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Dear TPN Reader

Welcome to the Spring 2016 issue which includes eight articles with new information relating to nursery production and outplanting of trees and shrubs for reforestation, restoration, and conservation.

This issue contains three articles focused on seed germination. Morgan and Zimmerman (page 4) compared the effectiveness of five stratification treatments on seed germination of *Bursera simaruba* (a species native to South America and the Caribbean). In another article, Devine and Harrington (page 23) assessed seedfall and germination of coast Douglas-fir (*Pseudotsuga menziesii*) as influenced by canopy gap size with implications for natural regeneration in coastal forests. In another seed study, Sheridan et al. (page 33) describe a study to evaluate whether mulching and shade affected direct-seeded western redcedar (*Thuja plicata*).

The other articles in this issue cover a range of topics. South (page 11) gives an overview of weed management practices in bareroot nurseries in the Southern United States, including various methodologies and considerations. Oster and DeBell (page 42) provide step-by-step instructions for construction of a backpack-mounted pollen vacuum that they have used successfully in the Meridian Seed Orchard (Olympia, WA) and which may have potential for seed collection from native plants. Doggett et al. (page 48) examined black walnut allelopathy effects on planted loblolly pine (*Pinus taeda*) with recommendations for future plantation establishment. Haase et al. (page 52) studied chilling hours, daylength, storage, and seed source as they related to cold hardiness and other seedling quality parameters. Finally, Overton et al. (page 64) describes the current propagation protocol for whitebark pine (*Pinus albicaulus*) at the U.S. Department of Agriculture, Forest Service, Coeur d'Alene Nursery (Idaho).

I'm still determined to complete TPN's State-by-State series. Since 2011, 20 States have been profiled. It can be a challenge to find authors to take time from their busy schedules and write these articles, but I intend to eventually profile all 50 States and the U.S.-affiliated islands. If you would like to volunteer to write the paper for your State (or to nominate someone), please contact me.

Warm Regards,

Diane

He that plants trees loves others beside himself. ~ Thomas Fuller

***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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Germination Rates of *Bursera simaruba* Seeds Subjected to Various Scarification Treatments

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Abstract

Bursera simaruba (L.) Sarg. seed were subjected to five scarification treatments to determine their efficacy on subsequent germination. Seeds that were scarified with sandpaper had the highest mean germination, although it was not statistically different than the untreated control. Those treated with hot water had significantly lower germination than the control, suggesting that temperatures may have been too hot. These results indicate that mechanical scarification may improve germination of this species but that further research is needed to refine treatments.

Introduction

Bursera simaruba (L.) Sarg., known as turpentine tree or gumbo-limbo, is easily recognized by its reddish, papery bark and the smell of turpentine when its leaves are crushed or branches are cut (figure 1). The species epithet *simaruba* references the Taino name for the tree (Nellis 1994). The Tainos, also called Arawaks, were the people Columbus encountered on the Caribbean islands when he claimed the Americas for Spain in 1492.

Figure 1. *Bursera simaruba* tree at Buck Island National Monument off the coast of St. Croix, VI. Note the thin, coppery bark and the large crooked branches. (Photo by Michael Morgan, 2014)

Distribution and Characteristics

Bursera simaruba is native to northern South America and the Caribbean Basin (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964). The species is abundant in the U.S. Virgin Islands and Puerto Rico. It has also become naturalized in south Florida, but some discussion remains regarding whether *B. simaruba* is an introduced species to Florida (Navarrete-Tindall and Orellana-Nuñez 2002, Nelson 1994). *B. simaruba* is very tolerant of salt, wind, and drought, making it well adapted to the semiarid Virgin Islands environment. It is found close to the sea and on hilltops, and it is native to limestone-derived soils (Kirk 2009). Although found in primary forests (such as xeric ridgetops), it is more common in secondary forests (Navarrete-Tindall and Orellana-Nuñez 2002).

On a good site, *Bursera simaruba* trees can grow up to 100 ft (30 m) in height and 36 in (1 m) in diameter; however, heights of 30 to 45 ft (10 to 15 m) are more typical. Leaves are alternately pinnate and compound. The length of the whole leaf is 6 to 8 in (15 to 20 cm); the whole leaf consists of leaflets, 1.2 to 1.6 in (3 to 4 cm) long by 0.4 to 0.8 in (1 to 2 cm) wide, occurring in one to three pairs and a single one at the tip. The leaflets are dark green on the upper side and lighter below. Leaves tend to be bunched at the end of the branches, making for an open crown made conspicuous by large, crooked branches (Jones 1995). The bark is thin, reddish or coppery, and peels away, showing green bark below. The small, white flowers are borne in spikes on the branch ends. The species is dioecious; male and female flowers are borne on separate trees. Some flowers, however, are bisexual. The fruit are capsules resembling small, three-sided olives and are green when young but reddish when mature (figure 2). The fruit opens when ripe, revealing a three-sided seed

Figure 2. Closeup view of *Bursera simaruba* leaves, unopened purple seed capsules, and naked seed covered in red pulp in center of photograph. (Photo by Michael Morgan, 2014)

covered by a thin, red pulp (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964, Nelson 1994).

Phenology

The physiography of Maryland combines features from both The phenology of *Bursera simaruba* is interesting. It is briefly deciduous, losing its leaves during the dry season months of January and February. Then, the tree flowers before or at the same time as new leaves appear around March (Jones 1995). Pollination of *Bursera* species is ambophilous; that is, it is performed by both insects and wind (Bullock 1994). Wind pollination is favored in the absence of rain, low relative humidity, and good air movement—common conditions in tropical dry forests (Bullock 1994). These weather conditions are the exact conditions on the island of St. Croix, VI, when *B. simaruba* flowers (M. Morgan, personal observation). Regarding insect pollination of this species, Nellis (1994) stated, “While flowers only last one day, they begin nectar production before dawn leading to intense honey bee activity at day break.”

After flowering is complete, new fruits form and grow to almost full size within days of pollination, although the seeds take nearly a full year to ripen (Kirk 2009). Fruit become ripe in ones and twos, and they do not ripen off the tree. This uneven ripening makes collecting enough seeds for experimental purposes or for nursery production a challenge. It can take several weeks or months of repeat visits to fruiting trees to collect sufficient seed to conduct an experiment or to produce several dozen seedlings in a tree nursery. Although production from seed can be a challenge because of difficulties in obtaining sufficient seed and variable germination rates, *B. simaruba* is easily propagated by both large and small cuttings.

Products and Uses

Many products are derived from *Bursera simaruba*, especially from its resin. The resin, or the “turpentine,” from the wood is collected by slashing the tree’s trunk and allowing the resin to drip out. The resin is known by various names: cachibou, chibou, copal, gomart, or gum elemi (Nellis 1994). This last name is likely the source from which the common English name gumbo-limbo is derived. The resin is used for incense, insect repellent, varnish, various home remedies, and glue. The Mayans have been using the resin for ceremonies of the tree for centuries, both before and after the arrival of the Spanish to the Americas. Another closely related species, *B. graveolens* (Kunth) Triana and Planch, from the dry coasts of Ecuador and Peru, is used in a similar manner, but, instead of burning blocks of pure resin, pieces of resin-impregnated heartwood are burned. Its Spanish name is Palo Santo, which translates to "holy tree" or "holy stick" (Morgan and Jose 2013).

Perhaps the most interesting use of *Bursera simaruba* resin is that of birdlime. The use of birdlime is an ancient, worldwide (and usually illegal) practice of smearing a sticky substance on a tree branch to capture birds. Many sticky substances besides *B. simaruba* resin are used as birdlime. Birds land on the branch and are trapped. The hunter then collects the birds.

Bursera simaruba wood is soft and not durable, unless it is treated with preservatives. It has been used for interior carpentry, crates, match sticks, fire wood, and charcoal (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964, Navarette-Tindall and Orellana Nuñez 2002). As plywood and veneer, it is sold under the commercial name of Mexican White Birch (Longwood 1961). More unusual uses for the wood are the construction of coffins, canoes, carousel horses, and voodoo drums (Nellis 1994).

Because the species is easily propagated via cuttings, both large and small, the tree is also used as living fence posts. One can plant large branches upright in the ground as fence posts and string wire between the posts. After a short wait and some rains, the posts resprout, creating a living fence (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964, Navarette-Tindall and Orellana Nuñez 2002). Often times on the island of St. Croix, where the authors live, a straight row of evenly spaced trees grows in the woods, suggesting a former field or property boundary created from cuttings. These fence line trees provided seed for the new forest that developed after the field was abandoned.

A more recent use of *Bursera simaruba* is that of landscape plantings because of its attractive coppery bark.

Seed Dispersal and Scarification

Throughout the wide geographical range of *Bursera simaruba*, various bird species disperse the seeds. The birds like to eat the pink, lipid-rich flesh that surrounds the seeds. They swallow the fruit, and seed within it, whole. As the aril's flesh is digested, the seeds pass through the bird's stomach and intestines. The seed ideally is deposited far from the parent tree to generate new seedlings (Gibney 2004, Jones 1995, Kirk 2009, Little and Wadsworth 1964). The number of bird species inhabiting the island of St. Croix is rather impoverished compared with other Caribbean islands and the North and South American mainlands. The primary author of this article, who is an avid birdwatcher, suspects that *B. simaruba* seeds are dispersed by pearly-eyed thrasher (*Margarops fuscatus*) and the gray kingbird (*Tyrannus dominicensis*). Both species feed primarily on insects but supplement their diets with fruit (Bond 1992, Evans 1990). These two bird species are so common on St Croix that one would not even bother to remark on their presence. They are seen repeatedly throughout the day, around houses, in open areas, and in the woods.

Seeds with physical dormancy, such as those of *Bursera simaruba*, have hard seedcoats that need to be cracked to allow for the entrance of water and air so the seed embryo can imbibe water and start metabolizing. Many of these seeds are animal dispersed. The seedcoat protects the seed embryo while the overlying fruit is being consumed, allowing for later dispersal. Passage through an animal's digestive tract often increases seed germination via exposure to stomach acid and the grinding actions of teeth or, in the case of birds, gizzards (Smith et al. 2002). This process serves to scarify the seedcoat, permitting the entrance of air and water to the seed embryo so that germination can begin.

Various scarification techniques can be used for seed propagation. One technique is to use sandpaper on a seed until it loses its shine, a result of abrading away the oily lipids that seal the seed to water. Another is to crack the seed with a hammer (Smith et al. 2002) or nick the seedcoat with a knife. Acid baths and hot-water soaks have been used to imitate stomach acids (Smith et al. 2002) and allow for scarification of many seeds at once. The problem with this method is that the seed embryo can be damaged or killed by soaking too long (i.e., be cooked). It is also potentially dangerous because hot water and acid can spill and burn nursery staff. A safer method is to soak seeds in cool water for several hours or even days to leach out chemicals that inhibit germination. With this method, the water must be changed daily to get rid of leachate or pathogens (Smith et al. 2002). If seeds soak too long, however, they may rot. For example, Cascol (*Caesalpinia paipai* Ruiz Lopez and Pavon), is a tropical dry forest tree with a hard-coated seed. Because the cascol seed is eaten and dispersed by ruminants, one would think that soaking overnight would be an appropriate pretreatment. Soaking for more than 4 hours, however, leads to rotting seeds (Morgan, n.d.). Less conventional pretreatments include feeding seeds to livestock, or even birds, and collecting the defecated or regurgitated seeds, setting fire to the seeds to burn off a thick pericarp, enabling ants to eat the pericarp, and even treating the seeds with fungal spores (CATIE 2000).

Scarification treatments may improve seed germination. In germination trials with the closely related *Bursera graveolens*, which also has bird-dispersed seeds, Morgan and Jose (2013) found that different scarification treatments (mechanical, chemical, and heat) increased seed germination compared with the untreated control. In that study, germination averaged 21 percent for untreated seeds and increased to 53 percent when seeds were immersed in water heated to 158 ºF (70 ºC) and allowed to soak for 24 hours as the water cooled. Using sandpaper to weaken the seedcoat before planting increased germination to 34 percent. The objective of this study was to determine if seed germination of *Bursera simaruba* could also be improved by scarification treatments before planting.

Materials and Methods

Seeds were collected from two *Bursera simaruba* trees growing approximately 10 mi (15 km) apart from each other on the island of St. Croix. Because of the uneven seed ripening of this species, seeds were collected weekly for 3 months (October, November, and December, 2014) to accumulate enough seeds for the experiment. Usually 12 to 20 seeds were collected on each visit. Pulp was removed from the seeds by washing the fruits in a 10-percent bleach and water solution. Then the seeds were allowed to air-dry before being stored in a small plastic box placed in a cool, dry room.

The experiment was performed in a glasshouse at the University of the Virgin Islands Agricultural Experiment Station on the island of St. Croix. Christiansted, the nearest town, is located at long. 64°43' W. and lat. 17°45'0l" N. (NOAA 2015). The glasshouse is screened on both the east and west sides to allow for air circulation. Winds predominantly blow from east to west in the Caribbean. Air temperatures range from 78 °F (24 °C) to 104 °F (40 °C) (Rhuanito Ferrarrizi, personal communication). Day length varies from 11 hours in the winter month to 14 hours in the summer months. At the time of germination, however, day length was 12 hours.

A total of 180 seeds were divided into five treatment groups of 36 seeds; each group was assigned a scarification treatment. Each treatment had three replicates of 12 seeds each. The five treatments were (1) soaking in concentrated sulfuric acid for 4 minutes, followed by a water rinse; (2) soaking in the growth hormone gibberellic acid for 4 hours; (3) mechanically scarifying the seedcoat with sandpaper until one side of the seed had been roughened to allow for the entrance of air and water to the seed embryo; (4) immersing in hot water heated to 158 °F (70 °C), followed by a 24-hour soak as the water cooled; and (5) maintaining an untreated control.

The seeds were sown in early March 2015 in 6-by-8 in (15-by-20 cm) unibody plastic trays filled with a 50:50 peat moss and perlite mix (figure 3). Each tray was randomly assigned a treatment, and a subset of seeds that received the assigned treatment was planted in that tray. The seeds were watered once daily. The number of germinated seeds in each treatment replication was recorded for the whole month of March, which was until 4 days after germination had ceased.

We had the opportunity to collect more seeds in September 2015 to conduct a second trial. By raking up leaf litter, we were able to uncover 150 seeds on the ground that had fallen from a *Bursera simaruba* tree. The seeds had either simply fallen off the tree and the aril rotted away, or birds had swallowed the fruit, digested the aril, and deposited the seeds in their droppings. An unusual drought in 2015 prevented the seeds from germinating. The seeds were estimated to have an age of 6 or 7 months.

Back in the laboratory, the seeds were washed in a 10-percent bleach solution and allowed to air-dry. Once dried, the seeds were divided into three treatment groups of 50 seeds. These seeds were not subjected to any pretreatment before sowing. On September 8, each group of 50 seeds was planted in a germination tray filled with 50:50 peat and perlite. The seeds were watered daily and monitored for germination as described previously in the March trial.

The number of germinated seeds by treatment was plotted over time. Analysis of variance was performed on the data

Figure 3. Germination of *Bursera simaruba* seeds treated with various scarification treatments was monitored for 30 days after sowing. (Photo by Michael Morgan, 2014)

using JMP® software, a menu-driven version of SAS (SAS Institute Inc., Cary, NC), to determine if a statistically significant difference occurred in germination among treatments and days since sowing. To meet assumptions of the model, the data were normalized before analysis by arcsine transformation. First, the raw data were converted into proportions. Then, the square roots of the proportions were calculated. Finally, arcsines were taken of the square roots (Chen and Maun 1999). A Dunnet's Post Hoc Test was used to compare the control with the different scarification treatments.

Results and Discussion

In the first trial, germination commenced 8 days after sowing and ceased 15 days later. Of the first three seeds to germinate, two had been subjected to the sandpaper scarification treatment and one was from the untreated control treatment. Seeds treated with the sulfuric acid and gibberellic acid treatments began germination 9 and 10 days after planting, respectively. Of the 36 seeds treated with the hot-water treatment, only 2 germinated during the study, 13 and 19 days after sowing. Germination ceased after 18 days for seeds treated with sandpaper scarification, 19 days for seeds soaked in gibberellic acid, 20 days for seeds subjected to the sulfuric acid treatment, and 23 days for untreated control seeds (figure 4). Overall, seeds scarified with sandpaper had the highest percentage of germinated seeds, although this did not differ significantly from those in the control treatment (figure 5). Seeds treated with hot water had significantly lower germination than those in the control treatment (figure 5). Seeds treated with gibberellic acid or sulfuric acid treatments had similar germination rates and did not differ from those in the control treatment. A statistically significant difference between timing of germination by treatment was noted 12 days after sowing (p = 0.04), indicating that seeds in all treatments, with the exception of the hot-water treatment, had begun to germinate. Average seed germination ranged from 6 to 44 percent across treatments. Note, however, that much variation occurred within treatment groups. For example, the three trays of 12 seeds assigned to the sandpaper treatment had 25, 33, and 75 percent germination (figure 6).

