treatments and without control (Devkota et al. 2018a).

The lesion length caused by two fungi varied among the four *Pinus taeda* families ($F_{(3,498)} = 5.8339$, $P = 0.0064$) (figure 4). *Grosmannia huntii* caused a relatively longer lesion than *Leptographium terebrantis* ($F_{(1,498)} = 21.085$, $P < 0.0001$) (figure 5). Overall, the lesions caused by the two inoculum treatments did not differ significantly between bareroot and containerized seedlings ($F_{(1,498)} = 1.0964$, $P = 0.29556$) or within each family ($F_{(3, 498)} = 1.2358$, $P = 0.29604$). Also, there was no significant three-way interaction among family, fungi, and stocktype. There was no variation in lesion length of bareroot and container-grown seedlings from the same family to *L. terebrantis* and *G. huntii* ($F_{(3,498)} = 0.178.08$, $P = 0.91125$).

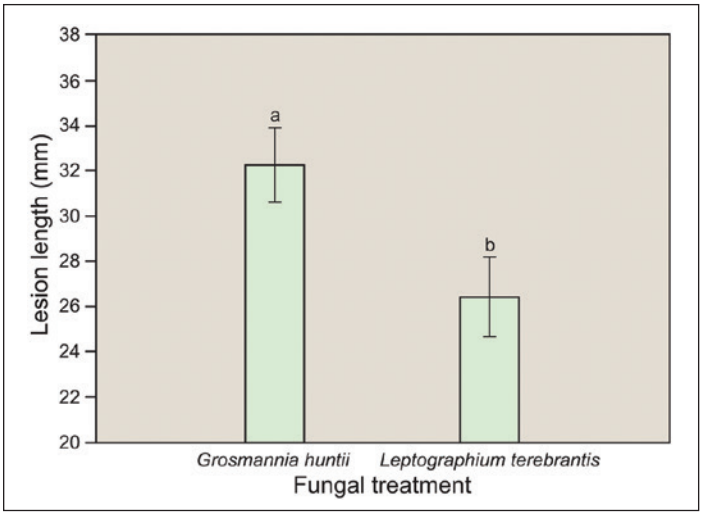

Figure 5. Lesion length caused by *Grosmannia huntii* and *Leptographium terebrantis* in *Pinus taeda* seedlings. Different letters indicate Tukey's Honest Significant Differences between two fungal treatments at $\alpha = 0.05$.

Overall, the occlusion length was significantly different among the four families ($F_{(3,477)} = 6.0584$, $P = 0.00047$; figure 6). There was, however, family and fungal interaction ($F_{(7,477)} = 17.384$, $P = 0.00004$). *Grosmannia huntii* caused significantly longer occlusion lengths compared with *Leptographium terebrantis*. Overall occlusion length did not differ significantly between the two seedling stocktypes within each family ($F_{(3,477)} = 0.06565$, $P = 0.97805$). Seedlings in the family L81 and L38 had the longest and shortest occlusion lengths, respectively.

Diameter growth differed significantly among the *Pinus taeda* families ($P < 0.00001$). Family L109 and L38 had the highest and lowest RCD increment, respectively. Both bareroot and containerized seedlings from family L09 had the highest RCD increment as compared to seedlings from other families. Diameter growth was significantly higher in containerized seedlings compared with bareroot seedlings ($P = 0.00432$; figure 7a).

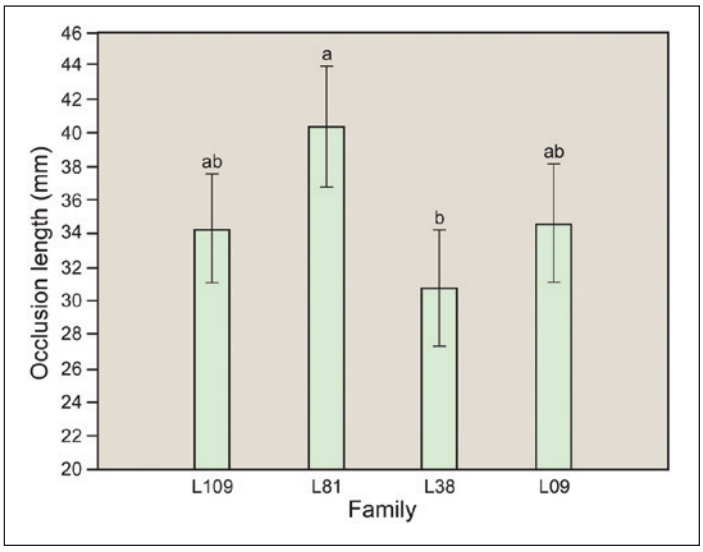

Figure 6. Occlusion length caused by *Grosmannia huntii* and *Leptographium terebrantis* in bareroot and containerized *Pinus taeda* seedlings. Different letters indicate Tukey's Honest Significant Differences among four families at $\alpha = 0.05$.

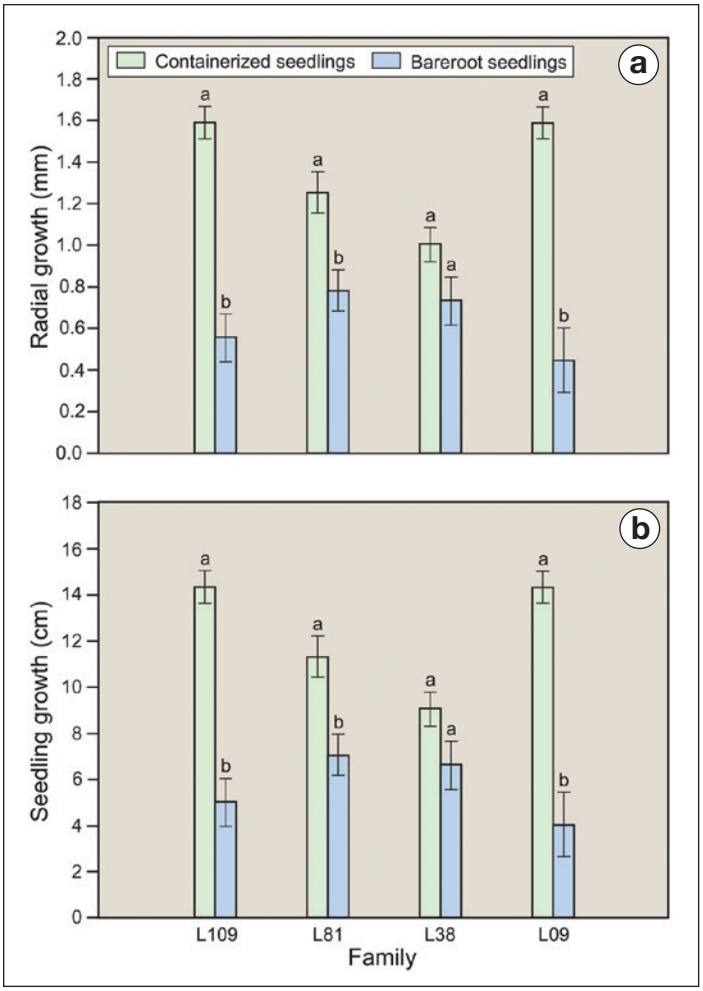

Figure 7. (a) Diameter and (b) height growth of bareroot and containerized seedlings from various *Pinus taeda* families. Different letters indicate Tukey's Honest Significant Differences between two seedling stocktypes at $\alpha = 0.05$.

Overall, seedling height growth differed significantly among the four families. Family L109 had the most growth, whereas L38 had the least growth. Height growth of seedlings inoculated with fungi was not significantly different from the control seedlings ($P = 0.85114$). Containerized seedlings for three of the four families had more growth compared with the bareroot seedlings ($P < 0.00001$; figure 7b).

Discussion

Our results show that intraspecific variation in tolerance of *Pinus taeda* to ophiostomatoid fungi is independent of seedling stocktype. Both seedling stocktypes from the same family responded similarly to *Leptographium terebrantis* and *Grosmannia huntii* (in terms of lesion and occlusion length). Therefore, either containerized or bareroot seedlings from each family may be utilized in screening *P. taeda* families to these fungi in the future screening studies. Our results suggest that this variation in susceptibility of *Pinus taeda* families to these fungi observed in different trials of Singh et al. (2014) may not be attributed to the stocktype differences but may be due to genotype x environment interaction. Containerized seedlings had more height and diameter growth than bareroot seedlings and less mortality in the 2 weeks after transplanting. Therefore, containerized seedlings may be a better choice for susceptibility screening though bareroot seedlings can also be used to accommodate necessary sample sizes.

Development of necrotic lesions and occlusions in the seedling root collar area 8 weeks following fungal inoculation serves as a reliable estimate of the host susceptibility (Matusick et al. 2010). The sizes of lesions and occlusions determine the susceptibility and tolerance of conifer hosts to the ophiostomatoid fungal pathogen. The fungal inoculation in *Pinus* spp. induces production of ethylene, which further regulates monoterpene production and influences lesion and occlusion formation (Devkota et al. 2018c, Paine et al. 1997, Popp et al. 1995). Accumulation of a high level of monoterpene causes heightened host response and longer lesion and occlusion. The trees with larger necrotic lesions have a greater decline in phloem non-structural carbohydrates and sapwood lipids. Larger lesions and occlusions cause disruption of conductive xylem tissue, decline in the radial growth, and tree mortality (Joseph et al. 1998, Krokene et al. 2008, Oliva et al. 2014).

Our study has some limitations. Early seedling performance in the weeks following infection may not necessarily be a predictor of longer term seedling performance on an outplanting site. Therefore, future studies should include field evaluations of both stocktypes. In addition, our conclusions were based only on four families of *Pinus taeda*. Therefore, future studies should focus on other *P. taeda* families as well.

Family genetics, but not the seedling stocktype, of *P. taeda* appears to be an important factor in disease development and plant tolerance to ophiostomatoid fungi. Thus, both containerized and bareroot seedling stocktypes can be used interchangeably in screening studies such as the one described in this article. Deployment of these *P. taeda* families in the field, however, should consider factors such as soil

management, drought, and various other biotic and abiotic stressors associated with ophiostomatoid fungal-vectoring beetle attacks.

Address correspondence to—

P. Devkota, Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI 48824; email: devkotap@msu.edu.

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Effect of *Pisolithus tinctorius* Nursery Treatment on Long-Term Loblolly and Longleaf Pine Survival and Growth in the South Carolina Sandhills

Michelle M. Cram

Plant Pathologist, U.S. Department of Agriculture, Forest Service, Forest Health Protection, Athens, GA

Abstract

The long-term effects of artificial inoculation of southern pines with *Pisolithus tinctorius* (Pt) in the nursery were tested in a demonstration project established on the U.S. Department of Agriculture, Forest Service-Savannah River Site, South Carolina. Loblolly (*Pinus taeda* L.) and longleaf pine (*Pinus palustris* Mill.) bareroot seedlings were produced from 1987 to 1991 with either a vegetative Pt inoculum or no inoculum (NI) at Taylor Nursery, Trenton, SC. At the Savannah River Site, two sites were planted per year for a total of 10 demonstration plantings. In 1991, a containerized longleaf pine treatment was added with and without Pt spores. Survival and growth of the seedlings were monitored at planting, after 4 years, and when sites were 15 to 19 years old. The Pt inoculation of longleaf pine produced a negative effect in the survival of bareroot seedlings in two out of ten plantings after 15 years. The addition of the Pt to loblolly seedlings in the nursery increased diameter at planting for four sites; however, this 8- to 16-percent increase in size did not affect tree size or survival over time. The only positive long-term effect with artificial inoculation with Pt was an increase in overall pine survival for site 2. The containerized longleaf pine treatment, added to the last two sites planted, increased seedlings survival over the bareroot longleaf pine. The addition of Pt in containers had no effect after 15 years on longleaf pine growth or survival. Artificial inoculation of southern pines with Pt did not provide a positive effect to warrant its use for reforestation of the sandhills in South Carolina. This paper was presented at the Joint Annual Meeting of the Southern Forest Nursery Association and the Northeast Forest and Conservation Nursery Association (Pensacola, FL, July 17–19, 2018).

Introduction

In the eastern United States, interest in producing seedlings with *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt) ectomycorrhizae (figure 1) was initiated following observations of *P. tinctorius* associated with increased tree survival and growth on mine wastes (Lampky and Peterson 1963, Marx 1975). Controlled studies in the Southeast showed Pt pine seedlings on coal spoils grew better than pine seedlings with the

Figure 1. *Pisolithus tinctorius* ectomycorrhizae. (Photo by Michelle Cram, 2010)

more common ectomycorrhizal species, *Thelephora terrestris* Ehrh. Ex Fr. (Tt) (Berry 1982, Marx 1975, Marx and Artman 1979). Extreme conditions of mine spoils include high temperatures, low pH, and low organic matter. Mycorrhizal species adapted to these extreme conditions, such as Pt, can influence tree survival and growth (Danielson 1985). The increase in survival and growth of seedlings with Pt ectomycorrhizae in high heat (up to 40 ºC) in comparison to seedlings with Tt is particularly interesting to Southern reforestation, as soil temperatures can go above 40 ºC (Marx and Bryan 1971, Marx and Bryan 1975).

Several studies in bareroot nurseries found that loblolly pine (*Pinus taeda* L.) seedlings grown with mycelial Pt inoculum were often larger than seedlings grown in non-inoculated soils (Marx and Bryan 1975, Marx et al. 1976, Marx et al. 1978, Marx et al. 1979). Extensive testing of Pt inoculations in 33 nurseries on 11 different pine species found considerable variation in inoculum effectiveness, and seedling response; however, loblolly pine was often larger with the inoculant from the Institute for Mycorrhizal and Development (Marx et al. 1984). Outplanting on reforestation sites also had mixed results for both longleaf (*Pinus palustris* Mill.) and loblolly pine (Kais et al. 1981, Ruehle 1982). A high level of Pt colonization was found to be necessary for any chance of a positive growth response or survival effect on general reforestation sites (Kais et al. 1981, Marx et al. 1982, Marx et al. 1988) although high Pt root colonization does not always result in a positive effect (Berry and Marx 1980, Echols et al. 1990, Leach and Gresham 1983).

The Savannah River Site (SRS), a National Environmental Research Park located near Aiken, SC, consists predominately of upper coastal plain and sandhill physiographic provinces. This site is known to have periods of severe drought in 2 out of 10 years (Rogers 1990). An earlier study on the SRS by Hatchell and Marx (1987), found bareroot longleaf pine had better survival and growth than non-inoculated seedlings after 7 years. Loblolly pine with Pt ectomycorrhizae also had improved survival and growth, but only in the first year (Hatchell and Marx 1987). Based on these and other positive results with Pt seedlings in reforestation, the U.S. Department of Agriculture (USDA), Forest Service began a demonstration project on the SRS to operationally test the use of Pt-inoculated seedlings on 10 sites with deep sandy soils over a 5-year period. The purpose of this demonstration project was to determine if Pt ectomycorrhizae on longleaf and loblolly pine could improve tree survival and growth. The demonstration plantings (figure 2) were measured yearly over 6 years and the 4th year (5th year for site 5) data was reported by Cram et al. (1999). In 2007, a final measurement was taken of all 10 demonstration plantings at ages 15 to 19, and the survival and volume of longleaf and loblolly were compared (Cram et al. 2010). The purpose of this paper is to present the effects of the nursery-applied Pt mycorrhizae treatment over the long term.

Methods

Bareroot (1+0) loblolly pine and longleaf pine seedlings for the 10 demonstration plantings on the SRS were produced at the South Carolina Forestry

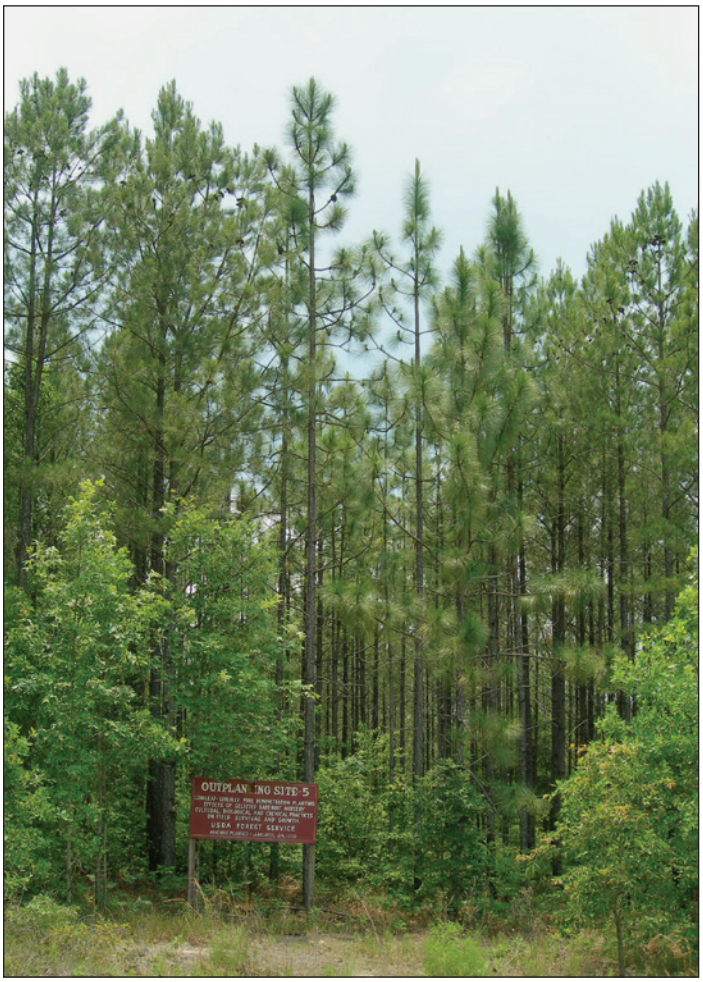

Figure 2. Demonstration site 5 on the Savannah River Site. (Photo by Michelle Cram, July 2007)

Commission's Taylor Nursery in Trenton, SC, to be outplanted from 1988 to 1992. The last year of seedling production also included container (98.32 cm^3) longleaf pine seedlings produced at the South Carolina State Creech Seed and Orchard-Container facility in Wedgefield, SC. Loblolly pine seed was sourced from genetically improved lots from the coast of South Carolina. The longleaf pine seed was from South Carolina and Georgia. The following method descriptions are taken from a more detailed description of the seedling production, inoculation, and planting documented in Cram et al. (1999).

Seedling Production and Inoculation

Bareroot seedling production and inoculation were applied in the same manner every year. Spring fumigation with 98-percent methyl bromide (394 kg/ha) was used prior to inoculation and sowing. The Pt inoculum was from a Georgia isolate that originated from loblolly pine and was produced as a vegetative mycelial product from Sylvan Spawn Labs in 1987 and from Mycorr Tech, Inc. in 1988–1991. Vegetative Pt inoculum was applied to loblolly and longleaf seedbeds at a rate of 0.28 l/m^2 prior to sowing. Pt inoculum for containerized longleaf pine in 1991 came from Pt fruiting bodies collected the previous year at Taylor Nursery from inoculated beds. The spores were applied at a rate of 0.5 g/1000 to emerging seedlings. Control beds or containers (1991 longleaf only) were not inoculated (NI), allowing for naturally occurring nursery mycorrhizae (predominately *T. terrestris*) to eventually develop.

All bareroot seedlings were laterally root pruned in early August and again in October. Before lifting, the Pt index was determined for each seedbed (Marx et al. 1984). The index was calculated by percentage of seedlings with Pt (average percent feeder root with Pt divided by average percent of feeder root with total ectomycorrhizae). Only beds with a Pt index of 50 or greater were used for Pt plots in the demonstration plantings. The Pt index was also assessed for treated container longleaf pine seedlings. Minimum culling standards were 0.32-cm root-collar diameter (RCD) and 15-cm root length for loblolly pine seedlings, and 1.0-cm RCD and 15-cm root length for longleaf pine. Seedlings were refrigerated at 4.4 to 7.2 °C after lifting and stored for less than 5 days before outplanting to the demonstration sites.

Demonstration Sites

Demonstrations sites were limited to sites that had been clearcut the year before and had deep, sandy soils with little slope. Two sites were selected each year for a total of 10. The soil series and site preparation of each site by year planted are listed in table 1. The experimental design for each site was a randomized complete block with species by inoculation treatment replicated 8 times. All seedlings were machine planted in treatment plots, each consisting of 3 rows of 50 seedlings spaced at 1.8 by 3.0 m. In the first 8

Table 1. Planting dates, soil series, and site preparation for loblolly and longleaf pine planting sites in South Carolina sandhills.

Site	Planting date	Soil series[1]	Sand depth (ft)	Site preparation
1	January 1988	Blanton sand	3.94	Chopped, burned, and hexazinone (1.5 lb/ac)
2	January 1988	Troup sand	4.43	Chopped, burned, and hexazinone (2.5 lb/ac)
3	January 1989	Lakeland sand	6.56	Chopped, burned, and hexazinone (2.5 lb/ac)
4	January 1989	**Wagram sand** Blanton sand	1.80 3.94	Chopped and burned
5	January 1990	Blanton sand	3.94	Sheared and raked
6	January 1990	Blanton sand	3.94	Chopped, burned, and hexazinone (2.5 lb/ac)
7	January 1991	Lakeland sand	6.56	Chopped, burned, and hexazinone (2 lb/ac)
8	January 1991	**Fuquay sand** Dothan sand	1.80 0.59	Sheared, raked, and hexazinone (2.5 lb/ac)
9	January 1992	Blanton sand **Lakeland sand**	3.94 6.56	Burned and partially raked
10	January 1992	Troup sand	4.43	Raked

[1] Sites with two soil series – Bold letters indicate the predominate soil type (Rogers 1990)

Table 3. Effect of *Pisolithus tinctorius* nursery treatment on loblolly pine growth over time.[1]

Site	Treat[2]	Diameter (cm)[3]			Height (m)		Survival (%)	
		0 yr	4 yr	16–19 yr	4 yr	16–19 yr	4 yr	16–19 yr
1	NI	0.36	3.7	14.5	3.29	14.32	95	92
	Pt	0.33	3.7	15.0	3.31	14.87	97	91
2	NI	0.41b	4.5	14.8	3.61	13.53	95b	81
	Pt	0.46a	4.5	15.1	3.76	13.62	99a	91
3	NI	0.33b	2.7	15.7	2.58	13.62	96	44
	Pt	0.36a	2.7	15.3	2.57	12.92	92	49
4	NI	0.36b	3.8	17.5	3.08	15.82	90	81
	Pt	0.40a	3.7	17.6	3.02	16.00	90	86
5	NI	0.37b	5.0b	15.3	3.82	13.62	90	78
	Pt	0.44a	5.2a	15.6	3.95	13.75	90	73
6	NI	0.43	3.2	14.5	2.88	13.44	90	81
	Pt	0.48	3.4	15.2	3.04	13.23	89	82
7	NI	0.43	1.7b	12.6	2.09b	10.48	91	84
	Pt	0.43	2.0a	13.2	2.23a	11.03	89	85
8	NI	0.42	3.2	15.9	2.86	13.47	93	60
	Pt	0.45	3.4	15.4	2.93	13.75	93	66

[1] Treatments within a site followed by a different letter are significantly different at the 0.05 level.
Data in years 0–4 taken from Cram et al. (1999); data in years 15–19 associated with Cram et al. (2010).

[2] Treatments = *Pisolithus tinctorius* (Pt) and not inoculated (NI).

[3] Diameters measured at the root collar year 0; all other diameters measured at breast height (DBH).

sites, the treatments consisted of longleaf and loblolly pine with artificial Pt inoculation or an NI control. Sites 9 and 10, had an additional pine species treatment of containerized longleaf pine. Although the first 8 sites were designed to include postplant herbicide with sulfometuron-methyl as an additional treatment in the study, only sites 3 and 4 received the treatment, which was applied in March 1989.

Data were collected on the middle row of each treatment plot at each site. Seedlings were measured at planting for RCD (excluding sites 9 and 10), and during the dormant season on the 4th year after outplanting (5th year on site 5) for diameter at breast height (DBH), total height, and survival. In 2007, a final measurement of all 10 sites (15–19 years since planting) was conducted prior to a planned thinning. None of the herbicide plots were included in the 2007 measurement. Final measurements consisted of DBH of all trees in the center row of treatment plots and height of every fifth live tree without a broken top. Trees with broken tops were skipped and the next live unbroken tree was measured for height instead.

Statistical Analysis

Data taken at planting and in the 4th year after outplanting were analyzed as described in Cram et al. (1999). Direct comparison of pine species was not done due to the grass stage of longleaf pine; therefore, the analysis of variance was within site and species. The plots designated for herbicide treatment that did not receive any application (sites 1, 2, 5, 6, 7, and 8) were treated as within-block replication. A 2 by 2 factorial analysis was used on data from sites 3 and 4 that received a postplant herbicide application. An analysis of covariance was also used to determine if initial RCD affected subsequent growth. On sites 9 and 10, longleaf pine was analyzed as a 2 by 2 factorial with contrasts due to the addition of the longleaf container treatment.

The data collected in 2007 were analyzed as described in Cram et al. (2010). A linear, mixed-model approach was used to analyze the DBH, height, and survival for each site. Significant differences were at the critical level $\alpha = 0.05$. The blocks were treated

as random effects, while the mycorrhizae and tree species were treated as fixed effects. The container longleaf pine was included as a species treatment for sites 9 and 10 and a Bonferroni correction was used to adjust each pairwise comparison to test at the 0.05/3 = 0.0167 level.

Results

Loblolly Pine

Pt inoculation of loblolly pine in the nursery resulted in 8- to 16-percent larger initial RCD at 4 out of 8 sites (table 2). This larger RCD at planting only persisted on one site after 4 years. Of those without an initial treatment difference in RCD, only inoculated loblolly pine on site 7 developed larger RCD after 4 years. After 16 to 19 years, there were no treatment effects on height, diameter, or survival for loblolly pine on any site (table 2).

Longleaf Pine

Pt inoculation of longleaf pine resulted in smaller RCD at planting on two sites (table 3). After 4 years, the RCD and height of inoculated longleaf pine was significantly less than the NI seedlings at sites 1 and 6. Four-year survival of inoculated longleaf pine was lower than NI seedlings on sites 6, 7, and 8 but higher on site 2. After 16 to 19 years, there were no significant treatment effects on any site (table 3).

Container and Bareroot Comparison

Seedling diameter of bareroot and container longleaf and loblolly pine on sites 9 and 10 were not affected by Pt inoculation in the nursery after 4 or 15 years (table 4). In the 4th year, height was greater for Pt containerized longleaf in site 9 and NI bareroot longleaf in site 10. No height differences were associated with the mycorrhizae treatments by the 15th year. In the 4th year, survival was significantly less for Pt bareroot longleaf than the NI bareroot at both sites.

Table 3. Effect of *Pisolithus tinctorius* nursery treatment on longleaf pine growth over time.[1]

Site	Treat[2]	Diameter (cm)[3]			Height (m)		Survival (%)	
		0 yr	4 yr	16–19 yr	4 yr	16–19 yr	4 yr	16–19 yr
1	NI	1.02a	6.0a	13.3	1.99a	14.36	90	87
	Pt	0.92b	5.8b	13.0	1.71b	13.66	91	84
2	NI	1.00	5.6	13.3	1.50	13.41	81b	77
	Pt	1.01	5.9	13.2	1.64	13.13	92a	88
3	NI	1.09a	4.7	12.6	1.17	12.19	88	76
	Pt	1.03b	4.5	11.7	1.10	12.01	88	84
4	NI	1.15	5.3	14.5	1.42	14.51	84	79
	Pt	1.19	5.4	13.9	1.47	14.26	89	79
5	NI	1.08	3.4	12.8	2.27	12.86	82	70
	Pt	1.01	3.3	12.5	2.23	12.13	81	74
6	NI	1.13	6.0a	13.3	1.72a	12.71	76a	72
	Pt	1.10	5.7b	13.2	1.55b	12.89	70b	64
7	NI	1.02	4.6	11.6	0.91	11.00	72a	71
	Pt	1.04	4.7	11.5	0.89	10.73	62b	61
8	NI	1.08	4.8	12.8	1.08	11.83	68a	49
	Pt	1.19	4.9	13.3	1.03	11.73	48b	42

[1] Treatments within a site followed by a different letter are significantly different at the 0.05 level. Data in years 0–4 taken from Cram et al. (1999); data in years 16–19 associated with Cram et al. (2010).

[2] Treatments = *Pisolithus tinctorius* (Pt) and not inoculated (NI).

[3] Diameters measured at the root collar year 0; all other diameters measured at breast height (DBH). Significant difference at the 0.05 level between the Pt and NI treatments within a site.

Table 4. Effect of *Pisolithus tinctorius* nursery treatment on growth over time for site 9 and 10.[1]

Site	Species (culture)	Treat[2]	Diameter (cm)[3]		Height (m)		Survival (%)	
			4 yr	15 yr	4 yr	15 yr	4 yr	15 yr
9	Longleaf (container)	NI	4.0	11.1	0.60b	10.37	90	87a
		Pt	4.4	12.0	0.79a	11.00	85	83a
	Longleaf (bareroot)	NI	3.7	11.6	0.54	10.83	65a	63b
		Pt	3.5	12.3	0.54	10.64	45b	43c
	Loblolly (bareroot)	NI	1.3	14.0	1.74	11.68	68	67b
		Pt	1.5	14.3	1.89	11.89	75	73ab
10	Longleaf (container)	NI	4.1	12.4	0.77	11.02	83	75a
		Pt	4.4	12.0	0.93	10.86	80	74a
	Longleaf (bareroot)	NI	4.1	12.8	0.78a	11.58	57a	51b
		Pt	3.9	12.8	0.62b	10.84	37b	35c
	Loblolly (bareroot)	NI	2.2	14.9	2.35	12.65	87	79a
		Pt	1.8	14.3	2.14	12.20	85	79a

[1] In the 4th year data (Cram et al. 1999) treatments within a site and species (culture) followed by a different letter are significantly different at the 0.05 level; in year 15 (Cram et al. 2010) treatments within a site followed by a different letter were significantly different at the Bonferroni adjusted 0.05/15 = 0.0033 level.

[2] Treatments = *Pisolithus tinctorius* (Pt) and not inoculated (NI).

After 15 years, survival of bareroot longleaf pine was significantly lower than container longleaf pine seedlings at both sites regardless of mycorrhizae treatment. Furthermore, inoculated bareroot longleaf pine had significantly lower survival than the NI bareroot longleaf pine (table 4).

Overall, data from the 10 demonstration sites showed only one site with a positive Pt treatment effect (tables 2 and 3). Although analysis of mycorrhizal treatment by individual pine species showed no overall survival effect, survival on site 2 was increased 10 percent (P = 0.016) with Pt inoculation (Cram et al. 2010).

Discussion

Success of nursery treatments for improving reforestation are rarely monitored long term. The 10 demonstration plantings on the SRS were unique in that Pt treatments in the nursery were subsequently monitored for 15 to 19 years after outplanting (Cram et al. 1999). Hatchell and Marx (1987) tested Pt as a nursery treatment to improve longleaf pine seedling establishment on the sandhills of South Carolina over a 7-year period. In a similar study, Marx et al. (1988) monitored loblolly pine with a Pt index greater than 58 for 8 years. These previous studies indicate that it is possible for nursery-applied Pt to have significant positive effects on survival and growth of longleaf and loblolly pine over the long term. The long-term results from these 10 demonstration plantings, however, revealed only one positive outcome in overall survival with the Pt treatment. All other individual positive effects at planting or after 4 years did not persist.

Large-scale testing of a mycorrhizal treatment is particularly important because of the symbiotic interaction between fungus and plant. Mycorrhizal fungi require carbohydrates from the host (Corrêa et al. 2006, Cairney and Chambers 1997) and the host obtains benefits from the mycorrhizal fungus, such as increased uptake of nutrients and moisture, which offsets the loss of photosynthate (Dosskey et al. 1990). This balance can change under different environmental conditions of drought and nutrient availability (Cairney and Chambers 1997). Most of the sites selected for the 10 demonstration plantings were on deep, sandy soils and expected to be drought prone (Rogers 1990); however, the monthly precipitation on the SRS during and following planting on the 10 sites did not indicate the occurrence of drought, and thus, could not be correlated with seedling performance (Cram et al. 1999). Soil depth was found to significantly affect height growth of both pine species, but there was no interaction with the mycorrhizae treatment (Cram et al. 2010). One factor that was thought to affect survival

of bareroot longleaf pine was the less-intensive site preparation, especially on sites 9 and 10, which can affect proper planting depth (Boyer 1988). Container longleaf pine are less affected by negative environmental conditions than bareroot stock (Boyer 1988, South et al. 2005); therefore, the relatively high survival rates of container longleaf on sites 9 and 10 were not surprising. Survival results indicate that bareroot longleaf seedlings were under greater stress than container seedlings, and the presence of Pt on these stressed seedlings had a negative effect. The Pt treatment appeared to act as a carbon sink for stressed seedlings, with little or no positive counterbalance, resulting in a significant loss.

Many other studies have demonstrated negative effects from artificial mycorrhizal inoculation when there is no counterbalance to the carbohydrate usage (Castellano and Trappe 1991, Corrêa et al. 2006, Dosskey et al. 1990, Echols et al. 1990). Individual isolates of Pt can have different levels of compatibility with host species, such that an isolate that performs well on one host could be less well-suited to another (Cairney and Chambers 1997, Marx 1981, Walker 2001). An example of this phenomenon is in a study by Marx (1981), where a Pt isolate (Georgia 227) colonized loblolly pine seedlings at high levels, but not longleaf pine. The commercially used Georgia Pt isolate had been tested for a wide range of host colonization (Marx 1981, Marx et al. 1984), but some species were not optimal hosts, as demonstrated by Castellano and Trappe (1991) with western conifers. The more negative than positive results with Pt inoculation of longleaf pine in our demonstration study might be the result of a less-than-optimal symbiotic relationship.

The results from the 10 demonstration sites show that, under operational conditions, the positive result of applying Pt to longleaf pine reported by Hatchell and Marx (1987) was not a typical outcome. Pt inoculation cannot be recommended for longleaf pine. Although Pt inoculation of loblolly pine had some early positive effects, these effects were lost after 15 or more years. The lack of a long-term effect with Pt inoculation of loblolly is similar to results obtained by other researchers (Echols et al. 1990, Leach and Gresham 1983). An earlier study of Pt-inoculated loblolly pine on the Savannah River Site also failed to show differences due to the natural colonization of the control seedlings by Pt within the first year of planting (Berry and Marx 1980). Mycorrhizal colonization of seedling roots were only examined prior to planting on the demonstration sites. Although we do not know the ectomycorrhizal species present on seedlings after planting, native mycorrhizae on a reforestation site would be expected to be present and colonizing new root tips (Miller at al. 1994, Pilz and Perry 1984, Tainter and Walstad 1977). The colonization of new roots by naturally occurring mycorrhizal fungi can occur within weeks of planting (Tainter and Walstad 1977), resulting in no growth differences between treatments over time.

The use of Pt inoculated loblolly and longleaf pine seedlings was found to be unnecessary for successful reforestation of the South Carolina sandhills (Cram et al 1999, Cram et al. 2010). In most cases, the presence of native mycorrhizal fungi in reforestation sites will make artificial inoculation of seedlings unlikely to provide enough positive effects to warrant the cost of the treatment. In harsh environments, especially where topsoil has been removed, the use of a particular mycorrhiza could make a sufficient difference to justify its use. This has recently been found to be true in other countries, such as China and Mexico, where Pt-inoculated seedlings performed well on abandoned mine sites (Gómez-Romeroa et al. 2015, Zong et al. 2015). In 1977, the Federal Surface Mining Control and Reclamation Act initiated changes in restoration of mined land that included replacing topsoil to cover the acid mining spoils. These changes created a less harsh environment for plants, and a quick return of mycorrhizae species diversity and population levels (Gould and Hendrix 1998), thus reducing the need for artificially inoculated seedlings. The use of Pt-inoculated seedlings in the United States is likely to be rarely justified as the cost outweigh the benefits.

Address correspondence to—

Michelle Cram, USDA Forest Service, 320 E. Green Street, Athens, GA 30602; email: michelle.cram@usda.gov; phone: (706) 559-4233.

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Questions and Considerations for the Next Generation of Seedling Fertilization Researchers

David B. South

Emeritus Professor, School of Forestry and Wildlife Sciences, Auburn University, AL

Abstract

Although 20th century researchers published numerous fertility trials, only a few bareroot nursery studies have been installed since 2000. Most seedling nutrition publications during the past 5 decades have involved either container-grown stock or stock grown in greenhouses. The next generation of researchers might consider testing old theories about bareroot nursery fertilization. Some long-held claims about nursery fertilizers were apparently based on faulty logic, while others were based solely on hydroponic research. This paper provides some questions that should be addressed by the next generation of researchers who choose to follow the scientific method. This paper was presented at the Joint Meeting of the Northeast Forest and Conservation Nursery Association and Southern Forest Nursery Association (Pensacola, FL, July 17–19, 2018)

Introduction

My first experiences with nursery fertilization trials began in 1973 as a graduate student at North Carolina State University. After I published a few papers (South and Davey 1983, Boyer and South 1985), I was confident that I knew something about fertilizers. The more I talked with nursery managers, however, the more I realized there was a lot I didn't know. I began to question some of the assumptions found in textbooks. The more I learned about problems with soil test interpretation and growing seedlings, the more questions I asked. For example, why do we rely so much on assumptions and opinions instead of relying on the scientific method? Why did we assume some nitrogen (N) and potassium (K) should be applied before sowing seed? Why did some say the optimum pH for growing hardwoods is pH 6 to 7? Why were these theories taken as facts? Why didn't anyone question some of the unfounded claims? After listening closely to first-hand experience provided by wise nursery managers, I realized there is a big difference between "book learning" and a "real world" nursery experience.

When questions about fertilizer practices are not answered, myths, mistakes, and stagnation will prevail. As a result, some 50-year old practices are still used because of tradition (figure 1). For example, it was

Figure 1. After sowing, some managers apply granular fertilizers (left) using equipment similar to that used during the first half of the 20th century. In contrast, about 87 percent now prefer to apply nutrient solutions using soluble fertilizers (right). As a result, some managers use granular diammonium phosphate (18-46-0) to stimulate seedling growth while others spray liquid polyphosphate (10-34-0). Due to a lack of solid scientific evidence, it is not known which method produces a more rapid growth response. (Photos by Warren Bryant and Michael Neel, 2018)

Table 1. A selected list of 52 reforestation nurseries in the Southern United States (2018) including location and initial year of production. Nurseries with an asterisk are members of the Southern Forest Nursery Management Cooperative.

State	Nursery	City	Stock type	Year	Ownership
Alabama	Selma*	Selma	Bareroot	1974	ArborGen
	White City	Verbena	Bareroot	1980	Summit
	Pine Hill*	Camden	Bareroot	1980	IFCO
	Elberta*	Elberta	Both	1991	Rayonier
	Westervelt*	Tuscaloosa	Container	1981	Westervelt
	Atmore	Atmore	Container	2017	PRT
Arkansas	Baucum*	North Little Rock	Bareroot	1958	State of AR
	Bluff City*	Bluff City	Bareroot	1980	ArborGen
	Magnolia*	Magnolia	Bareroot	1972	Weyerhaeuser
Florida	Buckeye	Perry	Bareroot	1956	Private
	Dwight Stansel	Wellborn	Bareroot	1986	Private
	Andrews*	Chiefland	Both	1956	State of FL
	Central Florida	Mayo	Both	1984	Private
	Superior Trees	Lee	Both	1953	Private
	Labelle*	Labelle	Container	2009	IFCO
	Blanton	Madison	Container	2001	Private
Georgia	Flint River*	Byromville	Bareroot	1987	State of GA
	Shellman*	Shellman	Bareroot	1996	ArborGen
	Jesup*	Jesup	Bareroot	1956	IFCO
	Native Forest	Chatsworth	Bareroot	1978	Private
	K&L Forest*	Buena Vista	Bareroot	1999	Private
	Pinecrest	Buena Vista	Bareroot	2007	Private
	Bell Farms	Bellville	Bareroot	1988	Private
	Rutland Forest	Lenox	Bareroot	1986	Private
	Bellville*	Claxton	Both	1957	ArborGen
	Moultrie*	Moultrie	Container	2003	IFCO
	Meeks' Farms	Kite	Container	1996	Private
	Forestate Growers	Douglas	Container	2001	Private
	Lewis Taylor	Tifton	Container	1997	Private
	Whitfield	Twin City	Container	1996	Private
	Zellner Farms	Culloden	Container	2010	Private
Kentucky	John Rhody	Kentucky Dam	Bareroot	1956	State of KY
	Morgan	West Liberty	Bareroot	1961	State of KY
Louisiana	Evans*	Deridder	Container	2014	IFCO
Mississippi	Shubuta*	Shubuta	Bareroot	1981	IFCO
	Delta View	Leland	Bareroot	1987	Private
	Pearl River*	Hazlehurst	Both	1998	Weyerhaeuser
North Carolina	Claridge*	Goldsboro	Both	1954	State of NC
	Washington*	Washington	Both	1970	IFCO
	Linville River*	Linville	Container	1970	State of NC
	Bodenhamer	Rowland	Container	2000	Private
Oklahoma	Engstrom*	Goldsby	Both	1947	State of OK
South Carolina	Blenheim*	Blenheim	Bareroot	1983	ArborGen
	Quail Ridge*	Aiken	Bareroot	1985	Weyerhaeuser
	Taylor*	Trenton	Both	1959	State of SC

Table 1 ***(continued)***

State	Nursery	City	Stock type	Year	Ownership
Tennessee	East Tennessee*	Delano	Bareroot	1989	State of TN
Texas	Bullard*	Bullard	Bareroot	1982	ArborGen
	Caddo*	Jasper	Bareroot	1976	TX Timber
	West Texas	Idalou	Container	1978	State of TX
Virginia	Augusta*	Crimora	Bareroot	1967	State of VA
	Garland Gray*	Courtland	Both	1986	State of VA

once believed that K applied in September would "promote pine seedling dormancy" (Sweetland 1978). In fact, out of the 37 bareroot nurseries in the Southern United States (table 1), about 29 still apply K in the fall to "harden off" seedlings (Starkey et al. 2015). This practice continues even though it does not "harden off" seedlings (Andivia et al. 2012, Benzian et al. 1974, Birchler et al. 2001, Bryan 1954, Dierauf 1982, Gleason et al. 1990, Hinesley and Maki 1980, Jokela et al. 1998, Rowan 1987, South and Donald 2002, South et al. 1993, Stone 1986). Unfortunately, research is of little use when it is ignored.