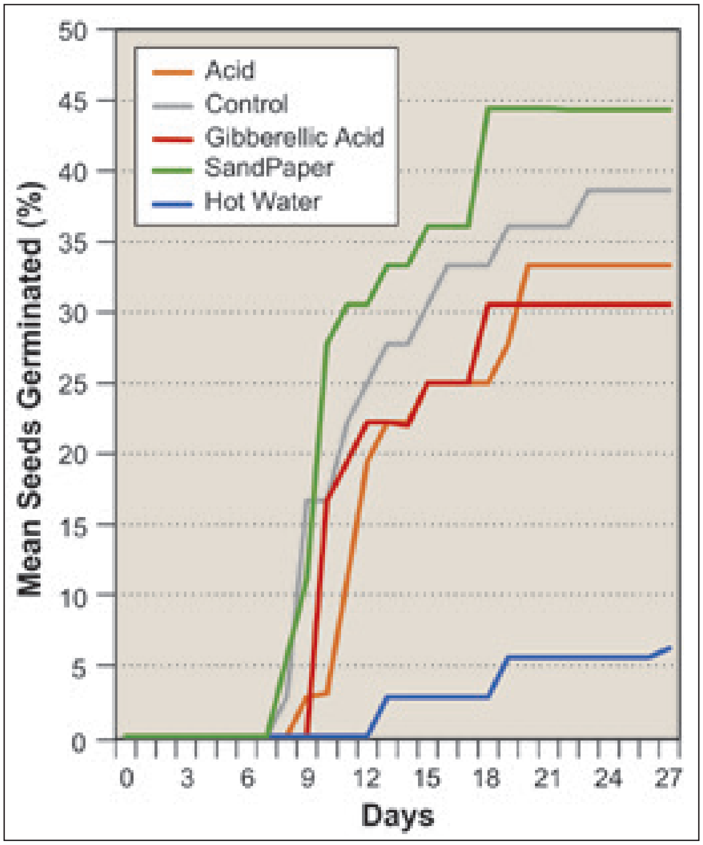

Figure 4. Average germination of *Bursera simaruba* seeds for each scarification treatment over time

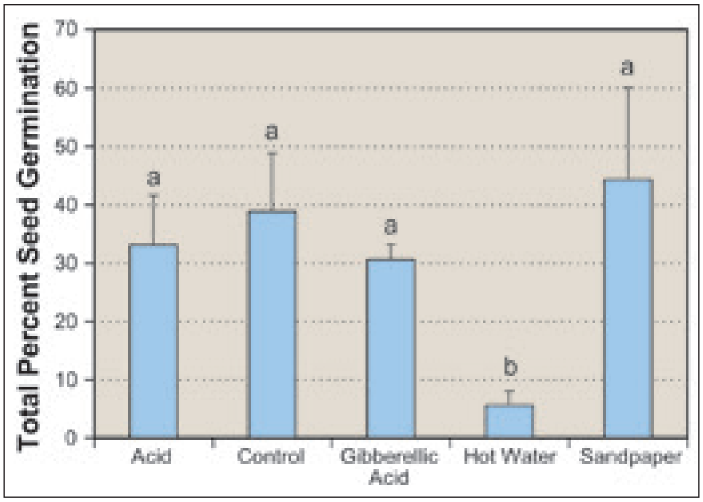

Figure 5. Total seeds germinated in each scarification treatment at the end of the study (30 days).

We suspect that the seeds in the hot-water treatment may have been exposed to too much heat, thus reducing the viability of the seeds. This result was surprising because a previous study with *Bursera graveolens* found that seeds immersed briefly in hot water (158 °F [70 °C]) had improved germination compared with untreated seeds (Morgan and Jose 2013), while those immersed in water heated to 219 °F (90 °C) were killed.

In the second trial, with seeds collected from the ground and sown untreated, germination began 5 days after sowing and ceased after 20 days, with a mean of 50 percent total germination. According to Navarrete-Tindall and Orellana-Nuñez (2002), 80 to 100 percent of *Bursera simaruba* seeds should germinate without any pregerminative treatment.

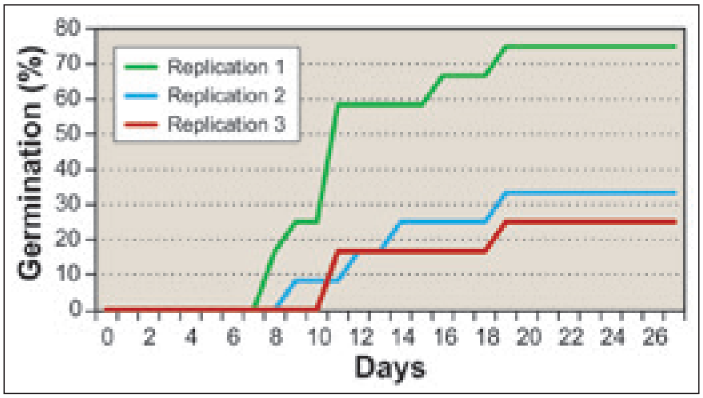

Figure 6. Germination was quite variable within treatments. This example shows germination among replications of sandpaper-scarified seed varying from 25 to 75 percent.

Our results using both fresh and older fresh seeds contradict this finding, with untreated seeds having 39 and 50 percent germination, respectively.

Murray and Russell (1994) conducted a study to determine if fruits of another bird-dispersed tree species (*Witheringia* spp.) had a laxative effect on the black-faced solitaire (*Myadestes melanops* Salvin), a type of thrush, while increasing seed germination. They found that the longer the seed was in the bird's digestive tract, the less likely it was to germinate. Of seeds that passed through the bird's stomach, however, 62 percent germinated compared with 51 percent germination for mature seeds just picked off the tree.

Although birds consume *Bursera* spp. fruit, it appears that this action serves primarily as a means of dispersal. In the trial with *B. graveolens* (Morgan and Jose 2013), germination of control seeds averaged 21 percent across four trials and did not differ greatly from the scarification treatments. Ortiz-Pulido and Rico-Gray (2006) similarly found that 17 percent of *B. fagaroides* (Kunth) Engl. seeds germinated if eaten and defecated by gray catbirds (*Dumetella carolinensis*) but no seeds germinated if eaten and defecated by white-eyed vireo (*Vireo griseus*), both of which were lower than the rate observed for seeds without any treatment (20 percent).

Conclusions

Sandpaper scarification treatment increased *Bursera simaruba* seed germination relative to the untreated control treatment. This result was not statistically significant, however, and notable variation was observed among replications. Because sanding each seed is labor intensive, the best method to propagate seedlings from seed is to sow the seeds without treatment after the seedcoat has been sterilized. Given these results and those of others, it appears that consumption of the aril and deposition of the seeds by birds is more important as a dispersal mechanism than as a scarification effect. Vegetative propagation is also an option for this species. At the University of the Virgin Islands, small cuttings of *B. simaruba*, 0.5 in (1 cm) in diameter and 12 in (30 cm) long, sprout new leaves and roots when placed in a container full of planting substrate and adequately watered (M. Morgan, personal observation).

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Weed Management in Southern Bareroot Hardwood Nurseries

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Abstract

Nursery managers in the Southern United States rely on chemical and nonchemical methods of weed control. Chemical treatments include fumigation with methyl bromide and chloropicrin in combination with selective herbicides. At nurseries where methyl bromide is not used, managers rely on herbicides and sanitation practices. Although several herbicides are registered for use on hardwoods, some formulations can injure seedlings if improperly applied to seedbeds. Most grasses can be effectively controlled with selective herbicides and many small-seeded broadleaf weeds can be suppressed when preemergence herbicides are applied before germination of weeds. Several preemergence herbicides may be legally applied either at time of sowing or after seedlings are established. Several nursery managers have fabricated shielded herbicide sprayers to apply herbicides between drills to certain difficult-to-control weeds. The most effective integrated weed management programs include rigorous sanitation practices and judicious use of efficacious herbicides.

Introduction

The hardwood nursery manager's primary objective is to produce morphologically improved stock as economically as possible. Morphologically improved hardwood seedlings have a minimum root-collar diameter of 10 mm, are grown at low seedbed densities, have a higher probability of survival, have a higher root-weight ratio (root dry weight/ seedling dry weight) often due to top-pruning, and have a greater root growth potential than smaller stock. The presence of weeds can be a major obstacle to this goal because they compete with seedlings for light, water, and nutrients. In addition, handweeders often pull up seedlings while weeding, reducing revenue from seedling sales. In some cases, weed populations will stunt seedlings and will cause large variations in seedling size at lifting.

To maintain a relatively weed-free nursery, most hardwood nursery managers implement a comprehensive, year-round weed control program. In the past, some seedbeds required more than 3,800 hours of handweeding per hectare (Abrahamson 1987). Today, many managers use an integrated weed management (IWM) program (Walker and Buchanan 1982), which includes sanitation, soil fumigation, and herbicide applications to keep weed populations low and minimize handweeding. As a result, several hardwood nurseries now require less than 60 hours of handweeding per hectare.

Weed Identification

To achieve good weed control, weed species must be accurately identified, especially when troublesome species are present. For example, some herbicides will suppress yellow nutsedge (*Cyperus esculentus* L.) but have little effect on purple nutsedge (*Cyperus rotundus* L.), even though the two species appear similar. Several online sites are available for identifying common weeds, and extension weed specialists should be able to identify rare species. Some of the more common weeds in southern nurseries, with their scientific names, are listed in table 1.

Sanitation

Preventing weeds from going to seed in the nursery is an important sanitation practice because weed populations in future years greatly depend on the number of seed produced during the current season. If one yellow nutsedge plant is allowed to mature, it can produce more than 2,400 seeds. A mature purslane plant (*Portulaca oleracea* L.) can produce more than 52,000 seeds and a single redroot pigweed (*Amaranthus retroflexus* L.) can produce 117,000 seeds or more (Stevens 1932). The importance of preventing a single weed from maturing and producing seed in the nursery cannot be overemphasized. A severe infestation of nutsedge can quickly result from the failure to control even a single plant. For example, one tuber of purple nutsedge produced 1,168 plants and 2,324 tubers after 6 months (Ishii et al. 1971). Weeds must be prevented from going to seed not only in the seedbeds, but also on the riserlines, fencerows, cover-crop areas, and fallow areas (Wichman 1982).

Table 1. Common names (Weed Science Society of America) for selected weed species in southern hardwood nurseries.

WSSA common name	Scientific name
Grasses	
Bermudagrass	*Cynodon dactylon* (L.) Pers.
Crowfootgrass	*Daclyloctenium aegyptium* (L.) Willd.
Hairy crabgrass	*Digitaria sanguinalis* (L.) Scop.
Sourgrass	*Digitaria insularis* (L.) Mez ex Ekman
Barnyardgrass	*Echinochloa crus-galli* (L.) P. Beauv.
Goosegrass	*Eleusine indica* (L.) Gaertn.
Sedges	
Annual sedge	*Cyperus compressus* L.
Yellow nutsedge	*Cyperus esculentus* L.
Purple nutsedge	*Cyperus rotundus* L.
Broadleaves	
Prostrate pigweed	*Amaranthus blitoides* S. Watson
Redroot pigweed	*Amaranthus retroflexus* L.
Spiny amaranth	*Amaranthus spinosus* L.
Sicklepod	*Senna obtusifolia* (L.) H.S. Irwin & Barneby
Eclipta	*Eclipta alba* (L.) Hassk.
Dogfennel	*Eupatorium capillifolium* (Lam.) Small
Spotted spurge	*Chamaesyce maculata* (L.) Small
Tall morningglory	*Ipomoea purpurea* (L.) Roth
Carpetweed	*Mollugo verticillata* L.
White clover	*Trifolium repens* L.

Irrigation Water

Irrigation water can be a major source of introduced weeds when the water is from a lake, pond, or river. The use of screens at the intake pipe can help filter out large-seeded weeds. Although the screens may require frequent cleaning, it is easier to remove the weed seeds from the screens than to remove weeds from seedbeds. When irrigating from ponds, it is best to keep the pond edges free of weeds. When installing a new nursery, a deep well is preferred over surface water sources.

Cover-Crop Seeds

Sowing weed seeds along with cover-crop seeds can be minimized by always using certified seed. At one nursery, the use of cheap, uncertified seed resulted in a large infestation of morning glory (*Ipomoea* spp.). Regulations require certified seed to be free of primary noxious weeds and to contain only small amounts of common weeds. The percentage of common weeds must be shown on the certification tag. It is best to buy seeds with the lowest percentage of common weeds.

Machinery

Weed seeds, rhizomes, and tubers are easily introduced by machinery. Frequent washings reduce the amount of weeds introduced by soil carried on tillage equipment, tractors, and vehicle tires. Weed seeds are often spread by combines during the harvest of cover crops. For this reason, it is better to leave cover crops unharvested unless combines are carefully cleaned before and after use.

Some weeds spread slowly by vegetative means alone. For example, nutsedge would spread less than 3 m per year without help from nursery workers and their cultivation equipment (Klingman and Ashton 1975). For this reason, special effort should be made to avoid spreading nutsedge. Infested seedbeds can be mapped in the summer to help identify areas in which to avoid soil movement (thus spreading nuts) in the winter after lifting. Nutsedge-free areas should be lifted first to avoid the spreading of tubers to noninfested fields. Time taken to prevent mechanical dissemination of nutsedge tubers will be repaid severalfold in the ease of eliminating nutsedge from a nursery.

Wind

Wind will constantly introduce weed seeds, but the impact may be reduced by planting windbreaks between the nursery and adjacent weed sources. Windbreaks will also help protect the nursery from high winds that blow mulch off beds, blow plastic off fumigated soil, and cause excessive drying of the beds.

Mulches

In the past, the use of straw mulches after sowing was a major source of introduced weeds (Bland 1974, Mullin 1965, South 1976). For example, at some nurseries, pine straw mulch increased time spent on handweeding by 260 to 500 hours per hectare (Bland 1974, South 1976). Due to the expense and introduction of weed seed, the use of straw mulches has declined over time. Several managers were using pine straw in 1980 (Boyer and South 1984), but today few use it due to the expense. New bark or sawdust mulches are relatively weed free (Stringfield 2005), but old, stockpiled supplies are often contaminated with weed seed. Several hardwood managers apply weed-free soil stabilizers after sowing. Most of these managers will forgo using mulch and, therefore, will apply additional irrigation.

Organic Amendments

In some cases, use of organic amendments will introduce weed seeds. In one nursery, rush (*Juncus* spp.) was introduced when an organic amendment was donated to the nursery. Yard litter and leaves collected by municipalities can contain many types of weed seeds. The value of these "free" amendments will depend on the increase in cost of subsequent weed control. Composting can help reduce the viability of many weed seeds, but some will likely remain viable.

Handweeding

Frequent weeding can be an important IWM tool. Handweeding is best conducted when the soil is moist and weeds are small (figure 1). Weeding small plants has two advantages: the weeds are often removed before they go to seed, and the weeds are easier to remove when the roots are small. In many cases, the total weeding cost is less than if weeding is delayed until the weeds are large and hard to remove.

The use of seasonal labor varies with each nursery. When using contract labor, the cost of 100 hours of handweeding might exceed $4,900 per hectare. Therefore, the use of herbicides depends, in part, on the cost of handweeding. At some nurseries, herbicides are used and minimal handweeding is required, but other managers rely on handweeding and, except for soil fumigants, do not apply herbicides to hardwood seedbeds. With an effective IWM program, hardwood seedbeds may require less than 60 hours of handweeding per hectare (South 2009).

Figure 1. When nursery managers adopt an effective integrated weed management program, the amount of handweeding can be kept to a minimum. Handweeding is most effective when weeds are small, before they go to seed. Weeding takes less time when the soil is moist and the weed has a small root system. (Photo by David South, 2015)

Mechanical Cultivation

Mechanical cultivation for weed control between seedling rows is feasible when the spacing between rows is 30 cm or wider (Barham 1980, Stanley 1970). Several types of seedbed and alleyway cultivators are available (Lowman et al. 1992). For example, a "brush-hoe" can be effective in reducing weeds in hardwood seedbeds (South 1988), although it has some drawbacks. To obtain a specified level of weed control requires a precise adjustment to ensure a proper working depth (Weber 1994). Weeds within the row remain uninjured. Any small error in alignment can damage hardwood seedling roots or shoots. In 2006, only 2 hardwood managers (out of 26) were using mechanical weed control between seedling rows (South 2009).

Living Mulch

The "living mulch" concept was used by the Virginia Department of Forestry during the 1980s. Rye (*Secale cereale* L.) seed were drilled into the sections immediately before sowing hardwoods in the fall. The "living mulch" protected the fall-sown seedbeds from injury by wind, rain, and frost. This system was also effective for fall-sown hardwoods in Illinois and Indiana (Stauder 1994, Wichman 1994). Nursery managers in Georgia and Tennessee currently sow wheat (*Triticum aestivum* L.), rye, or oats (*Avena sativa* L.) on prepared beds before fall sowing acorns (Ensminger 2002). The living mulch is then sprayed with an herbicide in February before emergence of oak seedlings. This system provides several advantages, including a retardation of weed growth.

Fall Sowing

Fall-sown hardwoods, such as red oaks (*Quercus* spp.) and black walnut (*Juglans nigra* L.), typically have fewer weeds the following year than spring-sown crops. This reduction in weeds is due to application of herbicides sooner in the spring and the fact that fall-sown crops typically achieve full canopy closure and shade out weeds sooner than spring-sown crops (figure 2).

Soil Fumigation

At many nurseries, effective soil fumigation with methyl bromide has been a cornerstone of a successful IWM plan. Several nursery managers contend that soil fumigation is more important when growing hardwoods because, when compared with conifers, fewer effective, registered herbicides exist (Murray 2009). It is relatively easy to justify soil fumigation, because it typically costs less than 6 percent of the

Figure 2. Weed control is typically easier when hardwoods are sown in the fall or winter, because the canopy closes sooner in the spring and the resulting shade reduces growth of various weed species. (Photo by David South, 2010)

wholesale value of the hardwood crop. For this reason, most managers in the South fumigate the soil before each hardwood seedling crop. Although dazomet is used in northern hardwood nurseries (Schroeder and Alspach 1995, Storandt 2002), hardwood managers in the South have traditionally relied on a combination of methyl bromide and chloropicrin to reduce weed, nematode, and fungi populations.

In the future, methyl bromide will continue to be produced by oceans, fires, and certain plants and fungi. It is possible, however, that production in the United States will decline due to regulations (Enebak et al. 2013) which might drive up production costs. If this occurs, some managers will likely switch to alternative fumigants, such as chloropicrin and dazomet, that have relatively low efficacy on weeds. Although dazomet can control certain soilborne pests, it is not effective in controlling nutsedge (Carey 1995, Carey and South 1999, Fraedrich and Dwinell 2003). If the use of effective soil fumigants declines, nursery managers will need to increase herbicide use to control weeds in fallow fields or cover crops.

Herbicide Use

The Weed Science Society of America (WSSA) sorts herbicides into 27 groups according to their chemical structure and activity. About one-third of these groups are used operationally in bareroot hardwood nurseries (table 2). Herbicides in the cyclohexanedione family (WSSA group 1) and dinitroaniline family (WSSA group 3) are commonly used in hardwood seedbeds.

Herbicides can be grouped into selective (usually not harmful to hardwood seedlings) or nonselective (can be harmful to hardwood seedlings if spray contacts bark and foliage). Glyphosate is typically a nonselective herbicide (kills both weeds and hardwoods) and sethoxydim is a selective herbicide (kills only grasses) (South and Gjerstad 1982). It is important to know the specific crop/weed system involved. For example, the herbicide clopyralid is a selective herbicide for black walnut, but it is nonselective when applied to black locust (*Robinia pseudoacacia* L.).

The terms "preemergence" and "postemergence" are used to describe when the herbicide is applied. For example, preemergence herbicides such as napropamide kill germinating weeds before they emerge through the soil surface. Some preemergence herbicides can be applied after emergence of the hardwood crop but before the emergence of the weed. Postemergence herbicides, on the other hand, are applied after the weeds emerge. When discussing herbicides, it is important to clarify if the application is to be made after the crop emerges and before the weeds emerge (e.g., pendimethalin, preemergence herbicide) or after weeds emerge but before the hardwoods emerge (e.g., glyphosate, postemergence herbicide).

Herbicide Applications in Cover Crops

The number of mature weeds in this year’s cover crop will determine the amount of weed seeds present in next year’s seedbeds. Some cover crops grow quickly and shade out the soil, thus reducing germination and growth of weeds. These cover crops are preferred over those that are sown at low densities and allow light to reach the soil. In the South, most herbicides used in cover crops will have no effect on seedling growth the following year. This is especially true when the herbicide is applied before July 1. It is best to check with nursery experts, however, to ensure that carryover from one season to the next will not be a problem. Some herbicide labels include information about the number of months required before sowing sensitive crops.

Cover-crop rotation provides an excellent opportunity to control weeds that are resistant to herbicides used in seedbeds. For example, if only diphenyl ether herbicides (WSSA group 14) were continually used on an area, resistant weed species such as prostrate spurge (*Euphorbia maculata* L.) could rapidly increase. By using an herbicide from a different herbicide family in the cover-crop area, however, the spread of troublesome weeds could be checked. Recommendations for using herbicides in cover crops vary, depending on the region

Table 2. Common names (WSSA) and trade names of selected herbicides used in southern hardwood nurseries.

Common name	Product name	Comment	WSSA group	REI* hours
		Soil fumigant		
Chloropicrin	Various	Good nutsedge control	-	>120
Dazomet	Basamid	Poor nutsedge control	27	>120
Methyl bromide	Various	Excellent nutsedge control	-	>120
		After sowing for oak, walnut, hickory		
Oxyfluorfen	Galigan, Goal, Goaltender	Field grown	14	24
		Very selective grass herbicides		
Clethodim	Clethodim, Select, Shadow	Grass control only	1	24
Fluazifop-p-butyl	Fusilade	Grass control only	1	12
Sethoxydim	Segment, Sethoxydim	Grass control only	1	12
		Herbicides with some selectivity when applied over established hardwoods		
DCPA	Dacthal	Found in groundwater	3	12
Dithiopyr	Dimension	Established plants only	3	12
Oryzalin	Surflan	May cause galls	3	24
Pendimethalin	Pendulum (Aquacap)	May cause galls	3	24
Prodiamine	Barricade	May cause galls	3	12
Trifluralin	Trifluralin HF	Certain labels only	3	12
Clopyralid	Lontrel	Will injure legumes	4	12
Oxyfluorfen	Goaltender	Field grown	14	24
S-metolachlor	Pennant	Active on sedge	15	24
Napropamide	Devrinol	Some grass control	15	12
		Granular herbicides—can be applied over transplanted stock		
Flumioxazin	Broadstar	Apply to dry leaves Do not apply to bedding plants	14	12
Oxadiazon	Ronstar	Apply to dry leaves	14	12
Oxyfluorfen + Pendimethalin	OH2	Apply to dry leaves	14 + 3	24
Dimethanamid + Pendimethalin	Freehand	May cause galls	15 + 3	24
		Granular herbicides—cannot be applied to seedbeds due to label restrictions		
Dichlobenil	Casoron	4 weeks after transplanting	20	12
Pronamide	Kerb	Not for use on 1-0 stock	3	24
Isoxaben	Gallery	Assume all risks	21	12
Simazine	Princep	Oak transplants	5	12
		Nonselective herbicides—applications must be directed away from seedlings		
Glyphosate	Roundup	Use shielded applicator	9	4
Pelargonic acid	Scythe	Use shielded applicator	27	12
Sulfosulfuron	Certainty	Avoid contact with leaves	2	12

WSSA = Weed Science Society of America.
*REI: Restricted-entry intervals for agricultural uses. Check the AGRICULTURAL USE REQUIREMENTS section of the label for required REI.

Figure 3. An effective way to control nutsedge on fallow ground is to treat emerged plants with glyphosate. Some managers treat nutsedge two or three times from June to September to reduce the population of tubers in the soil. (Photo by David South, 2012)

Figure 4. Controlling weeds adjacent to seedbeds is an important part of an integrated weed management program. Some managers prefer to apply preemergence herbicides to irrigation lines at time of sowing hardwoods, and others wait to treat emerged weeds with postemergence herbicides that have no soil activity. (Photo by Christine Makuck, USDA Forest Service, 2001)

and weed species to be controlled. Specific recommendations on herbicides and rates used can be obtained from the local extension service. Some genetically modified cover crops have a glyphosate-resistant gene that some managers use as part of an IWM program to reduce nutsedge in cover crops.

Herbicide Applications on Fallow Land

Weed control with herbicides is much easier on fallow ground (figure 3) than it is on hardwood seedling beds, because (1) a greater number of herbicides may be applied to fallow ground, (2) injury from drift is less likely, (3) multiple applications can be made, (4) timing of the application is not restricted to stage of hardwood growth, and (5) it is easier to see the weeds. For troublesome weeds like nutsedge, use of multiple applications of glyphosate on fallow ground is the preferred method to reduce the number of tubers in the soil (Fraedrich et al. 2003). At some nurseries, more glyphosate is used in fallow fields than is used in bareroot seedbeds (Juntunen 2001).

Herbicide Applications on Riserlines and Fencerows

It is important to control weeds on riserlines and fencerows, not only to prevent weeds from producing seed, but also to reduce the cover available for small rodents. Some managers apply a tank mix of two or three preemergence herbicides to riserlines at the time of sowing to prevent weeds from maturing and going to seed. Other managers will wait for weeds to develop and then apply a postemergence herbicide to kill emerged weeds. This type of application is often done with a shield designed to reduce drift to the hardwood crop (figure 4). Several types of shields can be used to reduce the potential of drift when applying herbicides to riserlines (Kees 2008). The number of herbicides that may be applied on riserlines is more than the number the Environmental Protection Agency allows to be applied to tree seedlings. To reduce the risk of injury, managers should avoid applying herbicides that are very water soluble (i.e., will move into adjacent seedbeds) or are very persistent in the soil.

Herbicide Applications in Seedbeds

At Time of Sowing

Several hardwood nursery managers do not apply herbicides at time of sowing because they typically sow on recently fumigated fields. Fumigated soils, however, can easily be contaminated with wind-blown seed; therefore, some managers apply herbicides at time of sowing (Jacob 2009, Murray 2009). Several preemergence herbicides can be applied at sowing to large-seeded species like oaks, black walnut, pecan (*Carya illinoinensis* L.), and hickory (*Carya* spp.). By contrast, only a few preemergence herbicides may be applied to small-seeded species like American sycamore (*Platanus occidentalis* L.). Managers who apply herbicides at time of sowing, in general, have less weeding time than those who rely solely on soil fumigants.

Oxyfluorfen is labeled for use on field-grown deciduous trees and has been used operationally as a preemergence herbicide (applied just after sowing) on large-seeded hardwoods (Jacob 2009, Murray 2009). Application should be made before seeds germinate because injury might occur if the herbicide contacts newly emerged tissues. Once oxyfluorfen is applied to the soil, large-seeded hardwoods can usually penetrate the herbicide barrier without much damage.

After the First True Leaves Have Formed

Herbicide selectivity is based on physiological or morphological differences between crop and weed. For example, a physiological difference between broadleaves and grasses is the basis of selectivity for clethodim, sethoxydim, and fluazifop-p-butyl. As a result, these postemergence herbicides typically do not cause injury to hardwoods after their first true leaves have formed. Preemergence herbicides (like prodiamine and pendimethalin) are active mainly on seed germination. These herbicides can also be applied after hardwood seedlings have germinated and have developed a few true leaves. The herbicide prodiamine is toxic to small hardwood seed, such as sycamore, if applied at time of seeding, but, when applied after the seedlings are 5 cm or taller, the chance of injury is greatly reduced. Although these herbicides will not control emerged weeds, they will help keep subsequent weed seed from germinating (South 1984b). Several nursery managers in the South successfully use this technique.

Some foliar-acting postemergence herbicides (like clopyralid) are selective and will affect the foliage of some weeds without harming certain hardwoods (Lawrie and Clay 1994, South 2000, Jacob 2009). Clopyralid, however, does have activity on legumes and, therefore, will injure eastern redbud (*Cercis canadensis* L.) and black locust. Injury has also been observed on black alder (*Alnus glutinosa* L.), hackberry (*Celtis occidentalis* L.), and dogwood (*Cornus florida* L.).

Granular Herbicide Formulations

The WSSA definition of *granular* is “a dry formulation consisting of discrete particles generally less than 10 mm3 and designed to be applied without a liquid carrier.” Granular herbicides are often used in horticultural nurseries and a number of granular herbicides are labeled for use on hardwoods. The cost of using granular herbicides, however, is more than the cost for using liquid formulations. The cost to treat with granular herbicides could exceed $300 per hectare, which may be 8 to 10 times the cost of applying the same active ingredient sold as a liquid formulation.

Figure 5. Although granular herbicides are typically more expensive, they often are less phytotoxic than liquid formulations. Granular herbicides should be applied to dry foliage, which allows most of the granules to roll off the foliage. Those that remain lodged in the foliage could be shaken off by dragging a cloth or bar over the foliage. (Photo by David South, 2007)

An advantage of granular herbicides is that when hardwood leaves are dry, the granules drop to the ground and do not affect the foliage (figure 5). When applied to dry foliage, herbicide granules of oxyfluorfen and oxadiazon may be less phytotoxic to foliage than liquid formulations (which may contain inert ingredients like naphthalene). For cases in which granules are lodged in the foliage, a sufficient amount of irrigation soon after treatment will reduce the chance of phototoxicity. For this reason, a wide variety of species are listed on granular herbicide labels. Granules of flumioxazin, oxyfluorfen, or oxadiazon could cause some temporary necrosis if they are allowed to remain on leaves.

Granular herbicides are not applied at time of sowing but are applied after the hardwoods have developed true leaves. Although effective weed control can be obtained with granular herbicides (Reeder et al. 1991), most nursery managers choose not to use granular formulations due to the added expense and because application is easier when herbicides are sprayed.

Managers should be aware that water dispersible granules (WDGs) do not fit the WSSA definition, even though they are called “granules.” Therefore, do not treat WDG formulations as though they were true granular formulations. WDG formulations should be mixed with water and applied as a liquid spray. Do not apply WDG formulations without following the label directions.

Figure 6. This photograph shows an example of a shielded herbicide applicator designed for hardwood seedbeds. The advantage of this model is that one person can operate it. (Photo by David South, 2010)

Directed Herbicide Application Using Shields

One way to provide selectivity is to ensure the herbicide does not come in contact with the hardwood foliage. Avoiding contact can be achieved with careful, directed applications by hand or by using shields when applying herbicides between drill rows (figure 6). To reduce the potential for seedling injury, most foliar-active herbicides should be directed away from the crop and toward the weeds.

Some nursery managers apply glyphosate "as needed" to control troublesome perennial weeds between rows using shielded sprayers (South and Carey 2005, Stallard 2005, Windell 2006). Glyphosate is a foliar-applied, nonselective herbicide with no soil activity. Glyphosate is bound tightly to soil particles and is unlikely to move off site. The relatively slow absorption of glyphosate into foliage causes efficacy to be reduced by rains within a couple of hours of application.

Herbicide Injury

Although many factors can injure seedlings, herbicides are often the first to be blamed. For example, herbicides have been blamed for injury caused by fertilizer. To reduce chances of a misdiagnosis, nursery managers should leave a few untreated areas in the seedbeds (i.e., check plots). The size of the check plots can be relatively small. These check plots are not only useful for diagnosing herbicide injury (figure 7), but they also provide a useful demonstration of what seedbeds would look like without the use of herbicides.

In some cases, herbicide injury will be minor and ephemeral. In fact, some herbicides might initially cause injury but eventually produce stock that is larger than untreated controls with no injury symptoms (Reeder et al. 1994). Therefore, most hardwood managers are more concerned with treatments that cause an "economic" injury to their crop than they are with one that causes a "cosmetic" injury to leaves, especially when hardwoods drop their leaves before lifting.

Economic injury occurs when an herbicide treatment reduces crop value (e.g., when the number of shippable seedlings produced per seedbed is reduced). The problem is determining which herbicides reduce seed germination before operational use. In some cases, herbicide trials are designed in such a

Figure 7. Use of untreated check plots can help to properly identify herbicide injury. Seedlings on both seedbeds experienced sandblasting during a May storm. Seedlings on the bed on the left were injured by sand that carried an herbicide, while those on the right were blasted with soil that did not contain a herbicide. In this case, injury was temporary and seedlings were fully recovered by July. (Photo by David South, 2010)

manner that even a 50-percent reduction in crop value would not be classified as "significant" injury (Garrett et al. 1991, South 1992). The low power of these experimental designs is due primarily to the high level of variability in many hardwood seedbeds.

Herbicide injury can result when the label instructions or precautions are not followed. It can also occur if the herbicide sprayer is not properly calibrated. Without regular calibration, uniformity may decrease and risk of injury may increase. In addition, it is wise to consult with nursery experts before applying the herbicide because new information may have occurred since the label was written. For example, some managers have observed injury to dogwood when a certain herbicide in WSSA group 1 was applied to newly emerged seedlings. These injuries occurred because one brand contained 65 percent solvent naphtha and 7 percent naphthalene (which can injure new foliage when applied under high temperatures). Consultation with an expert may have prevented injury if the expert has recommended a similar product that contained low amounts of naphtha and naphthalene.

Hardwoods occasionally have been injured when environmental conditions are right and the herbicide "lifts off" the soil within water vapor and then drifts over newly emerged hardwood seedlings (South 1984a), a process known as "*co-distillation*." This type of injury may occur soon after seedbeds have been treated with oxyfluorfen on warm, sunny days. The injury is usually just cosmetic—the new leaves turn brown. It is fortunate that the affected seedlings typically recover and grow normally.

At a few nurseries, the use of dinitroaniline herbicides (WSSA group 3) has injured certain hardwood species (Derr and Salihu 1996, Hood and Klett 1992, South 1992, Warren and Skroch 1991). In some cases, herbicide galls formed on the stem near the groundline (Altland 2005, South 2009). For example, sugarberry (*Celtis laevigata* Willd.) was injured after applying prodiamine and pendimethalin (figure 8). A postemergence application (after both weed and crop emergence) of oryzalin has caused injury and stem breakage on American sycamore, river birch (*Betula nigra* L.), yellow poplar (*Liriodendron tulipifera* L.), redbud, elm (*Ulmus* spp.), buttonbush (*Cephalanthus* spp.), plum (*Prunus* spp.), and black willow (Salix nigra L.).

In some cases, herbicide injury occurs when an herbicide applied to fallow ground carries over to the next year. For example, injury occurred when certain herbicides in the imidazolinone family were used the previous year on fallow land. The length of time that an herbicide remains biologically active in the soil is determined by a number of factors. In the South, most herbicides in WSSA groups 1, 3, and 15 do not persist long enough to affect hardwoods sown the next year. In regions where soils are cooler (e.g., Saskatchewan), however, herbicide carryover is more likely. This difference is primarily because the rate of microbial decomposition is slower in Saskatchewan than in Alabama or Georgia.

Figure 8. Some hardwood species are more tolerant of herbicides than others. For example, sugarberry (*Celtis laevigata* Willd.) can be injured by certain dinitroaniline herbicides. Photo by Chase Weatherly, Arborgen, 2008)

Herbicide injury will sometimes occur after a windstorm. For example, at one nursery, herbicide injury to sawtooth oak (*Quercus acutissima* Carruth.), swamp chestnut oak (*Q. michauxii* Nutt.), and persimmon (*Diospyros virginiana* L.) was noticed 2 days after seedlings were sandblasted (Skidmore 1966) with high winds. The herbicide (i.e., oxyfluorfen) was carried with the soil, and the abrasions allowed for the herbicide to enter the stem and foliage. Although oak seedlings in check plots were also sandblasted (figure 7), they were not injured, because the sand did not contain the herbicide. Use of a soil stabilizer would have reduced the amount of sandblasting and would have subsequently reduced this type of injury.

In some situations, injury to adjacent seedbeds has occurred when dazomet or metam sodium was applied without a tarp (Buzzo 2003, Scholtes 1989, Starkey 2011). Therefore, to reduce the potential for injury to adjacent crops, a plastic tarp is recommended when fumigating with these compounds. Some contractors now use a plastic tarp only when fumigating with metam sodium.

When using liquid fertilizers in returnable totes, it is wise to deal with a reputable dealer. Reputable dealers either do not refill herbicide totes with fertilizer solutions or they ensure the totes are thoroughly cleaned before they are refilled. At one nursery, injury resulted when a fertilizer dealer did not thoroughly clean out a tote that had previously contained triclopyr.