I have seen the origin of several other myths (Khan et al. 2014, South 1987, 2015, 2016, 2018), and I even assisted in keeping one alive for years (South 2013). It is easy to start myths, especially when applying precautionary principles to fertilization regimes and seedling quality and publishing it. Misinformation and myths can be stopped simply by asking the right questions and generating credible, scientific data. This article encourages the next generation of researchers to ask questions and test hypotheses to reevaluate unsubstantiated practices that have persisted for decades.

[Note: Except for years prior to 2000, nutrient levels mentioned in this paper were determined using the Mehlich 3 procedure. B = boron. Ca = calcium. Cu = copper. Fe = iron. kPa = kilopascal. Mg = magnesium. Mn = manganese. Mo = molybdenum. Na = sodium. P = phosphorus. S = sulfur. Zn = zinc. ppm = parts per million. CEC = cation exchange capacity. OM = organic matter.]

Researchable Questions

How Much N Is Really Needed?

Research has shown that N fertilization in the nursery affects tree growth after transplanting (Grossnickle and South 2017, van den Driessche 1991), which may explain why the application of N has increased over time (table 2). Even so, opinions can influence the rate of N fertilization. For example, some who want to avoid labor required for top-pruning believe that they can achieve this by limiting N fertilization. In contrast, those who practice top-pruning (South 1998) may apply additional N to increase wood production, perhaps as much as 14 percent at age 9 years (Jackson 2016).

N fertilizer regimes vary among nursery managers. For example, total amounts applied to pine seedbeds (over the growing season) can range from 56 kg N/ha (Kormanik et al. 1994, McNabb 1985) to 218 kg N/ha (Stone 1986) to more than 300 kg N/ha (Dierauf and Chandler 1995, Rodríguez-Trejo et al. 2003). Total amounts applied by researchers to grow pine seedlings in containers may vary by more than 700 kg N/ha (table 3). In addition, a few researchers recommend managers apply higher rates of N at the first spring fertilization than at applications made 4 to 6 weeks later (Birge et al. 2006, Timmer 1997). As a result, foliar N concentration of different genotypes and stock types vary during summer and early winter (figure 2). Although foliar N of longleaf pine (*Pinus palustris* Mill.) seedlings may be less than 1 percent when measured after September (Dumroese et al. 2005, Jackson et al. 2012, Rodríguez-Trejo and Duryea 2003, South et al. 2005), freeze tolerance is greater when levels are above 1.4 percent N (Davis et al. 2011, Dumroese et al. 2013). Growth after outplanting is also reduced when foliar N levels are

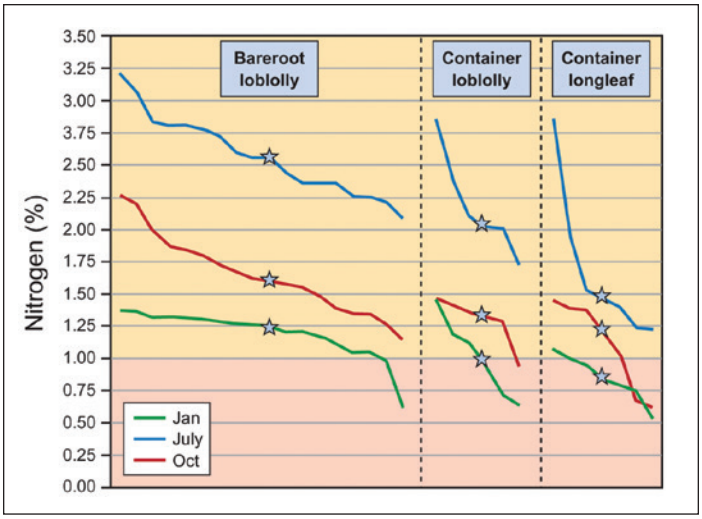

Figure 2. Foliar nitrogen (N) of pine seedlings declines over time, in part, due to carbohydrate dilution. It also varies by species, stocktype, and nursery. These data represent a range of N values in July, October, and January for 20 bareroot nurseries and 7 container nurseries, with the median value for each line marked by a star (adapted from Starkey and Enebak 2012).

below 1 percent (figure 3, Barker 2010, Jackson et al. 2012, Larsen et al. 1988). Excess N application can contribute to groundwater pollution (South 1994), while inadequate amounts can reduce seedling performance. These wide-ranging N application rates can occur due to species, soil conditions, growth stage, and target seedling specifications but can also be driven by unsubstantiated ideas about formulations, freeze tolerance, growth responses, and nutrient loading. Future research is needed to better define optimum N rates needed under varying circumstances.

Do Pines Really Need More K Than N?

Although most mineral soils contain 3,000 to 100,000 kg of K/ha (Sparks 2001), a sandy slash pine (*Pinus elliottii* Engelm.) nursery (20 cm deep) usually contains less than 200 kg/ha of available K. When soil tests indicate less than 60 kg K/ha, many managers in the Southern United States fertilize pine seedlings with more K than N (224 kg K/ha and less than 200 kg N/ha). The high use of K originated from Wilde (1958), who said a nursery soil should contain 4 times more K than N. There are no data, however, to show that pines need to be fertilized with more K than N. In fact, "some nursery researchers report that K fertilization is not needed in forest tree nurseries" (May 1984: 12-22) and others suggest K fertilization will likely not increase cover-crop yields (Khan et al. 2014).

At the time of lifting, 1-0 loblolly pine (*Pinus taeda* L) seedlings may contain 17 to 55 percent more N than K (Boyer and South 1985, Nelson and Switzer 1985). It is not clear, however, that pine seedlings need this much K to function effectively. The amount of K present in seedlings at lifting depends on how much K fertilizer is applied during the growing season and not on how much K is required for growth (Switzer and Nelson 1956). Therefore, when little or no K is applied during the growing season, seedlings lifted in January contain 100 to 300 percent more N than K (Danielson 1966, Miller et al. 1985, Sung et al. 1997, Switzer and Nelson 1956, Wall 1994). There is insufficient data to show that reducing K fertilization in the

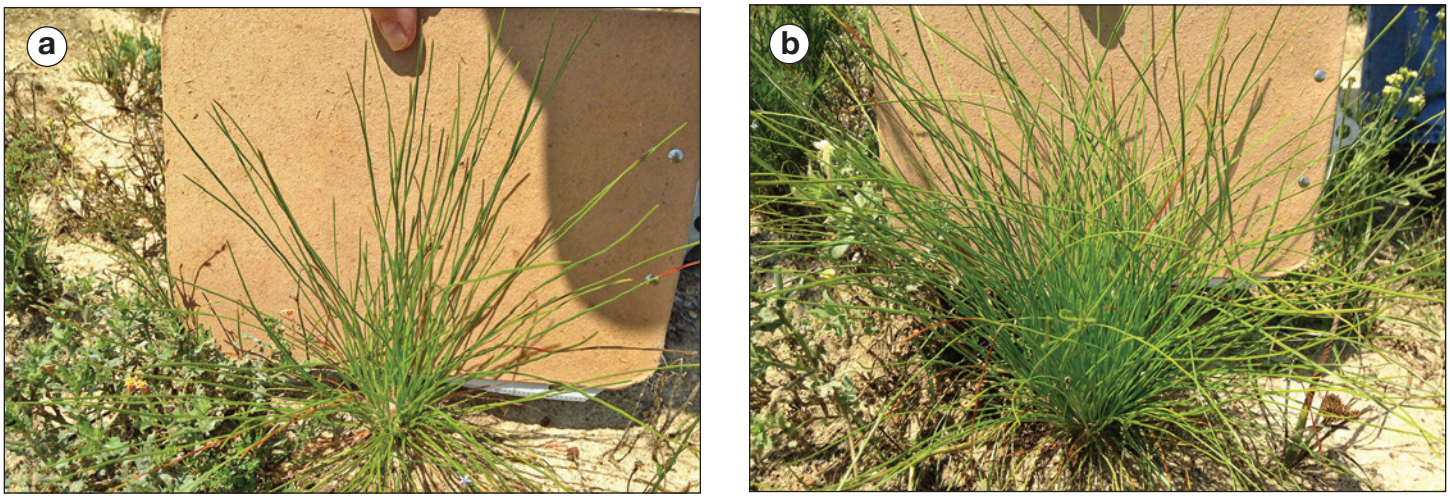

Figure 3. Both longleaf pine seedlings in these photos (June 26, 2016; 6 months after planting) were well fertilized and top-pruned multiple times in the nursery. The seedling on the right was grown with slow-release fertilizer in the container plug and therefore had about 119 percent more foliar nitrogen (N) applied than the seedling on the left (Starkey and Nadel 2017). At outplanting, the average root-collar diameter was the same (6.3 mm) for both seedlings but foliar N concentration of the seedling on the right was higher (1.5 percent) compared with the one on the left (1.2 percent). As a comparison, container-grown longleaf pine seedlings that are managed to produce short needles (that do not need to be top pruned) typically have foliar N levels in October (before outplanting) that are less than 1.0 percent. (Photos by Ryan Nadel, 2016)

Table 2. Examples of how nursery fertilizer practices for bareroot loblolly and slash pine seedlings have changed over time.

		Year:	1935	1958	1978	1998	2018	2018
		Sowing Date:	April 28	May 5	April 25	April 15	April 20	Cost
Fertilizer	Application Month		kg/ha	kg/ha	kg/ha	kg/ha	kg/ha	$/ha
6-10-7 (cover crop)	April		224					
4-10-7	April			896				
$MgSO_4$	March				112			
10-20-10	March				336			
$(NH_4)_2SO_4$	June				112			
$(NH_4)_2SO_4$	July				112			
$(NH_4)_2SO_4$	Aug				112			
KCl	Sept				112	112	112	77
$Ca(H_2PO_4)_2\ H_2O$	March					168		
$K_2Mg_2(SO_4)_3$	March					224	280	231
$(NH_4)_2NO_3$	June			56		56		
$(NH_4)_2NO_3$	July			56		56		
$(NH_4)_2NO_3$	July			56		56		
$(NH_4)_2NO_3$	Aug					100		
$(NH_4)_2NO_3$	Aug					100		
UAN 10-0-4 (4 percent S)	June-Aug						210 (10 sprays)	594
B	March					2.7	3.1	68
KCl	March						112	77
Gypsum	March						785	115
Fe - chelated	June			4.5			5	250
Cu - chelated	March						2.2	100
Zn - chelated	March						8	211
20-20-20 + micros	Summer						8 (5 sprays)	99
TOTAL N/ha			13	96	103	165	218	1,822

UAN = 50 percent urea and 50 percent ammonium nitrate

nursery has a negative effect on subsequent seedling field performance. Most reforestation sites have adequate K.

When soil K is low at time of sowing, can nursery managers fertilize conifer seedlings using an N/K ratio of 3? For loblolly pine, seedlings grew well when fertilized with a N/K ratio of 2.3 (figure 4), and a ratio of 4 resulted in maximum shoot growth in a greenhouse (Blackmon 1969). Ratios greater than 4 are sometimes used in bareroot seedbeds (table 4). Applying extra K (which decreased the N/K ratio to 1) had no effect on growth of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings (Shaw et al. 1998). Would applying only 70 kg/ha of K during the growing season (with 210 kg/ha of N) affect performance of pine seedlings? At one sandy loam nursery that contained 68 ppm exchangeable K at sowing, adding 300 kg/ha of K before sowing had no effect on seedling growth (Switzer and Nelson 1956). In another trial, irrigation leached K from the soil and yet seedling growth increased (figure 5). Early studies suggested that applying too much K to sandy nurseries "may result in a considerable loss by

Figure 4. Loblolly pine seedlings in this photo (July 25, 2018) were fertilized with 64 kg/ha of potassium (K) before sowing and received no additional K fertilization. Soil contained 24 ppm extractable K in May and 9 ppm K in October (Mehlich 3). These seedlings were fertilized with an N/K ratio of 2.3, and by October, needles contained 1.8 percent nitrogen (N) and 0.8 percent K. Assuming 10,000 kg/ha of seedlings were harvested (at 0.7 percent K for the total seedling), the amount of K removed at harvest would equal 70 kg/ha. (Photo by David South, 2018)

leaching, especially if heavy rains or excessive irrigation follow the application" (Wilde and Kopitke 1940: p. 331).

It may be that tradition, without sufficient scientific evidence, is the reason that growers apply more K than N. This practice needs to be investigated by the next generation to determine the appropriate levels of K to apply.

When Should We Apply Mg?

With the exceptions of N, Cl, Fe, Mo, and Na, soil tests may help determine when there is a need to fertilize seedbeds. "Trigger values" are used to determine when to apply P, K, Ca and Mg, but there is no consensus as to what these values should be (table 5) or how much of each element should be applied once the soil test value drops below the trigger value. For example, when a soil contains 34 ppm Mg (table 5), some experts may add Mg while others would delay fertilization until the value drops below 25 ppm. The cost of applying 35 kg of Mg (e.g., 350 kg/ha of Epsom salts) might exceed $150 per ha and, at some nurseries, this rate may result in no growth advantage (figure 6). A top-dressing rate this high might even reduce growth of some conifers (Ruter 1999). At the 25-ppm soil level, researchers have yet to report a response that justifies spending the extra time and money to apply Mg to pine seedbeds. At

Table 3. Nitrogen (N) fertilizer rates for several longleaf pine container studies. Rates assume all N applied enters the cell.

Cells/m^2	Container volume	N/cell	N/m^2	N/ha	Reference
#	cm^3	mg	g	kg	
530	95	40	21.2	212	Sung and Dumroese 2013
936	60	24	22.4	224	Dumroese et al. 2013
364	125	80	29.1	291	Davis et al. 2011
441	98	66	29.1	291	Jackson et al. 2012
581	98	63	>36.5	>365	South et al. 2005
441	98	66	38.3	383	Dumroese et al. 2005
441	98	88	38.8	388	Jackson et al. 2007
366	164	116	>42.5	>425	Sword Sayer et al. 2009
581	113	79	45.7	457	Figure 3- smaller seedling
364	164	112	59.0	590	Haywood et al. 2012
213	336	274	58.4	584	Dumroese et al. 2013
441	144	159	84.0	840	McGuire and Williams 1998
581	113	164	95.5	955	Figure 3 – larger seedling

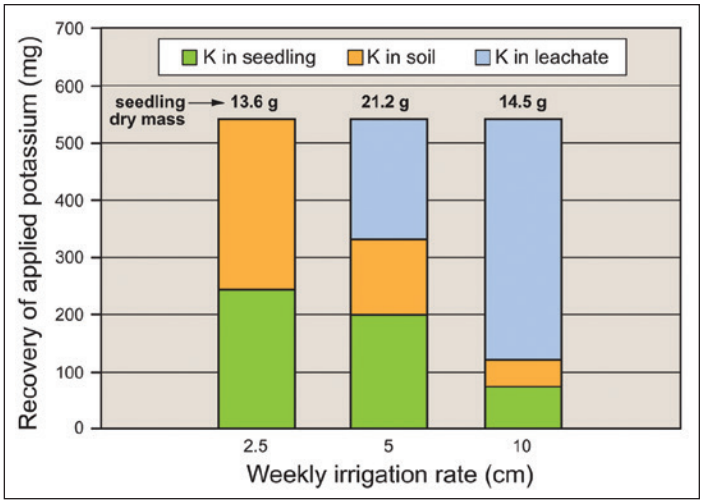

Figure 5. Irrigation rate influenced the amount of potassium (K) leached from containers filled with sand in a greenhouse. The slash pine seedlings grown with the low irrigation rate contained more K but seedlings grown with the middle irrigation rate had more growth (values above bars indicate seedling dry mass). (Adapted from Bengtson and Voigt 1962).

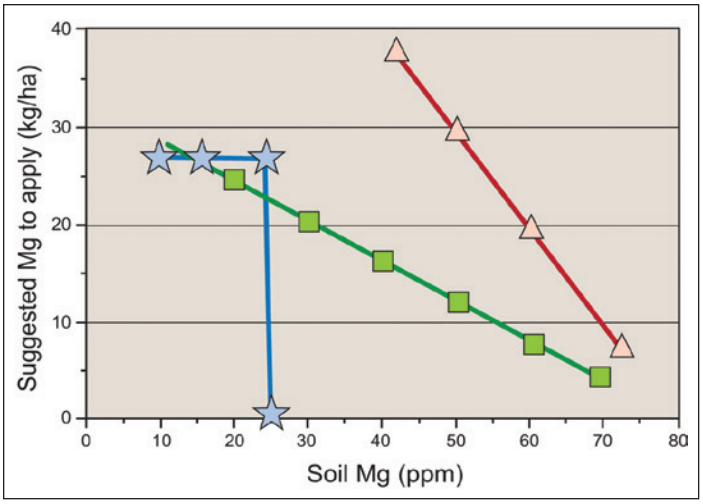

Figure 7. This figure compares three opinions as to how much magnesium (Mg – Mehlich 3) should be applied to the soil before sowing pine seed. Growth of pine seedlings is sometimes unaffected by increasing soil available Mg above 15 to 31 ppm (Edwards et al. 1991, Wall 1994). One professor (stars) recommends applying Mg only when soil tests indicate less than 25 ppm available Mg. In contrast, when soil contains more than 25 ppm available Mg, two agronomists recommend various rates of Mg. For example, when the soil Mg is 50 ppm, one agronomist (squares) recommends applying 12 kg/ha and another (triangles) recommends 30 kg/ha.

some sandy nurseries, pines have been grown in soil that contains only 8 ppm Mg (Munson 1982). Even so, some agronomists recommend adding Mg to pine seedlings when tests indicate the soil contains 50 ppm (figure 7). Clearly, there is a wide range of recommendations and there is a need for more science-based input regarding Mg fertilization.

What Is the Optimum pH for Growing Hardwood Seedlings?

In the past, bareroot hardwood seedlings were thought to grow best in soil ranging from pH 6 to pH 7 (Briggs 2008, Tinus 1980). I, however, reject that theory, since seedling mass of several species can increase when the pH drops below 5.0 compared with higher pH values (Wright et al. 1999, figure 8). Although some species grow well at pH 6 (DesRochers et al. 2003, Melhuish et al. 1990, Sparks 1977), several hardwood species grow well between pH 4 and pH 5.5 (Han et al. 2016, Hauer and Dawson 1996, Herendeen 2007, Lee and Weber 1979, Lutter et al. 2015, Ouimet et al. 1996, Rikala and Jozefek 1990, Salifu et al. 2006, South 1992, South 2019, Villarrubia 1980). "Assessment of a desirable pH range of a given species is quicker and easier than many growth factors often investigated for improving plant growth and should be one of the first factors investigated" (Bryan et al. 1989: p. 64). Hopefully, the next generation will establish empirical, species-specific trials to determine optimum nursery pH for hardwoods.

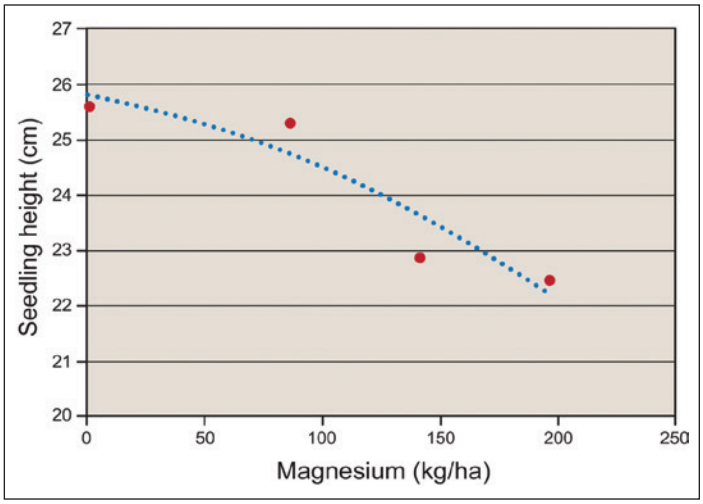

Figure 6. Effect of top-dressing of Epsom salts on height of pine seedlings at the Indian Mound Nursery in Texas (Wall 1994). A traditional F-test indicated no treatment effect ($\alpha = 0.05$).

How Much Irrigation Is Really Needed?

Insufficient irrigation can reduce seedling growth (Dierauf and Chandler 1991, Haase and Rose 1994, May et al. 1961, Pessin 1938, Shi et al. 2018, Williams et al. 1988). Likewise, excessive soil moisture for too long reduces seedling growth (Bengtson and Voigt 1962, Retzlaff and South 1985, South and Carey 1999, South and Starkey 2010). When managers use the precautionary principle, overirrigation can occur

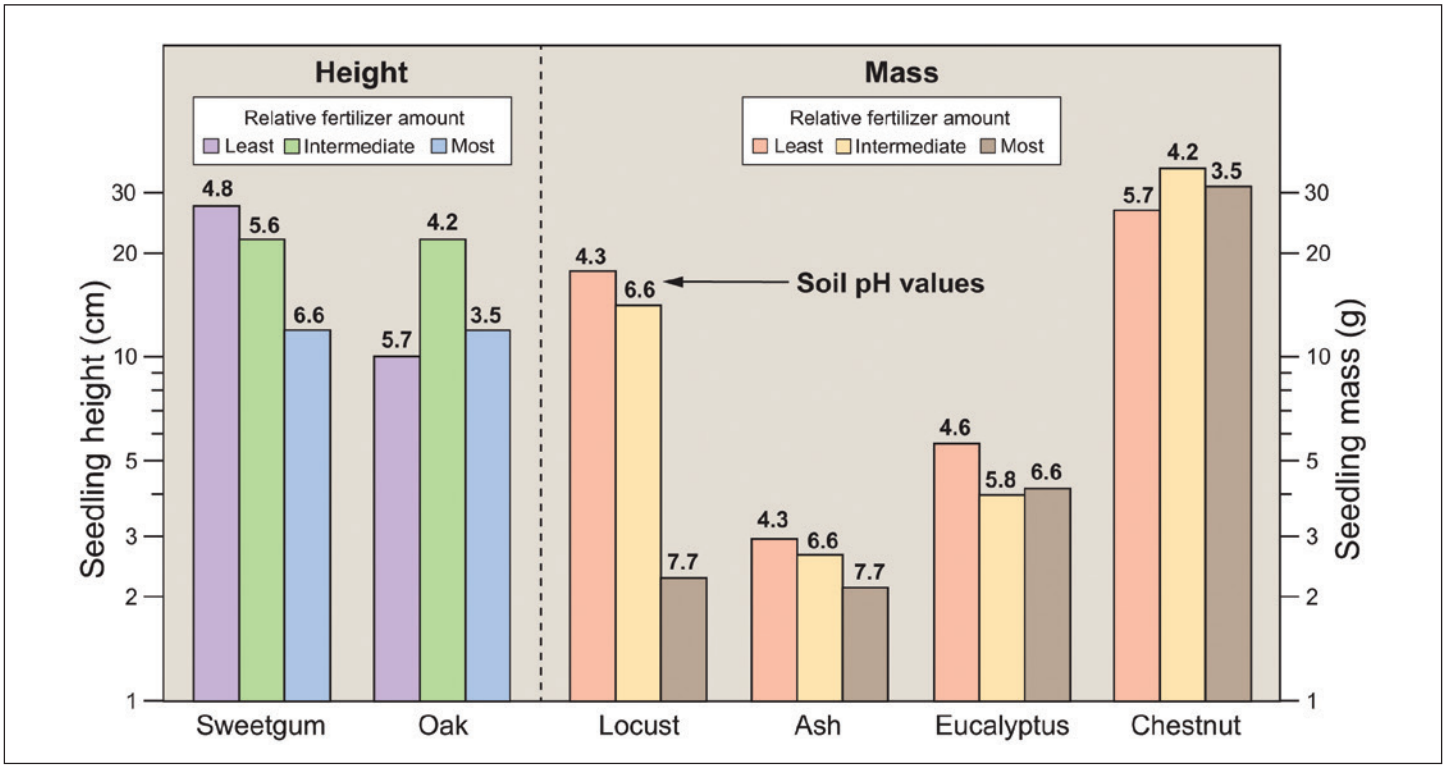

Figure 8. Growth of oak (*Quercus rubra* L.) and chestnut (*Castaneta dentata* Mill.) can be reduced by adding too much aluminum sulfate (see bars with pH < 3.6) while too much lime (see bars with pH > 6.0) can reduce growth of sweetgum (*Liquidambar styraciflua* L.), black locust (*Robinia pseudoacacia* L.), green ash (*Fraxinus pennsylvanica* Marshall) and *Eucalyptus urophylla* S. T. Blake). Absolute amounts of aluminum sulfate (for studies with oak and chestnut) and lime were reported by Yawney et al. 1982 (sweetgum), Davis 2003 (oak), McComb and Kapel 1942 (locust and green ash), Aggangan and Malajczuk 1996 (Eucalyptus), and Herendeen 2007 (chestnut).

in both container nurseries (Dumroese and Haase 2018) and bareroot nurseries (Johnson 1986, Retzlaff and South 1984). For example, applying more than 51 mm of irrigation after June reduced shoot mass of pine seedlings at nurseries in Alabama and Georgia (May et al. 1961). When compared to no irrigation, shoot mass at the Alabama nursery was 11 percent less when seedbeds were irrigated with 6.7 mm/week. Although several managers of pine nurseries in the Southern United States target about 25 mm/week (irrigation plus rainfall), some may apply three times that rate during hot periods in the summer. Future research may find that managers who fertilize with more N (table 2) do so because they apply more irrigation than needed.

The optimum combination of N and irrigation varies with soil texture (Pham et al. 1978, Sloan 1992), nursery location, mulch type, rainfall, and target seedling size. In addition, there likely is an interaction between irrigation rate and N rate (Bumgarner et al. 2008, Cabello et al. 2009, Dierauf and Chandler 1991, Gagnon and Girard 2018, Shi et al. 2018). Applying too much irrigation can leach N (Bengtson and Voigt 1962) and produce needles that are not as green (figure 9). If this interaction affects seedling performance (Dierauf and Chandler 1991), then it will be important for the next generation of researchers to provide details of N rates, irrigation rates, and rainfall rates.

Do Organic Matter Additions Improve Economic Returns?

Sandy nursery soils in the Southern United States average about 1.6 percent OM (South and Davey 1983, Starkey et al. 2015), though some nursery soils produce large seedlings with less than 0.8 percent OM (South et al. 2017). The amount of organic amendments applied to fallow or cover-crop fields is about 115 m^3/ha (Starkey et al. 2015) applied once every 3 to 5 years. In the past, OM was also added as a mulch to seedbeds, but since about 78 percent of managers now use soil stabilizers, only a few still apply sawdust or bark mulch after sowing.

Although there are several biological benefits from increasing soil OM, few studies provide the

Table 4. Selected examples of the ratio of nitrogen (N) and potassium (K) used to grow pines in research trials.

Species	Units	N	K	N/K ratio	Reference
Container					
Pinus taeda L.	ppm	250	40	6.2	Marx et al. 1989
Pinus taeda L.	ppm	100	30	3.3	Woessner et al. 1975
Pinus elliottii Engelm.	ppm	264	86	3.0	Samuelson 2000
Pinus palustris Mill.	mg	80	33	2.4	Davis et al. 2011
Pinus taeda L.	ppm	575	353	1.6	Ruehle and Marx 1977
Pinus tabuliformis Carr.	mg	150	100	1.5	Shi et al. 2018
Pinus palustris Mill.	mg	66	50	1.3	Jackson et al. 2012
Pinus palustris Mill.	g	684	538	1.3	Haywood et al. 2012
Pinus taeda L.	ppm	20	17	1.2	Marx and Barnett 1974
Pinus taeda L.	mg	155	129	1.2	Williams and South 1995
Pinus palustris Mill.	ppm	350	329	1.1	Barnett and McGilvery 1997
Pinus rigida Mill.	g	812	939	0.9	Helm and Kuser 1991
Pinus palustris Mill.	mg	78	120	0.6	Dumroese et al. 2013
Pinus elliottii Engelm.	ppm	80	132	0.6	DeWald et al. 1992
Bareroot					
Pinus taeda L.	kg/ha	185	24	7.7	Greene and Britt 1998
Pinus taeda L.	kg/ha	218	39	5.6	Stone 1986
Pinus taeda L.	kg/ha	205	46	4.4	Marx 1990
Pinus palustris Mill.	kg/ha	392	90	4.4	Hinesley and Maki 1980
Pinus palustris Mill.	kg/ha	250	66	3.8	Hatchell 1985
Pinus strobus L.	kg/ha	125	48	2.6	Bickelhaupt et al. 1987
Pinus elliottii Engelm.	kg/ha	106	41	2.6	Marx et al. 1989
Pinus taeda L.	kg/ha	143	88	1.6	Leach and Gresham 1983
Pinus taeda L.	kg/ha	110	60	1.8	VanderSchaaf and McNabb 2004
Pinus taeda L.	kg/ha	179	108	1.7	South et al. 2017
Pinus elliottii Engelm.	kg/ha	215	123	1.7	Simpson 1985
Pinus strobus L.	kg/ha	180	112	1.6	Dobrahner et al. 2004
Pinus palustris Mill.	kg/ha	352	227	1.6	Rodríguez-Trejo et al. 2003
Pinus caribaea Morelet	kg/ha	188	120	1.6	Ward and Johnson 1985
Pinus taeda L.	kg/ha	171	112	1.5	South and Donald 2002
Pinus elliottii Engelm.	kg/ha	67	51	1.3	Marx et al. 1986
Pinus taeda L.	kg/ha	157	156	1.0	South et al. 2015
Pinus elliottii Engelm.	kg/ha	101	167	0.6	Munson 1982
Pinus elliottii Engelm.	kg/ha	50	88	0.6	McNabb 1985

Figure 9. Loblolly pine seedlings were irrigated when soil tension (6 cm depth) reached either 8 kPa (left) or 30 kPa (right) at the New Kent Nursery (Dierauf and Chandler 1991). Over a 19-week period, the average weekly irrigation applied was 9.9 mm (left) and 2.8 mm (right). By October, the plots receiving less irrigation were a deeper green color. (photo by David South, 1985)

economics of adding OM (Blumenthal and Boyer 1982, Low and Sharpe 1973, Muntz 1944, Rose et al. 1995). Adding too much OM before sowing can be expensive and might reduce seed germination (which might appear to increase seedling mass). In some cases, applying too much OM may reduce seedling growth (Bickelhaupt et al. 1987, Davey 1953, Dierauf 1991, Koll 2009). Application costs are easy to determine (e.g., compost ranges from \$30 to \$200/m^3), but the economic gains from increasing OM by 1 percent (e.g., 13,000 dry kg/10 cm/ha) have not been well documented. Economic returns may not occur when OM has no effect (α=0.05) on conifer seedling size (Barnard et al. 1997, Dierauf 1991, Jacobs et al. 2003, Koll 2009, Mexal and Fisher 1987, Munson 1982, Sloan 1992) or when the amendment reduces subsequent plantation survival (Coleman et al. 1987). At one hardwood nursery in Indiana, applying 200 m^3/ha of compost increased both OM (+0.9 percent) and seedling size (α=0.1) (Davis et al. 2006). Unfortunately, it is not known if the increase in seedling size was caused by a reduction in density (e.g., Mañas et al. 2008). If a reduction in seedling production did occur, the cost of applying compost (e.g., \$10,000/ha) would have resulted in a reduction in profits. Economic analyses on short- and long-term effects of soil OM amendment are needed to determine whether the benefit/cost ratio is greater than 1.

Does Calcium Actually Harden Seedlings?

Some researchers claim that applying Ca nitrate helps bareroot seedlings develop strong cell walls and leaf waxes to protect seedlings during freezer storage (Jacobs and Landis 2009). There appears to be a lack of scientific evidence, however, to support this claim. Although Ca nitrate (Ca $(NO_3)_2$) and Ca ammonium

Table 5. Fertilizer regimes for bareroot loblolly pine seedbeds (> 80 percent sand) differ among individuals who prescribe fertility ranges (Davey 1991, Kormanik et al. 1994, May 1984, Steinbeck et al. 1966) and among individuals who prescribe fertilizers based on Mehlich 3 soil test results. Phosphorus values in bold are for the Brey II method of extraction.

	Desired fertility ranges				Soil test	Fertilizer rates prescribed by		
	Steinbeck	May	Davey	Kormanik	pH 5.7	Professor	Agronomist	Nursery manager
Element	ppm	ppm	ppm	ppm	ppm	kg/ha	kg/ha	kg/ha
Nitrogen		700				168	112	218
Phosphorus	25-38	25-50	**25-200**	**80**	100	0	0	0
Potassium	75-100	37-63	80-	80-90	39	196	93	162
Calcium	300-600	200-300	200-	350-400	150	112	0	173
Magnesium		25-30	25-	50-	34	0	20	31
Boron			0.3-	0.5-1.2	0.3	2.2	1.1	3.1
Zinc			1-30	3-8	1.4	0	2.2	8
Copper			0.8-8	0.3-3	0.7	3.3	1.1	2.2
Manganese			5-200		8	0	6.7	0
Sulfur					12	0	10	84
Iron					100	0	0	5

nitrate ($5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$) are sometimes used to increase shoot growth of container-grown seedlings (Dumroese and Wenny 1997, Holopainen et al. 1995), Ca nitrate does not increase freeze tolerance of pine seedlings (Christersson 1973, 1975, Montville et al. 1996) and may decrease freeze tolerance of some agronomic crops (Dexter 1935). In Washington, 2-0 seedlings fertilized with urea survived the winter better than seedlings fertilized with Ca nitrate (Radwan et al. 1971). This should not have happened if Ca nitrate really does produce stronger cell walls. More research is needed to define any relationship between calcium and cold hardiness.

Other Questions

Will Researchers Test "Snake-Oil" Products?

Several "snake-oil" products have been sold to farmers and nursery managers; the industry is "plagued" by such products (Cóndor Golec et al. 2007, Underwood 2000, Wagner-Döbler 2003). Promoters for these products boast of their amazing benefits to soil and plants. Most of these products purportedly have profound effects at low dosages. For example, one product (which costs about $62 to apply 0.14 kg/ha) is supposed to aid in the breakdown of OM and enhance micronutrient uptake while improving soil moisture. However, many view such treatments equivalent to a snake-oil remedy (Lazarovits 2001). The more benefits listed, the more likely the product does not work as promised. "Something about high fertilizer prices brings the snake oil salesmen crawling out from the woodwork looking for a quick dollar from folks trying to reduce the cost of raising crops" (Smith 2010: p. 1).

Alleged miracle products typically contain more than 90 percent inert ingredients, with the price of the active ingredients often greater than $150/kg. The benefit/cost ratio is low and the implied activity is very high. The recommended rates are miniscule and yet they supposedly will affect seedling physiology. Before purchasing a product that contains more than 90 percent water, one should search the web for independent publications with valid scientific testing to show the product works as intended. Unfortunately, many products have not been adequately tested (McFarland et al. 2002). One reason is because many researchers (like me) read the product label, calculate the math, and then see no need to test products that are applied at such minuscule rates. Also, some journal editors are prone to reject papers that do not demonstrate a significant treatment effect (Fanelli 2012). Fortunately, some researchers (with other funds) will test and expose products that do not work as advertised (Dumroese et al. 1996, Elegba and Rennie 1984, Miller et al. 1991, Starkey and Enebak 2009, Wolkowski et al. 1985).

Will We Learn Anything Useful from Hydroponic Studies?

Sometimes researchers conduct nutrient trials in hydroponics, since travel is not required and they do not have to deal with "real-world" variables such as rain, hail, irrigation irregularities, and interactions with soil organisms. Unfortunately, conclusions drawn from hydroponic studies often do not apply to bareroot seedbeds (Crannell et al. 1994). For example, the concept of exponential fertigation arose as a hydroponic method for maintaining the relative growth rate (South 1991) of seedlings that were less than 6 weeks old (Ingestad 1982). A constant mean relative growth rate, however, is not an objective of nursery managers and it may not work for older, bareroot seedlings (Birge et al. 2006, McAlister and Timmer 1998, Salifu et al. 2008, Wall 1994). Although researchers have conducted many exponential fertilization trials in pots and containers (where the highest dose of N is applied on the last day of fertilization), this fertilization method is not used in nurseries in Finland (Juntunen and Rikala 2001) nor at the nurseries listed in table 1. Most managers see no disadvantage of achieving sigmoidal seedling growth using conventional fertilization/top-pruning regimes.

Sometimes hydroponic trials have been used to determine which N fertilizers are best for growing conifers in nursery seedbeds. For example, some say that nitrate is not a good source of N and yet some trials cast doubt on that assumption (figure 10). Results from hydroponic trials may favor ammonium sulfate, but after correcting for the beneficial effect of lowering pH in soils, there may be no difference in seedling mass when comparing Ca nitrate with ammonium sulfate (van den Driessche 1971).

Why Do Lab Tests Vary So Much?

Different methods will result in different estimates of both foliar nutrients (Colbert and Allen 1996)

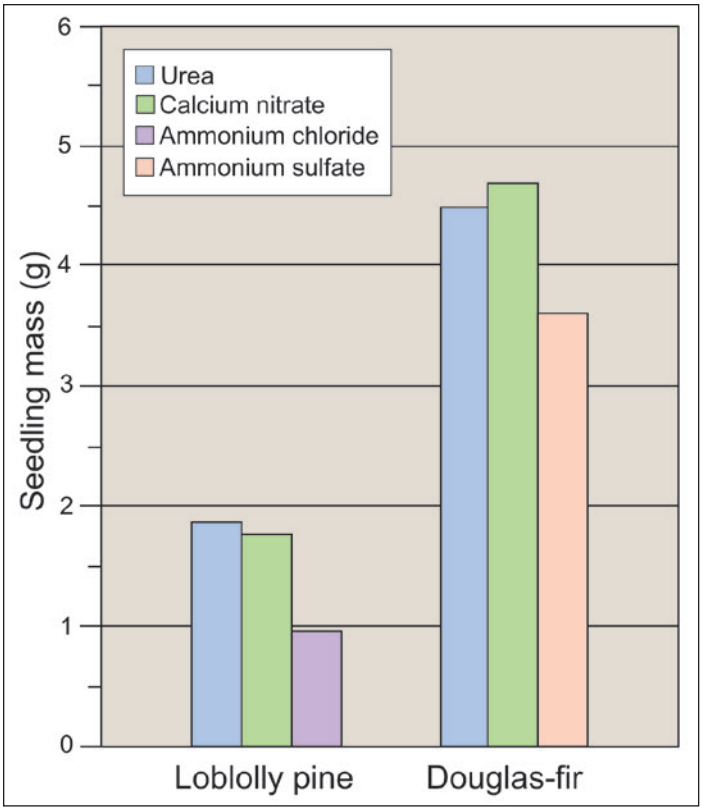

Figure 10. Nitrate fertilizers can produce acceptable growth for conifer seedlings. Loblolly pine seedlings were grown in sand in a greenhouse (Pharis et al. 1964) and fertilized with 75 ppm nitrogen (N) and 200 ppm calcium, then measured 4.5 months after sowing. In a different study, Douglas-fir seedlings were fertilized in a bareroot nursery in May and September with 56 kg N/ha for each application then measured in November (Radwan et al. 1971).

Table 6. Soil test results from the Mehlich 3 extraction procedure vary by laboratory.

Description	Laboratory A	Laboratory B	Laboratory C
pH (water)	5.2	5.1	-
pH (calcium chloride)	-	-	4.2
Buffer pH	7.9	6.9	-
CEC (meq/100g)	2.7	1.0	-
Organic matter (%)	0.48	0.7	-
	ppm	ppm	ppm
Phosphorus	65	44	22
Potassium	34	20	22
Calcium	308	93	63
Magnesium	29	8	34
Sulfur	6	2	-
Boron	0.16	0.5	0.7
Zinc	2	1.6	1.0
Manganese	32	11	3.5
Copper	1.5	0.7	0.5
Iron	120	97	34
Sodium	-	6	343

and soil nutrients (Davey 2002). Furthermore, when using the same soil extraction procedure (table 6), different labs will produce different results (Cools et al. 2004, Jacobsen et al. 2002). For this reason, the "Southern Forest Nursery Soil Testing Program" uses a single laboratory so that soil test results can be compared among different years and different nurseries (South and Davey 1983).

Which Fertilization Philosophy Will the Next Generation Adopt?

Currently, there are three fertilization philosophies: low, medium, and progressive. Some in the "low" group do not apply fertilizers (Hubbel et al. 2018), while others advocate reducing use of chemical fertilizers by 50 percent or more in hopes of benefiting mycorrhiza. Those in the "medium" group fertilize with the goal of producing seedlings that are easy to plant by hand (i.e., more than 80 percent Grade 2 seedlings [Boyer and South 1988]). Those in the "progressive" group adopt regimes to increase seedling growth after transplanting to the reforestation site. Stoeckeler and Arneman (1960: p. 132) said that "With a crop of such high value per acre, the progressive nurseryman also does not hesitate to provide whatever fertilizers or soil amendments are necessary to keep the trees in a state of active growth, high vigor, and good color. As a general rule, fertilized trees are larger and sturdier and have better survival than do unfertilized ones." Progressive growers produce "optimum" seedlings, which meets survival and growth goals (Grossnickle and South 2017) at the minimum cost of reforestation (South and Mitchell 1999). Based on field studies (Autry 1972, Irwin et al. 1998, Jackson et al. 2012, Kabrick et al. 2015, Larsen et al. 1988, South et al. 2015), seedlings (South et al. 2016) produced with the progressive approach can outperform those produced with the low or medium approach.