Economics

Some nursery managers base their weed management decisions on securing economic profits and on maintaining a good reputation for producing high-quality nursery stock. Their justifications for using herbicides include keeping seed efficiency high (South 1987) and production costs low. By contrast, other managers operate as a nonprofit entity and their primary objective is to achieve target production goals within a given budget. Both management systems can benefit when handweeding costs are reduced by using an effective IWM program.

The easiest way to justify the use of herbicides is to compare the cost of treatment with the cost of handweeding. For example, at a nursery where hand labor costs $15 per hour, an herbicide application that costs $30 per nursery hectare would be justified if it reduced handweeding by 2 hours or more. Thus, when seedbeds require 100 hours of handweeding per hectare ($1,500 total) to remove small grasses, 10 applications of herbicides (i.e., $300) could reduce weed control costs by as much as $1,200 (assuming the use of herbicides eliminated the need for hand weeding the grass).

Another method for justifying herbicide use is to determine how many seedlings are lost to weed competition and to handweeding. If a nursery loses $0.30 every time a weeder inadvertently pulls up a seedling, then saving 100 seedlings could justify an herbicide treatment that cost $30. Therefore, even in rare cases in which use of herbicides does not reduce the annual cost of weed control, their use could still be justified when seedling sales are increased. An examination of a hardwood nursery budget might reveal that herbicide treatments amount to less than 0.5 percent of the retail value of the crop (table 3). Therefore, use of herbicides may be justified when seedling production is increased by just 0.5 percent, which would be equivalent to selling 502,500 seedlings instead of 500,000 seedlings per hectare.

Table 3. Example of weed management costs in hardwood nurseries. Data assumes 444,600 seedlings per hectare and a price of $0.30 per seedling.

Weed management practice	Active ingredient (kg/ha)	Dollars per thousand seedlings	Percentage of total crop value
Herbicides in seedbeds	2.24	0.50	0.2
Herbicides on fallow ground	—	0.70	0.2
Handweeding—$15 per hour	—	2.08	0.7
Soil fumigation	392	17.22	5.7
Total	—	20.50	6.8

Conclusions

Because of the numerous species involved, a single herbicide regime (e.g., South 1992) is unlikely to be effective for all hardwood species. Weed species, hardwood species, soil types, and labor costs vary with nursery; therefore, weed management regimes vary with nursery. The most effective IWM programs, however, include a rigorous sanitation program and judicious use of efficacious herbicides.

Disclaimer

The mention of commercial products is solely for the information of the reader. Endorsement is not intended.

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Seedfall and Seed Viability Within Artificial Canopy Gaps in a Western Washington Douglas-Fir Forest

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Abstract

Seedfall of coast Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) has been studied at the forest edge-clearcut interface and in small canopy gaps, but it has not been evaluated in gap sizes that would be typical of a group-selection method of regeneration. In a mature Douglas-fir forest in the Puget Sound lowlands of western Washington, seedfall was measured by month in artificially created circular gaps 0.1, 0.2, 0.3, and 0.4 ha (0.25, 0.5, 0.75, and 1.0 ac, respectively) in size and in the forest matrix. Seedfall was assessed 1 year before and 2 years after gap creation, and a germination trial was used to detect potential gap and seasonal effects on seed viability. Seedfall density was not significantly affected by the presence of gaps up to 0.4 ha (1.0 ac). Germination percentage and germination rate did not differ between seed collected in gaps and that collected in the forest matrix. Seed weight and germination percentage both were highest for fall collections and declined for collections taken throughout winter and spring. We found no evidence that seed dispersal or viability would be a limiting factor in natural regeneration of Douglas-fir under a group-selection system that created gaps up to 0.4 ha (1.0 ac) in size.

Introduction

In recent decades, increasing attention has been focused on silvicultural systems for public lands that promote multiple age classes and structural diversity in production forests that traditionally have been managed under single-age systems (Aubry et al. 2009, Guldin 1996, Malcolm et al. 2001, Reutebuch et al. 2004). For tree species of low or moderate shade tolerance, including many of the important conifer timber species in North America, single-tree-selection systems do not create an understory environment with sufficient light for successful regeneration (Harrington 2006, Miller and Emmingham 2001). By contrast, a silvicultural system, such as group selection, which harvests and regenerates areas typically 0.04 to 0.8 ha (0.1 to 2.0 ac), creates gaps with sufficient light to potentially regenerate species that are less shade tolerant (Smith 1986).

Whereas economic or regulatory reasons often spur the planting of seedlings following group-selection harvests, natural regeneration of the canopy species is sometimes a viable alternative, owing, in part, to the close proximity of seed-producing trees bordering the small gaps. If the dominant canopy tree species are shade intolerant, it is unlikely that significant advance regeneration of these species will be present at the time of gap creation, leaving coppice regeneration or seed as the primary source of natural regeneration for the canopy species. Natural regeneration has been studied in group-selection silvicultural systems in a variety of forest types worldwide (Gagnon et al. 2004, Kinny et al. 2012, Stephens et al. 1999); however, much remains unknown about seedfall within the harvested patches. Although many studies have investigated seedfall in canopy gaps of tropical forests (Augspurger and Franson 1988, Connell 1989, Denslow and Gomez Diaz 1990), only a few have measured seedfall under a group-selection system in temperate forests (Gray 1995, McDonald and Abbott 1994).

In the case of coast Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*), one of the world's most important timber species and the subject of the present study, seed does not retain viability in the forest floor or soil past the year in which it falls (Isaac 1935). Therefore, under a group-selection system, seedfall rate in gaps—and seed viability—during the initial years following gap creation is of key importance, particularly because vegetative competition grows rapidly in newly created gaps (Spies and Franklin 1989). The small, single-winged seeds of Douglas-fir are dispersed by gravity and wind, with dispersal distance influenced by parent tree height and cone position, wind velocity, and other factors (Isaac 1930, Willson 1993). The seed shadow of Douglas-fir was quantified based on all existing dispersal distance data (Willson 1993); however, all these data were derived from virgin forests bordering clearcuts. Seed dispersal in smaller openings and in younger stands may differ, given potential differences in tree height and wind currents.

This 3-year study examined seedfall in a western Washington Douglas-fir forest to assess seedfall in harvested gaps 0.1 to 0.4 ha (0.25 to 1.0 ac) in size and to compare these values with seedfall within the forest matrix. A germination trial was conducted to assess potential gap and seasonal effects on seed viability. Hypotheses were (1) seedfall in gaps is negatively associated with gap size owing to proximity to seed source; and (2) seed viability is negatively associated with gap size because lighter seeds, including unfilled seeds (i.e., without a developed embryo), are expected to travel greater distances.

Methods

Study Area

The study was located on Joint Base Lewis-McChord, a U.S. military base in the Puget Trough physiographic province of southwestern Washington at an elevation of 106 to 139 m (350 to 460 ft) above sea level. The study area was anthropogenically maintained prairie and savanna before European settlement, which occurred in the mid-1800s. After settlement, Douglas-fir density increased in waves from 1878 to 1938 associated with low-intensity fires with fire return intervals of 10 to 91 years (Peter and Harrington 2014). The study area includes six forest stands, each characterized by a Douglas-fir overstory. Although these stands had been subjected to low-intensity thinning (15 to 20 percent of basal area removed per entry) two or three times, the overstory consisted of the original cohort of trees that colonized the area. The understory in the study area consists largely of western swordfern (*Polystichum munitum* [Kaulf.] C. Presl) and salal (*Gaultheria shallon Pursh*), with a lesser amount of Oregon grape (*Mahonia nervosa* [Pursh] Nutt.), California hazel (*Corylus cornuta* Marshall), oceanspray (*Holodiscus discolor* [Pursh] Maxim.), and common snowberry (*Symphoricarpos albus* [L.] S.F. Blake).

The soils in the study area are Spanaway and Everett gravelly and very gravelly sandy loams (Typic Humixerepts and Humic Dystroxerepts, respectively), mapped as Humic Cambisols by the Food and Agriculture Organization of the United Nations (FAO 1995). These soils are formed in glacial outwash and are very deep and somewhat excessively drained (Soil Survey Staff 2006). The climate is characterized by warm, dry summers and mild, wet winters, owing to a maritime influence. In Tacoma, WA (15 to 25 km [9 to 16 mi] from study plots), mean annual precipitation is 1,008 mm (39.7 in), although cumulative precipitation from May 1 through September 30 averages only 166 mm (6.5 in) (WRCC 2015). Mean air temperatures in January and July are 6 and 19 °C (43 and 66 °F), respectively.

Overstory characteristics of each stand were assessed using prism sampling (5 m^2 ha^{-1} [21.78 ft^2 ac^{-1}] basal-area factor), with 28 grid points per stand sampled. Diameter at breast height (measured at 1.3 m [4.3 ft] above ground) was recorded for every “in” tree and total height and crown height were recorded for one typical dominant or codominant tree per plot. With the exception of a single ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson), all overstory trees inventoried were Douglas-fir.

Study Design and Data Collection

To assess seedfall in stands without gap creation, 24 sample locations (8 in each of three similar forest stands) were established on predetermined grid points during the study’s “pretreatment” season (September 2008 to March 2009). In September 2008, a 73.2-m (240-ft) transect was established at each sample location, running northeast-southwest and centered on the sample location. Seven seedtraps were installed along each transect at a 12.2-m (40-ft) spacing. Each circular seedtrap consisted of a shallow fiberglass screen cone, with a 1.0-m^2 (10.8-ft^2) opening at the top, suspended approximately 0.75 m (2.5 ft) above the ground on metal rods (figure 1).

Figure 1. Each seedtrap contained a circular frame, 1.0 m^2 (10.8 ft^2) in area, constructed of flexible plastic tubing. A semicircle of fiberglass screen was stapled to the frame to create a conical trap for collecting Douglas-fir seeds, cones, and other forest litter. The trap was suspended on rods approximately 0.75 m (2.5 ft) above the ground. (Photo by Timothy B. Harrington, 2008)

Owing to operational constraints associated with the timing of overstory treatments (i.e., gap creation and thinning), it was not possible to measure posttreatment seedfall in the same three stands assessed for pretreatment seedfall. Instead, seedfall data were collected during two posttreatment seasons (September 2009 to March 2010 and September 2010 to March 2011), following the same sampling protocol, in three other similar stands that were part of the same study area (table 1). For this reason, we interpret pretreatment data with the caveat that they were collected in different stands from the posttreatment data. It should be noted, however, that pretreatment and posttreatment stand characteristics differed very little (table 1).

Table 1. Characteristics (mean ± standard error) of two groups of stands used in the Douglas-fir seedfall study. Three stands were assessed before any treatment (pretreatment stands), and three stands were assessed 2 years after a treatment that consisted of gap creation and thinning (treated stands).

Variable	Pretreatment stands	Treated stands
Height (m)	47.3 ± 2.3	48.9 ± 1.8
Crown base height (m)	24.9 ± 2.1	27.8 ± 1.9
Quadratic mean DBH (cm)	69.5 ± 4.5	68.9 ± 6.2
Stem density (trees ha^{-1})	141.1 ± 29.8	147.6 ± 40.5
Stand basal area (m^2 ha^{-1})	34.4 ± 3.5	32.9 ± 4.1
Stand density index (percent of maximum)	31.8 ± 2.9	30.4 ± 2.6

DBH = diameter at breast height.

Conversions: 1 m = 3.281 ft; 1 cm = 0.394 in; 1 tree ha-1 = 0.405 tree ac^{-1}; 1 m^2 ha^{-1} = 4.356 ft^2 ac^{-1}.

Treatments consisted of circular canopy gaps, 0.1, 0.2, 0.3, and 0.4 ha (0.25, 0.5, 0.75, and 1.0 ac) in size (figure 2). Gaps were created at the same time as a stand-level operational thinning having a target residual density of 30 percent of maximum Stand Density Index for Douglas-fir (Reineke 1933). Of the eight transect locations in each stand, four were centered in gaps of each of the four sizes ("gap locations") and four were located in nongap locations within the thinned forest matrix ("matrix locations").

For both pretreatment and posttreatment transects, the content of each seedtrap was collected at an approximate 1-month interval during each season. Contents (needles, cones, seeds, and other materials) from the 7 seedtraps at each transect location were composited in the field (figure 3). A total of 432 seedtrap samples were collected (18 sample dates [6 per year] x 3 stands x 8 locations). At the laboratory, cones were separated from other sampled materials, and both of these portions from each composite sample were air-dried in separate paper bags for 6 to 8 weeks. After drying, seeds were removed from cones and the total number of cone seeds per sample was recorded.

Seeds that fell individually (i.e., not in cones) were separated from all other materials in the seedtrap by handsorting and

Figure 2. Example of a 0.4-ha (1.0-acre) circular canopy gap that was created by harvesting trees within a mature stand of Douglas-fir at Joint Base Lewis-McChord. Seven seedtraps were placed within each gap at 12.2-m (40-ft) spacing along a northeast-southwest transect that intersected the gap center. (Photo by Timothy B. Harrington, 2009)

Figure 3. The contents of each seedtrap (Douglas-fir seeds, cones, and other forest litter) were collected monthly from September to March during the year before gap creation (2008 to 2009) and during the 2 years after gap creation (2009 to 2010 and 2010 to 2011). (Photo by Timothy B. Harrington, 2010)

were counted for six sample locations in each season. For the individually fallen seed samples in the posttreatment, a stratified random selection procedure was used to select one transect location from each of the four gap sizes and two from the forest matrix locations, with a total of two selected locations in each of the three stands. Thus, a total of 108 samples of individually fallen seeds were collected (18 sample dates [6 per year] x 3 stands x 2 locations). After sorting and counting, all seeds were placed in storage at -18 °C (0 °F) until the germination trial.

To achieve adequate sample sizes for the germination trial, all seeds (i.e., cone-origin and individual) from each sample date during the pretreatment collection were composited by stand. For the posttreatment samples, all seeds collected at each sample date within each stand were composited into two groups based on treatment: (1) forest matrix and (2) the four gap treatments. Samples were combined for the first two sample dates in posttreatment season 2 because an insufficient number of seeds were collected on those dates for separate germination tests; thus, the total number of samples in the germination test was 102 (17 sample dates x 3 stands x 2 treatment groups). Each sample was weighed (nearest 0.1 mg [0.000004 oz]) and counted to calculate average seed weight.

Before the germination trial, 100 seeds from the pretreatment season, 100 seeds from the posttreatment season 1, and 50 seeds from the posttreatment season 2 samples (owing to a limited number of seeds collected during that season) were randomly selected from each sample, soaked in deionized water for 24 hours, and then cold stratified (>0 °C [32 °F]) for 40 days. Following stratification, seeds were placed on moistened filter paper within a plastic germination box and placed in a germinator with 20/25 °C (68/77 °F) night/day (14 hr/10 hr) temperatures and observed for germination during a 30-day period. Observations were daily for the first 5 days and every 2 days thereafter.

Data Analyses

For each of the three sample seasons, seedfall data (number of cone seeds and individual seeds collected per sample) were analyzed using repeated measures analysis of variance (ANOVA), with sample date (i.e., days since beginning of study) as the repeated unit (Proc MIXED, SAS Institute Inc. 2008). For the two posttreatment sample seasons, the effect of gap treatment was included in the model, using gap size as a continuous variable (range = 0 ha [0 ac] in the forest matrix treatment to 0.4 ha [1.0 ac] in the largest gap treatment). Average seed weight per sample and total germination percentage after 30 days were also analyzed with repeated-measures ANOVA. In posttreatment models, gap treatment was included as a fixed effect with two levels (presence of a gap of any size vs. absence).

Cumulative distribution of germination over time was modeled for each sample using a four-parameter Weibull cumulative distribution function, as described by Brown (1987) (Proc NLIN, SAS Institute Inc. 2008):

$$F(t) = M(1 – \exp[–\{k(t–l)\}^{c}])$$

where F(t) is cumulative germination at time t (days), M is the maximum germination for the sample (germination at day 30), k is the rate of germination, l is the lag until germination initiates (days), and c is the shape parameter. After a function was fit for each sample, the k, l, and c parameters were analyzed using the same ANOVA model described for analysis of total germination percentage.

Residuals produced by ANOVA models were examined graphically and were tested using PROC REG and PROC UNIVARIATE (White's and Shapiro-Wilk tests) for variance and normality assumptions (SAS Institute Inc. 2008); no transformations were deemed necessary. Mean separation was performed using Tukey's HSD test. Significance was judged at a confidence level of 95 percent throughout the analyses.

Results and Discussion

Seedfall

Total seedfall varied tenfold among the three study seasons, from 211,000 ha^{-1} (85,400 ac^{-1}) to more than 2,165,000 ha^{-1} (876,100 ac^{-1}) (table 2). The proportion of seed dropped individually was 54 percent of the seed crop in the pretreatment season and 93 and 36 percent in posttreatment seasons 1 and 2, respectively. The number of seeds that fell individually varied widely among seasons, whereas seed extracted from dropped cones did not vary appreciably (table 2).

Douglas-fir seed production varies substantially among years, with heavy seed crops occurring every 2 to 11 years; at least some seed is produced in 75 to 80 percent of years (McDonald 1992, Stein and Owston 2008). An average of 800,000 seeds ha^{-1} (320,000 seeds ac^{-1}) has been suggested as necessary to produce an adequately stocked stand of Douglas-fir (Isaac 1943); however, this estimate varies

Table 2. Total estimated seedfall in 3 years (mean ± standard error). Estimates for posttreatment seasons 1 and 2 are an average across five treatments: forest matrix (thinned) and 0.1-, 0.2-, 0.3-, and 0.4-ha canopy gaps.

Year	Seedfall (thousand seeds ha^{-1} yr^{-1})		
	Individual seed	Seed in cones	Total crop
Pretreatment	182 ± 62	158 ± 89	340
Posttreatment season 1	2,017 ± 563	148 ± 178	2,165
Posttreatment season 2	76 ± 24	135 ± 91	211

Conversions: 1 ha = 2.47 ac; 1,000 seeds ha^{-1} = 405 seeds ac^{-1}

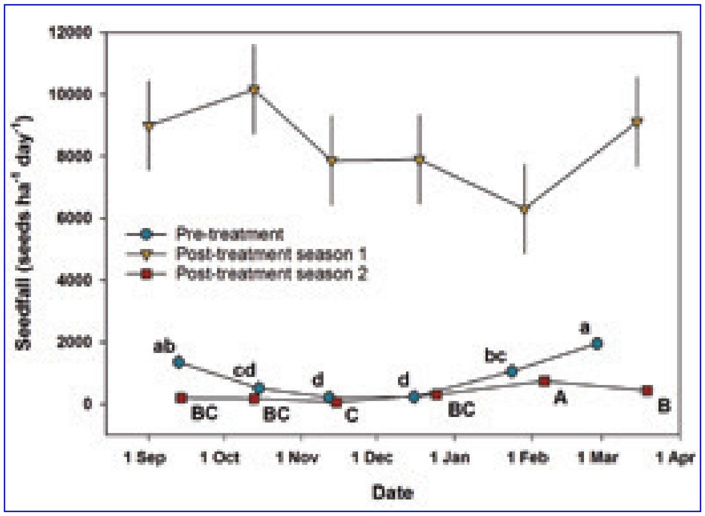

Figure 4. Mean Douglas-fir seedfall rate (± standard error) for seeds dropped individually (i.e., not in cones) by sample period in three seasons. Means accompanied by the same lowercase letter (pretreatment season) or uppercase letter (posttreatment season 2) do not differ significantly by sampling period (alpha = 0.05). No differences were observed among sampling periods for posttreatment season 1. Conversion: 1,000 seeds ha-1 day-1 = 405 seeds ac^{-1} day^{-1}

widely by site and may be significantly lower for favorable seedbeds (Isaac 1943, Minore 1986). Germination and subsequent seedling establishment success vary substantially among sites; success is highest on mineral soil with germinants protected from direct sunlight and where seed predation and vegetation competition are low (Gray and Spies 1996, Minore 1986). Thinning increases stand-level seed production (Reukema 1961), and it is possible that gap creation would increase seed production of bordering trees. Such an increase in seed production, however, would likely not lead to increased seedling establishment in gaps unless the increase occurred before substantial growth of competing vegetation.

Seedfall rate differed among sample periods for individually dropped seeds during the pretreatment season and posttreatment season 2 (table 3; figure 4). Where significant differences occurred among sampling periods, seedfall was lowest in late fall/early winter and greatest in late winter/early spring. The number of seeds dropped in cones varied among sample periods in all 3 years, with the greatest number of seeds dropped during November or December in all years, likely coinciding with significant fall and winter storms (table 3; figure 5). Previous studies reported seed dispersal for Douglas-fir beginning around September, with most seed usually falling by early December and seedfall virtually completed by the end of March (Gashwiler 1969, Isaac 1943, Pickford 1929, Reukema 1982). Periods of warm temperatures are associated with the opening of cones and an increase in the release of seeds (Stein and Owston 2008). Unlike these previous studies, we observed no obvious seasonal decline in seedfall rate for individually dropped seeds. Although seedfall declined in winter in the pretreatment measurement, it increased again at the final sample period in March (figure 4).

No significant gap effect on the number of individually dropped seeds in either posttreatment season was

Table 3. Significance (Pr > F) of treatment effects from ANOVA models evaluating seedfall (individual seeds and seeds in dropped cones) analyzed during one season pretreatment (no thinning or gaps created) and during two posttreatment seasons (with thinning and canopy gaps of varying sizes). Sampling period was September through March each season.

Variable	Effect[a]	d.f.	Pretreatment	Posttreatment season 1	Posttreatment season 2
Individual seeds (seeds ha^{-1} day^{-1})	Sample period	5[b]	< 0.001	0.387	0.014
	Gap size	1	—	0.284	0.713
	Sample period × gap size	5[b]	—	0.269	0.939
Seeds in cones (seeds ha^{-1} day^{-1})	Sample period	5[b]	< 0.001	0.002	< 0.001
	Gap size	1	—	< 0.001	< 0.001
	Sample period × gap size	5[b]	—	0.541	< 0.001

ANOVA = analysis of variance; d.f. = degrees of freedom; Pr > F = the p-value associated with the F statistic.

[a] Gap size was analyzed as a continuous variable (0 to 0.4 ha).
Conversion: 1 seed ha^{-1} day^{-1} = 0.405 seeds ac^{-1} day^{-1}.

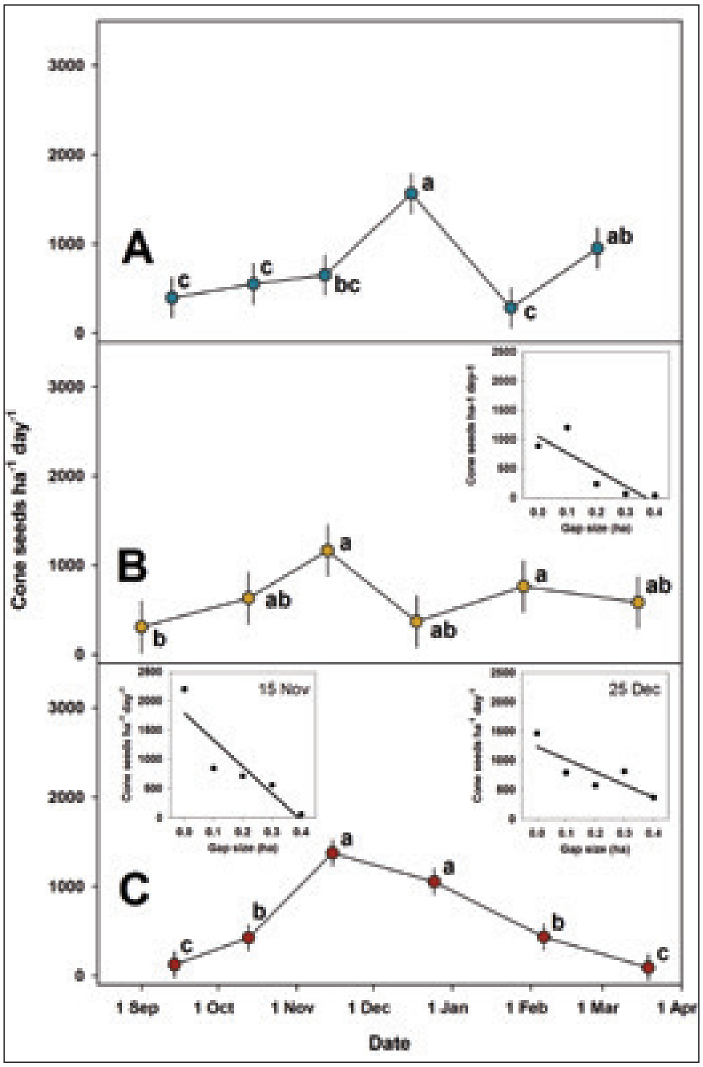

Figure 5. Mean Douglas-fir seedfall rate (± standard error) for seeds dropped in cones by sample period in three seasons: (A) pretreatment season, (B) posttreatment season 1, and (C) posttreatment season 2. In each season, seedfall differed among sample periods; points accompanied by the same lowercase letter do not differ significantly (alpha = 0.05). In posttreatment season 1, seedfall rate also differed significantly by gap size (table 3; inset in B). In posttreatment season 2, a sample period × gap size interaction resulted from significant gap size effects for 15 Nov and 25 Dec sample periods (table 3; insets in C) but not in the other sample periods. Conversion: 1,000 seeds ha^{-1} day^{-1} = 405 seeds ac^{-1} day^{-1}.

observed. A significant negative relationship existed, however, between gap size and cone seeds in posttreatment season 1, and a significant interaction existed between sample period and gap size in posttreatment season 2. This interaction resulted from a negative relationship between gap size and cone seed number in the November and December sample periods, when the overall number of cone seeds was highest. The number of cone seeds decreased linearly as gap area increased from 0 (forest matrix) to 0.4 ha (1.0 ac), probably because of associated increases in the distance from seed-bearing trees. We are not aware of any previous studies that separately assessed Douglas-fir seed that fell still attached to cones from seed that fell individually; however, it is clear that the dispersal distance of cones is less than that of individual seeds. In a heavy seed year, only a small proportion (7 percent) of total seedfall consisted of seeds in cones, and thus the lack of cones falling into larger gaps may not have a meaningful impact on regeneration. It remains unclear what fraction of the seeds that are dropped in cones germinate and establish as seedlings compared with that of individually dropped seeds.

Because no gap-size effect was observed on seedfall density for individually dropped seeds, in posttreatment season 1, even our largest gaps (0.4 ha [1.0 ac]) had seedfall densities well in excess of the densities suggested for successful stocking (355 seeds per seedling [Isaac 1943] and 75 to 190 seeds per seedling [Minore 1986]). We are not aware of any studies that have assessed Douglas-fir seedfall in larger gaps. One study examined smaller gaps in a mixed conifer-hardwood forest in northern California and found that seedfall did not vary significantly among gaps ranging from 9 to 27 m (30 to 90 ft) in diameter (0.007 to 0.059 ha [0.016 to 0.15 ac]) (McDonald and Abbott 1994). Several early studies measured seedfall in large clearcuts bordered by virgin forest (Isaac 1930, 1943; Pickford 1929). Seedfall in western Oregon clearcuts declined at a ratio of 5:2:1 at distances of 23, 69, and 114 m (75, 225, and 375 ft) from the forest edge, respectively (Gashwiler 1969). In another clearcut study, 44 percent of seed fell within 30 m (100 ft) of the forest edge and 83 percent fell within 152 m (500 ft) (Isaac 1943).

Seed Weight and Germination

Seed weight was approximately twice as great in the season of heavy production (posttreatment season 1) compared with the other seasons (figure 6). Seed weight in the year of heavy production was comparable or slightly less than values reported previously, whereas seed weight in the two light-production seasons was substantially lower (Stein and Owston 2008). Germination percentage was also substantially higher in the season of heavy seed production than in the other seasons, averaging more than 50 percent during the first four sample periods (table 4; figure 7). Germination was 10 percent or less throughout the other two seasons. The proportion of filled seed (i.e., seed with a normal embryo that is potentially viable) has been shown in several other studies to be positively correlated with the size of the

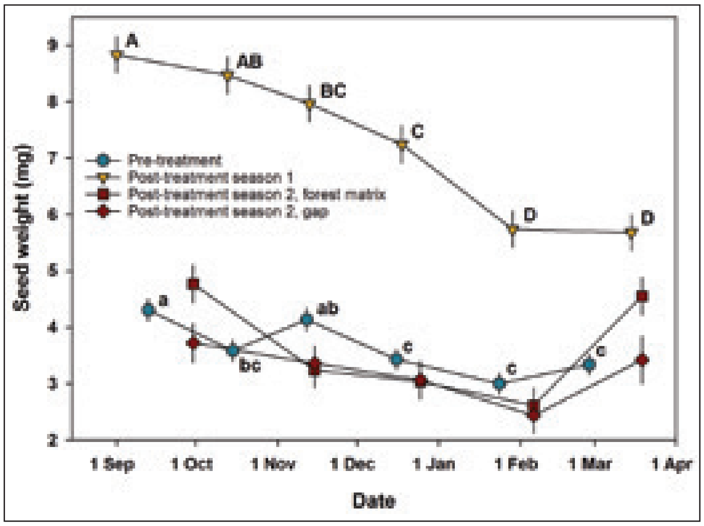

Figure 6. Mean Douglas-fir seed weight (± standard error) by sample period during three seasons. Sample periods differed significantly pretreatment and during posttreatment season 1 (table 4); means accompanied by the same lowercase letter (pretreatment season) or uppercase letter (posttreatment season 1) do not differ significantly by sampling period (alpha = 0.05). During posttreatment season 2, a significant sample period × gap treatment interaction was observed: seed weight was significantly greater in the forest matrix treatment during the first and last sample periods only. Conversion: 1 mg = 0.00004 oz.

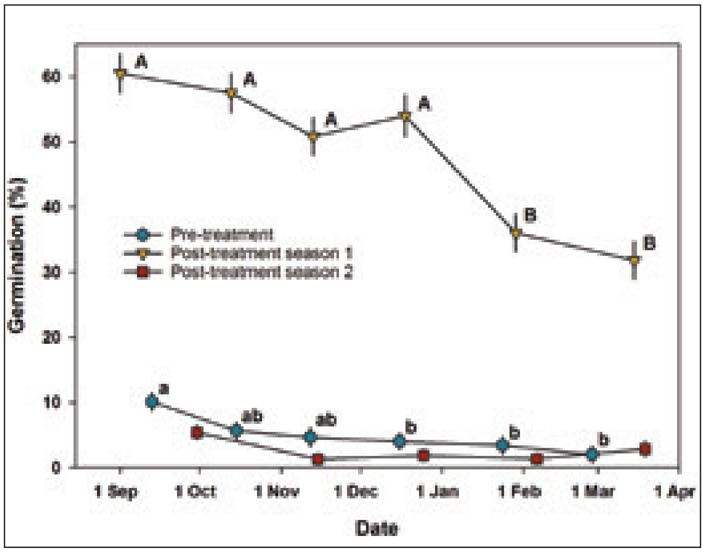

Figure 7. Germination percentage (± standard error) for Douglas-fir seed by sample period during three seasons. Germination differed within season pretreatment and within season during posttreatment season 1 (table 4). Means accompanied by the same lowercase letter (pretreatment season) or uppercase letter (posttreatment season 1) do not differ significantly by sampling period (alpha = 0.05).

annual seed crop, ranging from 1 to 54 percent (Garman 1951; Gashwiler 1969; Reukema 1961, 1982)

Seed weight followed a declining trend during each of the three seasons, with the exception of posttreatment season 2, when seed weight increased in the final sample period (table 4; figure 6). Within each season, seed germination percentage also declined for seeds collected later in the season, although the significance of this effect was marginal in posttreatment season 2. A similar trend was shown in two previous Douglas-fir seedfall studies, in which the seed that fell earliest had the highest viability (Gashwiler 1969, Reukema 1982). Within the year of heavy seed production (posttreatment season 1), a significant positive correlation between seed weight and total germination percentage was observed (figure 8), owing to the fact that both variables decreased similarly during the season.

Germination percentage did not differ between seeds collected in gaps and those collected in the forest matrix (table 4). No effect of gap presence on seed weight in posttreatment season 1 was observed, but, in posttreatment season 2, seed weight in the first and last sample periods in the forest matrix treatment was more than that of seeds dropped in

Table 4. Significance (Pr > F) of treatment effects from ANOVA models evaluating seed weight and total germination for seed collected in one season pretreatment (no thinning or gaps created) and during two seasons posttreatment (with thinning and canopy gaps of varying sizes). Sampling period was September through March each season. The gap presence effect compares forest matrix (thinned only) with a composite sample of seed collected in gaps of 0.1, 0.2, 0.3, and 0.4 ha.

Variable	Effect	d.f.	Pretreatment	Posttreatment season 1	Posttreatment season 2
Seed weight	Sample period	5[a]	0.001	< 0.001	< 0.001
	Gap presence	1	—	0.850	0.006
	Sample period × gap presence	5[a]	—	0.532	0.032
Total Germination (percent)	Sample period	5[a]	0.008	< 0.001	0.054
	Gap presence	1	—	0.616	0.252
	Sample period × gap presence	5[a]	—	0.137	0.276

ANOVA = analysis of variance; d.f. = degrees of freedom; Pr > F = the p-value associated with the F statistic.

[a] Degrees of freedom was 4 in posttreatment season 2.

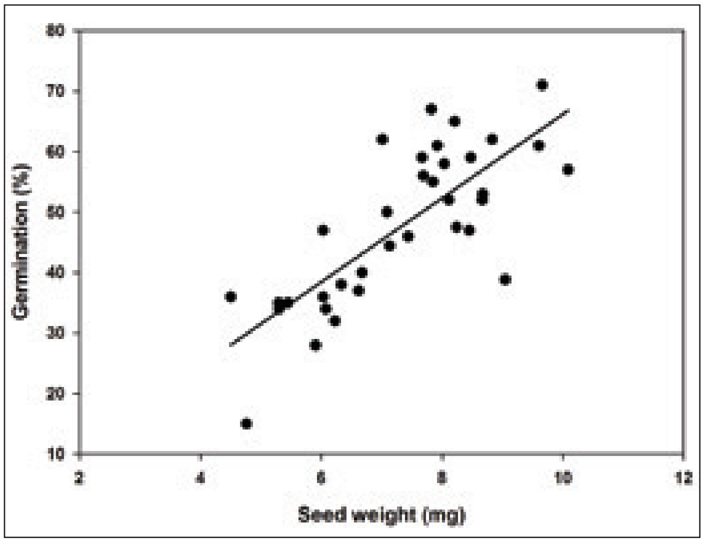

Figure 8. Relationship between germination percentage and seed weight for samples collected on three sites in posttreatment season 1 during six sample periods from September through March. Regression line equation is y = 6.93x – 3.16 (R^2 = 0.60). Conversion: 1 mg = 0.00004 oz.