Recommendations

I have some recommendations for the next generation of researchers. First, be aware of the most common statistical errors (Fowler 1990, Haase 2014, South and VanderSchaaf 2017) and then consult with an experienced statistician before designing your fertilizer trial. Ask for an experimental design with enough statistical power to detect an 8-percent difference in seedbed density and a 7-percent first-year height increase. The statistical power of some fertilizer trials is sometimes low (e.g., figure 6) and therefore variability might not be able to reject a null hypothesis even when a treatment caused a 100-percent increase in a seedling trait. If you do not already know, ask how to use contrast tests to examine linear and quadratic effects because these tests should be used for fertilizer rate trials. In toxicity trials, where the primary question is whether the treatment reduces growth, use a one-sided t-test (South and VanderSchaaf 2017).

When writing a study proposal, state the null hypotheses you wish to test. This might avoid embarrassment if the assumed outcome (i.e., alternative hypothesis) does not occur. Finally, when writing a thesis or dissertation, provide all the data (i.e., individual seedling measurements) in appendices (e.g., Olanin 2017) or in a digital data bank (South and Duke 2010). This will allow others the opportunity to collaborate by asking different questions that may produce additional insights.

Fertilizers typically represent a small percentage of the total growing costs in a nursery. When fertilizers cost $1,800 per ha (table 2), the cost per seedling is less than 0.1 cent, which equates to a small percentage (e.g., 2 percent) of the retail price. Even so, researchers should be aware of fertilizer costs before designing fertilizer trials. In some cases, a chelated fertilizer can cost 90 times more than a non-chelated formulation. It will be a waste of time to conduct research on products that are cost prohibitive (e.g., benefit/cost ratio less than 0.5). Although nursery costs certainly impact profits, the next generation should include the economic effects of fertilizers on short- and long-term outplanting performance. In some cases, spending money for fall-applied fertilizers will reduce the cost per living seedling at the reforestation site (Hinesley et al. 1980, Irwin et al. 1998, Puértolas et al. 2012).

Address correspondence to—

David South, Emeritus Professor, School of Forestry and Wildlife Sciences, Auburn University, AL 36849: email: southdb@auburn.edu

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Evaluation of Sowing Methods to Determine the Role of Hypocotyl Lift in Longleaf Pine Seedling Development

Nathan G. Bolner, D. Paul Jackson, Rabiu Olatinwo, and James P. Barnett

Fiber Supply Associate, International Paper Company, Vicksburg, MS; Associate Professor, School of Agricultural Sciences and Forestry, Louisiana Tech University, Ruston, LA; Research Plant Pathologist U.S. Department of Agriculture (USDA), Forest Service, Southern Research Station, Pineville, LA; Emeritus Scientist, USDA Forest Service, Southern Research Station, Pineville, LA

Abstract

Sonderegger pine (*Pinus x sondereggeri* H.H. Chapm.) is a hybrid pine species of loblolly pine (*Pinus taeda* L.) and longleaf pine (*Pinus palustris* Mill.) that is culled when noticed during seedling processing and packing in the nursery. Early indication of the hybrid has been theorized as the absence of a seed wing stub or hypocotyl lift off of the growing medium surface, although neither of these theories has been proven. Two trials were conducted to determine the role of container cell color, growing medium depth in the cells, and the presence of a seed wing stub on longleaf pine hypocotyl lift. Seedlings grown in black container cells had increased hypocotyl lift and tendency for reductions in root-collar diameter growth regardless of seed wing stub presence. Genetic testing indicated that both wingless and winged seeds were true longleaf pine. This paper was presented at the Joint Annual Meeting of the Southern Forest Nursery Association and the Northeast Forest and Conservation Nursery Association (Pensacola, FL, July 17–19, 2018).

Introduction

Sonderegger pine (*Pinus x sondereggeri* H.H. Chapm.) occurs naturally where longleaf pine (*Pinus palustris* Mill.) and loblolly pine (*Pinus taeda* L.) are in close proximity and have the potential to cross-pollinate and hybridize. Traditionally, this hybrid species is considered to be of low quality, with poor bole formation and limb distortion (Wakeley 1954). In addition, the loblolly pine genes in the hybrid cause the terminal bud of Sonderegger pine to become extended, often resulting in the bud being in direct line with flames from prescribed burning, which is required for true longleaf pine ecosystems to thrive (Jose et al. 2006). Because of these observable qualities, Sonderegger pine seedlings are generally culled from the nursery in an effort to prevent them from being erroneously planted as pure longleaf pine.

Sonderegger pines are typically culled when noticed by nursery workers during lifting. The hybrid seedlings exhibit bud elongation among true longleaf pine seedlings (figure 1), which conversely, have no bud elongation and instead have resting buds near the medium surface in containers. In describing longleaf pine germination phases, Boyer (1990) stated that "newly germinated seedlings have virtually no aboveground hypocotyl, and the cotyledons are close to the ground line." A theory exists that the elongation of the longleaf pine hypocotyl or the lifting of the cotyledons from the medium surface are signs that hybridization has occurred in that

Figure 1. The red circle indicates a Sonderegger pine seedling growing among longleaf pine seedlings in a container nursery. (Photo by Paul Jackson 2018)

seedling. Often, however, true longleaf pine seedlings exhibit a slight hypocotyl lift in the nursery, and to our knowledge, there have been no research trials conducted to determine the cause of the lift and whether it is truly indicative of hybridization.

Another theory proposed to detect Sonderegger pine seedlings in the nursery is the absence of a wing stub on longleaf pine seed. Wakeley (1954) stated that "the seed wings of all southern pines except longleaf can be rubbed or broken cleanly from the dry seeds. No way of completely dewinging longleaf seed in bulk has been discovered; commercial 'dewinging' merely reduces the wings to stubs." Wakeley goes on to state that "when the wing of a seed of any southern pine except longleaf is thoroughly moistened, the two curved prongs which attach the wing to the seed straighten out within a few seconds and the seed falls away at a touch. Advantage is sometimes taken of this fact in dewinging species other than longleaf." Thus, it was theorized that longleaf pine seeds missing a wing stub may be expressing this loblolly pine characteristic and are hybrid Sonderegger pine.

Three longleaf pine trials were conducted at Louisiana Tech University in the 2018 growing season to test the two theories. The first trial's objective was to determine if container cell color and growing-medium depth in the container cells affect hypocotyl lift. This objective centers on the premise that the lack of light reaching deeper into the cell, the way light is reflected due to container color, or the heat generated around the seedling in black containers may cause hypocotyls to lift off of the medium. Germination of longleaf pine seed in darkness can cause hypocotyl extension to lengths of 1 in (McLemore 1967), thus, the idea that shallow-filled container cells may reduce light availability and subsequently contribute to hypocotyl lift. Even though containers are filled with growing medium at the nursery using mechanized equipment, the chance of a cell not reaching operational capacity exists, especially cells on the edges. The second trial's objective was to determine if container cell color, medium depth, and the presence of a seed wing stub affected hypocotyl lift. The third trial's objective was to compare DNA between winged and wingless longleaf seeds to determine if any tested positive for the loblolly marker.

Materials and Methods

Trial One

Longleaf pine seeds from a Florida source and with intact wing stubs were soaked in an aerated water bath for 12 hours and stratified in a refrigerator for 9 days before being sown into Ray Leach Cone-tainer™ cells (RL98 Stubby, Stuewe and Sons, Inc., Tangent, OR) on March 16, 2018, at Louisiana Tech University (figure 2). A peat moss based Pro-Mix® growing medium was used. Two container colors (white and black) and two fill levels (operational level or two-thirds of operational level) were evaluated in the trial for a total of four treatments (figures 3 and 4). Filling container cells to normal levels and to two-thirds of normal levels (referred to from this point as lower levels) resulted in root plugs that were approximately 5-in and 3-in (12.7- and 7.6-cm) deep, respectively. Each tray of 49 cells served as a replication, and there were three

Figure 2. Longleaf pine seeds were sown into white and black container cells. (Photo by Paul Jackson 2018)

Figure 3. Black container cells filled with growing medium to normal operational levels. (Photo by Paul Jackson 2018)

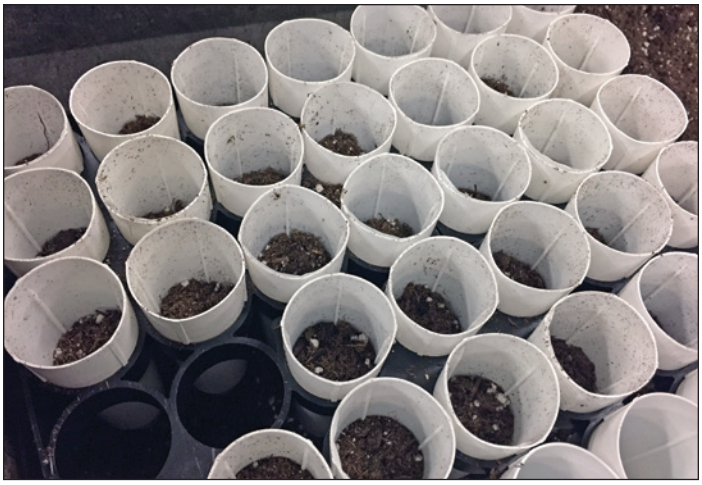

Figure 4. White container cells filled to two-thirds capacity with growing medium. (Photo by Paul Jackson 2018)

trays per treatment for a total of 588 container cells sown with longleaf pine seed. Trays were placed on greenhouse benches under misters that supplied water for 45 seconds every hour from 6:00 am to 6:00 pm. On April 9, 2018, the trays were moved outside to growing benches located in full sun and kept there for the duration of the trial. Seedlings were measured 98 days after sowing. Root-collar diameter (RCD), hypocotyl lift defined as the length of the hypocotyl lifted off of the growing medium (figure 5), and the length of the entire hypocotyl from the base of the cotyledons to the first divergence of a lateral root (figure 6) were measured on each seedling.

Trial Two

A second trial was installed on May 4, 2018, involving the same materials and methods as described previously, with the difference being that a third factor (longleaf pine seeds with or without an intact wing stub, figure 7) was added for a total of eight treatments. The same measurements as described previously were recorded 108 days after sowing on August 20, 2018.

Data Analyses

For both Trials One and Two, an analysis of variance was conducted using a General Linear Model (GLM) and multiple comparisons of means were conducted using Duncan's Multiple Range Test using SAS statistical software (9th ed., SAS Institute, Cary, NC).

Figure 5. Longleaf pine seedling showing hypocotyl lift off of the growing medium. This example is a seedling grown in a black container cell filled to two-thirds capacity with growing medium. Seedlings grown in cells filled with less medium averaged a root-plug depth of approximately 3 in (7.6 cm). (Photo by Paul Jackson 2018)

Trial Three

To determine if wingless seeds have a genetic predisposition to experience hypocotyl lift compared to winged seeds, deoxyribonucleic acid (DNA) from 20 winged and 20 wingless longleaf pine seed were analyzed and compared by using two chloroplast

Figure 6. A measurement of the entire hypocotyl on a seedling from the base of the cotyledons to the first lateral root (Photo by Paul Jackson 2018)

Figure 7. Longleaf pine seed with an intact wing stub (left) and missing the entire wing (right). (Photo by Paul Jackson 2018)

DNA markers with a set of specific primers for each (one for longleaf pine and one for loblolly pine). The DNA extracted from each seed was performed using the Qiagen DNeasy® Plant Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. The DNA from known longleaf pine and loblolly pine were included in the PCR amplification to serve as positive controls in evaluating the seed samples. Amplification of DNA was performed in 10 µl PCR reaction in an Eppendorf Mastercycler® Pro PCR machine (Eppendorf AG Hamburg, Germany). Gel electrophoresis was performed to examine amplified products by loading 5 µl PCR products on 1-percent agarose gels. The agarose was stained with ethidium bromide after 20 minutes of electrophoresis, and the resulting bands

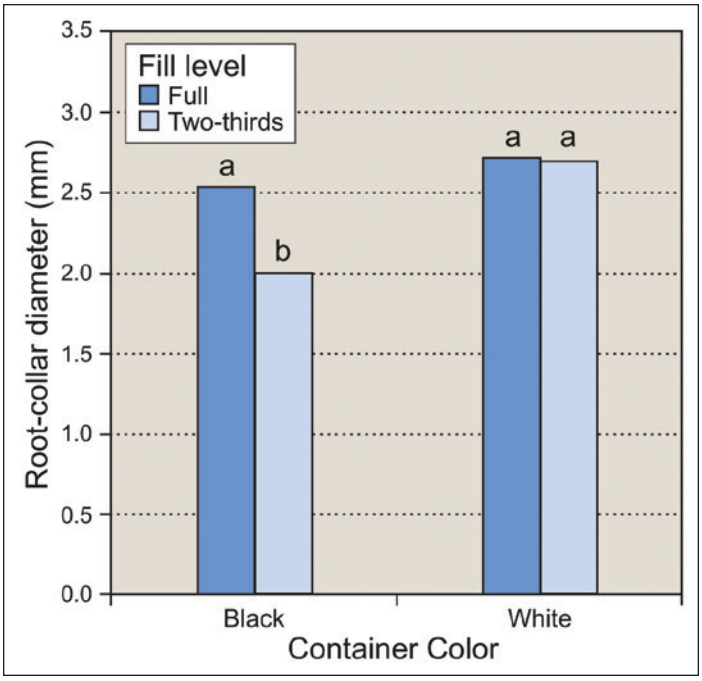

Figure 8. Mean root-collar diameter (RCD) was significantly smaller for seedling grown in black containers with less growing medium (two-thirds capacity) compared with all other treatments.

were visualized under ultraviolet (UV) illumination to confirm positive or negative amplifications. A band indicates positive amplification, while no band indicates negative. A positive with the longleaf marker identifies a sample as a longleaf pine, while a negative with longleaf marker indicates the sample is not a longleaf pine. Similarly, a positive with the loblolly marker identifies a sample as a loblolly or a Sonderegger pine, while a negative with the loblolly marker indicates the sample is not a loblolly or a Sonderegger pine. Therefore, if a sample is longleaf-marker positive, it is not a Sonderegger hybrid.

Results

Trial One

Longleaf pine seedlings grown in black container cells filled with lower levels of medium had significantly smaller RCDs compared with all other treatments (table 1, figure 8). Seedlings grown in black cells had more hypocotyl lift compared to those grown in white cells, and seedlings grown in cells filled with less medium experienced more hypocotyl lift compared to cells filled to operational levels (figures 5 and 9). Among all treatments, there were no differences in total hypocotyl length (figure 9).

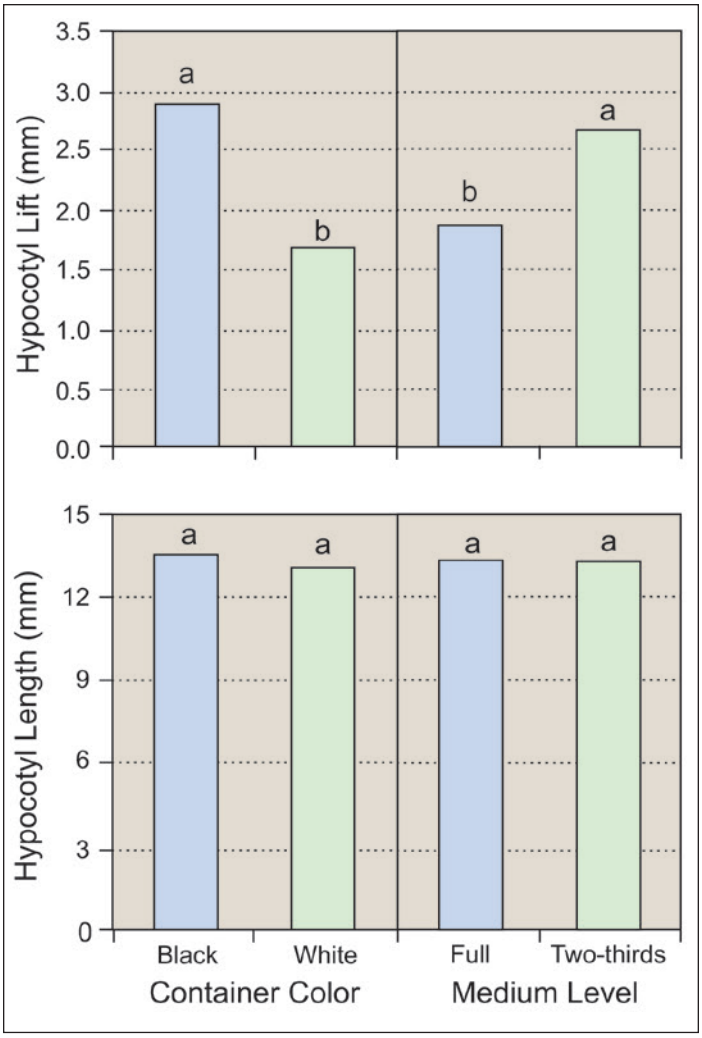

Figure 9. In Trial 1, mean hypocotyl lift off of the growing medium differed between longleaf pine seedlings grown in black and white container and between seedlings grown in normal operational levels of growing medium or two-thirds of normal levels. Mean hypocotyl length, however, was not influenced by either treatment factor. Means for each treatment factor were compared using Duncan's Multiple Range Test.

Table 1. Probability values for treatment main effects and interactions for Trial 1.

Treatment	Root-collar diameter (mm)	Hypocotyl lift (mm)	Total hypocotyl length (mm)
		Main Effects (P > F)	
Color	0.0003	0.0211	0.1014
Medium Depth	0.0058	0.0978	0.8309
Color*Medium	0.0080	0.2570	0.3018

Trial Two

Longleaf pine seedlings grown in black cells with less growing medium had increased hypocotyl lift for both winged and wingless seeds (table 2, figure 10). The amount of hypocotyl lift that occurred between winged and wingless was similar (figure 11).

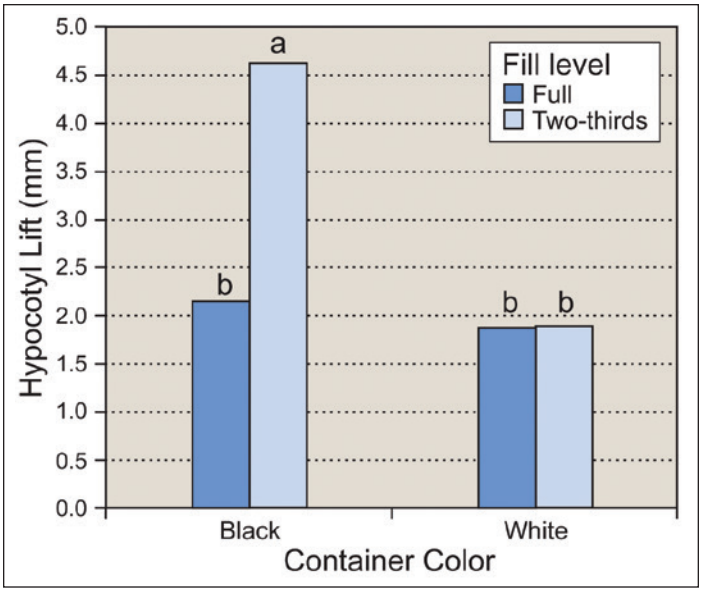

Figure 10. Mean hypocotyl lift off of the growing medium in Trial 2 had a significant interaction between container color and amount of growing medium.

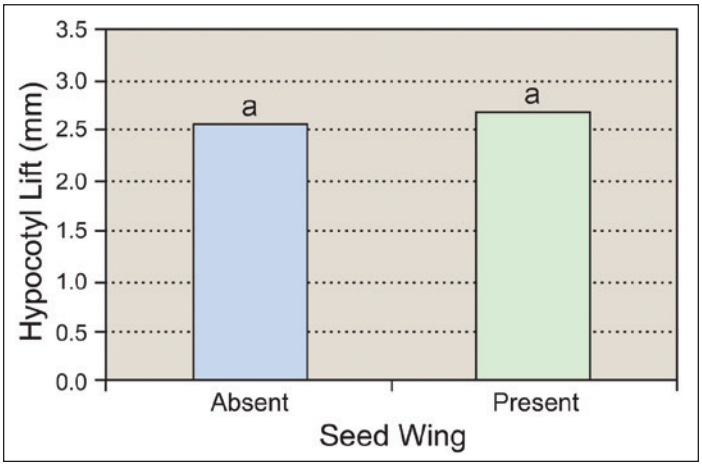

Figure 11. Mean hypocotyl lift did not differ between seedlings grown from seed with and without wing stubs in Trial 2 based on Duncan's Multiple Range Test.

Hypocotyl length in seedlings grown from wingless seeds was shorter compared to those on seedlings grown from winged seeds (table 2, figure 12). Seedlings grown in black cells had smaller RCDs compared to seedlings grown in white cells (table 2, figure 13).

Trial Three

All 20 wingless and 20 winged longleaf pine seeds tested positive with the longleaf pine marker along with the known longleaf positive control, while all 20 winged and 20 wingless DNA samples evaluated tested negative with the loblolly pine marker.

Table 2. Probability values for treatment main effects interactions for Trial 2.

Treatment	Root-collar diameter (mm)	Hypocotyl lift (mm)	Total hypocotyl length (mm)
	Main Effects (P > F)		
Color	0.0218	0.0016	0.1452
Medium Depth	0.6358	0.0064	0.2648
Seed	0.7740	0.7553	0.0008
Color*Medium	0.1385	0.0071	0.4830
Seed*Medium	0.1250	0.9082	0.2888
Seed*Color	0.5625	0.5582	0.6093
Seed*Color*Medium	0.2074	0.9603	0.2343

Discussion

With none of the wingless longleaf pine seeds testing positive to the loblolly pine DNA marker and no seedling differences found in Trial Two between seed types, the theory of wingless longleaf pine seeds being an indicator of hybrid Sonderegger pine does not hold true. In another trial conducted in 2016, out of 343 seedlings grown from winged longleaf pine seed and 392 seedlings grown from wingless longleaf pine seed, only three seedlings developed into true Sonderegger pines: one from a winged seed and two from wingless seeds (unpublished data). The occurrence of wingless seeds in a seedlot may relate more to how seeds from certain sources respond to seed extraction and processing.

In the nursery, slight hypocotyl lift is often observed on longleaf pine seedlings. The elongation of the hypocotyl either ceases and normal longleaf pine development occurs or the elongation of the hypocotyl continues and a Sonderegger pine seedling develops. In both trials, seedlings in all treatments had some level of hypocotyl lift. Seedlings grown in black container cells had more hypocotyl lift (both trials) and smaller RCD (Trial One only) compared with those grown in white container cells. In a study with red maple (*Acer rubrum* L.) and bush beans (*Phaseolus vulgaris* L.), black containers generated more heat and caused substrate temperatures to increase (Markham et al. 2011). Neither substrate temperature nor ambient temperature at the container cell level was recorded in these trials though we speculate that higher temperatures in the black cells stimulated hypocotyl lift and caused reductions in RCD growth.

Total hypocotyl length did not differ among treatments, but hypocotyl lift off of the growing medium tended to be more when container cells were filled to two-thirds capacity. After testing hypocotyl extension in known longleaf pine, loblolly pine, and hybrid seeds, Brown (1964) stated that "the intensity of light and varying temperature conditions during the period of hypocotyl elongation has little effect on final hypocotyl length in longleaf pine, whereas either light or temperature greatly influences the rate and duration of hypocotyl elongation in loblolly pine. The hybrid population lies between these extremes." Brown's assertion may have given more insight to the findings in these trials had there been an indication that any of the seeds (winged or wingless) compared positively to the loblolly

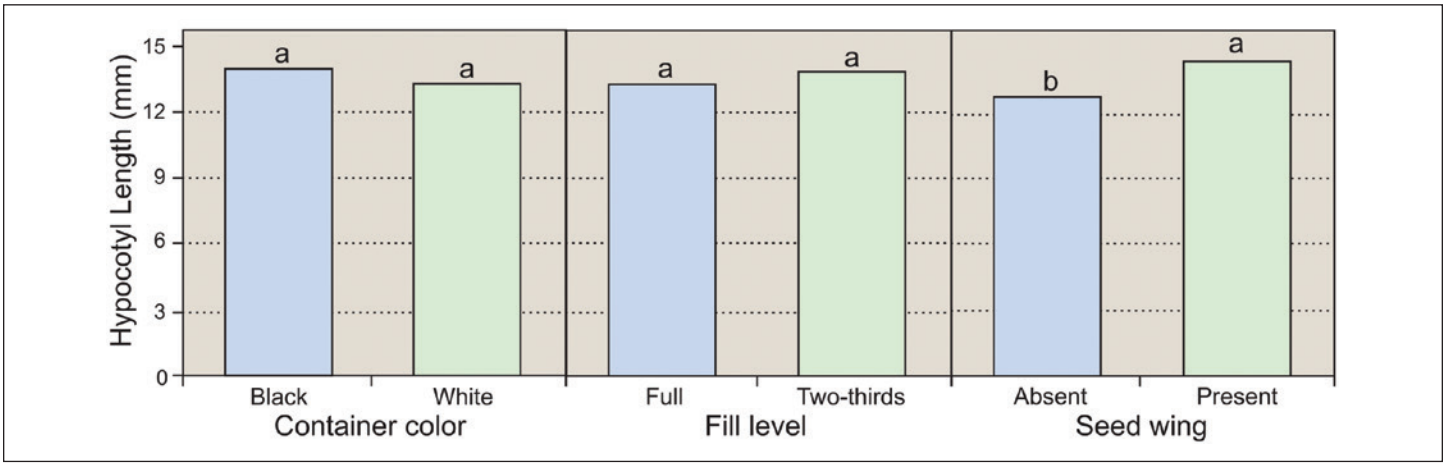

Figure 12. In Trial 2, mean total hypocotyl length of longleaf pine seedlings was unaffected by container color or the amount of growing medium in the container but was significantly longer for seedlings grown from winged seeds compared with wingless seeds. Means for each treatment factor with the same letter are not significantly different based on Duncan's Multiple Range Test.

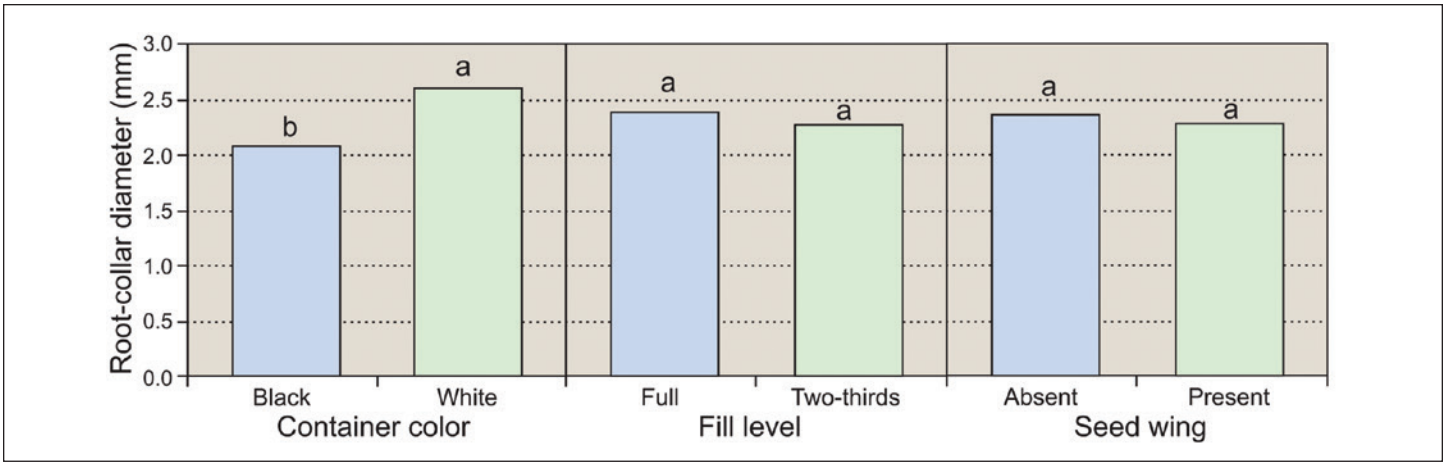

Figure 13. Root-collar diameter (RCD) of longleaf pine seedlings in Trial 2 was significantly influenced by container color but was unaffected by the amount of growing medium or the presence of wings on the seed. Means for each treatment factor with the same letter are not significantly different based on Duncan's Multiple Range Test.

pine genetic marker in the DNA comparison test. Based on our DNA comparisons, all were likely true longleaf pine. Thus, hypocotyl extension off of the growing medium in cells filled to two-thirds capacity is most likely a result of environmental factors such as light availability or light reflection in the container cell.

Future Research Direction and Considerations

Longleaf pine seedlings are traditionally grown in black containers in nurseries across the South. Future research trials to evaluate potential indicators of Sonderegger pine should include measurements of substrate temperature, ambient temperature near the growing-medium surface, and light intensity. These measurements could then be related to morphological data observed during seedling development. Trials could also be developed to administer differing levels of light and/or expose seedlings to certain temperatures in controlled settings. Testing other species such as loblolly pine or slash pine (*Pinus elliottii* Engelm.) would also be useful in comparison to longleaf pine results.

Address correspondence to—

D. Paul Jackson, Associate Professor, School of Agricultural Sciences and Forestry, Louisiana Tech University, P.O. Box 10198, Ruston, LA 71272; email: pjackson@latech.edu; phone: 318–257–2412.

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Genetic Integrity of Longleaf and Shortleaf Pine Seed Orchards and Seed Banks

Barbara Crane, Valerie Hipkins, Sedley Josserand, and Craig Echt

Regional Geneticist, U.S. Department of Agriculture (USDA), Forest Service Southern Region, Atlanta, GA; Director, National Forest System Genetics Lab, USDA Forest Service, Placerville, CA; Biological Technician, USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, Saucier, MS; Conservation Geneticist, USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, Saucier, MS

Abstract

Longleaf pine (Pinus palustris Mill.) and shortleaf pine (*Pinus echinata* Mill.) are priority species targeted for increased restoration on the national forests in the Southern Region of the U.S. Department of Agriculture, Forest Service. The genetic integrity of both species is important to ensure adaptation, survival, and resilience of future forests. Longleaf x loblolly pine hybrids (*Pinus ×sondereggeri H.H. Chapm. ex Sudw. [palustris × taeda]*) and shortleaf x loblolly pine hybrids are known to occur in the general forests, but at a rate of less than 5 percent. Climate change can trigger extreme fluctuations in temperatures, which could influence flower receptivity and result in greater potential for increased inter-species hybridization. This hybridization may compromise the genetic purity of a species and present challenges to successful restoration. It is important to know the genetic identity of the seedlings we are deploying in operational plantings, and the seed being sold to State partners. The Southern Region National Forest System Genetics program chose to DNA fingerprint longleaf and shortleaf pine parents (clones) in the regional seed orchards to assess genetic purity. Final results showed no hybrid fingerprint for the 250 longleaf clones tested and a hybrid fingerprint for 17 of the 619 shortleaf clones tested. The regional seed bank inventory for longleaf and shortleaf pines was also DNA fingerprinted. The seed tested had been collected across multiple years and seed zones. Final results showed a hybrid fingerprint for less than 3 percent of the seed. This paper was presented at the Joint Annual Meeting of the Southern Forest Nursery Association and the Northeast Forest and Conservation Nursery Association (Pensacola, FL, July 17–19, 2018).

Introduction

The U.S. Department of Agriculture, Forest Service National Forest System (NFS) in the Southern Region (R8) provides oversight for the management of approximately 800,000 ac (323,750 ha) of longleaf pine (*Pinus palustris* Mill.) and 1,440,000 ac (582,750 ha) of shortleaf pine (*Pinus echinata* Mill.), across 13 southern national forests. Approximately 97 percent of the longleaf pine ecosystem and 53 percent of the shortleaf pine ecosystem have been lost over the past century (Wear and Greis 2013). A range map for longleaf and shortleaf pine reflects the current geographic distributions of each (figure 1). There is a priority emphasis on accelerated restoration of these species and associated ecosystems on R8's national forests. Multiple agencies, organizations, and partners are also engaged in restoration of these species, such as the Longleaf Alliance (https://longleafalliance.org/), America's Longleaf (http://www.americaslongleaf.org/), the Shortleaf Pine Initiative

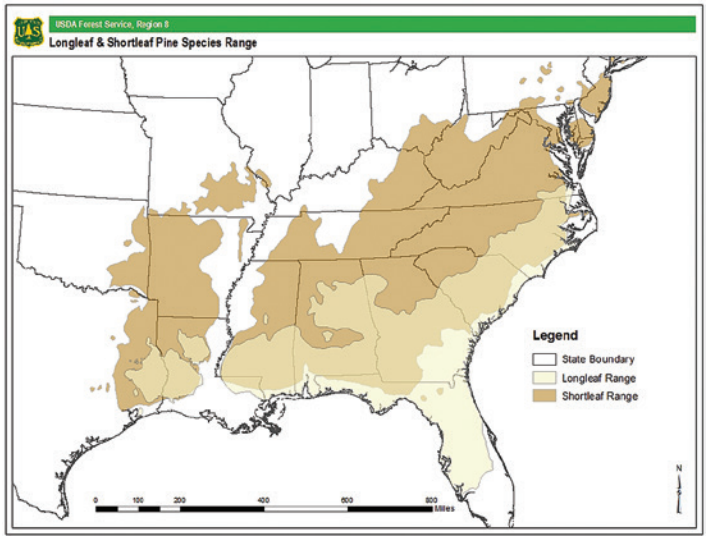


Figure 1. Current geographic range map of longleaf and shortleaf pine. (Created by Chelsea Leitz, USDA Forest Service, 2019)

(http://www.shortleafpine.net/), The Nature Conservancy (https://www.nature.org/), and others.

R8 NFS's reforestation trends reflect approximately 50 percent artificial regeneration and 50 percent natural regeneration. Artificial regeneration activities are expected to increase at an accelerated pace to support increasing restoration targets, therefore more seed will be needed. Genetic integrity (purity) of a species is important to ensure adaptation, survival, and resiliency of forests. If the genetic purity of a species has been compromised, this may present challenges to successful restoration and resiliency (Ledig and Kitzmiller 1992). Both current and future planted forests in R8 are often managed on a 100-year rotation cycle, so adaptation, survival, and resiliency are critical.

Within the past several years, a southern nursery experienced an increase in unusual pine seedling morphologies. Concerns and questions arose about the genetic purity and identity of the seed, and hybridization was suspected. Research to assess suspected increased hybridization in the general forests, seed orchards, and seedling crops was already ongoing (Tauer et al. 2012, Stewart et al. 2016). Longleaf x loblolly pine hybrids (*Pinus ×sondereggeri H.H. Chapm. ex Sudw. [palustris × taeda]*) and shortleaf x loblolly pine hybrids are known to occur in the general forests, but at a low rate of less than 5 percent (Chapman 1922, Tauer et al. 2012). Climate change can trigger extreme fluctuations in temperatures, which could influence flower receptivity and result in the potential for increased inter-species hybridization. The suspect seed did not come from R8 seed orchards; however, are the concerns over seed purity lead the R8 NFS Genetics program to initiate a project to validate the genetic purity of our germplasm (i.e., orchard trees and seed bank inventory). These genetic resources represent multiple seed sources and seed collections spanning 25 years. The objective of the project was to DNA fingerprint longleaf and shortleaf pine parents in all the seed orchards and the seed bank inventory to assess genetic purity and identify any hybrids that may exist.

Questions and Concerns About Hybrid Seedlings

Several questions and concerns have arisen regarding establishment of hybrid seedlings on the landscape. These questions include the following:

- Will hybrid seedlings adapt or be maladapted? Will they survive and reproduce? Is there hybrid vigor?
- What are the growth rates? What is the wood quality? What is the longevity/life span?
- Will the seed physiology change, e.g., germination, viability, stratification requirements?
- Will the hybrid seed provide adequate sustenance for the wildlife that depends on this food source?
- Will red cockaded woodpeckers build cavities in a hybrid tree?
- Will hybridization increase as climate change and extreme fluctuations in temperatures occur? What are the effects on phenology and flower receptivity? How will this affect orchard management?
- Are we looking at future forests that contain more natural hybrids? Will this support or deter forest resiliency? Will hybrids be as resilient as their progenitors to catastrophic weather events in the South, e.g., hurricanes, tornadoes, ice/snow?
- Will silvicultural methods need to be modified if hybrids increase on the landscape? Both longleaf and shortleaf pines are fire-dependent species. Currently, prescribed burning is the most economic and efficient silvicultural tool used to manage these forests. Loblolly pine is not fire tolerant, so then will the hybrids survive fire?
- Longleaf and shortleaf pines occupy very different geographic sites and soil types, from extreme coastal to Piedmont to mountain geographic regions, respectively. Will hybrids adapt, migrate, or die on various sites?

The seed harvested from the orchards and stored in the seed bank is used in restoration on the national forests. Excess seed from this seed bank is occasionally sold to State agencies. R8 NFS Genetics program manages the highest percentage of known longleaf and shortleaf genetic resources (seed orchards, seed production areas, progeny tests) that exist in the South (table 1). Scion material is shared with external partners who are establishing seed orchards. It behooves our program to know the genetic purity of the orchard trees and seed, so that the identity of the seedlings being planted on Federal and non-Federal forested lands is also known.

Table 1. Summary of longleaf and shortleaf pine genetic resources (seed orchards, seed production areas, progeny tests) acreage in the South.

Agency	# Longleaf pine seed orchards / seed production acres	# Shortleaf pine seed orchards / seed production acres	Number of progeny tests
Forest Service	540 / 272	527 / 0	35 longleaf 155 shortleaf
State	225 / 0	70 / 0	unknown
Industry	47 / 125	0 / 0	unknown
Private	unknown	Unknown	unknown

Methodology

Field Collections

R8 NFS longleaf pine seed orchards are located near Benton, LA, Wiggins, MS (figure 2), and Mt. Pleasant, SC. Shortleaf pine seed orchards are located near Mount Ida, AR (figure 3), Benton, LA, Wiggins, MS, and Murphy, NC. Seed orchard and field personnel collected needle samples from two ramets of each clone (family) in each orchard. Needle samples were taken from first-year needles in the top third of the crown. Samples were collected from a total of 250 longleaf pine clones and 619 shortleaf pine clones. Longleaf sources represented Alabama, Florida, Louisiana, Mississippi, South Carolina, and Texas. Shortleaf pine sources represented Alabama, Arkansas, Georgia, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, and Virginia. Needle tissue was shipped overnight to the National Forest System Genetics Lab (NFGEL) at the Institute of Forest Genetics, Placerville, CA.

In addition to needle samples, longleaf and shortleaf pine seed samples from the R8 NFS Ashe seed bank (housed near Wiggins, MS, figure 4) were shipped to NFGEL for DNA testing. Seed sources tested represented Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas, and Virginia. Seed collection years spanned 1987 to 2017, and approximately 200 seed from each source and year were shipped (table 2).

Figure 2. Longleaf pine seedling seed orchard in Mississippi (Photo by Barbara Crane, 2012)

Figure 3. Second-generation shortleaf pine seed orchard in Arkansas. (Photo by Barbara Crane, 2016)

Figure 4. Ashe seed bank inventory drums in Mississippi (Photo by Barbara Crane, 2015)

Laboratory Work

Simple sequence repeat (SSR) DNA markers were used to fingerprint the orchard needle samples and seed bank samples. The Southern Research Station Southern Institute of Forest Genetics (SIFG), Saucier, MS developed the longleaf pine markers (Echt and Josserand, 2018). The shortleaf pine markers were developed in collaboration with Oklahoma State University (Stewart et al. 2012). Three markers were developed from GenBank chloroplast DNA sequences that together identify species-specific profiles (haplotypes) among longleaf, shortleaf, and loblolly pines. In addition to distinguishing among species, these markers allow easy and fast assays to identify longleaf x loblolly pine and shortleaf x loblolly pine hybrids with a high degree of confidence, because chloroplast DNA is only inherited through pollen in pines. Loblolly pine chloroplast DNA, as the pollen parent, was the differential indicator marker for detecting hybrids in the samples.

SIFG invested substantial time and work in the initial development of the markers that could be used in the DNA fingerprinting and hybrid identification. Over 2 years, prior to the NFGEL work, many samples had to be initially screened to find relevant markers that would differentiate the species. Needle and seed samples for more than 2,000 longleaf pines, more than 1,000 shortleaf pines, and nearly 300 loblolly pines were screened (Echt et al. 2013). We have seen only one shared haplotype at 1 percent frequency in longleaf pine and 0.1 percent in shortleaf pine. There were no shared haplotypes with loblolly pine. These results indicate that these chloroplast markers are useful to estimate proportions of pollen contamination in seed lots and identify orchard trees that are likely to be hybrid. To estimate the full extent of species specificity, additional sampling and testing is being considered for each species.

NFGEL staff extracted DNA from the seed orchard needle samples. For the seed samples, the seed was first germinated, then the DNA was extracted from both the megagametophytes and the embryos. Qiagen DNA kits (https://www.qiagen.com/us/) were used for extracting the DNA. Applied Biosystems ABI machines were used to run the DNA samples with the markers. An example of an SSR marker profile to identify the different DNA fingerprint for loblolly pine, longleaf pine, and longleaf x loblolly suspected hybrid can be seen in figure 5.

Table 2. Summary results of DNA fingerprinting on longleaf and shortleaf pine families and seed to assess genetic purity and identify hybrids.