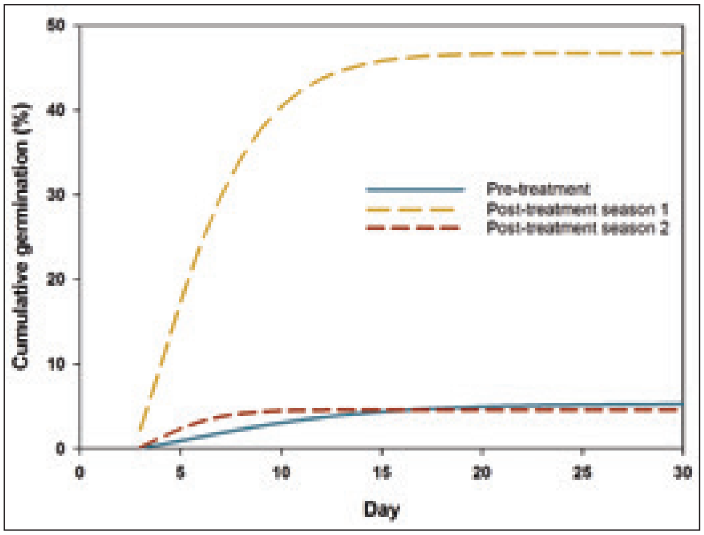

Figure 9. Germination functions for Douglas-fir seed collected during three seasons. Sample period and gap presence did not significantly affect the functions' germination rate, lag, or shape parameters; thus, the functions shown here are for the pooled germination data from each season.

gaps (figure 6). An initial concern with natural regeneration in larger gaps was that heavier seed—presumably the seed with higher viability—would be less likely to fall near the center of the gaps owing to greater distance from the parent tree. Because seed weight and germination percentages of seed collected in gaps were generally similar to that of the forest matrix, however, gaps of the size range in this study are not likely to incur that problem.

Within each season, the parameters describing the Weibull distribution did not differ significantly by sample period or gap presence. Thus, overall cumulative distribution curves describing germination in each season are shown in figure 9. Germination reached an asymptotic level around day 15 during each season.

Conclusions

Our findings, in general, do not support our hypothesis that seedfall in gaps is negatively associated with gap size. The only negative effect of gap size on seedfall occurred for seed dropped in cones. The impact of this phenomenon on total seedfall may be meaningful in a light seed production year, but, in a year of heavy seed production, its relative impact is minor. We found no evidence to support our hypothesis that seed viability is negatively associated with gap size. Although germination was correlated with seed weight, this relationship was a function of sample period rather than of gap presence.

We found no evidence that seed dispersal or viability would be limiting factors in natural regeneration of Douglas-fir in circular gaps up to 0.4 ha (1.0 ac) in size, assuming an adequate seed crop was produced soon after gap creation (i.e., before significant growth of competing vegetation). Thus, with sufficient seedfall and seed viability, establishment of natural regeneration in created gaps is more likely to be limited by other factors, such as light availability, seedbed conditions, seed predation, desiccation of germinants, and vegetative competition (Gray and Spies 1996, Isaac 1943, Minore 1986). Success of Douglas-fir seedlings and saplings as a future canopy cohort will likely require relatively large gaps; 2-year-old Douglas-fir seedlings regenerated from seed were significantly larger near the center of 0.2-ha (0.5-ac) gaps compared with seedlings in smaller gaps (Gray and Spies 1996). Measurements of photosynthetically active radiation (PAR) in this study (unpublished data) showed that PAR was similarly high in gaps from 0.2 to 0.4 ha (0.5 to 1.0 ac) but decreased sharply in the 0.1-ha (0.25-ac) gaps and in the forest matrix. Whereas the present study shows propagules are not limiting in any of the gap sizes tested, 0.2 ha (0.5 ac) may be a realistic minimum gap size for successful natural regeneration of Douglas-fir under a group-selection system.

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Mulching and Shade Effects on Emergence and Survival of Direct-Seeded Western Redcedar (*Thuja plicata*)

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Abstract

Western redcedar (*Thuja plicata* Donn ex D. Don) is an important forest species valued by foresters for its timber value and by the public for its beauty. Regeneration of this tree species, however, is threatened by difficulties in plantation establishment and by predicted climate change. Western redcedar trees are one of the most shade-tolerant species in northwestern forests, but regeneration requires sufficient light and moisture. Previous attempts at direct seeding the species have been mostly unsuccessful. We modified environmental conditions of direct-seeded western redcedar in two ways: we altered (1) light with wire hardware cloth and (2) soil moisture with two types of mulch or no mulch. The treatment without mulch had significantly higher emergence, but seedlings in all treatments did not survive through the first season. Additional environmental factors and establishment strategies need to be considered for successful direct seeding of western redcedar.

Introduction

Western redcedar (*Thuja plicata* Donn ex D. Don) is an ecologically important and economically versatile species. The species grows in a variety of forest types and provides habitat and browse for animals (Minore 1990). Western redcedar has long been an important timber species (Haig et al. 1941); the wood is workable and durable, making it useful in a wide variety of applications, from roofing shingles to decorative chests (Nystrom et al. 1984, Minore 1990). Western redcedar is also valued for its beauty by the general public (Sharpe 1974). Despite this species' environmental, economical, and aesthetic value, establishing plantations or managing natural stands to increase the number of western redcedar trees can be challenging (Nystrom et al. 1984). In addition, predicted climate changes will shift the region of suitable growing conditions for western redcedar, which will require careful consideration of replanting schemes involving this species (Hebda 2009). These changes will force foresters to plan for a dynamic context and may require assisted migration of some species (Williams and Dumroese 2014).

Western redcedar is found on the Pacific Coast and in the Inland Northwest, with little overlap between the two ranges. In the Inland Northwest, the species grows from lat. 54°30' N. in British Columbia and south into Montana and northern Idaho (Minore 1990). Along the coast, its range extends farther south into California (lat. 40°10' N.) and north into southeast Alaska (lat. 56°30' N.). In the central part of its Pacific range, the species grows inland as far as the western slopes of the Cascades (Minore 1990). Western redcedar is distributed across a range of environmental conditions but grows best on moist, humid sites (Fan et al. 2008), such as in stream bottoms, moist flats, and north-facing slopes (Brand and Schopmeyer 2008). Precipitation within the coastal range for western redcedar ranges from 890 mm to 6,600 mm (35 in to 260 in), mostly as winter rain; the interior range receives 710 to 1,240 mm (28 in to 49 in) annual precipitation, as snow and rain (Minore 1990). Western redcedar is one of the most shade-tolerant species in northwestern forests (Coates and Burton 1999, Ferguson et al. 1986) and can grow on a variety of soils across a range of elevations (Brand and Schopmeyer 2008), although sedimentary bedrock can increase mortality (Moore et al. 2004). Western redcedar does not commonly grow in pure stands but grows readily within mixed stands (Sharpe 1974).

Western redcedar is present in all stages of forest succession (McKenzie and Tinker 2013), but natural regeneration depends on well-disturbed mineral soil and canopy gaps in established stands (Clark 1970, Gray and Spies 1996). Remnant individuals in old-growth stands provide sources of seed for regeneration (Keeton and Franklin 2005). Western redcedar can be a prolific, although erratic, seed producer (Gashwiler 1970, Minore 1990). Survival of seed through its first winter can exceed 90 percent

(Gashwiler 1967). The seed has low survival in storage for 3 months at 2.0 °C (35.6 °F), however, suggesting that naturally dispersed seed will not be viable for more than one season (Terskikh et al. 2008). Western redcedar seed is less susceptible to predation than other, larger conifer seeds (Gashwiler 1970). The seed may be less palatable because of its pungent odor (Gashwiler 1967). Vegetative reproduction can also occur in some stands (Parker 1986).

Understanding the conditions under which western redcedar regenerates requires consideration of both the establishment phase and the growth phase (Ferguson et al. 1986). Natural regeneration can occur on disturbed areas, indicating that western redcedar is exposure tolerant (Wang et al. 1994). Initial seedling survival, however, requires a balance between light and moisture (Carter and Klinka 1992). Mortality of naturally regenerating seed can be high soon after peak emergence, but, after September, additional losses are minimal (Gashwiler 1971). The seedling first grows primary needle leaves before growing secondary, scale-like foliage, which may correspond to decreased mortality later in the growing season (Weber et al. 2003). If seedlings establish in full sunlight, abundant moisture is required for survival (Weber et al. 2003). Conversely, western redcedar seedlings exhibit greater shade tolerance on sites of low water availability (Harrington 2006). High temperatures, drought, and frost-heaving are major causes of seedling mortality (Brand and Schopmeyer 2008, Gashwiler 1971, Soos and Walters 1963).

Some western redcedar seedlings can survive at 10 percent of full sunlight, but seedling mortality tends to be higher at low light levels (Harrington 2006, Soos and Walters 1963). Seedling growth responds positively to increasing light and soil disturbance (Carter and Klinka 1992, Weber et al. 2003), with maximum growth rates occurring at 30 percent to more than 40 percent full sunlight (Harrington 2006, Wang et al. 1994). At high light, however, seedlings are susceptible to sun scorching (Wright et al. 1998). Western redcedar seedlings are particularly vulnerable to drought during the first 2 years (McKeever 1942). Height growth is slow during the seedling's first 5 years and peaks during the sapling's second decade (Nystrom et al. 1984). Ungulates are known to browse western redcedar repeatedly and severely, dramatically decreasing the number of leaved shoots per individual and increasing mortality (Burney and Jacobs 2010, Martin and Baltzinger 2002). Once established, western redcedar stands can have low mortality for several decades (Lutz and Halpern 2006).

Public concern about the decline of western redcedar in the Northwest has existed since the early 1970s (Sharpe 1974). Foresters are keen to promote western redcedar regeneration because of the tree's value. In intact stands, however, intense competition from overstory trees and understory vegetation limits seedlings' access to light, soil water, and nutrients (Harrington 2006). In gaps and larger openings such as clear cuts, natural regeneration requires seed sources that are within 100 m (330 ft), and several seed crops may be needed to fully stock the site; good seed crops can be expected only every few years (Clark 1970). Open environments present other challenges to the seedling, including competition, browsing, and sun scorching. Artificial regeneration using direct seeding or planting may be required to achieve reforestation objectives. Planting seedlings can be a way to avoid the stochastic events surrounding natural seedling establishment (Coates 2000). Seedlings need to be appropriately hardened for field conditions (Major et al. 1994). Direct seeding may be a low-cost option for regenerating western redcedar if successful techniques can be developed.

Successful direct seeding for any species requires proper timing, sufficient seed, predation and competition control, a suitable seedbed, and adequate soil moisture (Farmer 1997). Direct seeding has been used to reforest large areas of land in the American Southeast and has been particularly useful in large, remote, or low-productivity sites (Barnett 2014). Efforts to direct-seed western redcedar have generally proved unsuccessful, with lower germination and survival in western redcedar than Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson), and grand fir (*Abies grandis* [Douglas ex D. Don] Lindl.) (Engstrom 1955, Loewenstein and Pitkin 1966). Direct seeding has been most successful on north-facing sites with some shade and little competition; even under these conditions, however, the results have been only moderately successful (McKeever 1942). Direct seeding in fall may result in higher survival than in spring, although total survival through the first growing season was low in both treatments (Loewenstein and Pitkin 1966).

The objective of this study was to evaluate environmental influences on establishment success of direct-seeded western redcedar. We modified the environment using wire hardware cloth and mulch. Wire hardware cloth limits access by herbivores to the seeds and small seedlings (McKeever 1942) and hardware cloth increases shading on the seed by 15 to 21 percent (Minore 1972, Strothman

1972), which may help reduce mortality caused by high surface temperatures (Fowells and Arnold 1939). Mulch has a lower thermal admittance than bare soil, thereby helping to mitigate soil temperature and moisture stresses to newly germinated seedlings (Campbell and Norman 1998). The mulch retains moisture, which may also reduce water stress in the seedling. We hypothesized that seeds in the mulch and hardware cloth treatments would have higher emergence than the treatment with no environmental modifications.

Materials and Methods

This study was conducted on a relatively level, tilled agricultural field with coarse, loamy soil at the University of Idaho's Pitkin Forest Research Nursery (46°43' N, 116°57' W). The site receives an average of 600 mm (23.6 in) of precipitation annually, and the average summer and winter temperatures are 18 °C and 0 °C (64 °F and 32 °F), respectively (Western Regional Climate Center 2005). No persistent vegetation existed at the site.

Northwest Seed (IFA Nurseries, Canby, OR) supplied the seed on behalf of Potlatch Corporation. The seed was collected at 883 m (2,900 ft). The seed arrived at Pitkin Forest Research Nursery in sealed pouches on October 16, 2013, and was stored dry in a cooler at 0 to 1.5° C (32 °F to 35 °F) for 4 weeks until direct seeding. The seed was not soaked or cold stratified before direct seeding, because stratification does not change germination capacity in western redcedar (Khadduri 2007).

Five frames were constructed from plywood and placed on top of the soil at the research site (figure 1). Each frame was divided into six 15-by-15 cm (5.9-by-5.9 5.9 in) sections. Within each frame, six treatments were randomly assigned to the sections (three mulch treatments by two screening treatments). Mulch treatments consisted of no mulch, pine mulch, or straw mulch. Screening treatments consisted of wire hardware cloth or no wire hardware cloth. The pine mulch was aged pine needles collected from a stand of ponderosa pine adjacent to the field site. The straw mulch was from baled wheat straw. The pine and straw mulch pieces were similar in size with a maximum length of 12.7 cm (5 in) and interspersed smaller pieces. The screened sections were covered with 6.35-mm (0.25-in) hardware cloth, which sat on top of the frame, about 10 cm (3.9 in) above the soil. The unscreened sections were left uncovered.

Figure 1. To assess environmental influences on direct seeding of western redcedar, five wooden frames were constructed, each with six treatment sections. After sowing, seed were subjected to three mulching treatments (pine, straw, or no mulch), with or without wire hardware cloth screening. (Photo by Rebecca Sheridan, 2013)

Before direct seeding, a minimal number of weeds were hand weeded from the site and the ground was lightly scarified with a rake. The seeds were sown on November 15 and 17, 2013. In each section, 100 seeds were surface sown in a 10-by-10 grid, spaced 1.27 cm (0.5 in) apart. In the mulched treatments, the respective mulch was spread across the section approximately 2 cm (0.78 in) deep. No followup treatment was done to ensure seed-soil contact; however, the soil was wet at the time of sowing, and the seed stayed in contact with the soil once sown. The site received no maintenance from the time of seeding until the seed began to germinate. The plots were hand weeded through the spring and summer.

Because, western redcedar germination is epigeal, seedling emergence was defined in this study as the presence of the hypocotyl hook above the soil surface (figure 2). In April and May 2014, the plots were checked weekly for newly emerged and newly dead seedlings. From May to October 2014, the plots were checked monthly. Each newly germinated seedling

Figure 2. Seedlings were counted as emerged when the hypocotyl hook was visible above the soil surface. Emerged seedlings were marked with color-coded pins. (Photo by Rebecca Sheridan, 2014)

Figure 3. Seedling emergence was monitored from March through October 2014. On each monitoring date, different colored pins were used to mark newly emerged seedlings. Dead seedlings were marked with a black pin. By October 2014, all seedlings in the experiment had died. (Photo by Rebecca Sheridan, 2014)

was marked with a colored, ballpoint pin, with a different color used each week. When a seedling died, it was marked with a black pin (figure 3).

In addition to the field study, a germination test was conducted with four replications of 100 seeds each. The seed was soaked in cold, running water for 24 hours and then was cold stratified for 1 month at 0 to 1.5 °C (32 to 35 °F) (December 18, 2013 to January 15, 2014). Seeds were then placed on moist germination paper under a full-spectrum light for approximately 12 hours daily (Karrfalt 2008). The temperature fluctuated several degrees around 21 °C (70 °F). The seed was misted three times per day. Germinated seeds were counted every 7 days for 28 days. Germination was defined as the presence of a 5 mm (0.2 in) radicle (Baskin and Baskin 2014).

Statistical analyses were done in R, version 3.1.1 (R Core Team 2015). The experimental design consisted of a factorial (three mulch treatments by two hardware cloth treatments) completely randomized design with five replications. An analysis of variance was performed to test the treatment effects on the total number of emerged seeds in the field trial. Differences among treatment means were determined using Tukey's range test at the $\alpha \leq 0.05$ level. Diagnostic plots for equal variance and normality were examined and no data transformations were deemed necessary. Overall germination average and standard error were determined on the germination test data using Microsoft Excel statistical tools.

Results

In the germination test, the average germination was 81 percent (n = 4, standard deviation = 6.7 percent). In the field planting, however, average emergence across all treatments was 31 percent (n = 30, standard deviation = 7.5 percent). Emergence was quantified in the field planting rather than germination because the radicle was not visible on seeds in the field.

The first seedlings emerged by April 12, 2014, which was defined as week 1. Seedling emergence occurred earlier in the bare soil plots than the plots with mulch (figure 4). Seedlings began dying by the second week of observation, well before emergence was complete (figure 5). More than one-half of the seedlings were dead by week 8 (May 27, 2014). Some seedlings survived into September (figure 6) but, by week 27 (October 20, 2014), all seedlings in all treatments died and monitoring ceased. Dead seedlings were most often found intact and standing upright, with no sign that the cause of death was a pathogen or herbivore.

The highest total emergence occurred in the nonmulched with wire screening treatment (38.6 percent) and the lowest total emergence occurred in the straw mulch with no wire screening treatment (24.4 percent) (table 1). Seed in the nonmulched treatments had significantly higher total emergence than seed in the needle mulch or straw mulch treatments ($p < 0.01$). Emergence did not differ significantly between the two mulch types. No significant interactions occurred between the screening and mulching treatments nor was a significant difference observed between total emergence in screened and nonscreened treatments.

Table 1. Average total seedling emergence percent and standard deviation by treatment (n = 5). Seed in the nonmulched treatments had significantly higher total emergence than those in the mulched treatments ($p < 0.01$). Emergence did not differ significantly between the two mulch types or between screened and nonscreened treatments. No significant interactions occurred between the screening and mulching treatments.

Variable	No mulch		Pine mulch		Straw mulch	
	Without screening	With screening	Without screening	With screening	Without screening	With screening
Percent emergence	36.40	38.60	31.00	28.60	24.40	29.00
Standard deviation	1.95	9.02	4.95	8.56	1.95	6.44

Discussion

This experiment modified the seedbed environment to reduce light (screening treatment) and increase available soil moisture (mulch treatment). These modifications, however, were not sufficient to ensure western redcedar seedling survival past the establishment phase. More than one-half of the seedlings died before July and August, the hottest months of the year, at the field site (Western Regional Climate Center 2005). Soil temperature, soil moisture, and shade levels were not directly measured, but the seedlings likely died from high temperatures and low soil moisture. In a similar way, natural regeneration of western redcedar has been unsuccessful on high fire-severity sites, with high temperatures and low moisture conditions (Larson and Franklin 2005).