Species	Number of seed orchard families tested	Sources tested (orchard families and seed)	Seed years tested*
Longleaf	250 (2 ramets each)	AL, FL, LA, MS, SC, TX	1981 – 2017
Shortleaf	619 (2 ramets each)	AL, AR, GA, KY, LA, MS, MO, NC, SC, TN, TX, VA	1981 – 2017
Results: how many families had a hybrid fingerprint?	0 longleaf in all sources 0 shortleaf in all sources except LA 17 families shortleaf LA source	Less than 3% of the seed in each species	

*Not every year of seed tested due to lack of seed.

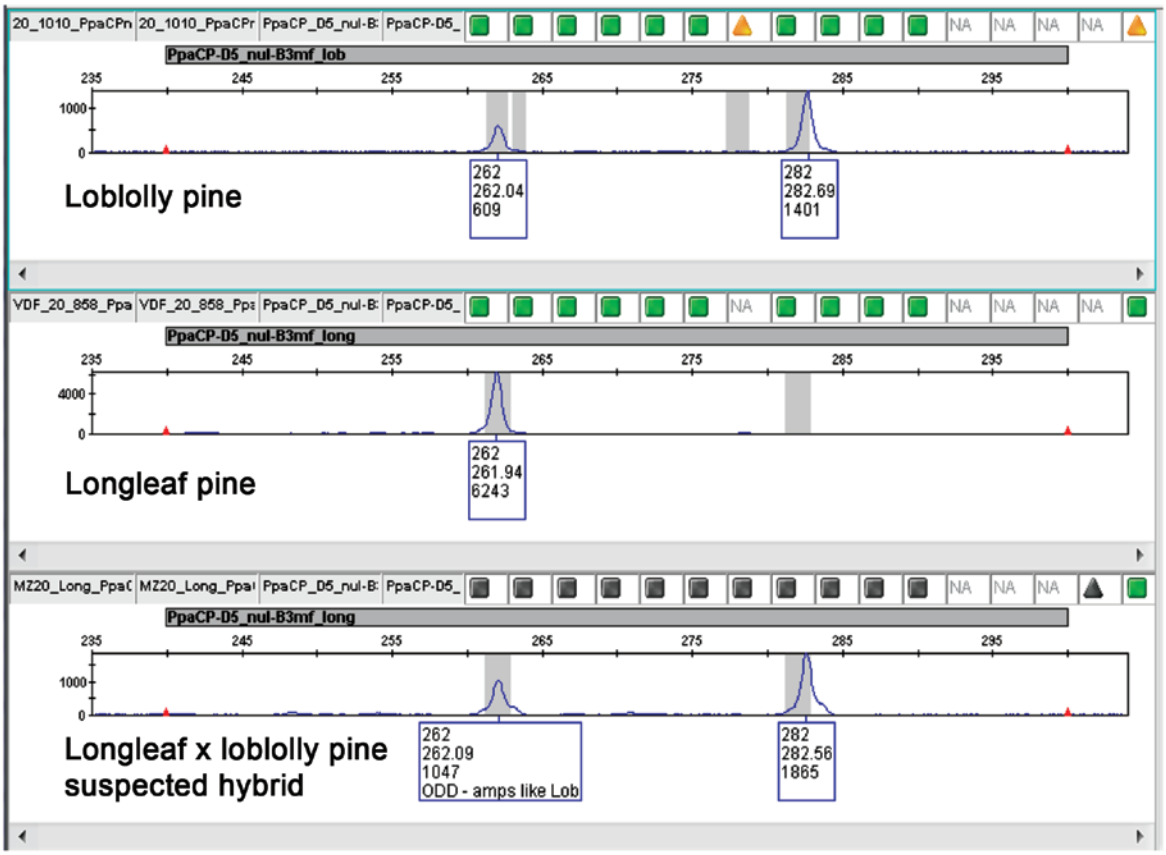


Figure 5. SSR marker profile to identify DNA fingerprints for loblolly, longleaf, and the longleaf x loblolly hybrid. (By Sedley Josserand, 2017)

Results

The objective of the project, to DNA fingerprint orchard trees and seed bank inventory for genetic purity and identify any hybrids, was accomplished. R8 NFS Genetics program, in cooperation with NFGEL and SIFG, used three DNA markers to fingerprint all longleaf and shortleaf pine clones in the seed orchards (two ramets from each clone).

Longleaf Pine Germplasm

All 250 longleaf clones, representing six seed sources, in the Louisiana and Mississippi orchards showed no hybrid DNA fingerprint (table 2). Twelve additional trees in the Louisiana longleaf seed orchard, with the unusual branchy "wolf tree" phenotype (suspected of being hybrids, figure 6), were also tested and showed no hybrid DNA fingerprint.

For the seed tested, less than 3 percent showed a hybrid DNA fingerprint. It is reasonable to surmise that there was minor pollen contamination in the longleaf orchard from the loblolly pine orchard. The phenology window for late-ripening longleaf flowers and early ripening loblolly pollen most likely coincided, thereby creating a hybridization event. Cones are collected by breeding zone or source rather than by mother tree, so the seed is bulked. This collection method maximizes genetic diversity in the seed. Phenology data for the late-flowering longleaf clones and loblolly clones with early pollen

Figure 6. Trees with branchy "wolf" phenotypes at the longleaf pine seed orchard in Louisiana. (Photo by Barbara Crane, 2014)

maturation will be reviewed. The R8 NFS seed bank is not concerned with this very small percentage of hybrid seed, because nursery practices include culling unusual longleaf seedling phenotypes (e.g., elongated stem potentially indicative of a hybrid) at the grading table.

Shortleaf Pine Germplasm

All shortleaf clones, representing 12 sources, in the Arkansas, Mississippi, and North Carolina orchards, showed no hybrid DNA fingerprint, for both first- and second-generation orchards, for a total of 602 pure orchard clones (table 2). The shortleaf pine results are updates to the findings by Stewart et al. (2016).

Approximately 17 shortleaf clones located in the Louisiana orchard showed a hybrid DNA fingerprint. Those clones have since been eliminated from the orchard. It is reasonable to suggest that during the 1960s superior tree identification campaign, some chosen candidates were hybrids but were mistaken for shortleaf pine. Additionally, during first-generation tree breeding activities in the 1980s, it is possible that loblolly pollen was mistakenly used on shortleaf mother trees, resulting in hybrids in progeny tests. If those individuals from the progeny tests were then selected to be grafted in the second-generation orchard, that may explain why some hybrids showed up in the second generation shortleaf orchard as well.

For the seed tested, less than 3 percent showed a hybrid DNA fingerprint. It is reasonable to surmise that there was minor pollen contamination in the shortleaf orchard from the loblolly pine orchard. The phenology window for late ripening shortleaf flowers and early ripening loblolly pollen most likely coincided, hence a hybridization event. As with the longleaf germplasm, phenology data for the shortleaf clones with late flowering and loblolly cones with early pollen maturation will be reviewed. R8 NFS seed bank is not concerned with this very small percentage of hybrid seed, since nursery practices include culling unusual shortleaf seedling phenotypes (e.g., elongated stem potentially indicative of a hybrid) at the grading table.

Discussion

Longleaf and shortleaf pine ecosystems have been identified as top priorities for restoration in the Southern Region, per each National Forest System Forest Plan. Accelerated restoration will require increased seed supplies, with seed of known quality, source, and genetic integrity. Quality seed is an important factor in the production of quality seedlings and field survivability (Barnett et al. 2002). Knowledge about the source and genetic identity of the seedlings will support successful restoration, when planting on the appropriate sites for both Federal and non-Federal forested lands. The genetic integrity of a species will favor survival, adaptation, and resiliency of the future forests. National and regional policy states that locally adapted, genetically appropriate seed sources are best to use for now. But as climate change impacts increase, the seed sources may be combined and seed movement guidelines will change to accommodate updated deployment strategies (Crane et al. 2011, Erickson et al. 2012).

The longleaf and shortleaf pine first-generation orchards were established in the 1960s, and the second-generation shortleaf orchards were established in the 1980s. Seedling seed orchards and progeny tests for both species were established throughout the 1980s and 1990s. Seed has been harvested from all orchards since the 1970s. A continuous seed supply, for multiple species, has been banked for use in reforestation and restoration on the southern national forests.

Quality seed is needed to support accelerated artificial regeneration efforts for both longleaf and shortleaf pine. Seed is often scare and in high demand. The cone cycle frequency of both species further complicates seed availability. Longleaf pine has a bumper cone crop approximately every 5 years (figure 7), and shortleaf pine bumper cone crops occur every 5 to 7 years. Bumper cone crop years yield seed that is high quality, high vigor, and has excellent germination (Barnett et al. 2002). Cone harvest methods differ for each species. Longleaf pine cones are collected using a tree shaker (cones fall to the ground), whereas shortleaf pine cones require the use of bucket trucks or lifts to cut the cones from branch tips. Cones then have to be transported to a cone extractory, where seed is removed. Seed extraction from the cones requires experience and skill with the cone dry kilns, seed gravity tables, and X-ray machines. Longleaf pine cones must be processed and seed extracted within 2 weeks of collection; otherwise the seed will begin

Figure 7. Longleaf pine bumper cone crop in Louisiana. (Photo by Barbara Crane, 2014)

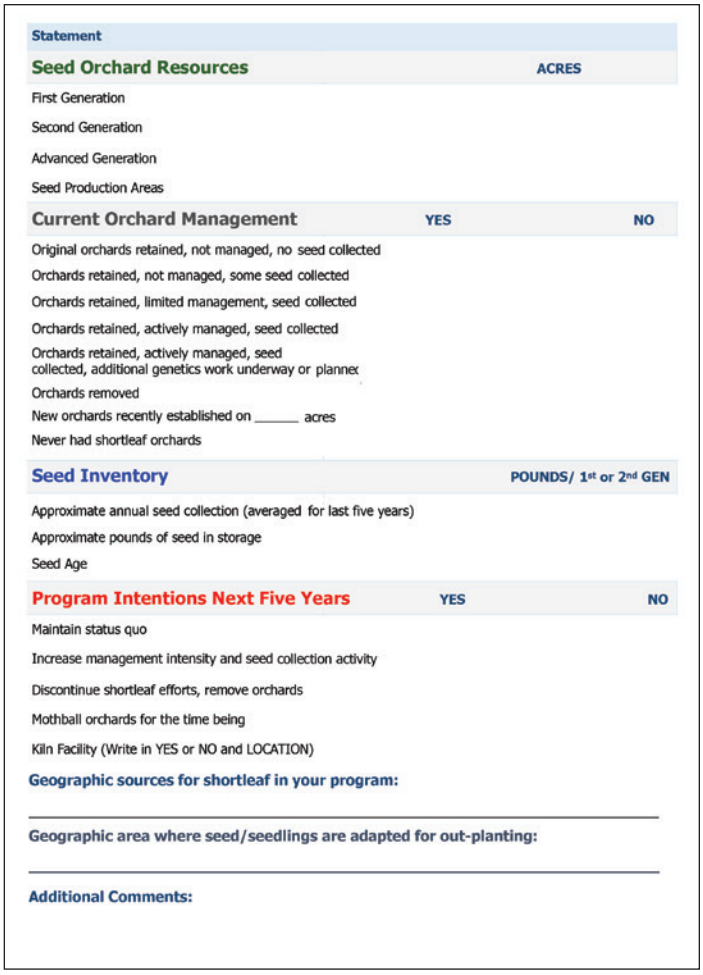
to degrade because of its thin seed coat (Barnett et al. 2002). By comparison, shortleaf pine cone processing follows a more routine protocol, with a more flexible timeframe in which to process cones and still extract quality seed.

Most pine seed must be dried down to the proper moisture content of less than 10 percent to ensure storage longevity (Barnett et al. 2002). If properly handled and processed, longleaf pine seed can be stored for 10 to 15 years and shortleaf pine seed can be stored for 15 to 20 years. The R8 NFS Genetics program ships samples of the newly harvested seed to the National Seed Lab (Dry Branch, GA; https://www.fs.usda.gov/nsl/) to be tested for initial germination and viability. At 5-year intervals after that, the seed bank re-tests seed lots for germination and viability. This testing protocol allows us to track seed quality and degradation over time. After the seed has reached it maximum shelf life, it is disposed of according to agency regulations. Most seed bank, seed orchard, and nursery personnel are aware that the infrequency of cone crops, improper handling or processing of cones or seed, and limited storage shelf life of seed can contribute to seed scarcity and compromised seed quality.

As plans for accelerated restoration move forward, it is important to be cognizant of the genetic resources (i.e., seed orchards, seed production areas, and progeny tests) available that can provide a sustainable supply of seed (Crane and Barbour, 2009, Crane et. al. 2015). This knowledge will help assess the capacity, identify needs, and ensure availability of multiple seed sources to plant in various seed zones. A survey template to assess southern genetic resources was developed by R8 NFS Genetics program, for both longleaf and shortleaf pine (figure 8). Surveys were circulated over the past decade to a number of participants, including Federal and State agencies, universities, nongovernmental organizations, private industry and private nurseries, and tree improvement and nursery cooperatives. The survey results were summarized (table 1), and the information will help us address questions such as the following:

- Does the South have enough seed to support accelerated restoration efforts?
- Who has ownership of the genetic resources? Private, public, Federal, State?
- What is the quantity and condition of these resources? What is the age of the resource? Are the resources being managed? Have they been mothballed? Or have they been abandoned?
- Are all seed zones covered? Are any seed zones missing?
- Eastern seed zones are being updated; how will this affect seed supply and deployment e.g., Eastern Seed Zone Forum (http://eszf.sref.info/)?
- Are there challenges to seed processing and kiln capacity? Are there bottlenecks? Are there adequate facilities? Storage shortfalls?
- Are skills being retained? Is there succession training to develop new personnel and provide continuity of experience and skills?
- What about climate change? Are there enough seed sources to address changing climates and subsequent changing seed zones? How will this affect deployment and what will be the guidance for deployment?

In the long term, options may be considered for additional DNA marker development and more intensive DNA testing of orchard trees and future seed crops. These options, however, are expensive and time-consuming. Neither the longleaf nor shortleaf pine genomes have been mapped. Genome mapping, especially for outbred organisms that have high genetic diversity, like longleaf and shortleaf pines, will take several years and more than $1 million. Questions remain about the number of genes in each genome and what those genes control.



Statement

Seed Orchard Resources — ACRES

First Generation

Second Generation

Advanced Generation

Seed Production Areas

Current Orchard Management — YES — NO

Original orchards retained, not managed, no seed collected

Orchards retained, not managed, some seed collected

Orchards retained, limited management, seed collected

Orchards retained, actively managed, seed collected

Orchards retained, actively managed, seed collected, additional genetics work underway or planned

Orchards removed

New orchards recently established on _____ acres

Never had shortleaf orchards

Seed Inventory — POUNDS/ 1st or 2nd GEN

Approximate annual seed collection (averaged for last five years)

Approximate pounds of seed in storage

Seed Age

Program Intentions Next Five Years — YES — NO

Maintain status quo

Increase management intensity and seed collection activity

Discontinue shortleaf efforts, remove orchards

Mothball orchards for the time being

Kiln Facility (Write in YES or NO and LOCATION)

Geographic sources for shortleaf in your program:

Geographic area where seed/seedlings are adapted for out-planting:

Additional Comments:

Figure 8. Longleaf pine genetic resources survey template. (By Barbara Crane, 2010)

Conclusions

There is uncertainty with climate change, and what the impacts will be on future forests. Will there be more natural hybrids? No one knows for sure. There are several management strategies in play that can deter hybrid seedling establishment on the forested landscape:

- R8 NFS Genetics program will continue to provide guidance on deploying the most appropriate genetic material, using pure seed, adhering to appropriate seed zones and seed movement guidelines (both current and updated), and monitoring future seed crops for any signs of hybridization. The program, in cooperation with partners, will work on increasing the genetic resources (seed orchards, seed production areas, seed banks) and support general forest collections to augment seed orchard collections.
- R8 NFS Silviculture programs will continue to use prescribed burning as a management tool, and monitor any evidence of hybrid seedlings during seedling survival checks.
- Nursery personnel will continue to cull any unusual seedling phenotypes at the grading tables.

One additional paramount management strategy is succession planning and training of new people in forest genetics. Genetics programs are critical in supporting successful reforestation and restoration by providing genetics expertise and genetically appropriate quality seed. Unfortunately, many challenges exist in maintaining the agency's forest genetics skills and expertise and its tree improvement and nursery programs. These challenges include: declining resources (funding, personnel); loss of skilled seed orchard, seed bank, and nursery personnel; aging seed orchards and/or lack of seed orchards representing all seed zones and seed sources; loss of forest genetics, tree physiology, and seed biology expertise; lack of, or failing, infrastructures; and loss of nurseries (Wheeler et al. 2015).

Summary

As practitioners struggle with how to restore and manage populations that are threatened with climate change, applied-academic partnerships can achieve both restoration and research goals. Through translational collaboration, we can increase the impact of our work by combining our resources to get projects done while also studying their efficacy. Engagement of professionals interested in forest genetics is an important component in this effort because it increases opportunities to collect more extensive and longer term data using different cohorts over time. However, the greatest benefit may be to stimulate interest in a diverse cadre of students to encourage them to continue on to professional careers in our disciplines and become a component of an informed citizenry.

Address correspondence to—

Barbara Crane, Regional Geneticist, USDA Forest Service, Southern Region, 1720 Peachtree Road NW, Suite 816N, Atlanta, GA 30309; email: barbara.crane@usda.gov; phone: 404–347–4039.

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Challenges and Opportunities for Maintaining Ponderosa Pine Forests in the Southwestern United States

Thomas Kolb, Aalap Dixit, and Owen Burney

Professor, School of Forestry, Northern Arizona University, Flagstaff, AZ; Graduate Research Assistant, School of Forestry, Northern Arizona University, Flagstaff, AZ; Associate Professor and Superintendent, John T. Harrington Forestry Research Center, New Mexico State University Mora, NM.

Abstract

Deforestation caused by wildfire and bark beetle attacks in southwestern ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson.) forests has increased over the past century due to climate warming. Continued warming is expected to increase deforestation. Ponderosa pine regeneration after deforestation often is inadequate in the region. Opportunities exist for active management to mitigate deforestation. First, planting can promote reforestation, but survival of planted seedlings is generally poor and highly variable among sites. The region needs more research about improving early seedling performance. Secondly, improving aridity adaptation of planted seedlings by seed source selection may improve outplanting performance. New common garden studies of seedling aridity adaptation of Arizona and New Mexico provenances suggest genetic variation in aridity adaptation among populations. Early results show genetic variation in survival under extreme drought conditions. Greenhouse experiments are investigating genetic variation in mechanisms of aridity tolerance. Promotion of forest recovery using these emerging approaches will be critical for sustaining forests in the increasingly arid Southwestern United States. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25–26, 2018).

Introduction

Ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson.) has the largest geographic range of any pine in the United States, occurring in 14 Western States (Hardin et al. 2001). It is a large tree at maturity with valuable wood in commercial quantities throughout its range. Moreover, it dominates forests in upland watersheds that supply clean water for human consumption and agriculture. Ponderosa pine forests provide numerous ecosystem services, including wood products, wildlife habitat, carbon sequestration, clean air, and temperature amelioration. Yet these services are threatened by deforestation resulting from increases in drought, wildfire, and bark beetle attacks (Kolb et al. 2016a, Williams et al. 2010, Williams et al. 2013). The threat is particularly severe in the Southwestern United States, where ponderosa pine is the dominant tree in most upland watersheds. Forests in this arid region are scarce compared to other U.S. regions (only about 27 percent of Arizona and New Mexico are forested), and consequently are disproportionally important for ecosystem services.

Threats to Ponderosa Pine Forests

The Southwestern United States has experienced a century of warming and unusually high tree mortality. Directly measured temperatures from weather stations show warming throughout the region of 2 to 5 ^{o}F (1 to 2.5 ^{o}C) since 1901 in both maximum and minimum air temperatures (Garfin et al. 2013). A pulse of ponderosa pine mortality occurred during the latter part of the century of warming. Since the mid-1980s, between 11 and 18 percent of ponderosa pine trees died in Arizona and New Mexico from drought-associated wildfire and bark beetle attacks (Hicke et al. 2016, Williams et al. 2010). Area burned by wildfire increased during this period of warming (Westerling et al. 2006), which includes the largest forest fires in the recorded history of Arizona (Wallow Fire, 469,300 ac [190,000 ha]) and New Mexico (Whitewater-Baldy Fire, 297,635 ac [120,500 ha]) in 2011. Moreover, forest area

attacked by bark beetles also increased in the Southwestern United States during this period of warming (Hicke et al. 2016, Raffa et al. 2008).

Ponderosa pine regeneration has been meager and slow after severe stand disturbance during this period of warming. This slow regeneration is due to lack of seed trees and the presence of harsh abiotic conditions (Puhlick et al. 2012) and is exacerbated by ponderosa pine's lack of fire-adapted, serotinous cones and lack of vegetative resprouting (Burns and Honkala 1990). Natural regeneration of ponderosa pine in deforested areas can occur on moister sites when seed trees are within about 500 ft (164 m) of openings (Bonnet et al. 2005, Haffey et al. 2018, Haire and McGarigal 2010, Owen et al. 2017). Many recent wildfires in the Southwestern United States, however, have produced large openings on dry sites that are significantly distant from seed trees, thereby limiting ponderosa pine regeneration (figure 1). Many recent studies report inadequate ponderosa pine regeneration after intense burning, leading to transitions from forests to grass- or shrublands (Allen and Breshears 1998, Chambers et al. 2016, Dore et al. 2012; Haffey et al. 2018, Ouzts et al. 2015, Roccaforte et al. 2012, Rother and Veblen 2016, Savage et al. 2013). Similar findings of a decrease in forest resilience to severe burning have been reported for multiple forest types in Western North America (Stevens-Rumann et al. 2017).

All of the aforementioned threats to southwestern ponderosa pine forests are expected to increase in the future with continued warming. Climate models for the Southwestern United States predict further increases in mean annual and maximum summer temperatures and more frequent severe droughts (Garfin et al. 2013, Seager et al. 2007). Future changes in precipitation are poorly understood, but winter precipitation in montane watersheds is expected to shift from snow to rain (Garfin et al. 2013). Forest area burned in the Southwest is also projected to increase in the future (Flannigan et al. 2013, Littrell et al. 2009, Xu et al. 2013). Aridity stress to forests caused by warming will be especially severe in semi-arid forests of the Southwestern United States because productivity is already strongly constrained by low precipitation (Boisvenue and Running 2006, Williams et al. 2012). Greater future aridity is projected to reduce establishment and growth and increase mortality of tree species in semi-arid forests such as in the Southwest (Adams and Kolb 2005, Bell et al. 2014, Petrie et al. 2017, Puhlick et al. 2012, Rother et al. 2015, Wu et al. 2011). Tree mortality due to bark beetle attacks in the region is expected to increase in the future as warming makes droughts more intense (Kolb et al. 2016a). Consequently, climate-change and climate-envelope models project substantial loss of ponderosa pine forests in the Southwestern United States over the next century (Rehfeldt et al. 2006, Williams et al. 2013).

Figure 1. Examples of no ponderosa pine regeneration several years after severe forest burning in northern Arizona. (Photo by Thomas Kolb, 2013)

Opportunities for Reforestation by Planting

Artificial regeneration by planting has the potential to slow recent losses of ponderosa pine forests in the Southwestern United States. The backlog of understocked areas that previously supported ponderosa pine forests in the Southwest is, however, formidable. The amount of U.S. Department of Agriculture (USDA), Forest Service lands in the Southwestern Region needing active planting of tree seedlings after wildfire was estimated to be 159,418 ac (64,542 ha) in 2015 with a projected cost of approximately $79M (USDA Forest Service 2016). This amount is a conservative estimate because of the increasing occurrence of wildfire. Most of these planting needs are in ponderosa pine forests, which represent over 60 percent of the forest cover in the Southwestern Region.

Establishment of ponderosa pine seedlings by planting in the Southwestern United States is difficult due to short frost-free seasons, dry spring weather, and

extreme variation in temperature and precipitation. Research about artificial regeneration of ponderosa pine in the Southwest is scant and most studies were performed decades ago (Schubert et al. 1970, Schubert 1974). Recent studies have confirmed the difficulty in establishing ponderosa pine by planting in the region. For example, survival of ponderosa pine seedlings planted recently in the Davis Mountains in West Texas ranged between 22 and 34 percent in fall plantings, and between 9 and 25 percent in late-summer plantings (Vickers et al. 2018). Physical weed control increased survival in both seasons. The most important mortality agents were pocket gophers and desiccation. A recent survey of ponderosa pine plantings in severely burned areas in Arizona and New Mexico reported average survival of 25 percent over eight sites (5 to 8 years after planting), with high variation among sites; some sites had no survival, others had survival between 10 and 40 percent, and the best site had 60 percent survival (Ouzts et al. 2015). The sites with no survival of planted seedlings also had no evidence of natural ponderosa pine regeneration and were converted from pine-dominated forests to shrublands or grasslands.

Ouzts et al. (2015) also addressed whether planting resulted in enough established seedlings to put the stand on a trajectory towards recovery of a ponderosa-dominated forest. They assumed a regeneration target of at least 49 established seedlings per ac (120 per ha) to produce a low-density ponderosa pine overstory, with the expectation that survival between the established seedling and mature tree stages would be 44 percent based on DeWald and Mahalovich (2008). The regeneration target is based on ecological restoration principles derived from the historical range of tree-density variation in southwestern ponderosa pine forests exposed to frequent, low-intensity burning (Reynolds et al. 2013). Planting seedlings produced close to this regeneration target at five of the eight sites (figure 2). This result shows that successful post-wildfire plantings of ponderosa pine can put stands on a trajectory towards forest recovery. Obviously, low survival of planted seedlings in the Southwest should be anticipated and compensated for by planting more seedlings than ultimately desired.

Opportunities exist to improve survival and performance of planted ponderosa pine seedlings in the Southwest although little contemporary research has been done. Approaches needing new research include:

- Planting season (e.g., Vickers et al. 2018)
- Stocktype (e.g., Pinto et al. 2011)
- Nursery conditioning for drought tolerance (e.g., Trickler et al. 2013)
- Physical protection of stems and roots from animals (e.g., Engemann et al. 1999)
- Irregular, cluster planting designs (e.g., North et al. 2019, Vickers et al. 2018)
- Nucleation plantings in favorable microsites (e.g., North et al. 2019)
- Facilitation by living (e.g. other plants) and non-living objects (e.g., logs, rocks) (e.g., Gómez et al. 2004; Burney et al. 2007)
- Selection of arid-adapted seed sources (e.g., Kolb et al. 2016b)

Opportunities to Improve Seedling Aridity Adaptation by Seed Source Selection

Recommendations to use strictly local seed sources in reforestation are likely outdated for the future with continued climate warming and increasing drought. Instead, reforestation projects should emphasize the planting of seedlings that are adapted to forecasted warmer and drier conditions (Williams and Dumroese 2013). Much evidence indicates that natural selection has produced populations that are preadapted for future arid sites (Alberto et al. 2013). For example, seedlings of several tree species from arid/warm locations performed better at warm sites than seedlings from wetter/cooler locations (Bingham and Simard 2013, Drake et al. 2015, Taibi et al. 2014). Past recommendations to use local seed sources in active reforestation of ponderosa pine in the Southwestern United States were based on common-garden tests of widely separated geographic sources planted many decades ago during cooler, wetter periods (DeWald and Mahalovich 2008). Such recommendations are likely no longer valid given rapid climate change. Instead, recent recommendations based on the concepts of preadaptation and assisted population migration call for use of arid-adapted genotypes in reforestation projects, especially at trailing-edge sites (e.g., low-elevation and southern range edges), to reduce adaptation lag times, and to reduce the number of generations required for evolution to produce populations attuned for warmer climates (Rehfeldt et al. 2014a, 2014b; Williams and Dumroese 2013, Taibi et al. 2014). These recommendations and supporting research have

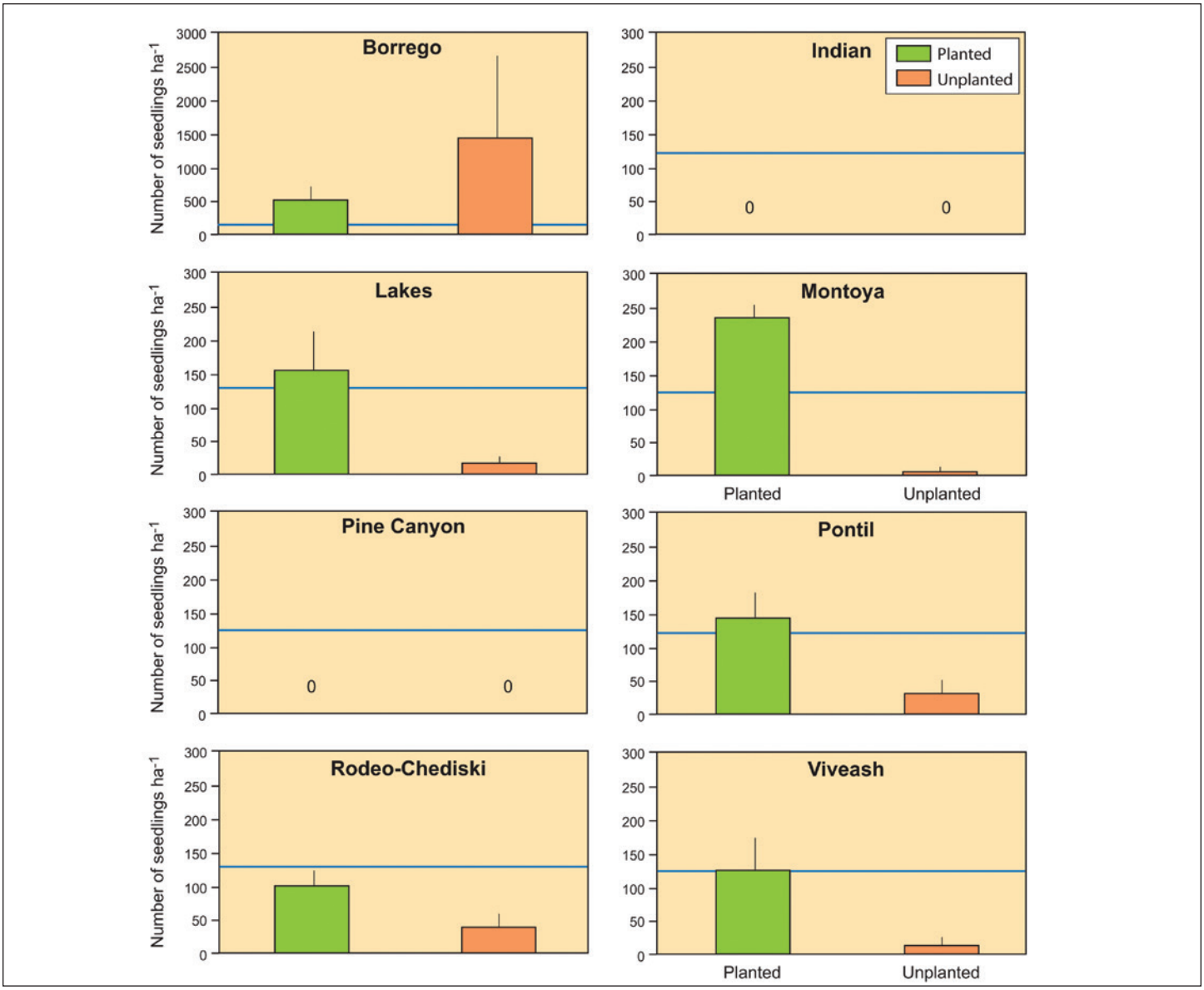

Figure 2. Ponderosa pine seedling density at eight severely burned sites in plots planted with ponderosa pine seedlings, and paired unplanted plots. Density at the Borrego site was nearly 10 times more than the other sites. The horizontal line in each panel is the minimum target seedling density (49/ac [120/ha]) projected for producing an open-structure forest of ponderosa pine in the future. Vertical lines are 1 standard error. Modified from Ouzts et al. (2015).

led to the development of decision-support tools for selecting seed sources adapted to future climates, such as the seedlot selection tool (https://seedlotselection-tool.org/sst/). Predictions of this tool, which are based on climate-matching algorithms, rarely have been tested, especially for trees in the Southwest.

Evaluations of appropriate ponderosa pine seed sources for a more arid climate in the Southwest have started. Kolb et al. (2016b) reported that ponderosa pine seedlings from low-elevation, drier seed sources in northern Arizona had a more drought-adapted architecture (lower shoot-root ratio) and longer survival of experimentally induced drought in the greenhouse than high-elevation, wetter sources. A new investigation builds on this finding by expanding investigations of seedling drought tolerance to 21 seed sources from a broad gradient of elevation, temperature, and precipitation over Arizona and New Mexico (figures 3 and 4). Seeds used in the investigation were compiled from collections made over the last three decades by John Harrington (New Mexico State University), Phillip Patterson (Northern Arizona University), and the authors. The John T. Harrington Forestry Research Center in Mora, NM, produced the seedlings, which were planted in July 2018 at the onset of monsoon rains at three common-garden experiments across an elevational gradient: (1) a cool, high-elevation site

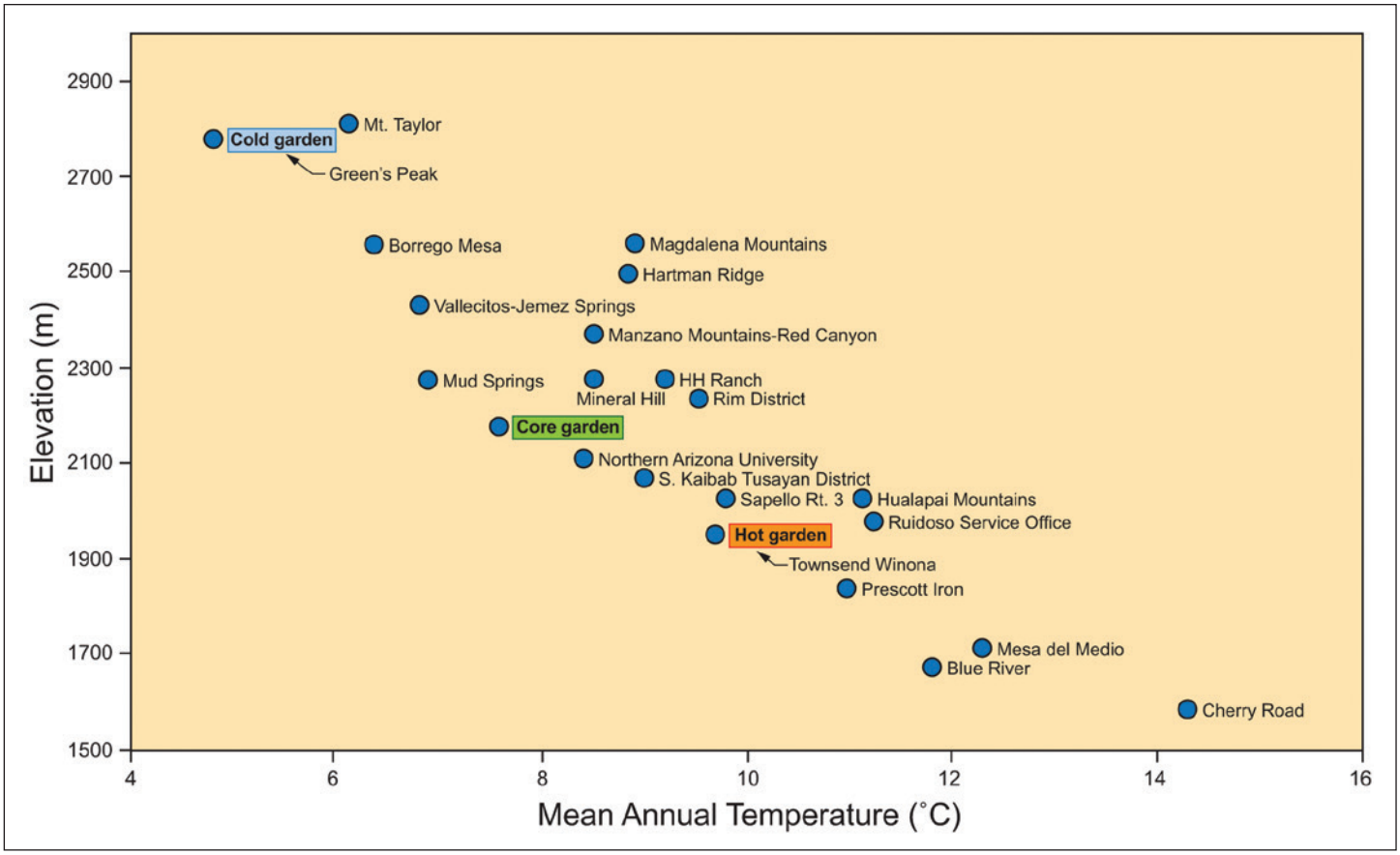

Figure 3. Elevation, mean annual air temperature (1981-2010 obtained from PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu), and site name of 21 seed sources from Arizona and New Mexico used in the new provenance experiments. The colored boxes are the three field common-garden sites where seed sources were planted in summer 2018.

currently supporting mixed conifer and aspen forests; (2) a moderate temperature, mid-elevation site currently supporting ponderosa pine; and (3) a hot, low-elevation site currently supporting pinyon-juniper woodland. A fourth common-garden experiment, located in the greenhouse at Northern Arizona University, will be used to investigate mechanisms of heat and drought tolerance (figure 5). Seedling survival has been high (greater than 95 percent) at the high- and mid-elevation sites 3 months after planting. In contrast, most seedlings died at the low-elevation site within 2 months after planting due to desiccation. Seedlings were planted at the low-elevation site in the middle of July, after the start of late-summer monsoon rains, but little rain fell for 3 weeks after planting and over 90 percent of seedlings died (figure 6). Interestingly, seedlings from

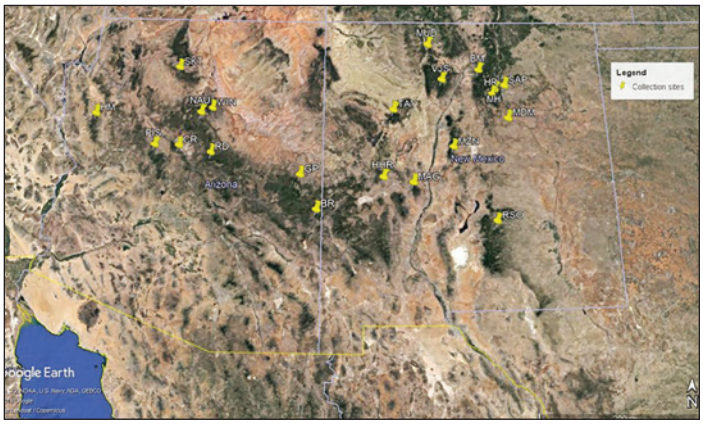

Figure 4. Location of the 21 seed sources from Arizona and New Mexico used in the new provenance experiments (Google Earth 2018).

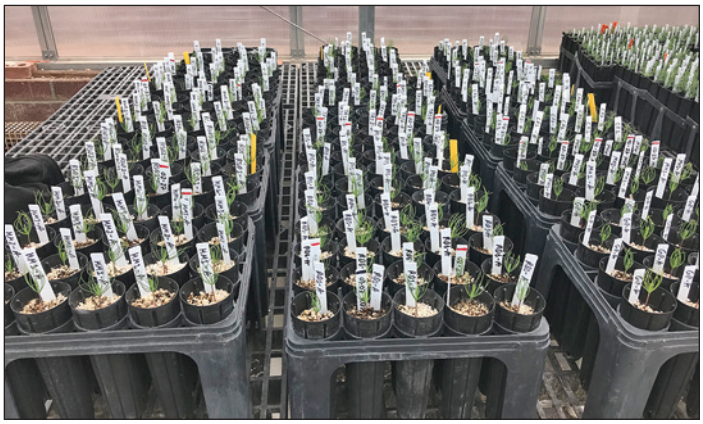

Figure 5. Ponderosa pine seedlings being grown in the Northern Arizona University Research Greenhouse for drought tolerance experiments. (Photo by Aalap Dixit 2018)

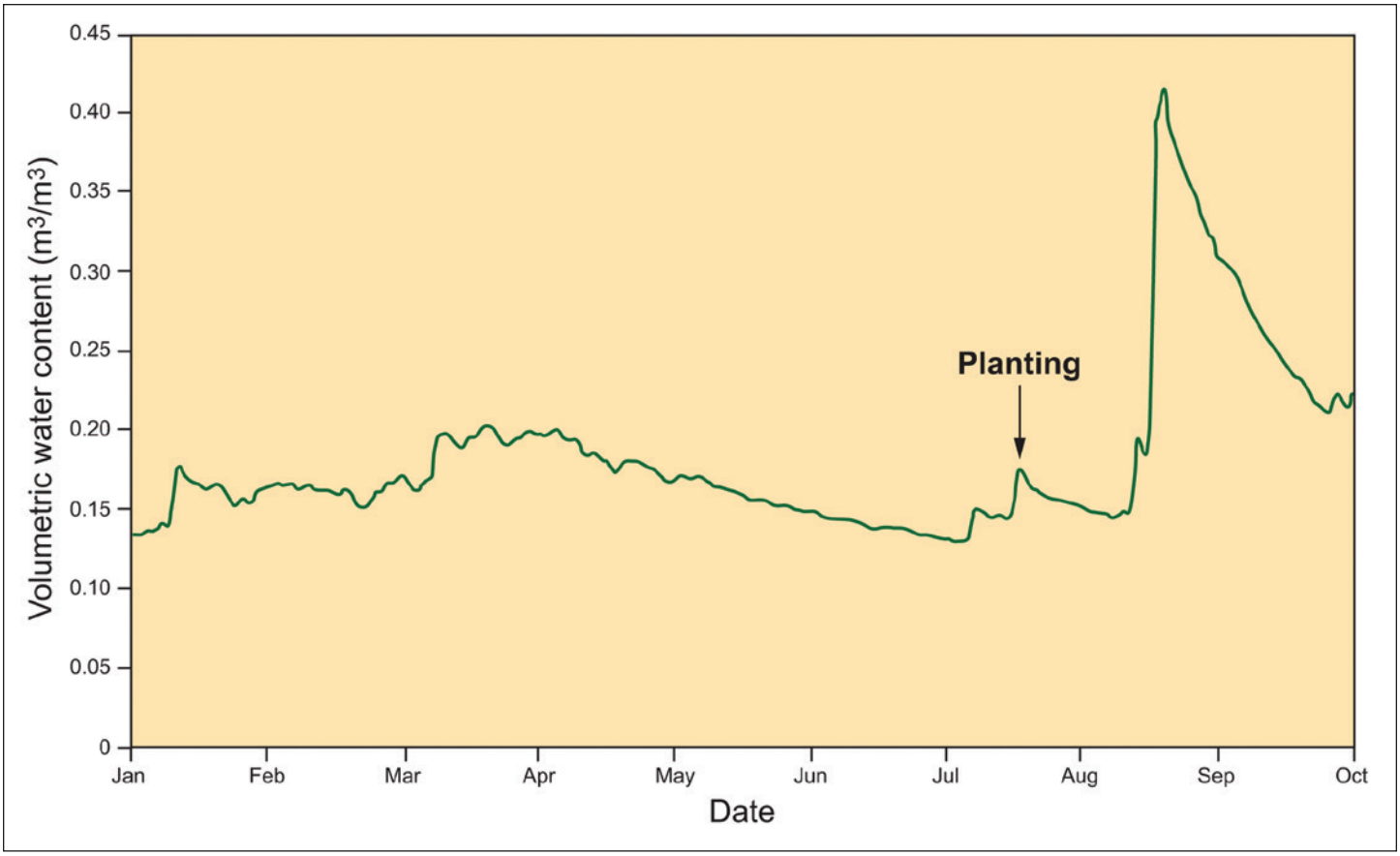

Figure 6. Soil volumetric water content (1 ft. depth) at the low-elevation test site in 2018. Seedlings were planted in mid-July when rains started, and most died in the next month when rains stopped for several weeks and soil water content dropped to about 0.15.

high-elevation (greater than 8,000 ft [2,438 m]) sources died fastest, and seedlings from low-elevation (less than 6,500 ft [1,981 m]) sources died slowest (figure 7). This result suggests greater inherent drought tolerance of low-elevation sources. Continuing measurements at the high- and mid-elevation field sites and greenhouse will provide information about trade-offs among heat-, drought-, and cold-tolerances of seed sources, as well as source by environment interactions that will inform revision of seed zones for the region.

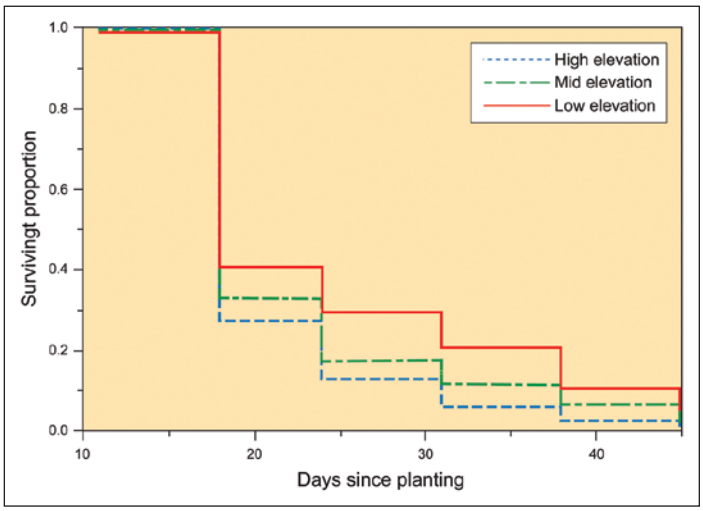

Figure 7. Surviving proportion of ponderosa pine seedlings planted in summer 2018 at the low-elevation, high-stress common-garden site from seed sources grouped by elevation (high = >8,000 ft [>2,438 m]); mid = 6,500 to 8000 ft [1,981 to 2,438 m]; low = <6,500 ft [<1,981 m]). The elevation groups differed significantly ($P < 0.05$) in survival duration.

Conclusions

We anticipate an increasing role in the Southwestern United States over the next few decades of tree-seedling production facilities, nursery cultural practices specific to semi-arid forest outplanting environments, genetic improvement of seedling drought and heat tolerances, and active reforestation by tree planting to help forests recover from severe disturbances. Such efforts are especially appropriate for publicly owned forests (e.g., Federal, State), which should be promptly regenerated after disturbance under current forest management laws. More research, such as the examples we describe here for ponderosa pine, is needed about improving the stress tolerance and performance of tree seedlings

on harsh sites. We call for a renewed focus on tree regeneration by forest managers, practitioners, and scientists to sustain forests in the increasingly arid Southwestern United States.

Address correspondence to—

Thomas Kolb, Professor, School of Forestry, Northern Arizona University, Flagstaff, AZ 86001; email: tom.kolb@nau.edu

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iFertigate: A New Mobile App to Assist With Fertigation Calculations

Daniel J. Drummond and Diane L. Haase

Information Technology Specialist and Mobile Applications Director, Southern Regional Extension Forestry, University of Georgia, Athens, GA; Western Nursery Specialist, U.S. Department of Agriculture, Forest Service, Portland, OR

Abstract

Nutrition management in nurseries is critical for growing quality plant crops. Applying soluble fertilizer through the irrigation system (i.e., fertigation) requires careful calculations of concentrated fertilizer products to create nutrient mixtures that will deliver target nutrition levels. The iFertigate mobile application (app), available for both iOS and Android platforms, allows the user to create and store calculations for specific nutrient tank mixes. The app provides information on macronutrient and micronutrient concentrations of each mix and gives an alert if there is a risk of precipitates. The user can set the language to either English or Spanish. This article provides background information about the app and describes how to use it. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25–26, 2018).

What Does It Do?

iFertigate was developed to assist with creating tanks mixes for nursery fertigation practices. With iFertigate, one can quickly and easily enter, calculate, and/or store multiple data for each tank mix:

- Water chemistry
- Macronutrient concentrations
- Micronutrient concentrations
- Nutrient ratios
- Precipitate potentials
- Preferred fertilizer products
- Past, current, and future fertigation costs

Where Did It Come From?

In 2014, the U.S. Department of Agriculture, Forest Service, Reforestation, Nurseries, and Genetics Resources (RNGR) team, in partnership with the Southern Regional Extension Forestry (SREF), began exploring the idea of developing a mobile application (app) specifically targeting a need within the forest and conservation nursery community.

Initially, a comprehensive review was launched to evaluate the cost, functionality, and overall benefits of all mobile apps that might be relevant to the forest nursery industry. Those findings were presented at the 2015 Joint Meeting of the Northeast Forest and Conservation Nursery Association and Southern Forest Nursery Association, in Kent Island, MD, and again at the 2015 Western Forest and Conservation Nursery Association Meeting, in Eugene, OR. These presentations were summarized in a 2016 article: Useful mobile applications for nursery and field personnel (Haase and Drummond 2016).

Following the presentation in Eugene, OR, the conference attendees participated in an open forum and gave their thoughts and suggestions regarding how a mobile app might be useful in the day-to-day life of a nursery manager. By combining those notes with the findings from the initial review, it became clear that there existed no mobile technology for assisting nursery managers with custom fertigation tank mixes. iFertigate was then developed to meet those needs.

How Do I Use It?

With simple data input, users can create and store any number of single- or multi-tank mixes and keep application quantities and costs in one place. After installing and launching the app, a navigation bar at

the bottom of the device screen displays buttons for the five main sections of the app: Mixes, Applications, Guide, About, and Settings (figure 1). These sections are used to tailor the app functionality for individual purposes and are described below.

Mixes

Most of the app interaction and output takes place in this section. The Fertigation Tank Mix Calculator is the first screen visible to the user under the Mixes main section. This screen displays a list of all previously made custom tank mixes, along with all included product quantities, product costs, and the total cost of the mix (figure 2a). Users can select previous mixes for review or edit, or can click the X beside any mix name to remove it completely from the app. Custom mixes can be added by clicking the Add Custom Mix button at the bottom of this screen.

From the main Fertigation Tank Mix Calculator screen, selecting an existing tank mix or the Add Custom Mix button will navigate the user to the Custom Fertigation Tank Mix screen (figure 2b). This screen allows the user to edit all information related to the custom mix and provides updated calculation results as new information is added.

The Custom Fertigation Tank Mix screen links to seven main areas:

1. Basic Information

This area displays the name of the mix, the crop to which it is to be applied, the dilution ratio of the fertilizer injector, and whether the mix is used for growing or hardening (figure 3a). Of this information, only the dilution ratio is used by any of the calculations. All other fields are optional, but are useful for keeping track of multiple tank mixes. Click the Edit Basic Information button to modify this information.

2. Water Quality

This area allows the user to enter custom water profiles (figure 3b). Each nursery has different water characteristics. Entering current water quality data enables the app to account for this in the fertigation output. Water profiles contain information about the nutrient concentrations, electrical conductivity, alkalinity, and pH of

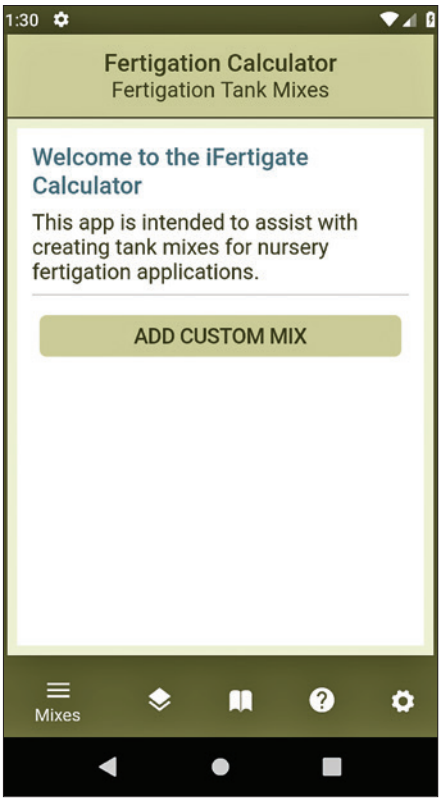

Figure 1. The iFertigate mobile app has five main buttons at the bottom of the screen. From left to right: Mixes, Applications, Guide, About, and Settings.

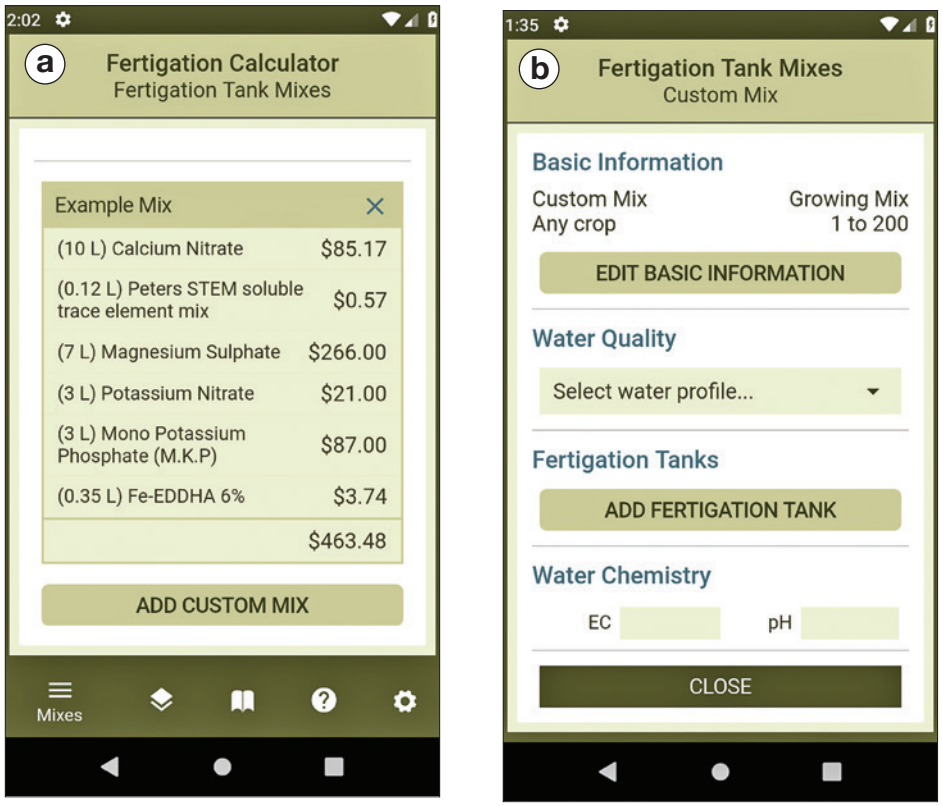

Figure 2. In the Mixes section of the iFertigate app, the user can view (a) all existing mixes stored in the app and their associated costs. By selecting "Add Custom Mix", the user can navigate to (b) the Custom Fertigation Tank Mix area of the app where the majority of data entry and app output occurs.

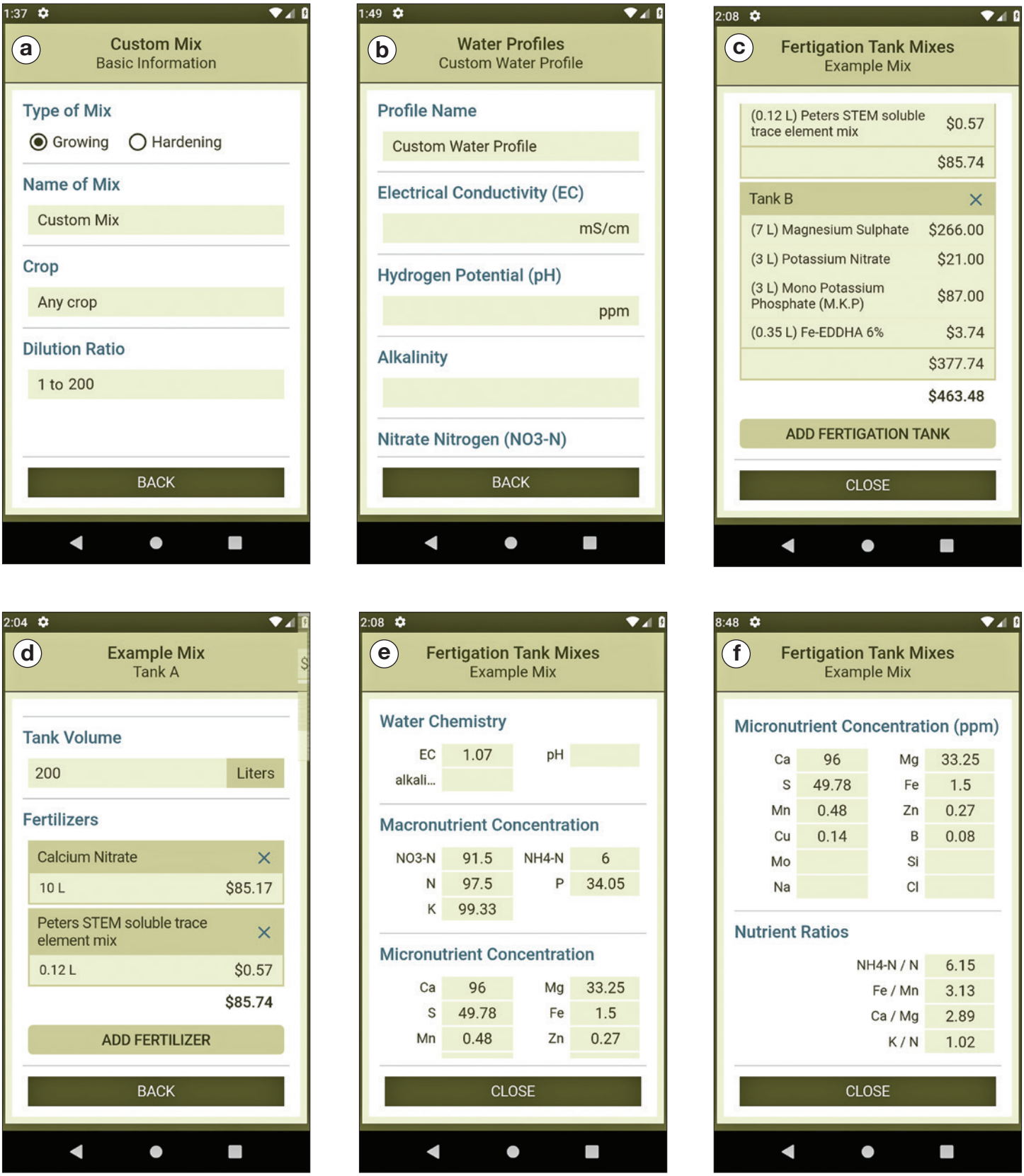

Figure 3. To use the iFertigate mobile app, the user starts by entering (a) basic information and (b) the irrigation water profile. The user can (c) view, add, or (d) edit tanks within existing mixes. For each mix, the app provides the resulting. [Additionally, these screenshots are taken from the phone application; the tablet application offers the same features but appears slightly different with more information visible on the screen at one time.] (e) water chemistry, macronutrient concentration, (f) micronutrient concentration, and nutrient ratios. [Note: these screenshots only show a portion of each page for description purposes; the user must scroll down to see all components. Additionally, these screenshots are taken from the phone application; the tablet application offers the same features but appears slightly different with more information visible on the screen at one time.]

the irrigation water prior to addition of fertilizer. These values are used in tank mix calculations. If no water profiles are available, a Create Water Profile button is shown instead. Water profiles are optional but can greatly impact the overall calculation results. Once one or more profiles are entered into the app, they will be available in this screen to select for specific tank mixes. Additional water profiles can be created or modified in the Settings section.

3. Fertigation Tanks

This area displays a list of fertigation tanks used in the mix, along with all included product quantities, product costs, the total costs of each tank, and the total cost of the mix (figure 3c). There is no limit to the number of tanks that can be included in a given mix. Warnings will appear in this area if any given tank has potential for precipitates caused by incompatible fertilizer products. Users can select a tank name to edit it or click the X beside any name to remove it completely from the mix. Fertigation tanks can be added to the mix by clicking the Add Fertigation Tank button at the bottom of this area.

Selecting an existing tank to edit it or choosing to add a new tank opens a screen that allows the user to edit/enter the name and volume (in liters or gallons) of a particular tank and to add fertilizers by clicking the Add Fertilizer button at the bottom of the screen (figure 3d). The screen displays a list of fertilizer quantities, fertilizer costs, and the total cost of the tank. Warnings will also appear in this area, if the mix in this tank could cause precipitates. Users can select a fertilizer name to edit it or click the X beside any name to remove it completely from the tank. There is no limit to the number of fertilizers that can be included in a given tank.

When adding a fertilizer to a tank mix, the app allows the user to select from a menu of available products, and enter a quantity (in liters, gallons, kilograms, or pounds), and optionally, product costs. A complete list of available fertilizer products can be modified in the Settings section.

4. Water Chemistry

Once a mix of one or more tanks is customized, this area displays the resulting electrical conductivity, alkalinity, and pH (figure 3e).

5. Macronutrient Concentration

This area displays the resulting concentrations (ppm) of nitrogen (nitrate and ammonium), phosphorus, potassium, calcium, and magnesium in a given mix (figure 3e). By examining these values carefully, the grower can adjust the products and amounts in the mix as needed to meet nutrition targets based on plant species, growing stage, and other factors.

6. Micronutrient Concentration

This area displays the resulting concentrations (ppm) of sulfur, iron, manganese, zinc, copper, boron, molybdenum, silicon, sodium, and chlorine in a given mix (figure 3f). Again, close scrutiny of these values can aid the grower in creating the optimum mix for the crop.

7. Nutrient Ratios

This area displays four common nutrient ratios: NH4-N/N, Fe/Mn, Ca/Mg, and K/N (figure 3f). Paying attention to these ratios can assist the grower in creating a balanced fertilizer regime.

Fertilizer Applications

The Fertilizer Applications screen displays a list of past and future applications of custom tank mixes, along with total application costs (figure 4). Users can select an application name to edit it or click the X beside any name to remove it completely from the app. There is no limit to the number of applications that can be recorded. Additional applications can be added by clicking the Add Application button at the bottom of this screen which allows the user to select from a menu of available custom tank mixes and enter a date of application.

Guide

This screen provides the user with helpful information regarding the iFertigate app and its use.

About

This screen provides information about the app, links to related resources, and contact emails. It also displays the following disclaimer:

This app was created for to use as a helpful tool with nursery fertigation practices. Every attempt has been made to ensure the accuracy of fertilizer data and associated calculations. Nonetheless, it is the app user's responsibility to confirm that the information generated by this app is correct and that the overall nutrition regime is appropriate for his/her crop(s). The information reported about specific fertilizer products does not imply recommendations or endorsements for their use.

Settings

The Settings screen is divided into four main areas:

1. Language

The app is available in English or Spanish. The user can use the radio buttons to change the language used throughout the app (figure 5).

2. Font Size

Font size can be adjusted by dragging the slider from left to right to reduce or enlarge the font size for readability (figure 5).

3. Products

This area provides the user with three possible actions (figure 5):

- Clicking Add New Product opens a screen where the name, cost, and nutrient information of a product can be entered (figure 6a). The new product will then be added to the list of available fertilizer products in the app.
- Clicking Edit Product List displays a list of available fertilizer products (figure 6b). Users can select a fertilizer product name to edit it or click the X beside any name to remove it from the app.
- Clicking Restore Defaults restores the list of available fertilizer products to its original settings.

4. Water Profiles

This area allows the user to Add Water Profile or to Edit Water Profile (figure 3b). There is no limit to the number of water profiles that can be added. Users can select a water profile name to edit it or click the X beside any name to remove it from the app. The user can enter the name, nutrient concentrations, electrical conductivity, alkalinity, and pH of a given irrigation water source.

Where Can I Find It?

Both iOS and Android versions are available on the iOS App and Google Play Stores, respectively. Links are also available at https://rngr.net/resources/apps/ifertigate. The app is available for both phones and tablets and will be updated periodically to address any glitches or inaccuracies.

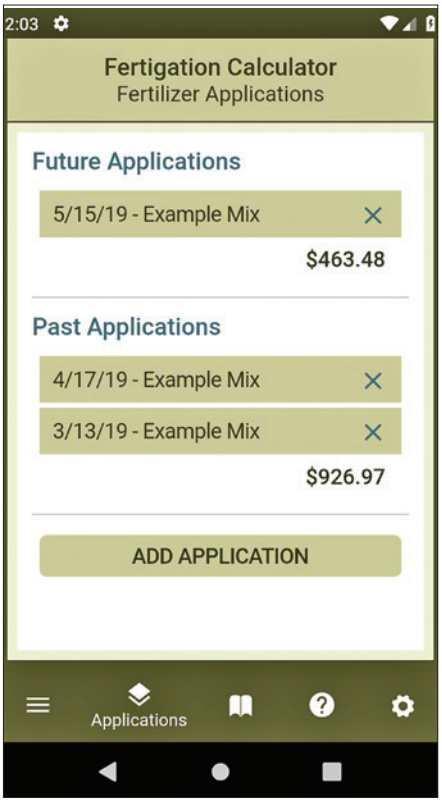

Figure 4. The iFertigate mobile app allows the user to record past and future fertigation applications and costs.

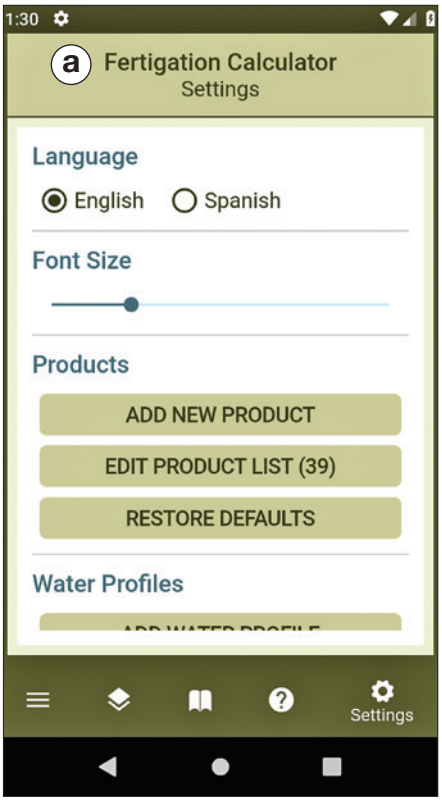

Figure 5. a) The iFertigate app settings allow the user to choose the language and font size as well as edit fertilizer products and water profiles. (b) The entire app is available in Spanish.

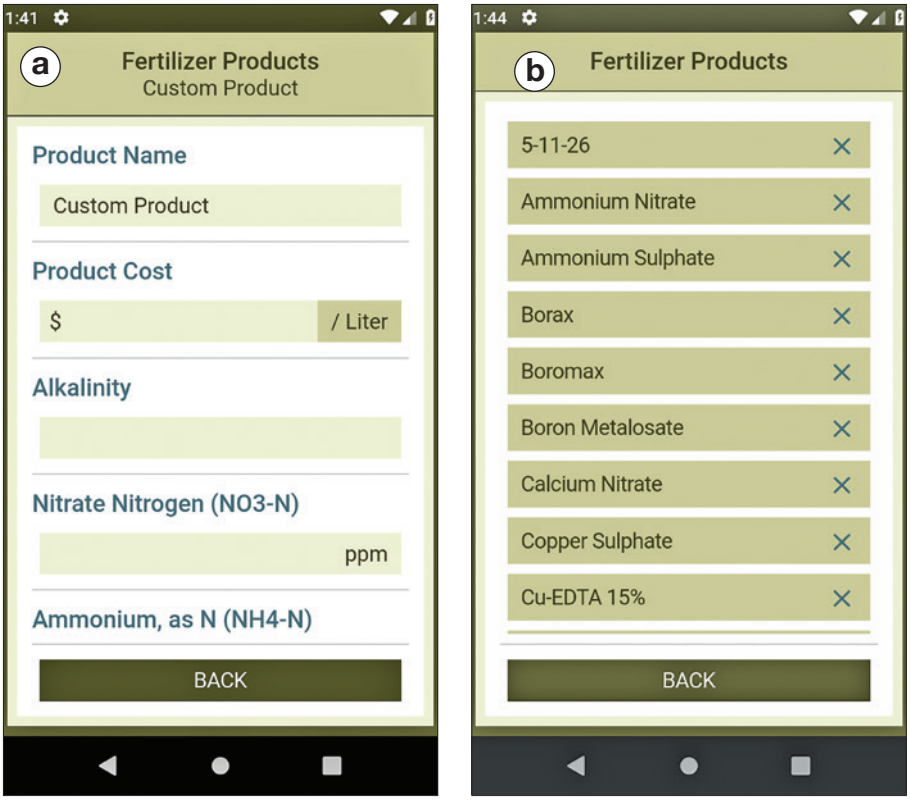

Figure 6. The iFertigate app comes pre-loaded with common fertilizer products. The user can (a) add additional products and (b) view, edit, or delete existing products.

Address correspondence to—

Daniel J. Drummond, Southern Regional Extension Forestry, University of Georgia, Warnell School of Forestry and Natural Resources, 4-430 Forest Resources, Athens, GA, 30602; email: sref@sref.info; phone: (706) 542-2955.

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Production of Genetically Appropriate Native Grass and Forb Seed at the USDA Forest Service Coeur d'Alene Nursery

Nathan Robertson

Bareroot and Seed Horticulturist,
U.S. Department of Agriculture, Forest Service, Coeur d'Alene Nursery, Coeur d'Alene, ID

Abstract

The U.S. Department of Agriculture, Forest Service, Coeur d'Alene Nursery is the tree seedling and native plant propagule production facility for the Northern Region of the Forest Service (Region 1). Although conifer seedling production is the nursery's primary focus, requests for native plant and seed production have risen over the last decade, partly in response to national efforts to use site-appropriate species and genetics for revegetation work on public lands. Region 1 botany staff and the Coeur d'Alene Nursery, in conjunction with partners, have worked for the past decade to establish empirical seed transfer zones for commonly used native forbs, shrubs, and grasses. This work is the primary component of the Region's effort to collect, increase, and furnish genetically appropriate native seed to revegetation practitioners. The nursery also provides seed increase services for individual projects and specialty native plant species from multiple ecosystems in the Western United States, with a focus on maintaining genetic integrity. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25–26, 2018).

Region 1 Seed Transfer Zone Development

Wildland Seed Use and Genetics

Historically, the U.S. Department of Agriculture (USDA), Forest Service and other Federal agencies involved in land management have relied heavily on the use of cultivars (cultivated varieties) for grass and forb reseeding work in wildlands (Burton and Burton 2002). These cultivars were developed by Federal, State, or private entities for revegetation efforts or range forage production. Typically, traits such as ease of culture, high seed yield, high biomass yield, and speed of growth and establishment were preferred when developing cultivars. Breeding programs focusing on such characteristics helped create low-cost, high-yield strains of useful revegetation species, but have rarely considered the genetic implications for long-term wildland establishment, adaptation to planting site climate and soil conditions, or impacts on local population genetics (Lesica and Allendorf 1999). These cultivars are often available in large, commercially produced quantities, whereas locally adapted alternatives are typically rare outside of small, wildland collections. Due to this rarity, revegetation practitioners often resort to using cultivars, despite sometimes tremendous geographic and climatic distances between revegetation project areas and the cultivar's genetic origin.

Species Selection and Seed Zone Establishment

Recognizing these challenges, practitioners in the Northern Region of the USDA Forest Service (Region 1) have been working for the past decade to research and develop locally adapted, genetically appropriate seed stores for commonly used shrub, forb, and grass revegetation species. This work involves three major components: (1) collect small volumes of genetically representative native wildland seed from across Forest Service lands in Region 1 for commonly used revegetation species; (2) establish, monitor, and collect data from common gardens grown from that wildland seed to develop area-wide genetic groupings (seed transfer zones); and (3) collect larger volumes of wildland seed

from within each of the newly developed seed transfer zones to use as seed stock for commercial-scale seed production. Using this method, species with previously poorly known or unknown genetic distributions can be mapped, sampled, bulk-produced, and incorporated into revegetation work without many of the genetic and ecological risks inherent to cultivars of the same species (Johnson et al. 2010).

Implementation

Region 1 Forest Service botanists and revegetation practitioners have systematically selected and studied two shrub species, four forb species, and eight grass species. These species were chosen based on the ubiquity of their distribution within the Region, their ability as early seral species to colonize disturbed areas and compete well with weedy invaders, and their ease of cultivation in large-scale seed production facilities. Several species are still in the process of development, but to date, seed transfer zones have been published for 12 native shrubs, grasses, and forbs (such as those shown in figures 1 and 2). Many of the 12 species are now being collected and used for commercial-scale seed production. Three of the grass species, bluebunch wheatgrass (*Psuedoregneria spicata* Pursh), rough bentgrass (*Agrostis scabra* Willd.), and tufted hairgrass (*Deschampsia cespitosa* L.), are currently in production and yielding significant volumes of zone-identified seed (figure 3). That seed is being incorporated into wildland seed mixes throughout Region 1 for revegetation efforts such as post-fire seeding, forest

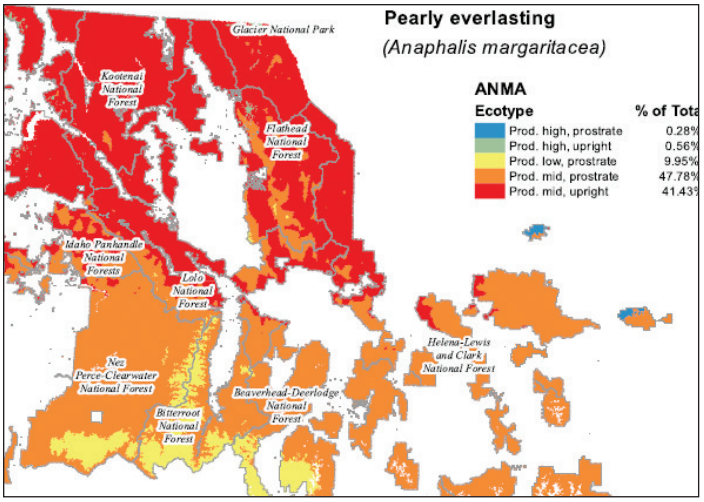

Figure 1. Seed transfer zone map for pearly everlasting (*Anaphilis margaritacea*, ANMA) showing seed zone distribution on USDA Forest Service Region 1 lands (Gibson et al. 2017a)

engineering projects, and wildlife/riparian restoration work. Some of the 12 species, such as pearly everlasting (*Anaphalis margaritacea* L.) and bluejoint reedgrass (*Calamagrostis canadensis* Michx.), are widely distributed across the Region and have high ecological value as native colonizers, but present cultural or seed processing challenges for large-scale seed production facilities (Flessner and Trindle 2003). In the future, some of these species may be bulk-produced by the USDA Forest Service Coeur d'Alene Nursery (CDAN). The primary avenue for Region 1 grass and forb seed production will, however, continue to be via contract growing with private-sector native seed production companies. Ideally, this scenario allows Forest Service revegetation projects to incorporate genetically

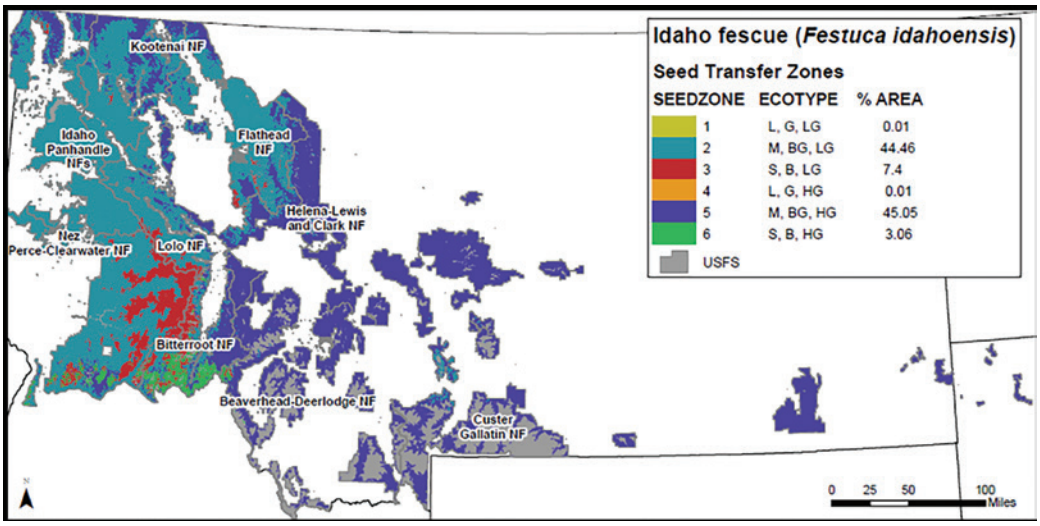

Figure 2. Seed transfer zone map for pearly everlasting (*Festuca idahoensis* Elmer, FEID) showing seed zone distribution on USDA Forest Service Region 1 lands (Gibson et al. 2017b)

Figure 3. Bags of source-identified bluebunch wheatgrass (*Pseudoroegneria spicata Pursh*) generated by a commercial grower for incorporation into Region 1 seed mixes. (Photo by Nathan Robertson, 2019)

appropriate seed in accordance with Federal guidelines, while simultaneously providing business opportunities for private-sector seed producers in lieu of cultivars of the same species.

Native Seed Production at the Coeur d'Alene Nursery

Historic Seed-Increase Production

Seed transfer zones have been used for decades to protect landscape-level genetic distributions in commercially valuable tree species. These zones have guided forest managers in the replanting of millions of acres of public and private forest lands. Adhering to seed transfer guidelines and sourcing genetically appropriate seed for tree species is now a well-accepted best practice, especially on publicly owned forest lands in the Western United States (Johnson et al. 2004). While the avenues for tree seed collection, processing, and storage, as well as orchard seed production, have been well studied, no such body of literature exists for most native grasses, shrubs, and forbs (USDA Forest Service 2012). This is especially true regarding native plant genetics (Bower et al. 2014). The Region 1 seed transfer zone establishment efforts described previously are an attempt to better understand and steward the use of native seed for commonly used revegetation species. The scope, extent, and cost of these studies, however, limits the number of species that can be practically evaluated. Practitioners often recognize that a particular native grass or forb has high value for restoration purposes, such as wildlife or pollinator habitat, rare or threatened status, or a specialized but important ecological niche (figure 4). Without the help of seed transfer zones to guide seed source selection, and a lack of availability of seed for some species, practitioners are left with few options for including such species in revegetation seed mixes. Often the best recourse in such cases is to collect propagules from undisturbed reference sites near the revegetation project area, then enlist a native plant nursery or seed producer to propagate and grow plants from that wildland collection (Hufford and Mealor 2014). Unlike trees, the typically short lifecycles of grasses and forbs allows large-scale harvest of seed from the original plants within a few years. Many private and some publicly owned nurseries and seed-increase facilities offer this service. Theoretically, the resulting seeds will be genetically appropriate to return to the revegetation project area (Shaw et al. 2005).

CDAN has been growing seed-increase plots of wildland-collected grasses, forbs, and shrubs for several decades. Over the last decade, CDAN has maintained an average of 140 different seed-increase plots per year. These plots average yields of more than 800 lbs (360 kg) of grass seed and more than 50 lbs (22 kg) of forb seed per year (figures 5 and 6). The bulk of seed weight yield comes from grass seed increase plots due to the typically larger and heavier nature of grass seed compared with forb seed. Additionally, grasses are often better colonizers of disturbed areas than more specialized native forbs. Forbs, however, represent a much greater diversity of species currently in seed production at CDAN (figure 7). Forbs typically produce smaller, lighter seeds than grasses, with some in excess of 9 million per lb (4.1 million per kg)

Figure 4. Source-identified showy milkweed (*Asclepias speciosa* Torr.), an important pollinator species, in flower at Coeur d'Alene Nursery. (Photo by Jasmine Drapeau, 2018)

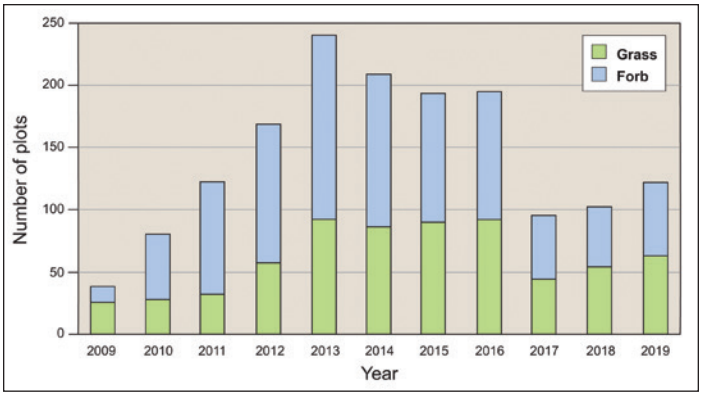

Figure 5. Number of seed increase plots over time at Coeur d'Alene Nursery. Each plot represents a distinct wildland collection of a single grass or forb species.

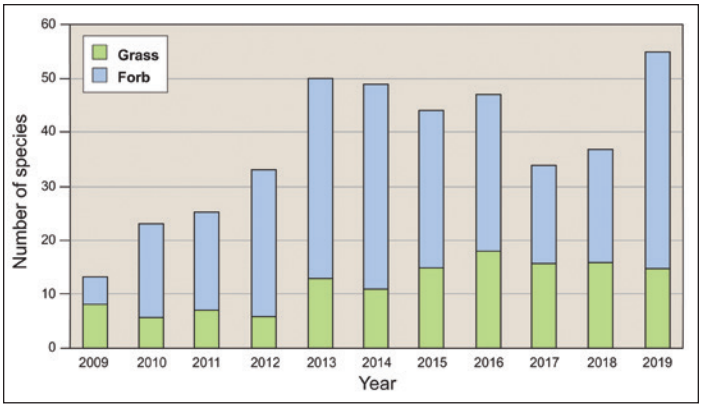

Figure 7. Species diversity in the seed increase program at Coeur d'Alene Nursery. Bars indicate the total number of distinct species in production during a given year.

(i.e., pearly everlasting). Although seed weight may be relatively small, actual seed yields can be impressive. Currently, CDAN has more than 60 forb seed-increase plots in production, an increasing trend over the last 3 years. Given the recent increased emphasis on using genetically appropriate wildland seed in the Forest Service and other public land management agencies, this upward trend is likely to continue, especially in Region 1 because of implementation of the newly developed seed transfer zones for wildland species.

Integration of Region 1 Seed Transfer Zones and Seed Increase

Private-industry seed growers are an invaluable resource for implementing wildland seed transfer zones

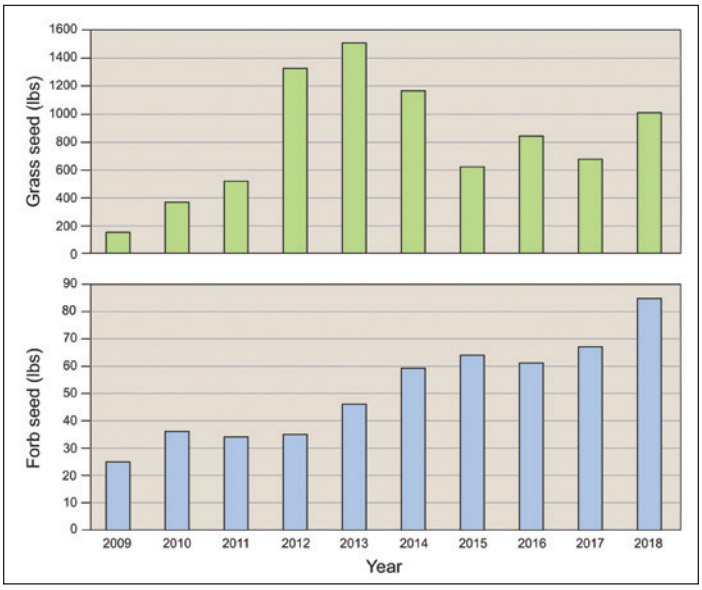

Figure 6. Volume of grass and forb seed production at Coeur d'Alene Nursery over time. Each year's yield represents cleaned seed volumes for all grass or forb species in production that year.