The wire hardware cloth was intended to provide some shade and also to limit access by herbivores to the seeds and seedlings. In southern pine forests, seed predation by rodents and birds is a major challenge to successful direct seeding (Barnett 2014). No significant effect of the wire hardware cloth was observed, however, suggesting seed herbivory did not occur in this experiment. Some seedlings were observed with damage from invertebrates, but no evidence suggested damage by vertebrate herbivore. If the seedlings had survived, however, herbivory would be a matter of concern for larger western redcedar seedlings (Stroh et al. 2008). In such cases, fertilization may aid in recovery from browse (Burney and Jacobs 2010).

Mulch can also help reduce the number of weeds on a site. In this experiment, the site was routinely weeded, so the ability of the mulch to suppress weeds was not quantified. If weeds had been present, they would have competed with seedlings for soil moisture. Removal of competing vegetation can lead to greater height growth of western redcedar than only removal of light competition, suggesting competition for water is more important than competition for light (Adams and Mahoney 1991). Weedy vegetation can also compete for soil nutrients, but western redcedar have deep-rooted, fine roots, which can reduce competition for nutrients (Messier 1993).

Both seedling emergence and mortality were observed earlier and at higher levels in the bare soil plots compared with the mulched plots (figures 4 and 5). It is important to note, however, that the absence of mulch in the bare soil plots made it easier to observe emerging seedlings. For eastern white-cedar (*Thuja occidentalis* L.), seed that falls on forest floor litter has a lower chance of survival than seed that falls on nurse logs or mineral soil (Simard et al. 2003). In this experiment, the seed was in direct contact with mineral soil and then was covered in mulch. Although great care was taken to count seedlings within the mulch, additional seeds may have emerged below the mulch and died before they were observed. The bare soil alternatively may have warmed earlier in the spring, allowing for earlier germination and emergence.

The seed was not stratified before planting but was subjected to cold, moist temperatures through the winter months. The need for cold stratification in western redcedar is debated, with some authors suggesting no stratification is needed (Brand and Schopmeyer 2008). Kolotelo (1996) observed no effect of a 3-week stratification period. We do not believe the lack of artificial cold stratification affected the experimental results.

Conclusions

It is important to understand the whole-plant response of seedlings to environmental factors such as light levels, water stress, and competition to choose the best method, species, and site combinations for successful regeneration projects (Coates and Burton 1999). These factors interact with one another in the field, impacting seedling development in complicated ways (Harrington 2010). In this experiment, germinated seedlings did not survive in spite of modifications to the microenvironment. If direct seeding is

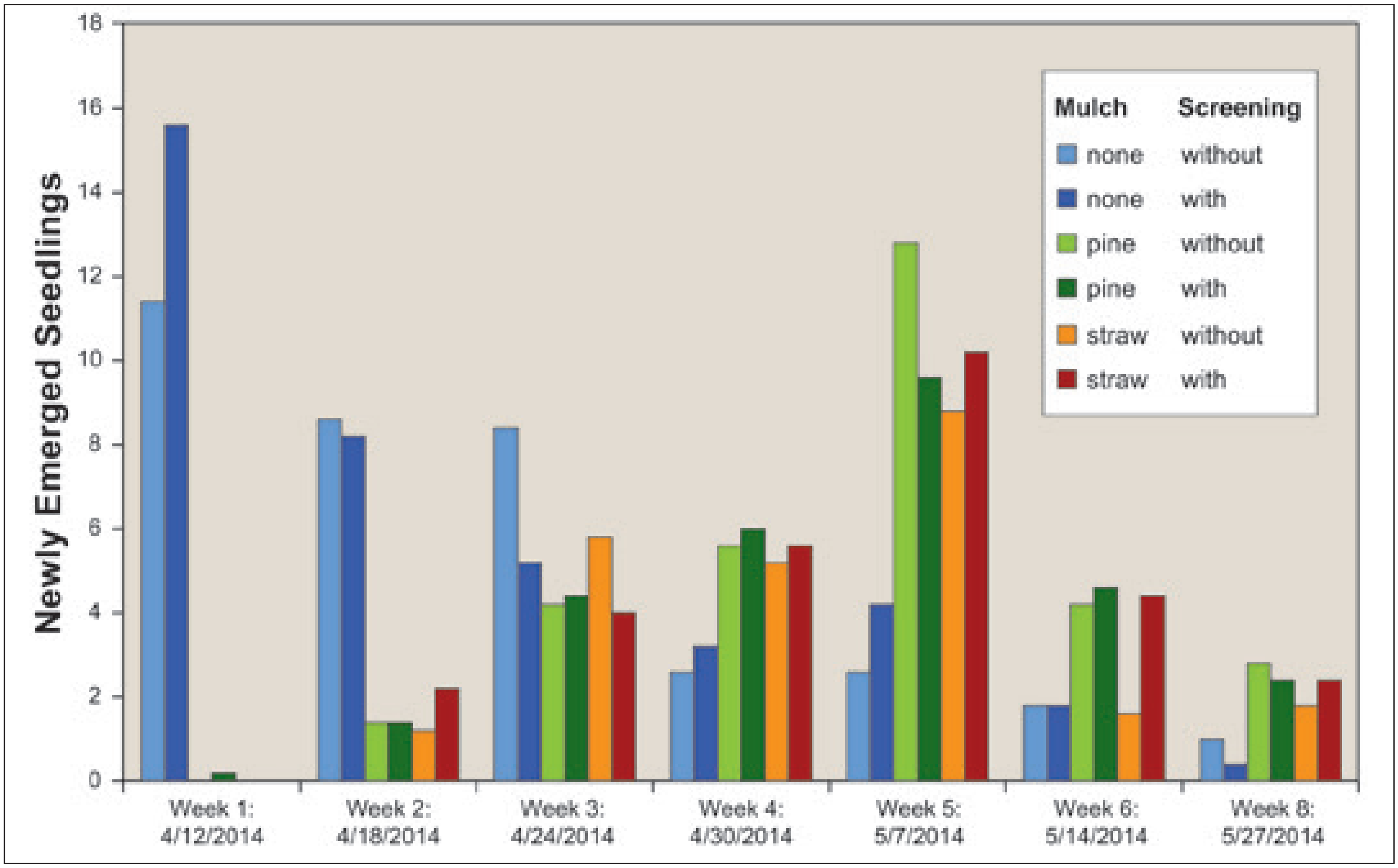

Figure 4. Number of newly emerged seedlings from April through May 2014, as affected by mulching and screening treatments. Seedlings were marked with a pin to ensure they were not recounted.

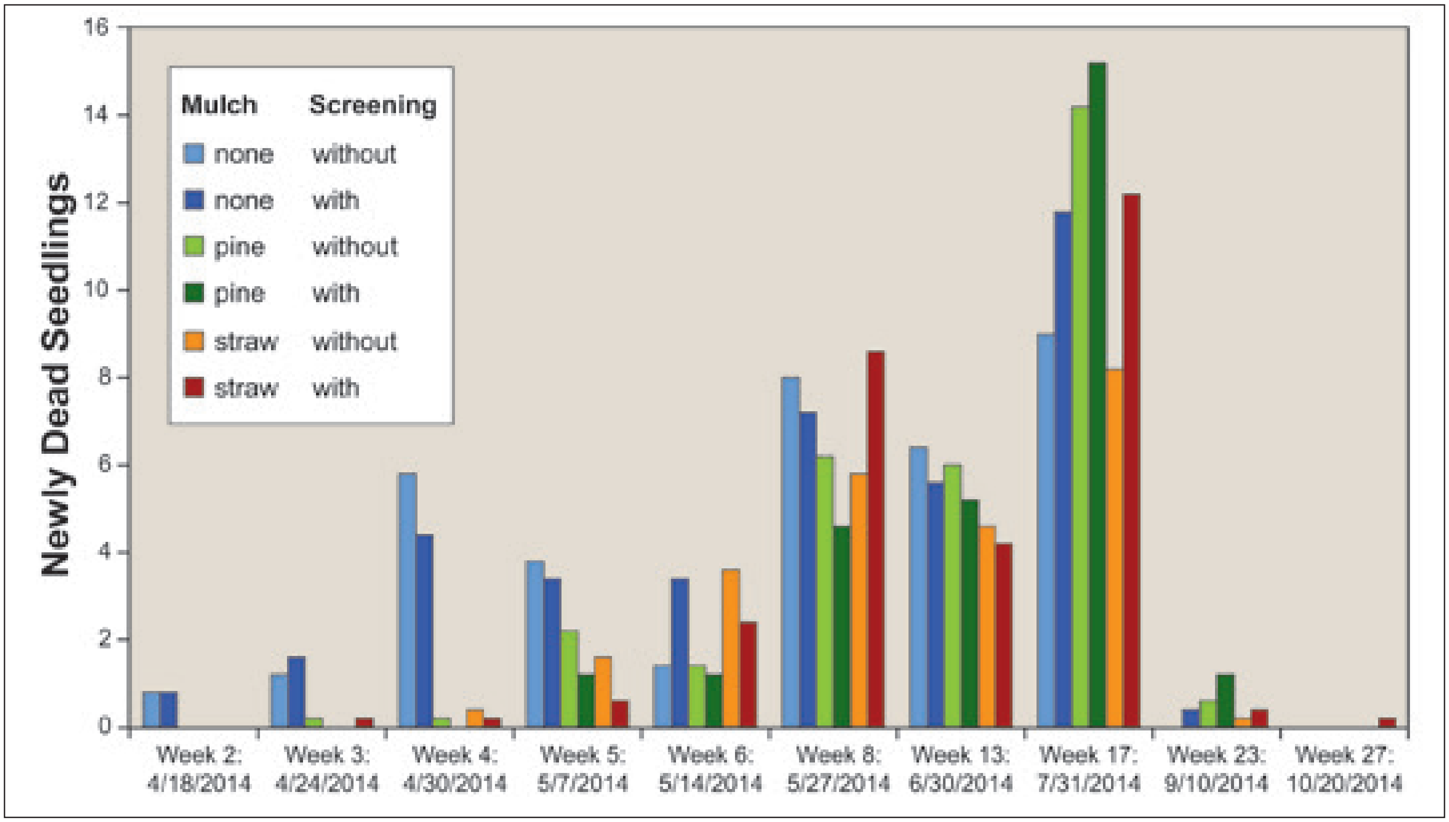

Figure 5. Number of seedlings that died, by week, starting on April 18, 2014. Dead seedlings were marked with a black pin to ensure they were not recounted.

to be successful with western redcedar, it can be considered only on carefully selected sites, and, even then, success is not guaranteed. Based on current approaches, planting seedlings is still the most successful method to ensure western redcedar establishment. Further investigation to develop strategies for direct seeding of this species, such as the use of pelletized seed (Khadduri 2007), is needed if direct seeding continues to be a desirable approach.

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Development of a Backpack-Mounted Pollen Vacuum

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Abstract

Supplemental pollination is regularly used in seed orchards to minimize gain dilution due to pollen sources outside the orchard. This practice requires large amounts of pollen. Standard pollen-collection methods can be labor intensive. This article describes parts needed and assembly steps for constructing a backpack-mounted vacuum system that is cord free and maintains suction efficiency without the need for filtration bags. This pollen-collection system has been used successfully at the Washington Department of Natural Resources Meridian Seed Orchard. The vacuum system also has potential for collecting native plant seed.

Introduction

Most Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings planted in western Oregon and Washington are grown using orchard seed from tree-improvement programs. The genetic gain level of orchard seed continues to increase as breeding and testing programs provide higher gain parents for inclusion in seed orchards (Jayawickrama 2005, St. Clair et al. 2004). As gain increases, the problem of gain dilution due to pollen contamination from sources outside orchard boundaries becomes more significant. One way to minimize gain dilution due to pollen contamination is to apply supplemental pollination, using pollen collected from high-gain parents. This type of pollen management is facilitated by the adoption of high-density orchard systems, similar to those used in fruit horticulture (Kolpak et al. 2015). These orchard systems keep crowns within reach from the ground, allowing for better access to pollen catkins and cone flowers and more rapid movement among trees. To take advantage of this improved orchard structure, however, better tools are needed.

Supplemental pollination programs require efficient collection of large amounts of pollen. The standard method of collecting Douglas-fir pollen for breeding work or supplemental pollination has been to collect branchlets of pollen catkins just before pollen shed, dry them under controlled conditions to enhance shedding, then sift the pollen through a sieve to remove needles, catkins, and other debris from the pollen (Webber and Painter 1996). With this method, however, both the collection and processing stages are labor intensive. Vacuuming the pollen from the trees is a more efficient process that eliminates the collection and processing of branchlets. The primary disadvantage of using a vacuum is that conditions must be favorable for pollen shedding, which usually occurs on dry afternoons. When weather is unfavorable, collecting branchlets will probably continue to serve an important role.

Pollen Vacuum Systems

Several vacuum systems were developed for orchard pollen collection in the 1990s by researchers at the U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station (Copes et al. 1991, Silen 2000). Although these and similar systems work, they are cumbersome to move around the orchard, particularly when visiting many small trees. Some orchardists have used electric backpack canister vacuums, such as those used by cleaning staff in large commercial buildings. While the units themselves are fairly light and mobile, workers are still tethered to an electrical cord plugged into a generator that must be moved through the orchard. Also, this style of vacuum typically uses a filtration bag to capture material. The bags tend to plug up fairly quickly, reducing suction and requiring frequent replacement. Some gas-powered leaf blowers come with adapters to allow them to be used as vacuums. Using this type of leaf blower would allow for freedom from electric cords but would still have the same issue with plugging the filtration bag.

Meridian Pollen Vacuum System

We combined a gas-powered leaf blower with a small cyclone separator to create a backpack-mounted vacuum system that is free from electric cords and maintains suction efficiency without the need for changing filtration bags. The key features are a fitting that captures the inflow to the blower so that it can be routed through a vacuum hose and a small cyclone separator that allows the pollen to fall out of the air stream before the air enters the blower and is expelled (figures 1 and 2). We tested the vacuum as described in this article and confirmed that essentially all pollen entering the vacuum is being captured by the separator, even at full throttle.

Vacuum Assembly

See table 1 for a list of parts needed. We provide brands and models of the components that we used simply because we tested the units using these components and verified that they work properly. Other brands and models may also work well, but we did not test other options. We did find that the cooling system of some blowers is not compatible with the airflow constriction inherent in this setup, which can result in engine overheating and failure.

Assembly Steps

[Conversions: 1 in = 2.54 cm; 1 ft = 30.48 cm]

1. **Assemble power unit.**
   a. Cut power unit shelf (9 by 13 in) from sheet of ¼-in plywood. On each 9-in side, cut a notch 3 in long and ½ in deep, centered between the corners.
   b. Attach blower to plywood shelf as follows:
      i. Blower should be positioned so that blower base under air outlet is 1.5 in from 9-in side of shelf, and black handle is flush with 12-in side of shelf; mark this position on the shelf with a pencil.
      ii. Make 2 straps, 2 holes long, from steel hanger strap; bend between the holes to form an "L" bracket. Place each L against the feet on the inlet side of the blower base along the 12-in side of the shelf, and mark the position of the strap hole on the plywood.

Table 1. Parts required for assembly of Meridian Pollen Vacuum System.

Power unit	Separation unit	Power unit to separation unit connection	Suction hose and funnel
1—pack frame (Alaskan Outfitter frame from Cabela's)	1—small cyclone separator (Dust Deputy from Oneida Air Systems; part #AXD001004)	1—4-by-2 in rubber coupling for ABS pipe	4 ft—1 ¼ in flexible vacuum hose (bought from local vacuum store)
1—gas-powered leaf blower (Echo ES-250)	1—clear plastic jar with 5-in diameter lid [it is somewhat difficult to find this size; we found one holding peanut butter-filled pretzels]	1—2 in PVC 90° elbow	1—2 ¼-by-1 ¼ in reducer (bought from local vacuum store)
1—2 -by-2 ft piece of ¼ in plywood (cut down to 9 by 13 in)	1—3 ft long by ¾ in wide by 1/8 in thick aluminum bar	1 ft—2 in Class 200 PVC pipe	1—right-angle hose adapter (from Oneida Air Systems, part #AHA000004)
1—3 ft long by ¾ in wide by 1/8 in thick aluminum bar	1—8-by-9 in piece of ¼ in plywood (cut from sheet listed under power unit list)	1—2 ½ in diameter by 5 ft long Dust Deputy flex hose with cuffs (from Oneida Air Systems, part #AXD250066)	2—hose clamps large enough to fit over 1 ¼ in hose
4—vibration dampeners; ¼ in male coarse thread by ¼ in female coarse thread (McMaster-Carr part #9378K31)	4—1 ½ in L-brackets (can also make these from aluminum bar)	1—right-angle hose adapter (from Oneida Air Systems, part #AHA000004)	1—large funnel, with outlet sized to fit snugly into 1 ¼ in hose
1 roll—3/4 in-by-10 ft galvanized steel hanger strap	12—¼ in bolts, 20 thread, ¾ in long	4—½ in sheet metal screws	1— 9-by-1 in PVC pipe
6—¼ in bolts, ¾ in long, coarse thread	4—¼ in bolts, 20 thread, 1 ½ in long	1 roll—duct tape	2—1 ½ in long, 20 thread, ¼ in bolts
4—¼ in bolts, 1 in long, coarse thread	4—¼ in flat washers		2— ¼ in lock washers
4—¼ in bolts, ½ in long, coarse thread	16—¼ in lock washers		2—¼ in nuts
16—¼ in flat washers			
18—¼ in lock washers			
10—¼ in nuts			
2—#8, ½ in long sheet metal screws			

ABS = acrylonitrile-butadiene-styrene; PVC = polyvinyl chloride. Conversions: 1 in = 2.54 cm; 1 ft = 30.48 cm.