(Shaw et al. 2005). Because of their experience in plant culture and seed production on a commercial scale, these growers will be the primary avenue for zone seed production in Region 1. Contract growing allows for competitive pricing, quality control measures, and a decreased burden on Government agencies to generate seed in-house. Unfortunately, not all wildland seed production efforts are financially desirable for private growers. Some species are too labor-intensive to yield profitable returns, require specialized equipment or practices for establishment and seed harvesting/processing, or are needed on such a small scale as to make commercial seed increase impractical. In instances such as these, Region 1 Forest Service seed-increase efforts are typically undertaken by CDAN. Several of the species with newly established seed transfer zones fall into this category. Forbs such as pearly everlasting and white spirea (*Spirea betulifolia* Pall.), and grasses such as bluejoint reedgrass present challenges to commercial growers due to indeterminate seed ripening and complex seed morphology. Because of these cultural complications, CDAN is conducting bulk production of zone-identified seed for these species. Additionally, CDAN is increasing the smaller zones of other species with seed store requirements typically below the commercial threshold.

Whether produced commercially by private growers or increased at CDAN, native seed for Region 1 is typically housed at CDAN for storage and distribution. The nursery serves as a seed cache, seed mixing facility, and distributor for Region 1 of the Forest Service and some neighboring public land agencies. Through these functions, CDAN can provide custom wildland seed mixes, while maintaining a high level

of quality control with regard to both seed source genetics and seed quality (purity, viability, weed seed contamination) (Vankus 2018). Nursery personnel work with national forest or district botanists and revegetation practitioners to determine which zone-identified seed is appropriate for the project area, and in which concentrations. CDAN then mixes, bags, and distributes the seed to project managers. In cases where zone-identified seed is unavailable, commercially available cultivars are used. When purchasing cultivars, nursery personnel seek out high-quality seed free of noxious weeds, ideally from parent sources that are close in geography and elevation to the project area. Through a combination of these three sources (wildland collected/increased seed, Region 1 seed transfer zone seed, and cultivars when needed), CDAN can provide land managers with quality wildland seed and seed mixes with considerations for both genetics and purity.

Seed-Increase Management Considerations and Challenges

Considerations

Cultural practices for forest seedling nurseries have been, and continue to be, studied extensively. Unfortunately, this information is of limited utility when considering the cultural needs of wildland grasses and forbs. Some grasses and a few forbs have been researched specifically for the purpose of seed production, given those species' utility for revegetation work. Species such as bluebunch wheatgrass, Idaho fescue, and mountain brome have well-established cultural practices (Bartow 2015). These species, however, are exceptions to the general rule of limited cultural information for wildland plants, which complicates seed-increase efforts for CDAN and other native seed production facilities. Fortunately, growth failures or poor performance for seed-increase plots can often be avoided by considering several broad-stroke cultural factors.

Ecotypic groupings of grass and forb species are often found growing together across their ranges, presumably due to similarities in growing conditions associated with climate and temperature (Bower et al. 2014). For example, species growing in shortgrass prairies in eastern Washington contain many of the same species as shortgrass prairies in northern Utah or eastern Montana. Although climatic tolerances (and associated genotypes) may or may not be very different between these communities, as a general rule, these groupings can be strong indicators of general cultural conditions for seed-increase efforts. Because water is typically the major limiting factor for plant community development in the West, CDAN personnel apply supplemental irrigation carefully to mimic ideal growth and reproductive conditions for species in seed-increase plots. When laying out plots, species with similar water needs are grouped together. Species from xeric or dryland ecotypes are grouped and planted in areas of the nursery with higher soil drainage and are irrigated minimally (figure 8). Mesic or riparian species are planted in fields with more moisture-retentive soils and irrigated regularly. In this way, cultural needs at the nursery are scaled out from a single plot to a grouping of ectopically similar species, with the results being increased efficiency and decreased water use.

In addition to ecotypic groupings, it is very culturally advantageous to group seed-increase plots by lifeform. Weed control is a major expense and limitation to seed-increase work (Bartow 2015). Standards of cleanliness for wildland seed used on public land are high, and distributing wildland seed contaminated with weed seed is either illegal or highly undesirable, depending on the species and State. Weed control is a foremost consideration for seed-increase work at CDAN. Most post-emergent herbicides fall into two classes: non-selective (indiscriminately affecting all plants), and selective (affecting either grasses or broadleaf plants, but not both). Although non-selective herbicides are invaluable for weed control in bare-ground areas and around seed-increase plots, they are of little use in controlling weeds in established plots. A tremendous advantage of grouping grass and forb seed-increase plots separately is the ability to safely apply broadleaf-specific herbicides to grass fields, and grass-specific herbicides to forb fields. If grass and forb plots are grown in close proximity, the risk of plant injury from spray drift or other contact is high, as is the complexity of the task for the applicator. Only after selective herbicides have controlled their target weeds are labor-expensive hand-weeding crews used to pull grassy weeds in grass increase plots and broadleaf weeds in forb-increase plots. The ideal scenario for seed increase at CDAN is to plant suites of ecotypically similar grass plots in

Figure 8. Source-identified seed-increase plots in an ecotypic grouping at Coeur d'Alene Nursery, including slender wheatgrass (*Elymus trachychaulus* Link)(center) and sulphur-flower buckwheat *(Eriogonum umbellatum* Torr.) (lower right). Similar cultural requirements allow for cultural efficiency. (Photo by Jasmine Drapeau, USDA Forest Service, 2018)

one field (figure 9) and suites of ecotypically similar forbs in another (figure 10). This management approach helps reduce labor and cost and increase plant growth and yield.

Genetic Considerations

In addition to plant culture, one of the most important considerations for seed-increase work at CDAN is the preservation of source genetics during seed production. Cross-pollination between plots of the same species is a high risk when grown in close proximity (Young et al. 2006). Cross-pollination between plots may result in offspring that lack the genetic fitness to survive and thrive on revegetation project sites, thus eliminating the advantage of the seed-increase effort. Managers at CDAN plan seed-increase plots with this risk in mind, taking care to separate genetically distinct plots of the same species by as much distance as possible. The risk of interspecies hybridization further complicates seed-increase efforts and must be taken into consideration. Several genera of commonly used grasses (i.e., *Elymus* sp. and *Festuca* sp.) and forbs (i.e., *Erigeron* sp. and *Penstemon* sp.) are known to produce interspecies hybrids (Culumber et al. 2013, Wilson and Vanesuela 2002). Growth in close proximity can encourage such hybridization, so increase plots of known hybridizing species are kept separated at CDAN. Before undertaking to grow a new species for seed increase, managers at CDAN research the possibility for hybridization with any currently growing species, and plan accordingly to preserve genetic integrity.

Figure 9. Newly germinated source-identified Sandberg's bluegrass (*Poa secunda* J. Presl.) seed-increase plot at Coeur d'Alene Nursery. Grass species grouped together allow for efficient herbicidal control of broadleaf weeds. (Photo by Nathan Robertson 2018)

Special Challenges

CDAN periodically receives requests for seed increase of species not typically conducive to agricultural conditions or bulk seed harvest. Often the highly valuable ecological role of a species, or its classification as rare or threatened, prompts restoration practitioners to pursue seed-increase efforts regardless of the cultural difficulties. When possible, CDAN undertakes to cultivate such species. Success with these especially challenging species has often depended on research and the amount of information available. Some of the more challenging species encountered are those with indeterminate seed ripening, symbiotic or parasitic needs, and/or very long or short lifecycles.

One of the biggest challenges to seed harvesting on wildland plants occurs when seed does not ripen uniformly. Genera such as *Thermopsis* sp., *Lupinus* sp., and many members of the Asteraceae family

Figure 10. Source-identified seed-increase plots at Coeur d'Alene Nursery, including white sagebrush (*Artemisia ludoviciana* Nutt.)(upper left), silky lupine (*Lupinus sericeus* Pursch.)(center), and Canada goldenrod (*Solidago canadensis* L.)(right). Native forb groupings allow for herbicidal control of grassy weeds in production plots. (Photo by Nathan Robertson 2018)

Figure 11. Source-identified showy fleabane (*Erigeron speciosus* Lindl.) flowers at Coeur d'Alene Nursery. Pappas-borne seeds from such Asteraceous species require specialized seed-collection efforts. (Photo by Nathan Robertson, 2018)

(figure 11) ripen and disperse seed throughout the growing season. Culturists are forced to either destructively harvest (combine) at a single point, thereby losing any further harvest of existing unripe seed, or to non-destructively harvest (hand collect, vacuum, etc.) throughout the season, which is typically extremely labor intensive. Although periodic collection methods can be very effective, the time investment translates to high cost per pound of seed yield. At CDAN, some collection, especially for asteraceous plants with a windborne pappas, is expedited through the use of a leaf blower that has been reversed and used as a motorized vacuum with a collection bag.

Some species present complications with basic plant establishment and growth in a horticultural setting. For example, most of the species in the genus *Lupinus* do not thrive and produce appreciable seed unless favorable conditions exist for root establishment and, possibly, inoculation with a compatible mycorrhizal root fungi occurs (Jones et al. 2018). These conditions and inoculation can occur in a cultural setting, but until they are met, individual plants often languish. On a plot-wide scale, the effect can be very frustrating for seed producers. Other species present similar problems. Plants in the genus *Castilleja* (Indian paintbrushes) are typically hemi-parasitic and depend on the root system of a neighboring grass or forb to thrive (Kaye 2001). For seed-increase efforts, *Castilleja* species cannot be planted as a monoculture, but must be grown in association with a suitable host plant. At CDAN, ideal host plants for *Castilleja* have very different seed sizes, and/or different seed ripening timelines. This difference helps prevent cross-contamination when harvesting *Castilleja* seed pods.

Ideally, a species used for seed increase reaches reproductive maturity quickly (within 1 to 3 years), and yields seed for multiple seasons without needing to be reseeded. Unfortunately, some very desirable species

do not fit this description, and must be managed differently. Annuals, biennials, and very short-lived perennials such as *Agrostis* and *Ipomopsis* species can be excellent colonizers on disturbed areas, but often require a higher cultural investment due to reseeding costs. Conversely, long-lived species can require years of cultural investment prior to producing significant seed yields. At CDAN, clients are frequently informed that timelines and cultural costs for slow-maturing species such as basin wildrye (*Leymus cinera* Scrib. & Merr.) and balsamroots (*Balsamorhiza spp*. Nutt.) may extend 3 to 5 years before seed yield even begins. Each species CDAN undertakes for seed increase is researched extensively to determine lifecycle timelines, expected seed yields, and any special cultural considerations inherent to that particular species.

Conclusions

Reliable access to genetically appropriate, site-adapted native plant seed stores is a challenge for revegetation practitioners working with disturbed public lands in the Western United States. Through the development of empirical seed transfer zones, Region 1 of the Forest Service has made strides to facilitate the development and availability of seed stores for commonly used native plants. Creating these stores is accomplished by both private-sector seed production facilities and the Coeur d'Alene Nursery. Because of these increase efforts, zone-identified native seed is now becoming available for incorporation into native seed mixes across the Region. For species without established seed-transfer guidelines, the Coeur d'Alene Nursery provides services for source-identified seed production, storage, and mixing. The nursery's cultural management approach helps ensure the genetic integrity of seed crops, efficiency of production, and high seed quality. These approaches will help the nursery maintain viable seed production services in response to rising future demands for site-appropriate native seed.

Address correspondence to—

Nathan Robertson, U.S. Department of Agriculture, Forest Service, Coeur d'Alene Nursery, 3600 Nursery Road, Coeur d'Alene, ID 83815; email: nathan.robertson@usda.gov; phone: 208–765–7387.

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Outplant Evaluation of Container Red Alder Grown with Bonzi® Plant Growth Regulator

Nabil Khadduri and Brian C. Morris

Nursery Scientist, Webster Forest Nursery, Washington Department of Natural Resources, Olympia, WA; Silviculture Scientist, Pacific Cascade Region, Washington Department of Natural Resources, Castle Rock, WA

Abstract

Red alder (*Alnus rubra* Bong.) seedlings are challenging to grow due to their tendency to reach excessive heights in nursery culture. We evaluated several Bonzi® (paclobutrazol) plant growth regulator frequency by rate applications on large plug seedlings (15 in3 [250 ml]) then compared the best treatment (three applications of 25 ppm Bonzi®) with our standard Plug+1/2 stocktype at two outplant sites. We also evaluated all nursery treatments, including non-treated plugs, at a garden plot. Large plugs, even without treatment, broke bud several days earlier than P+1/2s and this effect increased with increasing Bonzi® intensity. The P+1/2s had greater height and stem diameter growth at one location and greater height growth at the other. Survival did not differ between stocktypes. The nursery grower can use Bonzi® to produce a balanced plug that is easier to frost protect in the nursery and less susceptible to mechanical damage, with overall higher average nursery packout (yield). This study shows, however, that reducing treatment intensity may lead to better outplant success. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25-26, 2018).

Introduction

Red alder (*Alnus rubra* Bong.) production at the Washington Department of Natural Resources' Webster Nursery in Tumwater is currently grown as a Plug+1/2 (P+1/2) stocktype, where a small plug is started in the greenhouse then transplanted for the remainder of the growing season into a bareroot field. It is sometimes referred to as a "4 by 4," as the seedling spends 3 to 4 months in the greenhouse and an additional 4 months in the bareroot field prior to outplanting. The primary advantage of the P+1/2 is that transplanting to a lower density produces a seedling with a large stem diameter, an important characteristic for resisting freeze damage of thin alder bark, both in the nursery and up to the first 3 years in the forest (Dobkowski 1996). Large seedlings are also less likely to be overtopped by competing vegetation (Dobkowski et al. 2006). The P+1/2 stocktype also has its downsides, including some mortality at the time of transplant. Seedlings that withstand or avoid transplant stress within the nursery may grow taller than desired later in the season. Seedlings in excess of 3.5 ft (1 m) are more likely to suffer mechanical damage during nursery frost protection (figure 1). Alder stems and roots are relatively brittle compared to conifers and are prone to breakage in lifting and packing operations, as well as damage from handling in the woods.

We have trialed application of the plant growth regulator paclobutrazol (Bonzi®, Syngenta, Wilmington, DE) in the plug stage of the P+1/2 stocktype over the last several years (Khadduri 2015) to control excessive height growth and the drawbacks that go with it. Fine-tuning application rates and timing has led to improved packout due to better root-to-shoot balance of the plugs at the greenhouse-to-bareroot transition and less transplant stress in the bareroot field. Even with aggressive root culturing and reduced watering to limit seedling growth, however, the P+1/2 stocktype still has a tendency to have excessive growth late in the growing season, when temperatures decline, nighttime humidity rises, and moisture (precipitation) cannot be controlled.

Given these challenges, we decided in 2017 to evaluate large-plug production as an alternative to our standard P+1/2 stocktype. Whereas P+1/2s start with a

Figure 1. Bareroot alder seedlings can be prone to frost damage in the nursery. Excessively tall seedlings are particularly subject to damage, not only from ice accumulation during frost protection, but also during lifting, packing, and outplanting. (Photos by Nabil Khadduri, 2003)

small plug that finishes in a bareroot field, large plugs remain in greenhouse production the entire season, although they may be moved outdoors for hardening during summer months. Since they remain in containers, large plugs can be moved back under cover for both moisture control and frost protection in the fall (figure 2). The plugs are grown at a higher density than the P+1/2, 26.5 stems per ft^2 (284 per m^2) versus 6 stems per ft^2 (64 per m^2), respectively. Accordingly, plugs are not expected to reach the stem diameter of P+1/2s. The goal of growing a large plug is to attain a seedling with as much stem diameter as possible, but with good balance between shoot-to-root systems. A preferred shoot-to-root target for container seedlings should be 2:1 or less (Haase 2011). Even with better moisture and nutrition manipulation, growers may still struggle to control height in container production, leading to spindly seedlings with a poor sturdiness quotient (height-to-stem-diameter ratio) (Ahrens 2006). Some growers may top mow or pinch off seedlings to control height, but we have observed that this often leads to a loss of apical dominance and subsequent low forking of the tree structure. While this may be acceptable for alder used in restoration efforts, alder used for saw log production should be free of defects in the bottom portion of the tree (Plank and Willits 1994).

The objective of our study was to evaluate the effects of two rates of Bonzi® at various application frequencies on subsequent morphology of large plugs. Since we had never grown large plugs with Bonzi®, the goal was to identify the best treatment in the nursery, then compare it against the standard P+1/2 stocktype in outplant environments.

Materials and Methods

Seedling production

In 2017, we evaluated two red alder stocktypes in a nursery trial using the same seed source (Washington Seed Transfer Zone 05 [Upper Chehalis], 0 to 1,000-ft elevation, woods-run collection, 2009). We started our standard P+1/2 in a 2 in^3 (40 ml) 240-cell Styroblock™ Container (Beaver Plastics, Acheson, Alberta, Canada) in the greenhouse with subsequent transplant to a bareroot field for the remainder of the growing season. We sowed seed February 28 and inoculated with 0.035 oz (1 g) blended fresh alder nodules per 1,000 seedlings at germination. As per our new standard, we applied two spray-to-wet applications of 25 ppm Bonzi® solution at approximately 0.5 fl oz (15 ml)

Figure 2. Large plugs set bud sooner (foreground) and can be easily protected under cover. P+1/2s set bud later due to late-summer/early-fall precipitation and must be frost protected with irrigation. (Photo by Nabil Khadduri, 2018)

Table 1. Bonzi® application frequency, rate, and treatment date..

Number of Bonzi® applications	Rate	Treatment dates
0	N/A	N/A
2	25 or 50 ppm	May 7, June 4
3	25 or 50 ppm	May 7, June 4, July 20
4	25 or 50 ppm	May 7, June 4, July 20, Aug 17

ppm = parts per million

per cell in the container stage at week 8, and again at week 12, just prior to transplant. Plugs were lifted and transplanted May 24–25, grown in the bareroot field for about 6 months, lifted December 7, and stored at 30 °F (-1 °C).

For comparison with the standard P+1/2, we grew large-plug seedlings in 15 in^3 (250 ml) 60-cell Styroblock™ Containers with a range of treatments. We sowed seed March 13 and inoculated with 0.035 oz (1 g) blended fresh alder nodules per 1,000 seedlings at germination. We applied Bonzi® at two rates (25 ppm or 50 ppm) for 0 (non-treated control), 2, 3, or 4 times through the season (table 1). Spray volume for spray-to-wet application to large cells was approximately 0.5 fl oz (15 ml) solution per cell initially, and increased to 1.0 fl oz (45 ml) for the third and fourth applications to account for larger leaf area. Large plugs were lifted December 22 and placed in storage at 30 °F (-1 °C).

We noted widespread presence of nitrogen-fixing *Frankia* bacteria nodules on both stocktypes by early summer. Artificial inoculation of alder seedlings with *Frankia* bacteria has been linked to improved seedling growth (Martin et al. 1991) and improved field performance, particularly in nutrient poor soils (McNeill et al. 1989). While excessive nutrient load in the nursery can limit alder nodulation, we did not know what effect adding a plant growth regulator to a peat-only growing medium might have. We did not see nodule inhibition in this trial.

We recorded final seedling height and stem diameter for both Plug+1/2 and S-15 stocktypes the week before harvest. We conducted a factorial analysis (frequency by rate) on treatment responses using the R statistical package (R Core Team 2019). Means were subjected to Tukey's Honestly Significant Difference (HSD) test and considered significantly different at the $p<0.05$ level.

Nursery Results for Selection of Large Plugs for Outplant Evaluation

Bonzi® applications at the 25 ppm rate provided an effective seedling response (figure 3). Two applications slowed seedling height growth compared with the control, and three applications further retarded growth compared with two applications. Four applications, however, had no additional growth reduction compared with three applications. Bonzi® applications at the 50 ppm rate (data not shown) proved to be excessive. Unlike the lower rate, the 50 ppm Bonzi® treatments significantly reduced both height and stem diameter in comparison with the 25 ppm rate or non-treated seedlings at as few as 2 applications. Three applications of Bonzi® at 25 ppm significantly controlled height with minimal impact to stem diameter in comparison with non-treated seedlings (table 2).

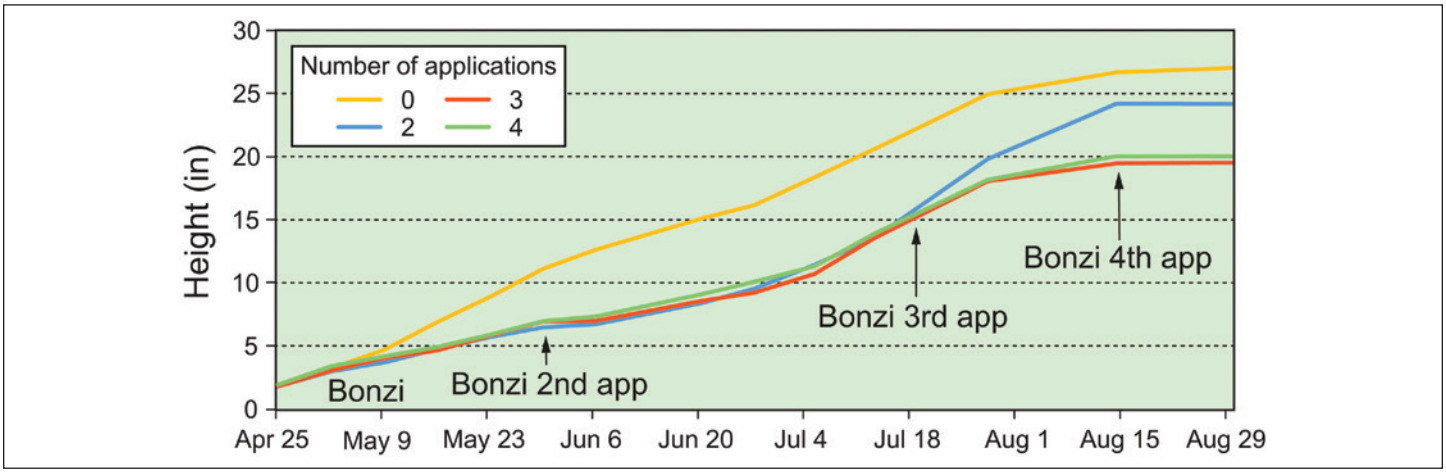

Figure 3. We applied Bonzi® at 25 and 50 ppm solutions 0, 2, 3, or 4 times through the growing season in response to height growth. For clarity, only 25 ppm rate is shown. Note that the 4th application applied late in the season did not have an effect on height growth as seedlings were in the process of shutting down on their own.

Table 2. Morphology averages for 25 ppm Bonzi®, applied 0, 2 or 3 times compared with the standard P+1/2 alder stocktype. Sturdiness ratio is height divided by stem diameter. Means with same letter are not significantly different at the p<0.05 level.

# Bonzi® apps	Height (cm)	Stem diameter (mm)	Sturdiness ratio (height/stem diameter)
0	68.6b	5.8b	11.8a
2	61.5c	5.7b	10.8a
3	49.5d	5.6b	8.8b
P+1/2 alder	102.9a	11.1a	9.3b

ppm = parts per million

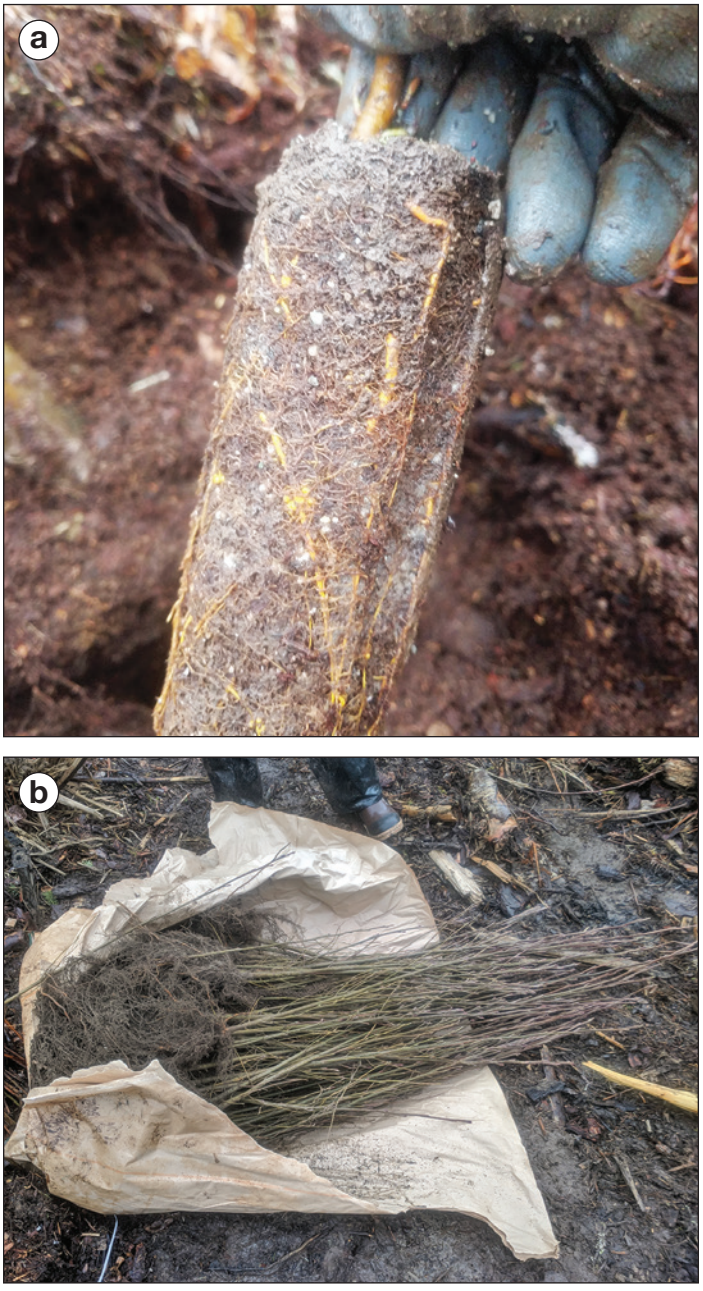

Figure 4. Shoot-to-root ratio averaged 1:1 for (a) large plugs (3 Bonzi applications at 25 ppm rate) compared with (b) 3:1 for P+1/2s. (Photos by Nabil Khadduri)

These data resulted in a superior sturdiness quotient (ratio of height to stem diameter in cm/mm) averaging close to 9 which is similar to the standard P+1/2 alder stocktype. Ahrens (2006) assumes a maximum sturdiness quotient of 10 for culling standards. In an effort to minimize the sturdiness ratio of the treated plugs, we selected the 3 application @ 25 ppm Bonzi® treatment for comparison with the standard P+1/2 alder stocktype for outplant evaluation.

Outplant and Garden Plot Evaluations

In 2018, we compared field performance between the P+1/2 and large plug stocktypes. We used a water displacement method to determine shoot and root volumes (all soil media or soil washed from roots). Shoot-to-root ratios (n = 30) averaged close to 1:1 for S-15 (3 apps at 25ppm) versus a more top-heavy 3:1 for the P+1/2 stocktype (figure 4). Seedlings were planted at two forest sites and a nursery garden plot (figure 5). The University of Washington Pack Forest site, near Eatonville, is at 1,100 ft (335 m) elevation, with an east aspect and a Scamman silty clay loam. The Louie site is outside of Castle Rock, WA, at an elevation of 750 ft (230 m), with a northeast aspect and an Olympic cobbly silt loam. The nursery garden plot is near Tumwater, WA, at 200 ft (60 m), with a flat aspect and Yelm sandy loam soils. Since the nursery site is a

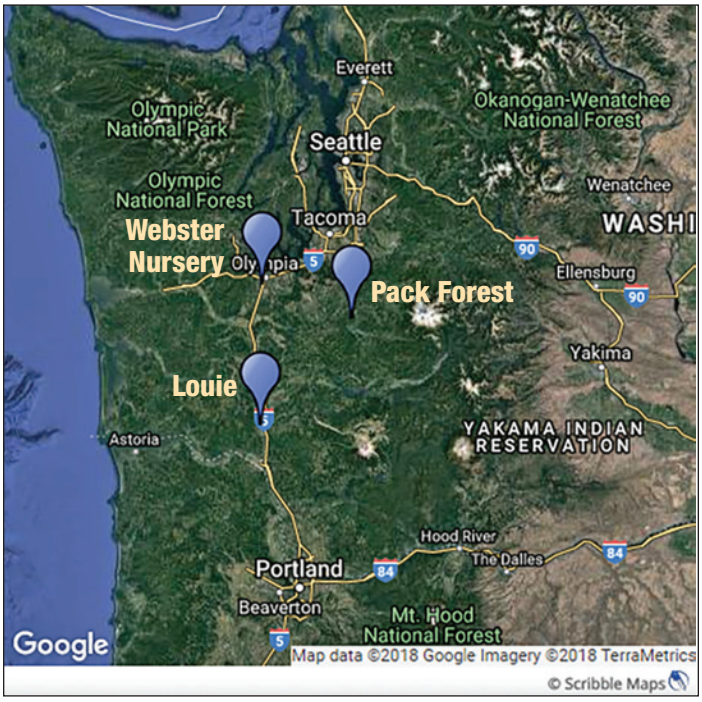

Figure 5. Two forest sites and nursery garden plot location of outplanted seedlings.

Figure 6. P+1/2 seedling at Pack Forest site at end-of-season measurement in October 2018. (Photo by Nabil Khadduri, 2018)

cultivated agricultural field, we placed fixed irrigation to avoid excessive drought mortality, but due to natural soil moisture conditions, it was not used.

We planted four replications (blocks) of 25 seedlings per stocktype at Pack Forest and Louie for a total of 100 seedlings of each stocktype per site. We planted 30 seedlings, without blocking, of every container treatment at the nursery site along with the P+1/2 stocktype (table 1).

We started thawing seedlings on March 10 by moving stock to a cooler kept at 36 °F (2 °C) until planting. We planted the nursery garden plot March 20, Pack Forest on March 22, and Louie on April 17.

At the garden plot, we evaluated budbreak three times weekly on all seedlings for 5 weeks, from the end of March through April. We measured chlorophyll fluorescence and chlorophyll content at all three sites the last week of August with a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL). Thirty seedlings were measured per treatment. We placed three leaf clips (replicates) per plant and allowed foliage to adjust to baseline light levels for 20 minutes for stabilization before measurement. We measured predawn moisture stress (PMS Instrument Company, Albany, OR) on 12 seedlings per treatment at the nursery garden plot and Pack Forest the third week of August. We measured seedlings at all sites for height and stem diameter and tallied survival in early October (figure 6). We conducted ANOVA analyses using the R statistical package (R Core Team 2019) and subjected treatment means to Tukey's HSD test and considered means significantly different at the $p<0.05$ level.

Outplant Results and Discussion

Overall, container seedlings broke bud earlier than the bareroot stock, and tended to be earlier with increasing Bonzi® applications. While there were no differences in survival, the P+1/2 seedlings started and remained taller by the end of the first growing season, with significantly greater stem diameter at all three sites.

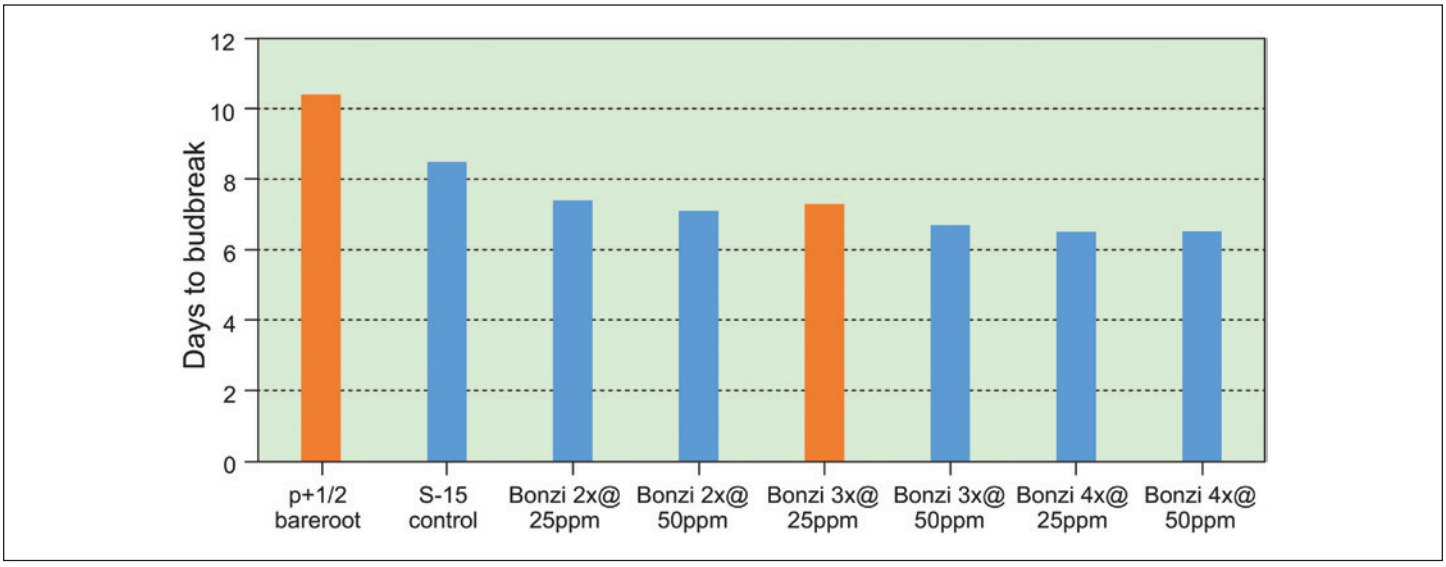

Figure 7. Day of budbreak in April by treatment at nursery garden plot following planting on March 20. Outplant-evaluated treatments are highlighted for clarity.

Budbreak

In the nursery garden plot, the P+1/2 stocktype broke bud later on average than all container treatments (figure 7). Within the plug treatments, increased Bonzi intensity (rate combined with application frequency) tended to have earlier budbreak (figure 7).

Budbreak was not measured at the forest sites. We did, however, note at Pack Forest that approximately 60 percent of large-plug stock had broken bud 3.5 weeks after planting whereas less than 20 percent of P+1/2 stock had broken bud at that time.

Dobkowski (2006) noted earlier budbreak in red alder container stock in comparison with bareroot stocktypes. This effect may be attributed to earlier budset in the nursery, or different hardening or de-hardening conditions while finishing in a greenhouse environment. We did not find mention in the literature of gibberellin-inhibiting growth regulators further hastening budbreak in Pacific Northwest tree species. Paclobutrazol has been shown to hasten flowering time in other plants, for example lupine (*Lupinus varius* L.) (Karaguzel et al. 2004). The important biological ramification is that alder seedlings breaking bud even a few days earlier risk increased exposure to spring freezes. Peeler and DeBell (1987) list spring freeze damage as one of the primary obstacles to successful alder seedling establishment.

Physiology

We saw no significant differences between stocktypes for chlorophyll fluorescence or plant moisture stress (table 3).

Chlorophyll content readings were significantly higher at Pack Forest for the large-plug stocktype compared with P+1/2. Paclobutrazol, the active ingredient in Bonzi®, has been shown to concentrate chlorophyll in other species, and this is attributed to a larger number of chloroplasts in a relatively smaller leaf area compared with non-treated plants (Khalil et al. 1995). Our data indicate that for red alder this effect may last late into the first growing season, but it is not clear what impact this may have had on growth or survival.

Survival

We saw no significant survival differences between stocktypes at any of the sites. Survival was 97 to 100 percent at Pack Forest and the nursery garden plot. At the Louie site, however, both stocktypes had less than 20-percent survival, which is most likely due to later planting and drought stress caused by an early dry season and heavy vegetation competition. Precipitation at the Louie site from the time of planting to the end of August was 4.1 in (10.4 cm) compared with 9.2 in (23.4 cm) and 8.6 in (21.8 cm) at the nursery garden plot and Pack Forest sites, respectively. A challenge for planting alder is to weigh the risk of late freezes into March against drought onset that can occur as early as May in some years. Tanaka and Dobkowski (unpublished data) found that seedlings planted in mid-March had approximately two and four times more roots in July, respectively, than seedlings planted in mid-April or early May.

Morphology

At all sites, P+1/2 seedling heights were initially taller at planting and remained significantly taller at the end of the first growing season (figure 8). At the Pack Forest site and nursery garden plot, P+1/2 seedlings had significantly greater height growth during the growing season. Similarly, P+1/2 stem

Table 3. Physiology measurements conducted in August of first growing season. Chlorophyll content (SPAD) readings were significantly higher for large plugs than the P+1/2 stocktype at Pack Forest.

State	Chlorophyll fluorescence (Fv/Fm)		Chlorophyll content (SPAD)		Plant moisture stress (Mpa)	
	P+1/2	Plug	P+1/2	Plug	P+1/2	Plug
Oregon	0.771 a	0.785 a	30.2 a	38.0 b	-0.36 a	-0.41 a
Washington	0.740 a	0.767 a	36.9 a	42.3 a	n/a	n/a
Washington	0.814 a	0.816 a	39.2 a	43.8 a	-0.43 a	-0.46 a

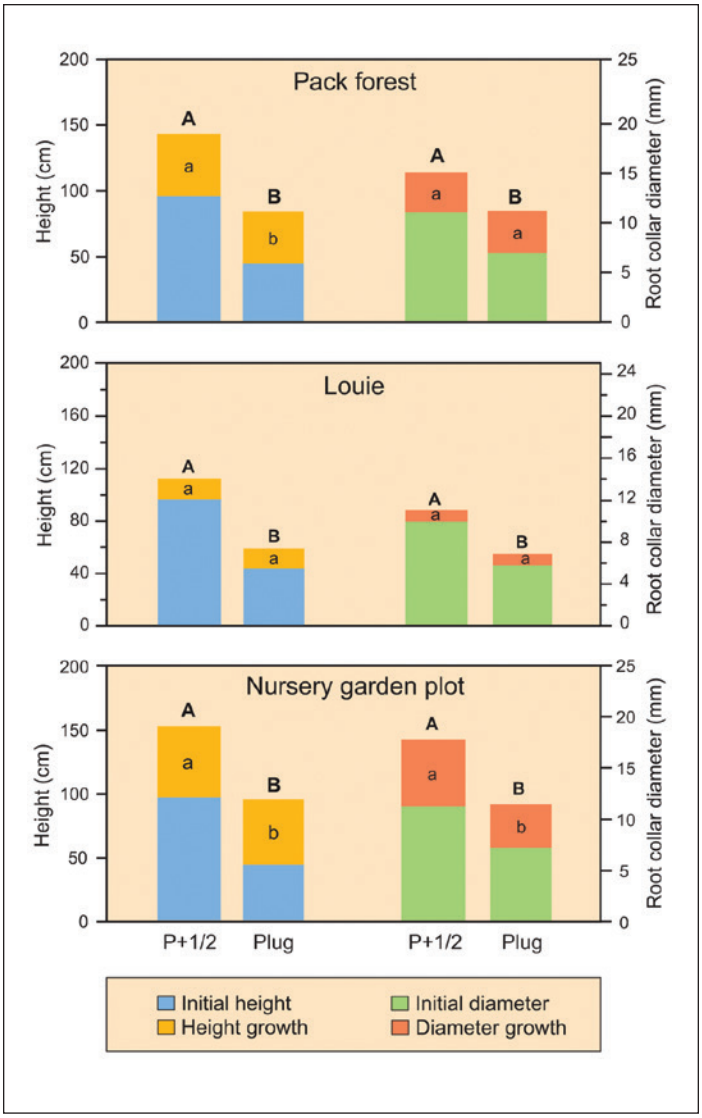

Figure 8. Final height and height growth were significantly greater for P+1/2 seedlings at Pack Forest. At Pack Forest, P+1/2 seedlings averaged greater initial and final root collar diameter, but we saw no significant difference in root collar diameter growth. At Louie, final height and root collar diameter remained significantly taller for P+1/2 seedlings, but we saw no difference in height and root collar diameter growth compared to plugs. At the nursery garden plot, P+1/2s had greater final height and root collar diameter, as well as greater root collar diameter growth compared to plugs. We saw no difference in height growth.

diameters started and remained significantly larger than large plug stem diameters on all three sites. Stem diameter growth did not differ among stocktypes at the Pack Forest or Louie sites but P+1/2 had greater stem diameter growth than large plugs at the nursery garden plot (figure 8).

All large-plug treatments were included at the nursery garden plot and showed a significant trend between Bonzi® plant growth regulator intensity (rate by frequency of application) and decreased stem diameter growth (figure 9). Anecdotal evidence in early Bonzi® studies on reforestation species suggested the plant growth regulator might stunt seedlings for several growing seasons. At least with the lower intensities applied in this trial, we did not observe dramatically negative effects in the first growing season. The correlation between paclobutrazol intensity and decreased stem diameter growth at the nursery garden plot, however, suggests limiting nursery applications to avoid negative impacts on outplant performance.

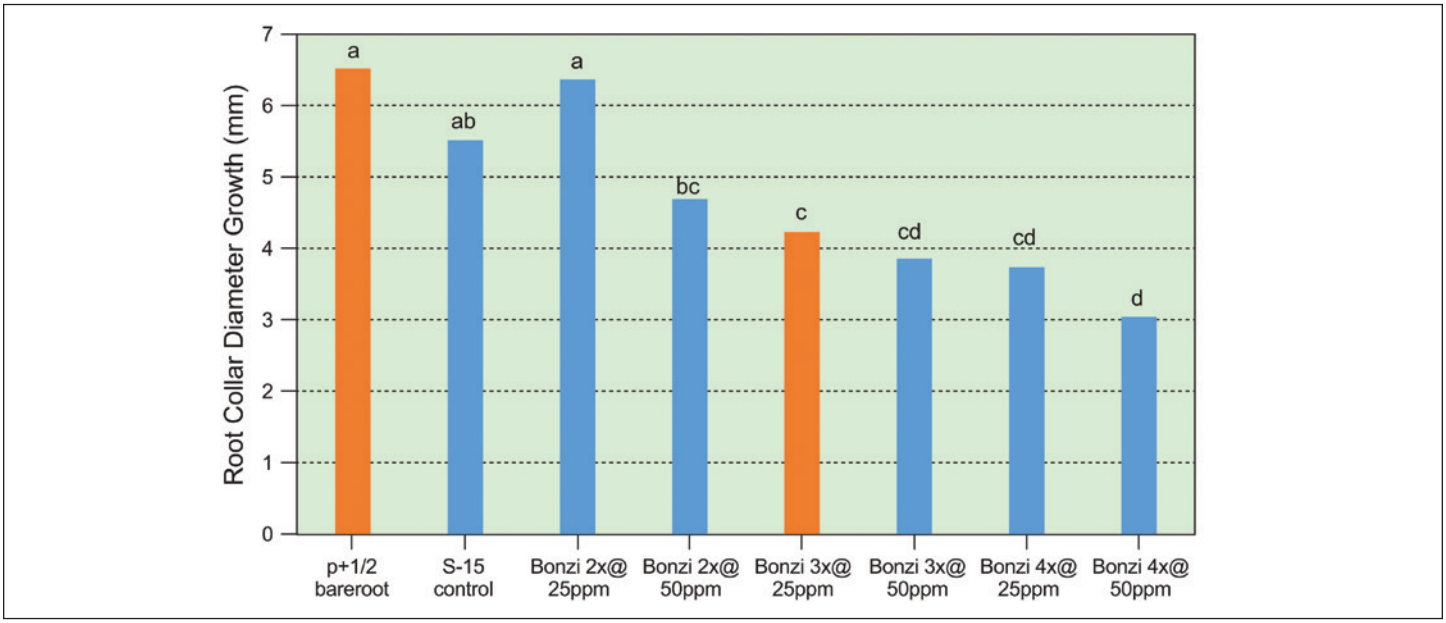

Figure 8. In the nursery garden plot evaluation of all treatments, increasing Bonzi® treatment intensity (rate by application frequency) significantly decreased root collar diameter growth. Outplant-evaluated treatments are highlighted for clarity.

Conclusion

We advise growers to minimize rate and frequency of Bonzi® applications to large plugs to effectively limit height growth while avoiding unnecessary side effects such as early budbreak or reduced stem diameter growth after outplant. Large plugs already tend to break bud earlier than bareroot stocktypes, and, as found in this study, a plant growth regulator can exacerbate that effect.

Despite these potential drawbacks, using a plant growth regulator for large-plug production can result in a seedling with a balanced root-to-shoot ratio and reasonable sturdiness. This balance may reduce stem breakage during handling and provide a more resilient plug root system for shallow soil or in mild drought. Although noted but not measured in this study, another benefit may be the increased density of buds along the stem in Bonzi®-treated large plugs. Large plugs without treatment had fewer buds, spaced farther apart along the stem. An increased density of buds on the lower portion of the stem has been attributed to a reduction of sunscald (Harrington et al. 1994), though we did not observe sunscald on any seedlings at our sites. Perhaps the greatest benefit of the large plug is an increase in expected nursery packout.

We emphasize the importance of appropriate outplant timing to avoid both late freezes and early drought. The current recommendation of mid-March to mid-April planting holds, but early drought years may pose as much or more of a risk than late frosts. It may be better to err on planting earlier in this window rather than later. As always, drought and/or severely frost-prone sites should be avoided altogether when planting red alder, especially with P+1/2s for the former and large plugs for the latter.

Address correspondence to—

Nabil Khadduri, Nursery Scientist, Webster Forest Nursery WADNR, PO Box 47017, Olympia, WA 98504; email: nabil.khadduri@dnr.wa.gov; phone: 360–902–1279.

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Sap-Flow Sensors for Small-Diameter Nursery Seedlings

Lloyd L. Nackley

Assistant Professor, Nursery Production, Horticulture Department, North Willamette Research and Extension Center, Oregon State University, Aurora, OR

Abstract

Sensors can be used to improve irrigation management decisions in nurseries. Optimizing irrigation efficiency aims to apply sufficient water for growth while reducing excessive leaching to reduce costs and environmental pollution. Pairing soil moisture sensors with plant sensors enables irrigation managers to quantify the volume of water to be applied that will directly affect crop productivity. Sap-flow sensors are considered a potential tool for irrigation management because they provide a real-time method to measure how plants respond to above- and belowground environments. This report provides detailed methods to build an external sap-flow sensor that can be used on small-diameter nursery seedlings and discusses how sap-flow sensors can be utilized with nursery seedlings to provide information about plant physiology, improve irrigation scheduling, and monitor outplanting success. This article will be useful to researchers and growers who previously associated sap-flow sensors only with large diameter trees by describing the opportunities for applying sap-flow methodology to small-diameter nursery plants. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25-26, 2018).

Introduction

Most greenhouse production systems grow plants in inert, well-drained soilless media, and apply pelletized or liquid fertilizer to deliver essential plant nutrients. In these conditions, water and nutrients are regularly flushed past the root zone when scheduled irrigations exceed plant demands. Leaching the container is important to prevent excessive salt build up. Nutrient-laden runoff from greenhouse production systems can, however, create significant environmental ground- and surface-water pollution. In addition to generating pollution, flushing nutrients also results in significant lost costs in terms of wasted fertilizer and wasted water. Irrigation best management practices for nursery plant production attempts to maximize irrigation efficiency and to minimize leaching and associated loss of nutrients (Yeager et al. 2010). Research has shown that reducing fertilization rates would likely have a substantial impact on both cost savings from reduced fertilizer use and an environmental benefit from reduced nutrient leaching, particularly from greenhouse and container nursery production, and, to a lesser degree, field nursery production (Majsztrik et al. 2018). In many locations, the expense of watering is primarily related to energy costs associated with diesel or electric pumps. Additionally, fertilizer represents one of the more expensive materials used in plant production (Ingram et al. 2016). Too little water can kill a crop; too much water wastes energy and fertilizer and can promote fungal pathogens (Dumroese and Haase 2018). Optimizing irrigation efficiency aims to apply sufficient water for growth while reducing excessive leaching to reduce costs and environmental pollution.

Sensors can be an important tool to improve irrigation management decisions (Lea-Cox et al. 2013). Soil moisture sensors (SMS) are commonly used in field and row crop production settings and can also be modified for greenhouse production systems. Some SMS measure the soil moisture tension, while others measure the volumetric water content of the soil. The merits of these different types of measurements have been debated (Jones, 2007). One common aspect for all SMS is that the moisture information is independent of the plant

responses. The implications are notable, considering that the lack of necessary information on plant responses to soil moisture is one of the major causes of inefficient irrigation application (Marin et al. 2016). Thus, using SMS is only half the solution.

Pairing SMS with plant sensors enables irrigation managers to quantify the water volume to be applied that will directly affect crop productivity. The combined plant-soil moisture measures can provide information about the moisture thresholds where plants do not suffer drought stress and irrigators do not excessively leach nutrients. Sap-flow measurement is considered a potential tool for irrigation management as it is a parameter indicative of the interactions between the amount of water available in the growing medium and the atmospheric water demand. Unlike other tools for measuring plant water status, such as leaf gas exchange or plant water potential, sap-flow sensors can be cheaply constructed, provide continuous data, and are non-destructive.

Methods for measuring sap flow were pioneered nearly 100 years ago by Huber and colleagues in the 1930s (Clearwater et al. 2009, Skelton 2017). The modified Huber method, now known known as Heat Pulse Velocity (HPV), calculates the velocity of a short pulse of heat carried by convection in the transpirational stream. The basic premise of HPV is that a short pulse of heat (1 to 6 sec) is released into the sap stream, and sapwood temperature is monitored at points upstream and downstream from the heater (Kirkham, 2014). Sap flow may already be familiar to foresters because some manner of this technique has been used in many studies of tree responses to drought and climate in mature timber stands (Simonin et al. 2007, Vanclay 2009). For example, sap-flow methods have been used to investigate how transpiration is affected by air turbulence near plantation edges, firebreaks, and streamlines, and how hydrology in mixed stands differs from hydrology in monocultures (Vanclay 2009). Alternatively, sap-flow methods have been used to identify differences in stand-level evapotranspiration (Simonin et al. 2007). These types of plot- or forest-scale investigations dominate the forestry sap-flow literature. While the theoretical underpinnings of forest-level sap-flow measurements are the same, the methods used for large trees are wholly inappropriate for seedlings in forestry nurseries. In particular, measuring sap flow on large, woody species involves inserting metal needles into the sapwood. This technique would critically damage vascular tissue and potentially destroy young nursery plants. Fortunately, non-destructive methods for sap flow have been developed for horticulture that are effective for small-diameter stems.

Sap-Flow Sensors for Nursery and Field Applications

The external sap-flow sensors we use on nursery seedlings were inspired by a system developed for measuring the pedicles of fruits (Clearwater et al. 2009). Commercially produced external sap-flow gauges may be purchased from a supplier. On the other hand, if you can solder, constructing your own sensor is relatively easy with some basic electronic supplies and parts from a hardware store (figure 1). The following is a description of the method we used to build sap-flow sensors.

Figure 1. Using basic supplies, growers and researchers can construct sap-flow sensors for small-diameter plants. This model is not described in detail but shows how growers can modify the design. For this sensor, we added Velcro® to attach to the stem, used thermistors instead of thermocouples, and used a pile resistor in place of the chip resistor. (Photo by Lloyd Nackley)

1. Create thermocouple
    a. Strip 5 mm of insulation from each wire 0.05-mm type T thermocouple wire and form the junction of the thermocouple twisting the two wires together; apply solder to make a reliable connection. Trim the soldered junction with a pair of snips to minimize the length of the junction to approximately 1 mm.
2. Assemble resistor chip
    a. Strip 2 to 3 mm of insulation from 30 AWG (American wire gauge) wire, twisting a small loop in the end, and tinning the wire so that the loop is filled with solder. Note that the loop should not be wider than the width of the chip resistor.
    b. Secure a chip resistor with cross-locking tweezers so that the contacts on the bottom of the resistor are accessible.
    c. To complete the joint, hold the loop of the tinned wire against the bottom surface of the solder point on the resistor and applied heat with a soldering iron. Repeat this step for the other wire. The wires should not extend past the top surface of the resistor, as this is the part of the resistor that will be in direct contact with the plant stem once installed.
3. Install the connector to the heating resistor (figure 2)
    a. Strip approximately 3 mm of insulation from each wire and solder the wires to the two pins for the connector. When the pins are cool to the touch, press each pin into the plastic housing of the connector until the pin clipped into place. To test that wires are locked, gently pull on each wire.
4. Mount heating resistor and thermocouples
    a. Tape the resistor and thermocouples to the 13-mm foam block insulation (figure 3) and mark the locations of the resistor and thermocouples with a fine tip marker, making sure not to dent the foam with the marker.
    b. Place the resistor against the foam with the face up and pressed lightly to make an indentation for the chip resistor.
    c. Route the thermocouple wires around the top and bottom of the foam block. Additional tape can then be added to hold the two wires for the resistor against the face of the foam block.

Sensor Data Analysis

Our analysis method (figure 4) examined temperature variation (ΔT_h) values measured at 10 and 90 seconds after the heat pulse and 100 and 180 seconds after the end of the heat pulse using the equation below.

$$\Delta T_h = \frac{(T_{90} - T_{10}) + (T_{180} - T_{100})}{2}$$

Where:

ΔT_h = Temperature changes in the plant during the pulse

ΔT_{10} = Temperature measured by the sensor at 10 seconds after the start of the pulse

ΔT_{90} = Temperature measured by the sensor at 90 seconds after the start of the pulse

ΔT_{100} = Temperature measured by the sensor at 100 seconds after the end of the pulse

ΔT_{180} = Temperature measured by the sensor at 180 seconds after the end of the pulse

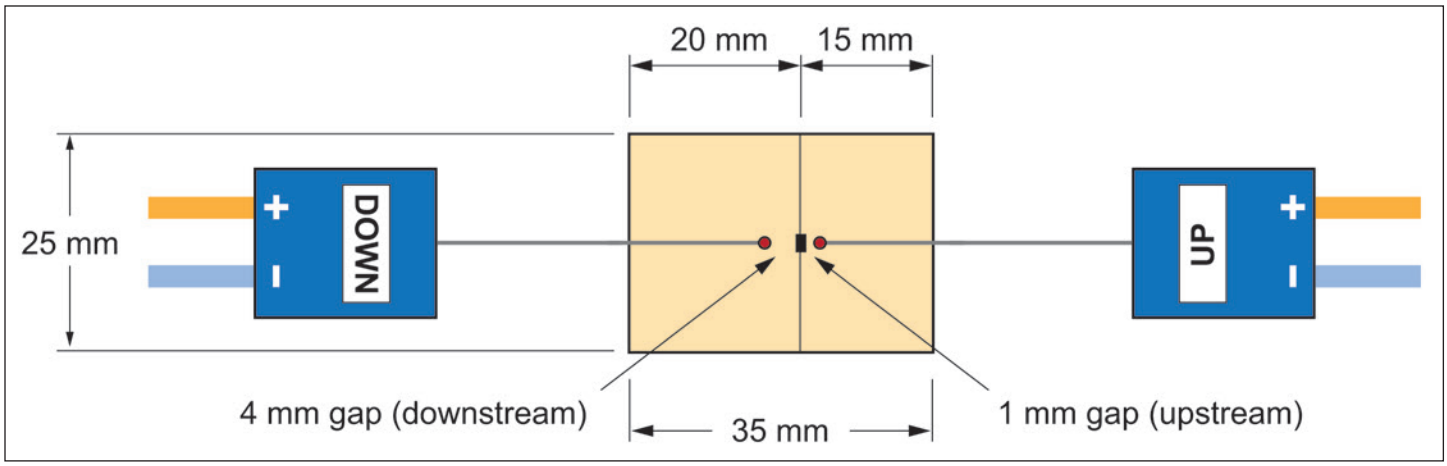

Figure 2. Schematic of the sap-flow sensor design. The foam block is represented by the square in the middle. The thermocouples are represented by the small circles and are spaced at 4 mm and 1 mm away from the small resistor chip (small black rectangle).

Figure 3. Sap-flow sensor constructed using foam insulation rather than a cork. The sensor is attached to an elderberry seedling (*Sambucus nigra* L.). The measurable sap flow is relative to the leaf area downstream (i.e., above) the sensor. Therefore, sensors should be located low on the seedling, or near to the main stem if placed on a lateral branch. (Photo by Lloyd Nackley)

The actual output of the sensor (Λ) was determined as:

$$\Lambda = 1 - \frac{\Delta T_h}{\Delta T_h^0}$$

Where:

Λ = Sensor signal (dimensionless)

ΛT_h^0 = Temperature range measured by the sensor installed in the plant at zero flow condition

ΛT_h = Temperature range measured by the sensor installed in the plant in one point in time during the day

Assuming a linear relationship between the signal measured by the sensor and the plant's sap flow, the amount of sap flow can be estimated as:

$$J = k.\Lambda$$

Where:

J = Sap flow density, in $m^3\ s^{-1}\ m^{-2}$

k = Coefficient on the basis of the thermal properties of the stem and the sap (diffusivity and thermal capacity), and the sensor geometry.

Application of Sap-Flow Sensors

Pairing sap-flow sensors with SMS has a number of promising applications in a forestry nursery setting. Three research areas that offer significant opportunity are: deficit irrigation scheduling, native plant eco-physiology, and outplanting performance evaluation.

Centuries of cultivation have made clear distinctions between wild-type and agricultural plant species. Over countless generations, the genetic variability of particularly prized plants has been reduced in favor of desirable traits such as flower size and color. In recent decades, seeds and cuttings of wild-type native plants have also been collected for propagation in nursery and greenhouse production facilities. Yet, the cultivation requirements for many native species remain largely unknown. People often incorrectly assume that cultivating native plants will be easy since native plants do not require human intervention to regenerate in natural conditions. Unlike commercially selected cultivars whose genetic profiles have been narrowed to emphasize specific traits, however, the horticultural needs of native plants can be obscured by genotypic and phenotypic plasticity. Natural selection processes like frost, flood, and fire have bred variability into the cultural requirements for wild-type native plants. Unpredictable growth habits of native plants can frustrate novice and experienced growers with failed attempts to propagate rare and endemic species,

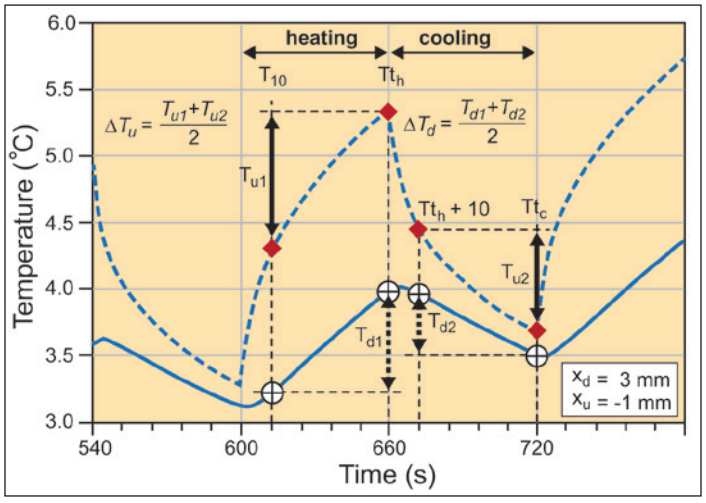

Figure 4. With the heat pulse method, temperatures, upstream (Xu) and downstream (Xd) of the heating resistor are measured by thermocouples. The (ΔTh) values are measured at 10, 90, 100, and 180 seconds after the start of heating supply. The heat pulse is evidenced by the wave form thermal signature.

stalled development following germination, and high mortality after outplanting in recently disturbed restoration conditions. The need for greater understanding of the optimal environmental conditions necessary for producing native plant nursery stock is ecologically important now because endemic species from isolated populations face increasing threats from catastrophic exogenous disturbance.

Pairing SMS with sap-flow sensors can provide a real-time method for examining how native plants respond to above- and belowground environments. Sap flow of small plants are more responsive to environmental cues compared to large trees that have stored water reserves and may experience considerable (i.e., hourly or daily) lags in sap-flow signals (Čermák et al. 2007). Linking SMS with sap-flow sensors allows researchers to measure the plant's transpirational pulse that is driven by the atmosphere and restricted by the rhizosphere. Taking the pulse of native plant species during nursery production provides fundamental insights about variability within a species and among populations from different regions. In ecology, location is sometimes used as a proxy for function. For instance, when a species expresses different morphological characteristics along a precipitation gradient, dry-side varieties have been considered discrete populations from wet-side varieties (Nackley et al. 2018). Concerns with this method suggest that ecotype comparisons are rarely conducted for a long enough time for long-lived species, and that the genetic basis of local adaptation and genetic associations with climate has rarely been identified (Galliart et al. 2019). Adding sensors to a nursery production system can elucidate if phenotypic differences between ecotypes are correlated with physiological differences. More specifically, sensor data can help determine if source material collected across a latitudinal (or precipitation) range needs to be cultivated differently, or if growers can apply the same irrigation to all plants within the same species, even between sub-species.

Pairing sap flow with SMS is an excellent way to optimize irrigation scheduling. Typically, growers tend to overwater in nursery production (Lea-Cox et al. 2013) because the direct consequences of under-watered plants are more immediate than the indirect consequences of overwatered plants, such as nutrient leaching and fostering conditions for moisture-loving pathogens (Dumroese and Haase 2018). Sap flow can be used to determine the safe threshold for deficit irrigation in two ways. First, this method can be used to determine the soil moisture level at which plants close stomata. This threshold would represent when irrigation should be turned back on. Growers could pair sap-flow techniques with gravimetric measures of soil water content to learn at what moisture contents (i.e., weights) plants stop transpiring. Gravimetric techniques for scheduling irrigation have previously been shown to be simple and effective (Dumroese et al. 2015). When plant stress is appropriately linked to soil moisture status, monitoring soil moisture becomes a suitable proxy that can be used to schedule irrigation to maximize water-use efficiency. Secondly, drought research can determine at what point after this low-moisture threshold is reached a plant can revive when re-irrigated. This point is also known as the permanent wilting point. The physiological consequences of deficit irrigation depend on the duration of the drought. Obviously, prolonged drought will kill a plant. Yet, less devastating effects include reduced leaf expansion and growth rate, and increased water-use efficiency and root-to-shoot ratio. Deficit-irrigated plants are comparably shorter than well-watered plants with smaller leaves or fewer leaves, or both (Hsiao 1973, Villar-Salvador et al. 2013). Deficit-irrigation strategies can be used as a form of moderate drought conditioning, which is a technique that has increased stress tolerance and seedling survival in semi-arid environments (Villar-Salvador et al. 2013). Sap-flow sensors can be invaluable in drought conditioning during which drought intensity and duration should be considered. In addition, the levels of stress applied should be species specific (Vallejo et al. 2012). Lastly, drought-induced smaller, thicker leaves may be less attractive to foliage-eating insects and herbivores.

Investigations of seedling physiology with sap-flow sensors highlights the components of the Target Plant Concept (TPC) that put an emphasis on seedling quality, which is measured by outplanting performance (Dumroese et al. 2016). Water stress is commonplace in afforestation, reforestation, and restoration sites where nursery seedlings are typically expected to survive without supplemental irrigation. The timing and degree of drought will dictate whether stocktype choice, deep planting, or adequate root growth will compensate for the low water potential conditions in the upper soil profile (Vallejo et al. 2012, Pinto et al. 2016). Water stress occurs when

plant leaf and stem evapotranspiration rates exceed water absorption by the roots. Water stress impairs plant processes and may cause vascular embolisms that can kill the seedling (McDowell et al. 2008). Revegetation in droughty environments prioritizes plant water conservation through site modifications such as micro-catchments for "run-off harvesting," mulching (Vallejo et al. 2012), temporary shade structures, and nurse planting (Badano et al. 2011). Soil moisture readings can be taken concurrently with plant metrics to develop a relationship between plant physiology and soil moisture status.

Investigating outplanting success of nursery-grown seedlings is another opportunity for pairing SMS with sap flow. In research and non-research contexts, binary plant survival monitoring, (e.g., "dead or alive") is commonly used to assess outplanting success. Although survival monitoring is better than no monitoring, it provides limited information about critical environmental gradients by conflating various environmental stresses. It is these same gradients and stressors that foresters and restoration ecologists can take advantage of to adaptively manage restoration projects and improve upon in future designs (Badano et al. 2011, Vallejo et al. 2012). For revegetation to succeed, it is imperative to describe the restored environment in terms of the factors that pertain to long-term plant growth, survival, and reproduction. The TPC calls for a strong a nursery-client partnership for circular feedback evaluation, generating more realistic expectations by both parties throughout the plant material ordering, production, and outplanting process (Dumroese et al. 2016). Pairing plant and soil moisture sensors can provide new insights about how moisture stress affects outplanting success, thereby providing greater clarity in the feedback evaluation for nursery growers.

This report is not an assertion that sap-flow is the only, or even the best, method for measuring plant moisture responses. It is, however, an under-utilized tool for growers, foresters, and ecologists working with small-diameter plants. Applying low-cost, data-intensive tools like sap-flow sensors to the production and revegetation system can help take the guesswork out of correlating environmental factors with plant performance. The methodologies described here provide a framework by which practitioners may consider physiological plant monitoring when working with stressful environments. Within this framework, installing plants appropriate to the region, ecosystem, and most importantly, project goals is required to prevent unnecessary plant death (Dumroese et al. 2016). Without installing plants suited to local conditions—plants whose physiological performance is matched to the site's potential performance—no amount of stress consideration or mensuration can help build a successful project.

Address correspondence to—

Lloyd Nackley, Oregon State University, North Willamette Research and Extension Center, 15210 NE Miley Road, Aurora, OR 97002; email: lloyd.nackley@oregonstate.edu; phone: 971–801–0385.

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Root Growth Potential Effects on First-Year Outplanting Performance of Inland Northwest Conifer Seedlings

Andrew S. Nelson

Tom A. Alberg and Judith Beck Endowed Chair of Native Plant Regeneration and Assistant Professor, Forest, Rangeland, and Fire Sciences Department, University of Idaho, Moscow, ID

Abstract

Root growth potential (RGP) is used to evaluate seedling vitality from nurseries prior to outplanting. Because results from previous studies indicate mixed results, there is still interest in exploring if a correlation between RGP and outplanting performance exists. This study tested RGP for 44 western larch (*Larix occidentalis* Nutt.) and 24 Interior Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *glauca* [Beissn.] Franco) seedlots using mist chambers followed by outplanting at three sites in the Inland Northwest. Survival exceeded 95 percent for both species at all three sites and was not related to RGP. RGP was not correlated with aboveground growth for western larch but was positively correlated for Douglas-fir at one site. Weather during early summer was suitable for new root growth (warm temperatures and average precipitation) and most likely caused the high survival and growth during the first year. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25–26, 2018).

Introduction

Root growth potential (RGP; root production under optimal controlled conditions) is one of many seedling quality tests used to assess vitality of seedlings grown in nurseries prior to outplanting (Haase 2008). RGP was first proposed by Stone (1955) to assess seedling physiology in response to claims that seedling physiological grades were equally or more important than morphological grades (Wakeley 1954). Stone's experiment was simple: he grew conifer seedlings in a greenhouse, observed their root development, and related root development to seedling survival. The idea that a simple test of seedling root development under controlled conditions may relate to field performance spurred rapid development of RGP research and methodologies from the 1970s through the 1990s and their applications continue today.

Literature reviews from the peak of RGP research show inconsistent correlations between RGP and outplanting performance that vary by species, RGP testing procedures, and outplanting site conditions (Ritchie and Dunlap 1980; Ritchie 1985; Ritchie and Tanaka 1990). Variability led to a debate about the relevance of RGP to predict outplanting performance given the other factors that can influence seedling outplanting performance such as site quality and climate (Simpson and Ritchie 1996). The debate continues today in the Inland Northwest and other regions as landowners contract with private nurseries to grow seedlings, with overall goals of improving seedling quality and outplanting success.

The ability of seedlings to produce new roots is strongly controlled by their physiology. Seedling physiological potential is developed in the nursery by manipulating nutrient inputs, watering regimes, light quality and quantity, temperature and relative humidity, and seedling dormancy. RGP, like dormancy, shows seasonal cycles that are regulated by internal factors. RGP typically peaks when shoots are not actively growing but dormancy intensity is weak, possibly due to available assimilates and hormonal signals to promote root elongation (Ritchie and Tanaka 1990). Villar-Salvador et al. (1999) found that aleppo pine (*Pinus halepensis* Mill.) seedlings hardened in the autumn under severely dry conditions produced 27 percent less new roots in RGP tests compared to seedlings hardened under no water stress. Even though they found RGP was lower in water-stressed seedlings, no significant differences were found in survival or

growth 2 years after planting. Similar results were found for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings (Tinus 1996).

Given the simplicity of measuring new root growth under controlled conditions, various methods have been developed to test RGP. Testing systems can be divided into three broad classes: (1) seedling potted in soil medium; (2) seedling placed into hydroponic water baths; and (3) seedlings suspended in aeroponic mist chambers where water is misted onto the roots to avoid desiccation. Even though the testing systems expose seedling roots to different environmental conditions, results between the three methods are often correlated (Rietveld 1989). The variety of testing systems, but also the diversity of testing regimes among investigators, makes a comparison of results among studies difficult. To make inferences on seedlot performance, it is thus best to use a consistent testing system and regime to ensure repeatability of results.

Mist chamber RGP is a desirable method since multiple seedlots can be tested within a compact space while being exposed to similar environmental conditions. The mist chamber method was first used by Lee and Hackett (1976) to examine root regeneration of Chinese pistache (*Pistacia chinensis* Bunge). The method was later adapted for conifer seedlings by Harvey and Day (1983) using a system that continuously misted roots with fine droplets of water recirculated within the chamber. The U.S. Department of Agriculture, Forest Service Lucky Peak nursery was one of the first to develop an operational mist chamber system (Hileman 1986). It tested seedlings during packing by misting roots for 10 days then counting new white root tips and measuring the length of the longest new roots (Dolata 1986). The system was further refined by Rietveld and Tinus (1987) to become portable and provide uniform conditions for the roots. The mist chamber method continues to be used to assess RGP (Tinus et al. 2000).

The outplanting environment substantially influences the relationship between RGP and seedling performance (Ritchie et al. 2010). Results typically show low RGP and poor site quality result in poor seedling performance, while high RGP and good site conditions results in good seedling performance. These generalities are pieced together from multiple studies where RGP testing procedures and species differed. Jenkinson et al. (1993) provides one of the most comprehensive examination of the topic, where seedlings were grown at the same nursery, RGP was tested using the same method, and seedlots with different RGP were planted at more than 30 sites across the Pacific Northwest. They classified sites based on critical RGP, where harsher site conditions exhibited higher thresholds of RGP for adequate seedling survival. Seedlots that did not produce RGP values above the critical RGP for the site did not have good first-year survival. Burdett et al. (1983) and Grossnickle (2012) found a similar positive correlation between RGP and seedling survival. In contrast, Ritchie (Simpson and Ritchie 1996) argued that RGP is not a good indicator of field performance. He used a dataset derived from Binder et al. (1988) to demonstrate poor correlation between RGP and first-year seedling survival. Binder et al. (1988) suggested the high variability in first-year mortality of seedlings of three seedlots with moderate RGP was due to microsite conditions such as location from a shading object and proper site preparation.

Silviculture and planting within proper microsite conditions has advanced substantially in the Pacific and Inland Northwest regions, prompting reexamination of the relevancy of RGP for predicting field performance within a contemporary reforestation context. Extensive research suggests RGP is not the "holy grail" for predicting early seedling outplanting performance, but testing can still be beneficial to evaluate if seedlings are physiologically damaged, and thereby assist in the prediction of seedling performance. RGP data can vary by individual seedling responses within seedlots, but especially among seedlots. Only rarely have numerous seedlots been tested simultaneously and then outplanted in common-garden experiments that minimize within-site variability. Therefore, the objective of this study was to examine the first-year survival and growth of western larch (*Larix occidentalis* Nutt.) and Interior Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *glauca* [Beissn.] Franco) seedlings in relation to RGP mist chamber results at three sites in the Inland Northwest.

Methods

Seedlings

Seedlings for RGP testing and outplanting were grown at various nurseries located in western and

central Oregon, western Washington, Idaho, and British Columbia. All seedlings were 1-year-old containerized seedlings grown in 91/130 Styroblock® containers (Beaver Plastics, Alberta, Canada) with cavity volume of 130 ml (7.9 in^3). Only seedlots derived from seed sources from the east side of the Cascade Mountains were tested. Seedlings were grown using operational growing regimes at different private nurseries. Minimum morphological specifications were 2.7 mm (0.1 in) stem diameter and 15 to 30 cm (5.9 to 11.8 in) tall.

Each nursery shipped 90 seedlings per seedlot for this study. Nurseries were instructed to randomly select the seedlings from across the crop to avoid sampling bias. Seedlings were immediately placed in freezer storage at -2.0 °C (28.4 °F) at the University of Idaho Center for Forest Nursery and Seedling Research (CFNSR) until testing. A total of 24 Douglas-fir seedlots from 10 nurseries and 44 western larch seedlots from 9 nurseries were used for this study. Of the 90 seedlings from each seedlot, 15 seedlings were randomly selected for RGP testing and 75 were reserved for outplanting.

Root Growth Potential

RGP was tested in mist chambers at the CFNSR Seedling Quality Lab starting in January 2018 using chest freezers with the lids removed (figure 1). The freezer's

Figure 1. Root growth potential chambers with seedlings suspended on top of the chambers in plastic slats and supplemental light-emitting diode (LED) light bars. (Photo by Andrew Nelson, 2018)

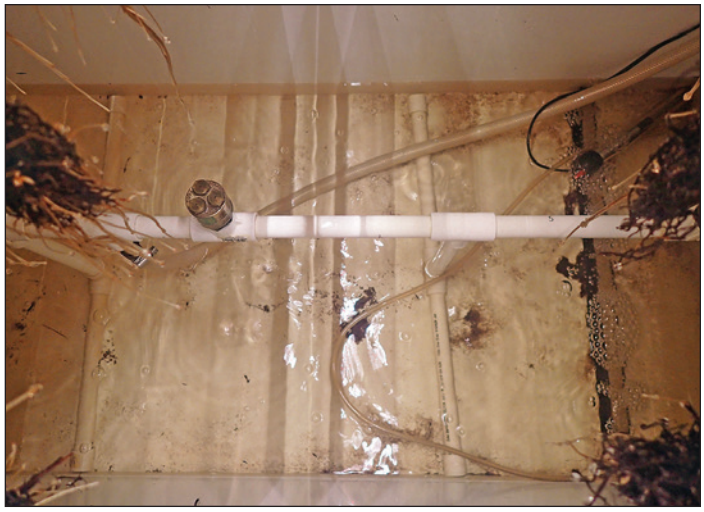

Figure 2. Three superfine misting nozzles attached to a polyvinyl chloride (PVC) frame were placed in the bottom of the chamber to continuously spray seedling roots. (Photo by Andrew Nelson, 2018)

internal dimensions were 137.2 by 50.8 by 71.1 cm (54 by 20 by 28 in), and external dimensions were 156.2 by 70.0 by 82.5 cm (61.5 by 27.5 by 32.5 in). Each chamber was filled with 76 to 113 L (20 to 30 gal) of water, which was recycled throughout the testing. A hose with an attached strainer was submersed in the water to pump water to three superfine misting nozzles (Fogg-it Nozzle Co., Belmont, CA) that sprayed 1.9 L (0.5 gal) of water per minute using a 115-volt diaphragm pump operating at 11.4 L (3 gal) per minute at a pressure between 276 and 345 kPa (40 and 50 lbs/in^2 [PSI]). The three nozzles were equally spaced 44.5 cm (17.5 in) apart and mounted to a polyvinyl chloride (PVC) frame that was centered in the chamber, approximately 25 cm (9.8 in) from all chamber walls (figure 2). The PVC frame was designed to be 31 cm (12.2 in) from the chamber floor and approximately 28 cm (11.0 in) from the bottom of the seedling root plug. The pump was plugged into a timer that misted for 5 seconds followed by 4 minutes and 55 seconds of no misting. The system ran 24 hours per day throughout the test.

Supplemental light was provided to seedlings using Phillips light-emitting diode (LED) linear light modules for 12 hours during the day. Each of the 16 lighting modules in the lab has 87 bulbs emitting 85:10:5 (red:blue:green) light (DR/W LED 120-110V, Phillips, Texas, USA). The lights were suspended 140 cm (55.1 in) above the tops of the chambers and were evenly spaced 12.7 cm (5.0 in) apart (figure 1). Blackout curtains were hung around the sides of the chambers from the ceiling to the top of the chambers to control light intensity and quality (figure 3).

Figure 3. Blackout curtains are suspended from the ceiling around all the chambers. (Photo by Andrew Nelson, 2018)

Figure 4. Example of western larch new root growth at the end of root growth potential testing in the mist chambers. (Photo by Andrew Nelson, 2018)

Air and water temperature were maintained at 21 °C (69.8 °F), and an airstone was inserted into the water at the bottom of the chamber to increase the amount of oxygen in the water. Between each round of testing, the chambers and pump system were sterilized using a 1:8 bleach:water solution that circulated within the system for 24 hours. Prior to suspending seedlings in the mist chambers, Styrofoam insulation boards were cut to the dimensions of the chambers and placed over the top, and the mist system was run for approximately 1 hour to raise internal relative humidity to 100 percent and water temperature to 21 °C.

Seedlings were removed from freezer storage and thawed in a refrigerator set to 4 °C (39.2 °F) for 2 days prior to RGP testing. Seedling roots were then washed in room-temperature water to remove soil medium, then measured for root-collar diameter (RCD; mm) and height from the root collar to the tip of the terminal bud (cm). Seedlings were suspended in the chambers in plastic slats with square rubber mats to hold the seedlings upright (figure 1). Slat dimensions were 57.1 by 7.6 cm (22.5 by 3.0 in) to align with the internal chamber width. Five circular (10.2-cm [2-in] diameter) holes were cut out of each slat. Rubber squares were 7.6 by 6.7 cm (2.6 by 3.0 in) with a slit cut halfway through the mat and a small hole cut out of the center for the seedling.

Douglas-fir seedlots were tested for 16 days and western larch seedlots were tested for 20 days, based on preliminary research to identify the minimum number of days required to achieve consistent seedling performance in the mist chambers. At the end of testing, seedlings were removed from the chambers and the number of new white roots 1 cm (0.39 in) long or longer were counted (figure 4).

Field Experiment

Three study sites were selected on private land in the Inland Northwest (figure 5) that had similar climate but different soil characteristics (table 1). All sites were harvested and treated with chemical site preparation the year before planting using standard operational mixtures to control shrubs, forbs, and grasses. The

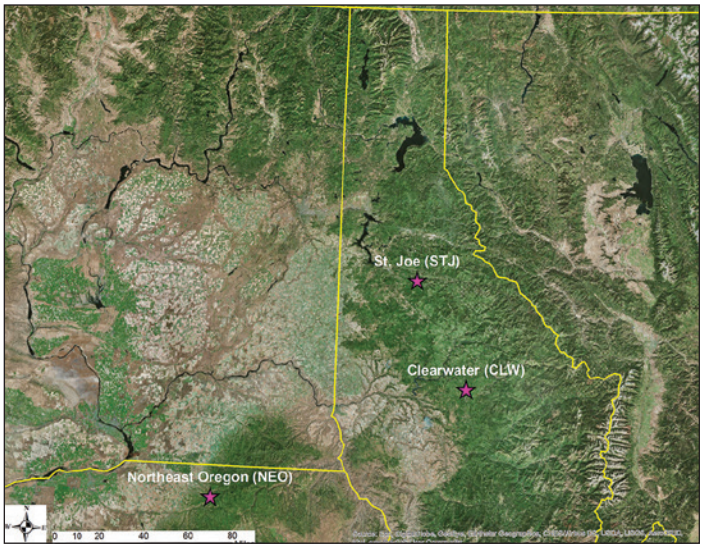

Figure 5. Location of the three RGP outplanting sites in the Inland Northwest. One site was in the Blue Mountains of northeastern Oregon, while the other two sites were located in northcentral Idaho.

Table 1. Thirty-year normal climate, planting season weather, and site characteristics for the three experiment sites in the Inland Northwest (Hegewisch and Abatzoglou 2019; Soil Survey Staff 2017). Planting season temperature and precipitation are shown for June, the month typically before the summer dry season begins.

Site	30-year norm			Planting season (June)			Elevation (m)	Soil parent material	Available water top 50 cm (mm)
	max. temp. (°C)	min. temp. (°C)	average precipitation (mm)	min. temp. (°C)	max. temp. (°C)	precipitation (mm)			
Clearwater (CLW)	26.5	-6.6	1139	6.4	19.9	71	1091	Ash over granite	121
Northeast Oregon (NEO)	24.2	-6.5	1246	7.5	20.8	46	1202	Ash over basalt	107
St. Joe (STJ)	26.9	-6.5	996	6.4	21.1	38	991	Ash over metased-imentary rock	107

1 °F = (°C × 9/5) +32; 1 inch = mm/25.4; 1 foot = m × 3.281

amount of slash left after harvest was minimal at all three sites.

Two days before planting, seedlings were removed from the freezer and thawed in a shaded warehouse with an air temperature of approximately 10.0 °C (50.0 °F). The 68 seedlots were planted in a completely randomized block design where each site served as a block (n=3) (figure 6). At each site, 15 seedlings from each seedlot were planted in a row with a spacing of 0.91 m (3 ft) between seedlings within a row and 1.22 m (4 ft) spacing between rows. Seedlots were randomly assigned to rows and all rows were oriented up-down the slope. Seedlings were shovel planted during a 2-week period starting 25 April 2018. Initial height and RCD were measured within 3 weeks after planting.

Figure 6. The Blue Mountain RGP site in northeast Oregon in April when seedlings were being planted. (Photo by Andrew Nelson, 2018)

Seedlings were remeasured at the end of September 2018. Mortality was also recorded. Seedlings that were missing and those that died due to animal damage were excluded from the analysis.

Data Analyses

Generalized additive models (GAMs) were used to examine the relationships between RGP and survival and RGP and growth. GAMs are semi-parametric extensions of generalized linear models (GLMs) (Hastie and Tibshirani 1990) and have been used extensively in ecology (Guisan et al. 2002, Yee and Mitchell 1991). GLMs examine the relationship between the mean of the response variable and the linear combination of explanatory variables using a link function, while GAMs use the link function to examine the relationship between the mean of the response variable and a smoothed function of explanatory variables. This makes GAMs very effective for analyzing nonlinear relationships.

Individual GAM models were developed using the data for western larch and Douglas-fir seedlings for both survival and growth. Survival models used a binomial link, since survival was a binary variable (alive or dead). Growth was expressed as the 1-year increment of volume index (cm^3) calculated as $RCD^2 \times$ height. Models tested the relationship for each of the three sites using a thin plate regression spline (Wood 2003) of RGP using the "mgcv" package (Wood 2019) in R version 3.4.3 (R Core Team 2017).

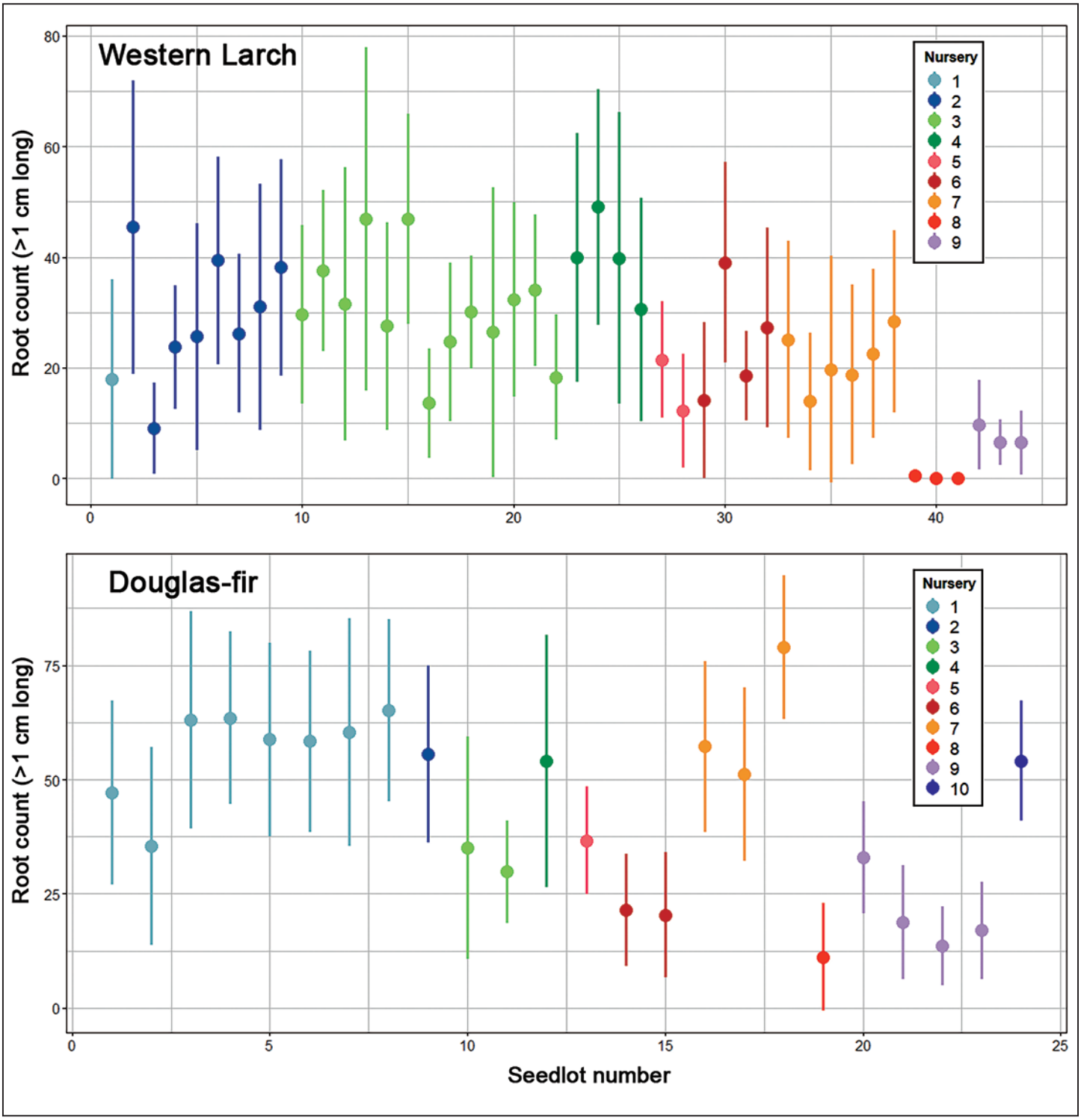

Figure 7. Average (circle) and one standard deviation (range of bar) of root growth potential measured as the count of new white roots greater than or equal to 1 cm long by seedlot and nursery. There were 44 western larch seedlots tested from 9 commercial nurseries, and 24 Interior Douglas-fir seedlots tested from 10 commercial nurseries.

Results

Root Growth Potential

RGP varied considerably among seedlots (figure 7). Average RGP of western larch and Douglas-fir was 25 and 43 new white roots, respectively. Even though RGP varied among seedlots within a nursery, nurseries 8 and 9 grew western larch seedlots with relatively low RGP, while nurseries 6, 8, and 9 grew Douglas-fir seedlots with low RGP (figure 7).

RGP Effects on Seedling Field Survival

RGP was a poor predictor of first-year survival of western larch and Douglas-fir seedlings. For example, nursery 4, which produced seedlots with higher RGP values (figure 7) had the lowest average survival, while nursery 9, which had the lowest average RGP, had higher survival (table 2). The western larch survival GAM model did not find a relationship between RGP and survival at the Northeast Oregon (NEO) and St. Joe (STJ) sites (estimated degrees of freedom [edf] = 1.000, $p \geq 0.376$), and only a slightly nonlinear relationship at the Clearwater (CLW) site (edf=1.048, p=0.056) (table 3). The deviance explained by the model was only 3.90 percent. The same was found for Douglas-fir, where the smoothed term for RGP had an edf of 1.000 for all three sites and only explained 0.23 percent of the deviance (table 3).

RGP Effects on Field Volume Index Growth

RGP had a greater effect on volume index growth than on seedling survival, but the effect was still small. RGP was not a significant smoothed term for western larch at any of the three sites ($p \geq 0.146$) and the deviance explained by the model was only 4.06 percent (table 4). Volume index plateaued at a RGP value of approximately 25 new roots at the CLW and STJ sites, while the relationship was flat at the NEO site (figure 8). Douglas-fir volume index was positively related to RGP at the CLW site (edf=1.296, p=0.021) with the model accounting for 14.5 percent of the deviance (table 4). Douglas-fir showed a continual increase in volume index with increasing RGP values at all three sites (figure 8).

Discussion

The relationship between RGP and outplanting survival and growth during the first year is not always consistent and often lacks correlation (Simpson and Ritchie 1996). The same was found in the current study for several western larch and Interior Douglas-fir seedlots planted at three sites across the Inland Northwest. This contrasts with other syntheses that found strong correlations between RGP and field performance. Ritchie and Dunlap (1980) reported that 85 percent of 26 papers reviewed showed a positive relationship, while Ritchie and Tanaka (1990) found 75 percent of 12 studies reported a positive correlation. Ritchie and Tanaka (1990) recognized, however, that a relationship does not always occur and postulated three reasons: (1) inadequate testing procedures, (2) poor seedling handling after leaving the nursery, and (3) site and weather conditions.

RGP testing procedures vary considerably among investigations, including differences in procedure with the same testing method. This makes it difficult to draw broad conclusions about the utility of RGP for assessing seedling vitality and outplanting performance. The aeroponic mist chamber RGP testing system used in this study is based on previous iterations of similar systems (Day 1982, Hileman 1986) and was designed to rapidly test multiple seedlots within a limited space. Most published studies that examined the relationship between RGP and seedling performance used potted RGP tests, especially studies comparing multiple

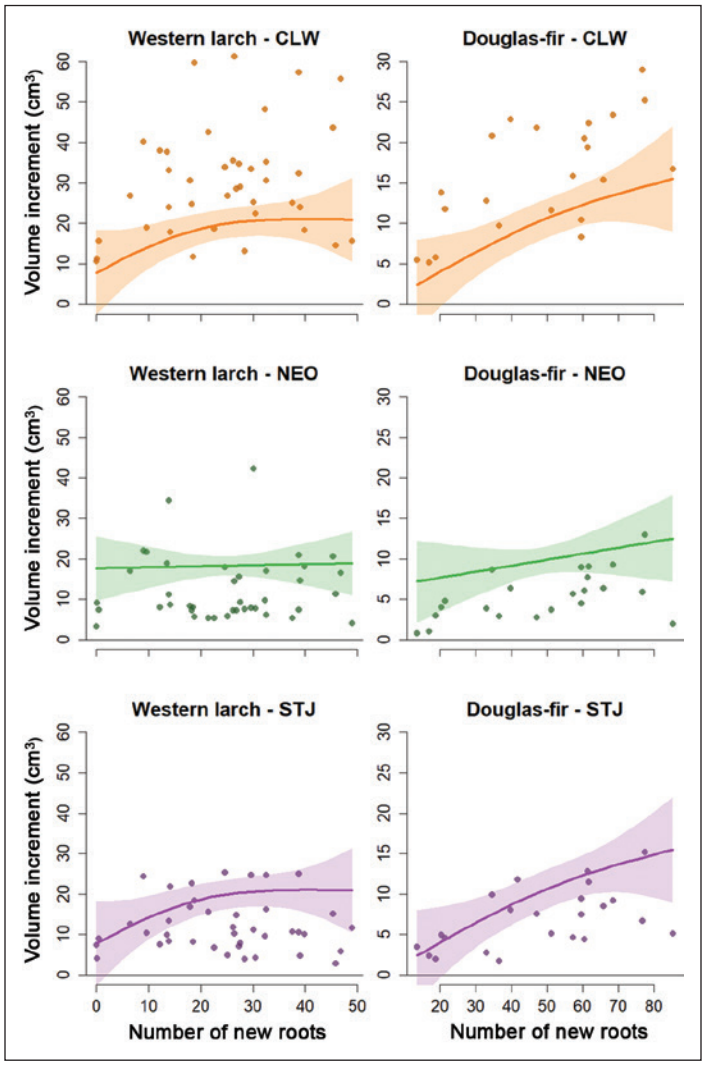

Figure 8. Correlation between RGP and volume index growth during the first growing season for western larch and Interior Douglas-fir at sites in northeastern Oregon (NEO), central Idaho (CLW), and northern Idaho (STJ).

Table 2. Average and one standard deviation of seedling size and survival at the beginning and end of the first growing season for western larch and interior Douglas-fir at three sites in the Inland Northwest.

Site	Initial			End of season			Survival (%)
	Height (cm)	Diameter (mm)	Volume index (cm^3)	Height (cm)	Diameter (mm)	Volume index (cm^3)	
Western Larch							
Clearwater (CLW)	33.6 (7.2)	3.8 (0.4)	5.1 (1.9)	52.3 (9.4)	7.6 (1.1)	32.3 (13.4)	95.7 (8.7)
Northeast Oregon (NEO)	32.4 (5.9)	3.8 (0.6)	4.9 (1.8)	47.6 (7.1)	5.7 (1.0)	16.3 (8.3)	96.7 (7.2)
St. Joe (STJ)	33.8 (6.7)	3.9 (0.6)	5.3 (1.9)	42.5 (8.1)	6.0 (0.9)	16.4 (7.3)	97.6 (4.0)
Interior Douglas-fir							
Clearwater (CLW)	32.1 (7.3)	4.3 (0.7)	6.3 (3.1)	40.2 (9.7)	7.0 (1.0)	21.3 (9.9)	98.5 (4.0)
Northeast Oregon (NEO)	30.8 (7.0)	4.3 (0.7)	6.0 (2.4)	38.7 (8.9)	5.1 (0.9)	10.7 (4.9)	95.7 (11.8)
St. Joe (STJ)	31.7 (7.2)	4.3 (0.7)	6.4 (2.9)	37.4 (8.4)	5.6 (1.0)	12.7 (6.0)	98.5 (2.8)

1 inch = cm/2.54; 1 inch = mm/25.4; 1 in^3 = cm^3/16.387

Table 3. Generalized additive model results testing the correlation between RGP and first-year survival for western larch and Interior Douglas-fir.

Western Larch				
Parametric variable	**Estimate**	**St. Error**	**t-value**	**p-value**
Intercept	0.966	0.001	156.2	<0.001
Smooth variables	**Est. DF**	**Ref. DF**	**F-value**	**p-value**
s(RGP-Count): CLW	1.048	1.093	3.77	0.056
s(RGP-Count): NEO	1	1	0.79	0.376
s(RGP-Count): STJ	1	1	0.004	0.952
Deviance explained:				3.90%
Douglas-fir				
Parametric variable	**Estimate**	**St. Error**	**t-value**	**p-value**
Intercept	0.977	0.001	108	<0.001
Smooth variables	**Est. DF**	**Ref. DF**	**F-value**	**p-value**
s(RGP-Count): CLW	1	1	0.108	0.743
s(RGP-Count): NEO	1	1	0	0.999
s(RGP-Count): STJ	1	1	0.036	0.85
Deviance explained:				0.23%

CLW = Clearwater; NEO = Northeast Oregon; STJ = St. Joe; DF = degrees of freedom; RGP = root growth potential.

species and seedlots (e.g., L'Hirondelle et al. 2007). Results from different testing methods are correlated, but mist chamber systems typically produce less new roots than potted tests under similar environmental conditions (Rietveld 1989). The differences in the number of new roots produced could be due to a lack of dissolved oxygen in the water sprayed onto the roots, as roots are usually coated with fine droplets throughout testing even though they are surrounded by oxygen in the aeroponic environment. To overcome this potential limitation, nozzles with larger droplet sizes can be used to increase oxygen to the roots or an aeration stone could be added to the water at the bottom of the chamber, as was done in this study, so that fine-droplet nozzles could still be used to maintain moistened roots.

RGP testing conditions intentionally diverge from field conditions, where seedlings are exposed to warm conditions that favor root proliferation. This led Ritchie to argue that the logic behind the RGP-outplanting performance relationship is flawed (Simpson and Ritchie 1996) since proliferative root production under warm, controlled conditions does not reflect root growth in the field, when soil temperatures are low. Soil temperature was not measured at the three outplanting sites in the current study, but the minimum and maximum air temperature in May when the seedlings were planted were 6.4 °C and 19.7 °C (43.6 °F and 67.5 °F), respectively, which is approximately 2.0 °C (1.8 °F) warmer than the 30-year normal (Hege-

Table 4. Generalized additive model results testing the correlation between RGP and first-year volume index growth for western larch and Interior Douglas-fir.

Western Larch				
Parametric variable	**Estimate**	**St. Error**	**t-value**	**p-value**
Intercept	18.248	1.174	15.55	<0.001
Smooth variables	**Est. DF**	**Ref. DF**	**F-value**	**p-value**
s(RGP-Count): CLW	1.689	2.062	1.878	0.146
s(RGP-Count): NEO	1	1	0.026	0.872
s(RGP-Count): STJ	1	1	0.012	0.915
	Deviance explained:			4.06%
Douglas-fir				
Parametric variable	**Estimate**	**St. Error**	**t-value**	**p-value**
Intercept	9.755	0.845	11.54	<0.001
Smooth variables	**Est. DF**	**Ref. DF**	**F-value**	**p-value**
s(RGP-Count): CLW	1.296	1.505	5.739	0.021
s(RGP-Count): NEO	1	1	1.11	0.296
s(RGP-Count): STJ	1	1	1.825	0.181
	Deviance explained:			14.50%

CLW = Clearwater; NEO = Northeast Oregon; STJ = St. Joe; DF = degrees of freedom; RGP = root growth potential

wisch and Abatzoglou 2019). Precipitation at the sites also persisted through the end of June, with average precipitation of 76 mm (3 in) during that month, which is the 30-year normal (Hegewisch and Abatzoglou 2019). The warmer-than-normal temperatures, typical early season precipitation, and deep surficial volcanic ash deposits that help maintain soil moisture during the summer may have resulted in conditions conducive to root growth and good seedling survival.

The hypothesized link between RGP and outplanting performance assumes that seedlings need to produce new roots following planting to absorb water from the soil. Although new root production is important for seedling survival (Grossnickle 2005), suberized roots can absorb water (Kramer 1946). Seedling morphology (e.g., height, stem diameter, and root mass) at the time of planting are typically positively related to aboveground seedling growth, while the relationship between RGP and shoot growth is more mixed (Grossnickle and MacDonald 2018). Grossnickle and MacDonald (2018) report that, of the 10 studies reviewed between 1991 and 2016, an equal split between positive and neutral responses was found. Our results align with the mixed results from other studies, where a significant relationship was not found for western larch at any of the three sites and for Douglas-fir only at the CLW site. In one of the few studies to examine western larch, L'Hirondelle et al. (2007) found a positive asymptotic relationship ($R^2 = 0.66$) between RGP and shoot dry mass of first-year coastal and interior western conifer species at moderately productive sites. When examined by species, however, the results were more variable: western larch seedlots that produced zero new roots in the RGP tests produced only about 10 percent of the maximum shoot dry mass, while seedlots that produced between 80 and 120 new roots had 80 percent of maximum shoot mass. This suggests a threshold value of RGP at which more new roots do not result in greater aboveground growth.

RGP varied considerably among seedlots and nurseries, but seedlings performed well overall across all three sites. It is unlikely that the lack of relationship between RGP and survival was caused by inadequate testing procedures or poor handling practices. The most likely reasons for the good performance were the favorable site and weather conditions during the period of observation. Mild site conditions can help seedlings overcome vitality issues because of fewer resource limitations (Burdett 1987; Ritchie et al. 2010; Ritchie and Tanaka 1990). Research with this mist chamber system will continue to refine assessment of seedling vitality, examine potential changes in the relationship between RGP and field performance in the second year after planting, broaden the scope of the outplanting sites to encompass a greater range of site quality in the Inland Northwest, and potentially observe the relationship during drier and warmer field seasons. Additional research on harsher site conditions is especially important as climate predictions suggest the region will experience increased mean temperatures and slightly lower precipitation in summer through 2100 (Joyce et al. 2018). Characteristics that define seedling quality may be revised to match site conditions as climate change progresses, including seedling physiology and specifically drought resistance. This may necessitate modifying nursery cultural practic-

es to adjust seedling physiology to withstand harsher site conditions. Since mist chamber RGP testing can produce results in a short time, the system could be used in future investigations to evaluate potential seedlot performance on harsh sites that may be common across the region in the future.

Address correspondence to—

Andrew S. Nelson, Forest, Rangeland, and Fire Sciences Department, University of Idaho, Mail Stop 1133, Moscow, ID 83843-1133; email: asnelson@uidaho.edu; phone: 208–885–1004.

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Seedling Performance Metrics: A Standardized Monitoring Approach

Abbie A. Acuff

Silviculturist, PotlatchDeltic, Lewiston, ID

Abstract

Planting seedlings is a significant investment. To assure success and minimize costs, the consumer needs to monitor seedling quality and field performance. A comprehensive monitoring program allows the consumer to identify issues in the nursery and the field and make adjustments as needed. PotlatchDeltic has established nursery inspections, root growth potential tests, box audits, garden plots, and field transects to evaluate seedling quality. The data generated from these monitoring activities is essential for assuring an efficient and successful reforestation program. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d'Alene, ID, October 25–26, 2018).

Introduction

Reforestation in the Inland Northwest is big business. Every year, industry, government, and private entities invest millions of dollars to grow and plant seedlings for reforestation and restoration. A common goal among them all is to plant high-quality seedlings that perform well in the field. There are several ways to ensure this goal, but the most important one is monitoring the crop.

It is important to monitor the production of seedlings from the time of ordering to establishment. The process involves several steps and without close monitoring, there is possibility of wasted seed, over production, and substandard seedlings. Each step allows the consumer to save money, but the consumer needs to be proactive.

PotlatchDeltic, headquartered in Spokane, WA, has an active container seedling-quality program that enables us to conserve our seed resources, monitor crop development, confirm that delivered seedlings meet contract specifications, and monitor seedling performance in the field. These basic principles may be customized to fit any consumer: industry, government, or private landowner.

Recommended Seedling Monitoring

Nursery Inspections

Nursery inspections are underutilized by the consumer, yet they are one of the most powerful tools available. PotlatchDeltic inspects nurseries twice a year. The purpose of the first inspection is to identify any seed issues, look for disease symptoms, and review the culturing regime with nursery personnel. Almost any issue with seed and early growth will show up as blank container cavities (figure 1). If there is an issue, the first inspection provides a good opportunity for a robust discussion with nursery personnel to determine if the problem is related to

Figure 1. Poor germination of lodgepole pine woods run seedlot (16 percent) resulted in blank container cavities and fewer net seedlings than ordered. (Photo by Abbie Acuff, 2013)

the seedlot or to nursery cultural practices, and if there is an opportunity to re-sow. By identifying an issue this early, the consumers have time to review potential impacts to their planting program and adjust accordingly. Also, a discussion regarding germination concerns can identify any seed issues and provide necessary information to adjust future seed calculations.

The purpose of the second inspection is to review seedling quality at the end of the growth cycle in the fall. At this time, the seedlings will have completed their active height growth, have lignified stems, have a good bud set, and should have roots filled out in the plug (figure 2). This is a good opportunity for the consumer to see the final product before it is packaged for cooler or freezer storage and to discuss cold hardiness, packing schedules, and anticipated shipping dates.

Root Growth Potential Tests

Root growth potential (RGP) tests are a quick, inexpensive way to evaluate the ability of seedlings to grow roots in an ideal environment. The idea is that the more roots seedlings grow in a test environment equates to better field performance. Some nurseries perform their own RGP tests. However, PotlatchDeltic works with Center for Forest Nursery and Seedling Research, Seedling Quality Lab at the University of Idaho (Moscow, ID) and has developed a consistent testing protocol and is participating in research to better understand the linkage between test results and field performance (figure 3). This allows for tests and research to be conducted on seedlings grown at all nurseries. PotlatchDeltic uses all available data to review seedling performance (table 1). If a potential issue is identified, then steps can be taken to minimize the impact on successful regeneration, such as block planting seedlings with lower RGP in one or two stands.

Box Audits

In too many cases, the first time consumers see their seedlings is on site when they are ready to plant. At this point, it is often too late to take a clinical look at seedlings or take measurements. To ensure delivered seedlings meet contract specifications and are of good quality, PotlatchDeltic instituted a box audit program several years ago. An independent auditor randomly measures height and stem diameter and assesses overall seedling quality shortly after delivery (table 2). By being proactive, foresters are made aware of any issues prior to planting and can adjust their planting program if necessary. Audit results are sent to nurseries weekly and any problems are addressed. For large seedlots, early notification of

Figure 2. Western larch seedlings grown in three different container sizes, all exhibiting roots that completely fill the plug. (Photo by Abbie Acuff, 2018)

Figure 3. Douglas-fir seedlings after 20 days in a root growth potential test at Center for Forest Nursery and Seedling Research, Seedling Quality Lab at the University of Idaho, spring 2018. (Photo courtesy of Center for Forest Nursery and Seedling Research, Seedling Quality Lab at the University of Idaho, 2018)

issues allows the nursery to review boxes still at its facility to determine if the problem is widespread or isolated. In addition, nurseries may be assessed penalties for poor audit results. Some of the issues identified by box audits include poor plug integrity, small-diameter seedlings, multiple tops, short seedlings, poor budset, disease, and active root or bud growth (figure 4).

Garden Plots

In 2013, PotlatchDeltic started planting samples of every seedlot and nursery combination in a garden plot at a common location (figure 5). The purpose of the garden plot is not to compare nurseries but to have one location to evaluate seedling performance in the field and to quickly identify issues.

Table 1. An example of root growth potential test results for seedlings planted in spring 2018. Seedlots with the same name were grown at different nurseries.

Seedlot	Size	Average height (cm)	Average RCD (mm)	Average root count	Average longest new root (cm)
DF-82-50 Blackwell Hump	8	35.87	3.79	57.2	7.1
DF-CL-Z1 Zone 1	8	28.45	3.85	32.9	7.7
DF-CL-Z1 Zone 1	8	37.36	4.86	47.1	7.5
DF-CL-Z2 Zone 2	8	34.89	4.77	39.9	10.4
DF-CL-Z2 Zone 2	8	41.65	5.03	59.6	8.2
DF-CL-Z4 Zone 4	8	33.93	3.89	21.4	7.2
DF-CL-Z4 Zone 4	8	42.94	4.74	61.7	7.4
DF-CL-Z5 Zone 5	8	39.71	4.69	60.4	6.7

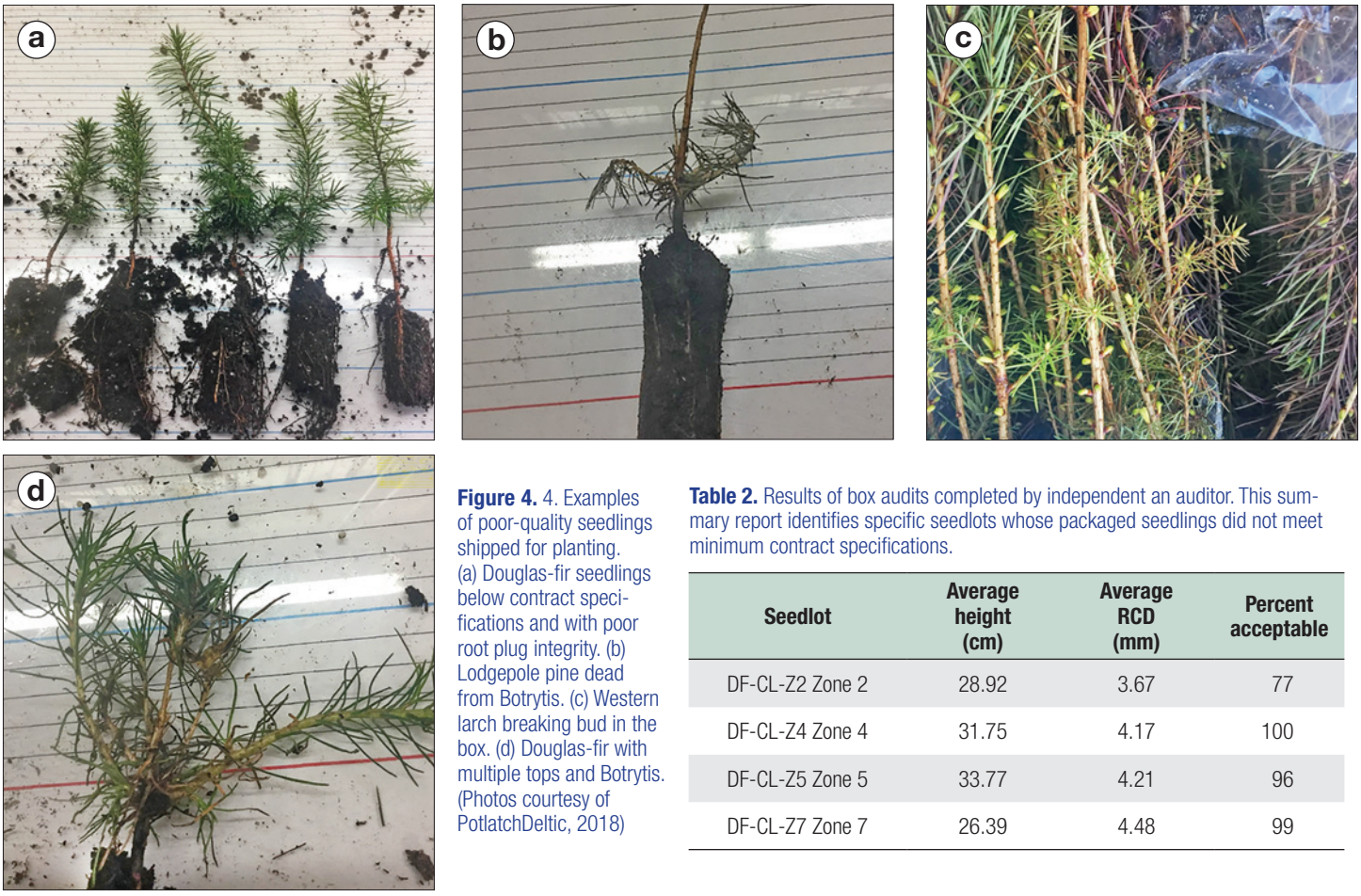

Figure 4. 4. Examples of poor-quality seedlings shipped for planting. (a) Douglas-fir seedlings below contract specifications and with poor root plug integrity. (b) Lodgepole pine dead from Botrytis. (c) Western larch breaking bud in the box. (d) Douglas-fir with multiple tops and Botrytis. (Photos courtesy of PotlatchDeltic, 2018)

Table 2. Results of box audits completed by independent an auditor. This summary report identifies specific seedlots whose packaged seedlings did not meet minimum contract specifications.

Seedlot	Average height (cm)	Average RCD (mm)	Percent acceptable
DF-CL-Z2 Zone 2	28.92	3.67	77
DF-CL-Z4 Zone 4	31.75	4.17	100
DF-CL-Z5 Zone 5	33.77	4.21	96
DF-CL-Z7 Zone 7	26.39	4.48	99

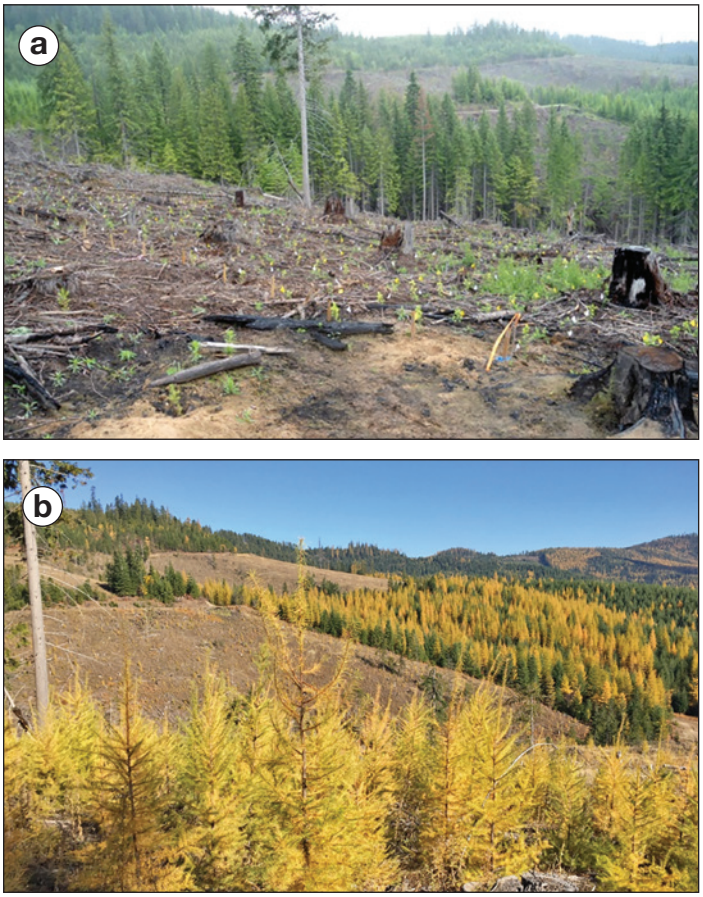

Figure 5. Garden plots are useful to evaluate field performance among seedlots and nurseries. (a) Staked rows of seedlings for garden plot. (b) Three-year old garden plot of western larch. (c) Labeled stake marking the end of a garden plot row and identifying the specific seedlot/nursery combination. (Photos by Abbie Acuff, 2017)

Figure 6. Sample of Douglas-fir root system at end of one growing season in a garden plot. (Photo by Abbie Acuff, 2017)

The rows of seedlot by nursery combinations are permanently marked at both ends and seedlings are flagged. Height and stem diameter measurements are taken at planting and the end of each growing season. Enough seedlings are planted so destructive sampling may be done to evaluate seedling growth (figure 6). The information garnered from garden plots not only alerts foresters to potential problems in the field but creates a database on early growth and survival of planted seedlings. The PotlatchDeltic program has evolved, and now each of the three Idaho Districts has a fenced site for planting and evaluating seedlings.

Transects

The purpose of the transect program is to quantify planted seedling mortality and associated causes within the first month of planting. The three main categories of cause are site conditions, site preparation, and seedling attributes. Evaluation of cause (figure 7) enables PotlatchDeltic to improve silviculture activities and

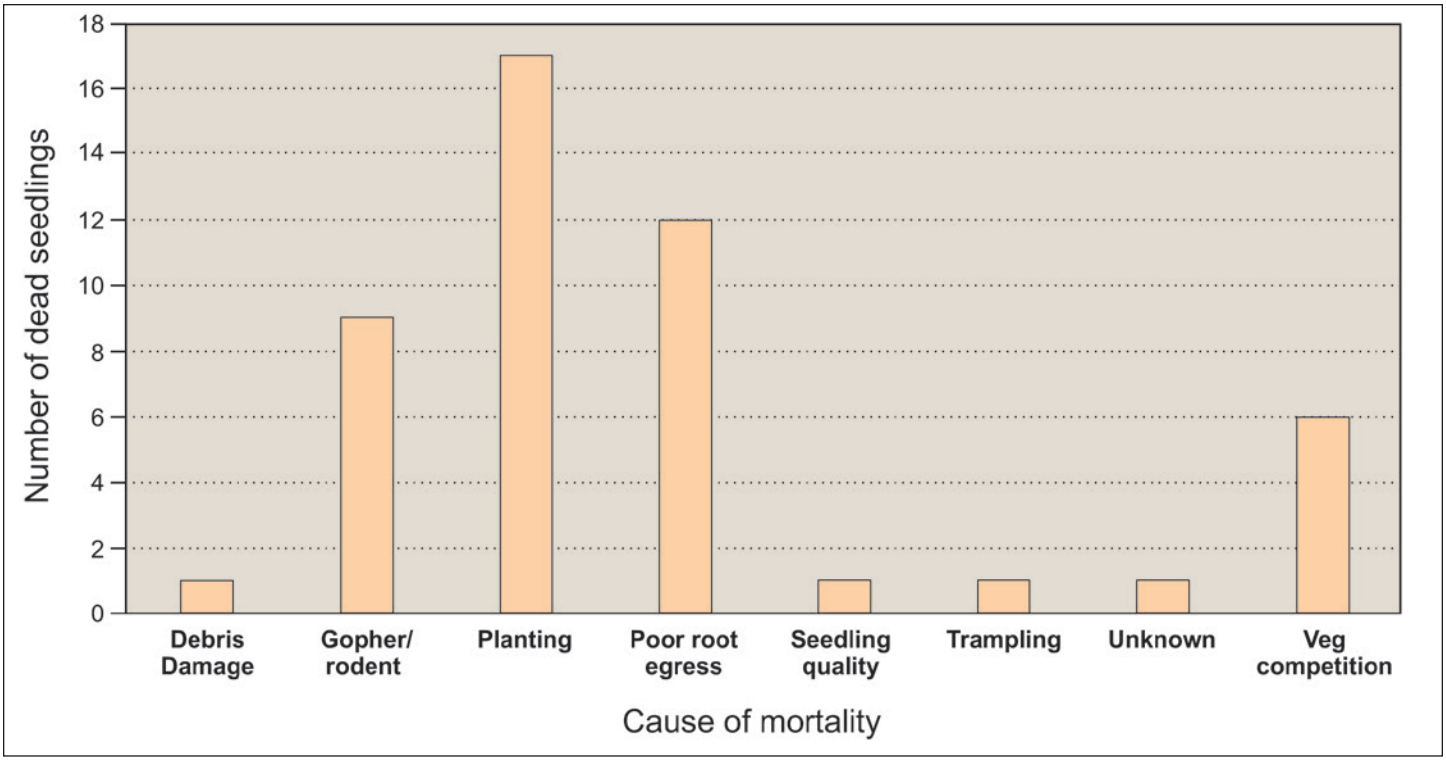

Figure 7. Example data showing causes of seedling mortality assessed using transects within 1 month of planting.

Table 3. Transect data collected as transect line is installed within 1 month after being planted.

Tree #	Species	Seedlot	Aspect (0-360)	Herbicide applied?	Vigor	Height (cm)	Caliper (mm)	Percent total vegetation cover	Dominant vegetation	Notes
1	DF	Mixed Z6, Z8	130	No	Average	27.0	4.7	<10	Grass	
2	DF	Mixed Z6, Z8	130	No	Average	30.0	4.1	10-19	Herbaceous	
3	DF	Mixed Z6, Z8	130	No	Average	29.0	4.5	10-19	Herbaceous	
4	DF	Mixed Z6, Z8	130	No	Average	20.0	4.0	10-19	Herbaceous	
5	DF	Mixed Z6, Z8	130	No	Average	22.0	3.0	10-19	Low shrub	
6	DF	Mixed Z6, Z8	130	No	Average	26.0	3.9	<10	Herbaceous	
7	DF	Mixed Z6, Z8	130	No	Average	19.0	3.2	20-29	Herbaceous	
8	DF	Mixed Z6, Z8	130	No	Average	28.0	4.0	10-19	High shrub	High shrub is natural regeneration
9	DF	Mixed Z6, Z8	130	No	Average	19.0	2.9	10-19	Grass	
10	DF	Mixed Z6, Z8	130	No	Average	32.0	4.8	<10	Low shrub	

fine-tune seedling specifications. Stands are semi-randomly selected to ensure major elevation bands, aspects, and districts are represented. Two transects per stand are permanently marked at each end and seedlings are flagged (figure 8). Each seedling's height, stem diameter, and health are recorded, as well as vegetation cover within 1 m2 (table 3). As the database increases with the addition of more transects, it will be used to confirm and drive future management decisions.

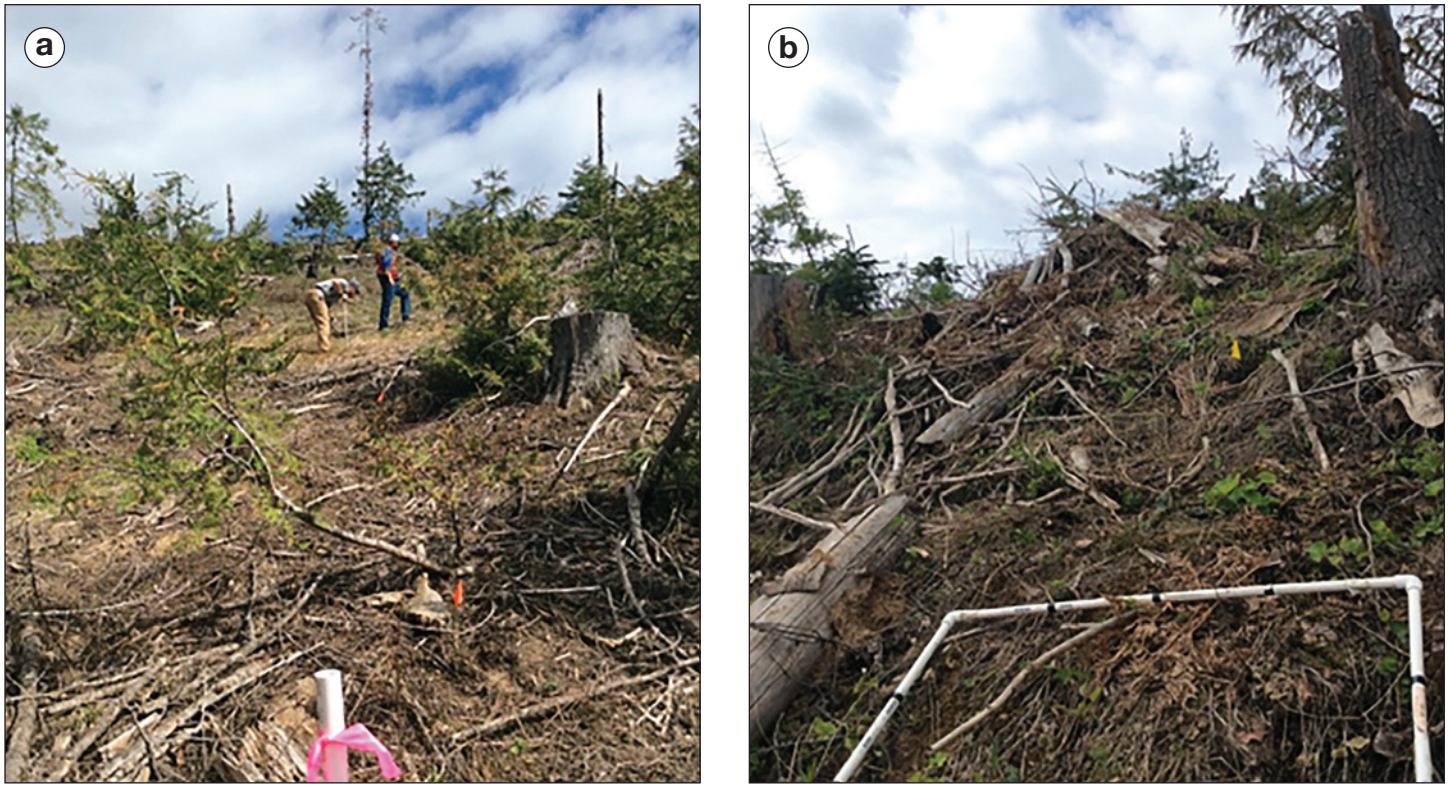

Figure 8. Transects can provide useful data in the early years after planting. (a) Data collecti on requires setting up transects, measuring seedlings, and (b) assessing vegetation cover. (Photos courtesy of Chance Brumley, Operations Manager, PotlatchDeltic, 2018)

Conclusions

Growing and planting seedlings is expensive (table 4). The best shot for minimizing costs is to have high survival the first time a unit is planted. To achieve this goal, care must be taken at each step to ensure the consumer is not wasting any resource, such as seed, seedlings, or site preparation. The consumer needs to be proactive and consistent with monitoring, otherwise any issues causing successes or failures will remain unknown. The consumer also needs to monitor every year to account for anomalies such as extreme weather. The monitoring systems outlined in this article are relatively inexpensive, easy to do, and provide a lot of valuable information to ensure a successful planting program the first time: one and done!

Table 4. Cost of planted seedlings shows how quickly costs increase with the planting program size. This table assumes 436 seedlings per acre (1,077 per ha), $350 per 1000 seedlings, and $100 for planting 1,000 seedlings.

Acres	Planted seedlings	Cost
500	218,000	$ 98,100
1,000	436,000	$ 196,200
2,500	1,090,000	$ 490,500
5,000	2,180,000	$ 981,000
10,000	4,360,000	$1,962,000
20,000	8,720,000	$3,924,000

Address correspondence to:

Abbie A. Acuff, PotlatchDeltic, 301 D Street, Suite A, Lewiston, ID 83501; email: abbie.acuff@potlatchdeltic.com; phone: 208–748–2027.