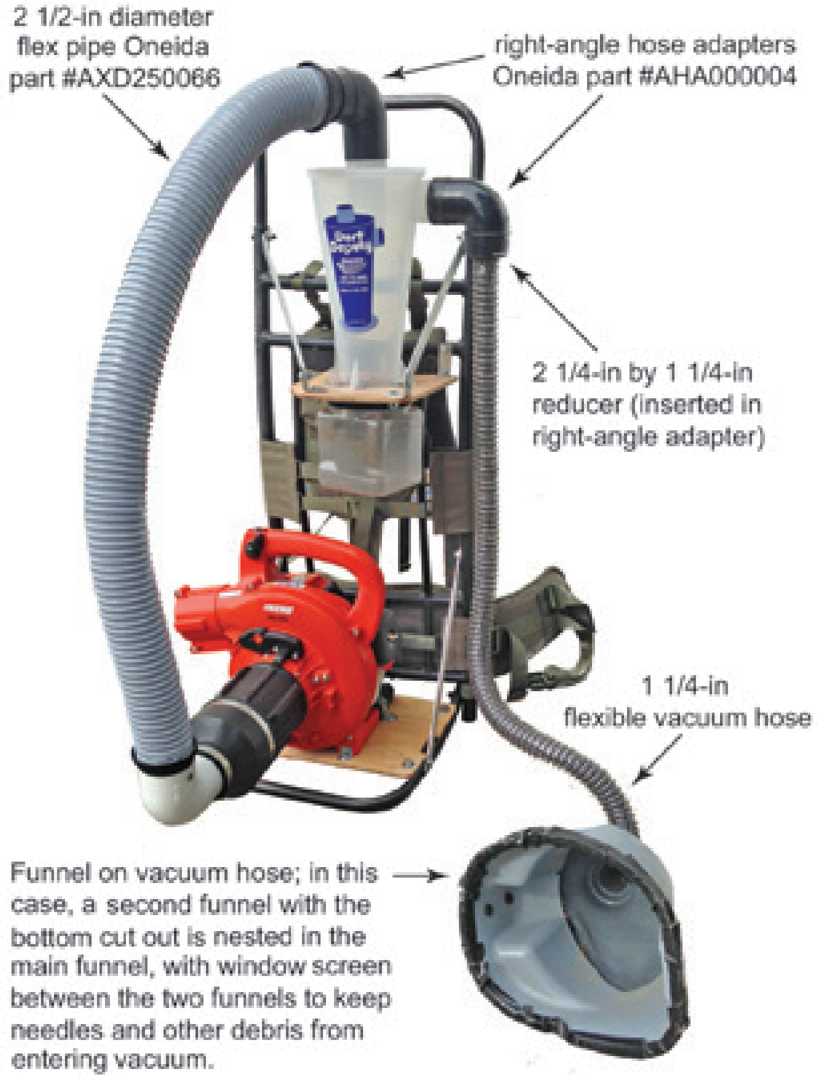

Figure 1. Rear view of Meridian Pollen Vacuum System. Conversions: 1 in = 2.54 cm; 1 ft = 30.48 cm. (Photo by Rocky Oster, 2015)

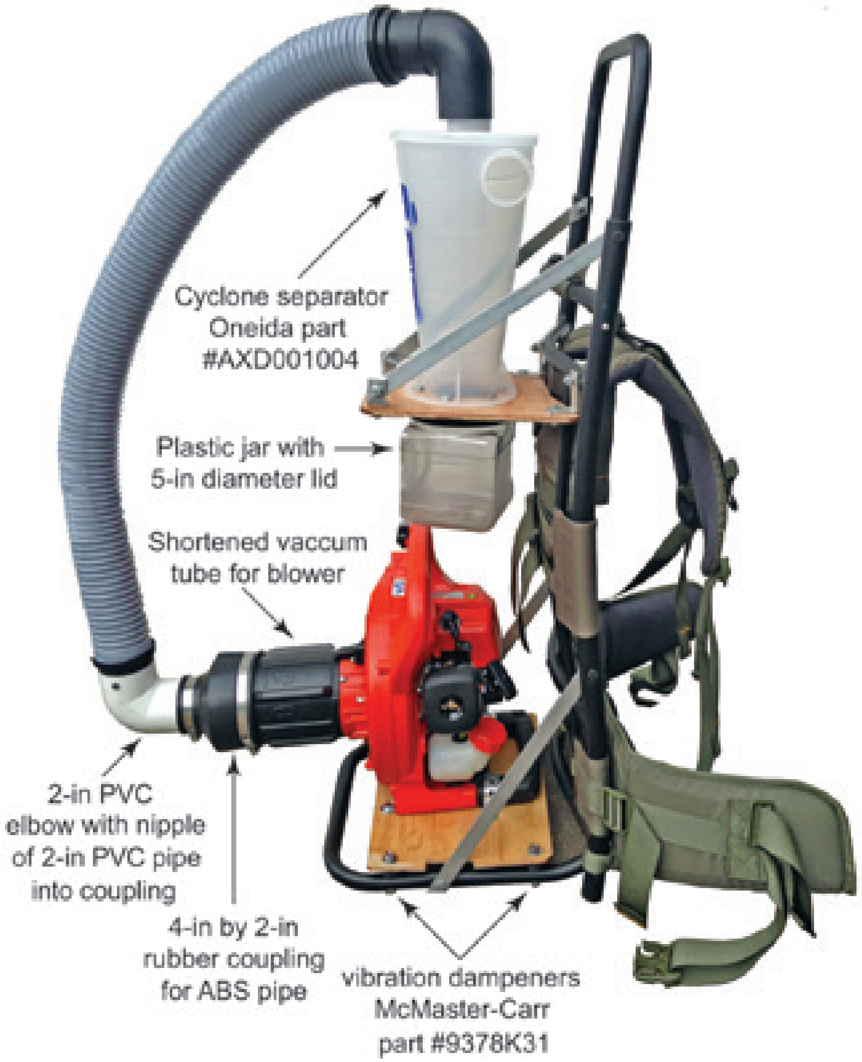

Figure 2. Side view of Meridian Pollen Vacuum System. Conversions: 1 in = 2.54 cm; 1 ft = 30.48 cm. (Photo by Rocky Oster, 2015)

   iii. Cut 2 straps, 8 holes long, from steel hanger strap. Bend these straps over black handle on blower base, and mark the positions of the end holes on the plywood.
   iv. Move the blower off the plywood base, and drill 5/16-in holes in the plywood at the 6 positions marked in steps ii and iii above.
   v. Place the blower back on the plywood base. Attach the straps over the black handles using ¼-in by ¾-in long bolts with flat washers on both sides of the plywood and lock washers between the flat washers and nut.
   vi. Attach the "L" brackets to the plywood with the same bolt/washer/nut combination in step v above; drill a 1/8-in hole through the hole in the "L" bracket into the plastic foot of the blower. Use ½-in long #8 sheet metal screws to attach "L" brackets to blower base.

2. **Mount power unit on pack frame.**
   a. Remove bolts holding bottom shelf on pack frame; separate shelf from frame, and remove cloth platform from shelf.
   b. Reattach shelf frame to pack frame using the original bolts.
   c. Drill a 5/16-in hole in the outer tube on each side of the pack frame, 11.5 in above the base of the tube.
   d. Drill a 5/16-in hole through each side of the shelf frame, 7 in from the pack frame.
   e. Cut two 15-in pieces of aluminum bar; drill holes near the ends of the bars, 13.25 in apart.
   f. Use aluminum bars to brace shelf to pack frame at points drilled in steps c and d above; use ¼-by-1.5 in long bolts, lock washers, and nuts.
   g. Place power unit on lower shelf and drill 5/16-in holes through plywood and metal tube of shelf at four corners of plywood. Attach plywood to shelf using vibration dampeners between shelf and plywood (figure 2), with male end of dampeners through plywood and ¼-by-1 in long bolt through lower shelf into female end of dampeners. Use flat washers, lock washers, and nuts on male end. Use lock washers on bolts into female end. To minimize vibration, be sure that neither plywood nor blower body contacts pack frame or aluminum braces directly.

3. **Assemble separation unit.**
   a. Cut separation unit shelf (8 by 9 in) from sheet of ¼-in plywood. Place an 8-in side against the pack frame at the cross bar behind the shoulder strap. Trace the curve of the cross bar on the plywood, and cut this curve into the plywood so the shelf can conform to the curve of the pack (figure 2).
   b. Refer to the lid template from the Oneida web site; use a compass to mark a hole of correct diameter (about 3 in) on the lid of the plastic jar and on the separation unit shelf. Cut the holes in the plywood and plastic lid.
   c. Look at the base of the cyclone separator. New holes need to be drilled closer to the walls of the separator so that the bolts will not interfere with the lid of the plastic jar that will be attached below. Following the same pattern as the existing holes, mark the placement of new holes that will be as close as possible to the walls of the separator while still allowing room for the heads of the bolts. New holes should be rotated so they are halfway between existing holes. After the new spots are marked, drill 5/16-in holes from below.
   d. Center the cyclone separator over the hole in the plywood shelf (figure 2). The inlet of the separator should be adjacent to the curved side of the plywood and pointing toward the adjacent 9-in straight side (figure 2). Mark the newly drilled holes in the separator base on the plywood. Drill 5/16-in holes at each of those positions.
   e. Center the plastic jar lid on the base of the cyclone separator, and mark the new holes from the separator on the jar lid. Drill the holes in the jar lid.
   f. Use ¼-in by ¾-in long bolts with lock washers and nuts to sandwich the plywood shelf between the cyclone separator and the plastic jar lid (figure 2). Apply caulk between the layers to ensure a good seal in case of any gaps.

4. **Mount separation unit on pack frame.**
    a. Use four 1 ½-in L brackets with ¼-in bolts, lock washers and nuts to attach back of upper shelf to cross bar on pack frame (1 ½-in long bolts through pack frame, ¾-in long bolts for L brackets to plywood). New holes need to be drilled in pack frame for this step.
    b. Use ¼-by-1 ½ -in long bolts to attach aluminum bars to pack frame. Use ¼-in by ¾-in long bolts to attach aluminum bars to plywood shelf using L brackets. Use lock washers at all nuts and flat washers with lock washers on plywood surfaces. New holes must be drilled for each of these bolts.

5. **Connect power unit to separation unit (see figure 2).**
    a. Cut blower vacuum tube so that tube extends 2 in beyond ridges at base.
    b. Remove cover on inlet side of blower and screw shortened vacuum tube into blower.
    c. Attach rubber coupling to vacuum tube.
    d. Glue 2.5-in long piece of 2-in PVC pipe into the PVC elbow.
    e. Insert PVC pipe extending from elbow into rubber coupling and tighten.
    f. Place the Oneida right-angle hose adapter on the top of the cyclone.
    g. Shorten the Oneida flex hose to 40 in, and use it to connect the elbow adapter at the top of the cyclone unit to the PVC elbow pointing up from the power unit (figure 1). The fitting on the bottom end of the flex hose needs to be wrapped with several layers of duct tape to fit snugly into the PVC elbow. To keep the connections between the vacuum tube and the blower, as well as the hose and the PVC elbow, from vibrating loose, drill and insert ½-in long #8 sheet metal screws.

6. **Connect suction hose and funnel (see figure 1).**
    a. Attach Oneida right-angle hose adapter to inlet side of cyclone.
    b. Fit 2 ¼-in by 1 ¼-in reducer into elbow.
    c. Slide 1 ¼-in vacuum hose over reducer and attach with hose clamp.
    d. Insert funnel into other end of vacuum hose and attach with hose clamp.
    e. Bolt 8-in long segment of 1-in PVC pipe to back of funnel to serve as a handle. Use 1 ½-in nuts, lock washers, and bolts for this.
    f. For pollen-collection use, consider installing window screen material over top of funnel to screen out needles, catkins, and other debris.

Figure 3. Using the Meridian Pollen Vacuum System, one person can collect more than 0.5 qt (500 cc) of pollen per hour under good shedding conditions. (Photo by Rocky Oster, 2015)

Operating the Meridian Pollen Vacuum

We have successfully used the vacuum described in this article to collect a large volume of pollen in a short time when shedding conditions are good (figure 3). Good shedding conditions typically occur on warm, dry afternoons when humidity is low. If pollen sheds easily when branches are lightly tapped, vacuuming conditions are good. It is important to focus on individual trees that are shedding heavily, because large tree-to-tree variation exists on any given day due to phenological differences. We found that, under good shedding conditions and focusing on the most productive trees, one person can collect more than 0.5 qt (500 cc) of pollen per hour.

Although our reason for developing this backpack vacuum was to collect pollen, we believe it may also have utility for efficient collection of some kinds of native plant seed. The system would need to be tested on each species, however, to confirm that the cyclone separator is effectively collecting the desired seed.

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Black Walnut Allelopathy in a 28-Year-Old Loblolly Pine Stand and Implications for Initial Plantation Establishment

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Abstract

Damaged and dying loblolly pine trees (*Pinus taeda* L.) were found around black walnut trees in a 28-year-old plantation in Chatham County, NC. The damage and mortality are attributed to disease caused by walnut allelopathy. Damage is first evident by the presence of resin exudation on the lower trunks of affected trees. As damage progresses, resin exudation occurs higher on the trunk, eventually reaching heights of more than 10 ft (3 m). The phloem and sapwood beneath the resin exudation are killed thereafter leading to termite invasion and bird predation. Affected trees eventually die. Sampling of black walnut trees and the affected pines in their vicinity indicated the following: most of the resin exudation (95.6 percent) is found on the side of the pines facing the black walnut tree, pine mortality increases as resin flow height increases, larger diameter black walnut trees result in farther damage extent, and damage extends beyond the dripline of the black walnut tree. Before establishing loblolly pine plantations, the planting area and vicinity should be surveyed for the presence of black walnut trees. It is recommended that no pines be planted within 35 ft (10 m) of driplines of established black walnut trees. If black walnut trees are found in the vicinity of a pine plantation, the plantation should be surveyed at least every 5 years to locate and eliminate any newly established black walnut seedlings.

Introduction

Black walnut (*Juglans nigra* L.) is an extremely valuable hardwood tree found in Eastern North America from southern Ontario, Canada, in the north to northern Florida in the south (Burns and Barbara 1990). Wood from this species is used in furniture manufacture, both as solid boards and veneer, and in gunstock manufacture. Black walnut is shade intolerant and develops best on moist, deep, well-drained soils, although it can survive and grow on a wide variety of other soils.

Black walnut is an allelopathic species. *Allelopathy* is defined as “any direct or indirect harmful effect by one plant on another through production of chemical compounds that escape into the environment” (Rice 1974). Allelopathy was first documented in the United States in Wisconsin in the late 19th century (Hoy and Stickney 1881) when black walnut was reported to cause adverse effects to other plants. Davis (1928) identified the causative allelopathic agent produced by black walnut as juglone (5-hydroxy-1, 4 napthalenedione). Appleton et al. (2009) summarized information on production of juglone and included a list of known susceptible and resistant plant species. Juglone is produced in walnut husks and leaves and is exuded by the roots. Once exuded, juglone remains in the soil around walnut roots and can injure roots of susceptible plant species within 0.25 to 0.5 in. (0.64 to 1.27 cm.) of a root. Injury to susceptible plants can include wilting, chlorosis, necrosis, or mortality. Pine (*Pinus* spp.) is a susceptible genus (Appleton et al. 2009).

Several studies have confirmed the allelopathic effect of black walnut on other species. Gabriel (1975) studied the allelopathic effects of walnut on white birch (*Betula papyrifera* Marsh) and observed that mortality of birch seedlings planted near walnuts began the first year after they were planted. He also noted that, as surviving seedlings grew older, their vigor increased as their distance from walnut trees increased. Fisher (1978) conducted a field study in a 22- to 25-year-old mixed plantation of red and white pine (*Pinus resinosa* Ait. and *P. strobus* L.) and black walnut in Ontario, Canada. The study compared pine growth and survival in the mixed pine-walnut stand between well-drained Brant soils, imperfectly drained Toscola soils, and poorly drained Colwood soils. The pines growing adjacent to the walnuts on the Brant soil showed no significant effect from walnut allelopathy, but the pines growing on the Brant soil had significantly poorer survival and growth. The pines on the Colwood soil that were adjacent to the walnuts all died.

Funk et al. (1979) conducted laboratory studies on the effects of juglone on seedlings of Japanese larch (*Larix kaempferi* [Lam.] Carrière), Norway spruce (*Picea abies* [L.] Karst.), white pine, and Scots pine (*Pinus sylvestris* L.). The seedlings were grown in a hydroponic system for 8 to 10 weeks in various juglone concentrations. At high concentrations, juglone was toxic to all species. At moderate concentrations, no visible injury to white pine was observed, but seedling growth was inhibited. Appleton et al. (2009) summarized information on production of juglone and included a list of known susceptible and resistant plant species.

The objective of this study was to determine if black walnut allelopathy caused observed damage to a 28-year-old loblolly pine plantation growing in proximity of black walnut trees and to document damage symptoms.

Materials and Methods

A 115-ac (46.5-ha) loblolly pine (*Pinus taeda* L.) stand was planted in Chatham County, NC, in 1986. The plantation site included an abandoned homestead surrounded by several black walnut trees and also scattered black walnut trees along a small stream. These black walnut trees were not removed before the pines were planted so they and the walnut seedlings established from their nuts competed with the pines. Because walnut seedlings were found at distances well beyond the driplines of the parent trees, the long-distance movement was probably caused by gray squirrels burying nuts resulting in randomly scattered walnut trees of various ages throughout the pine stand. Before the pines were planted, most of the property was in fescue pasture, which was furrowed before planting to reduce vegetative competition. The pine stand was thinned for pulpwood in 2001 and 2014. Before the 2014 thinning, an examination revealed that pine trees were dying and that the mortality appeared to be associated with the presence of black walnut trees. Affected pine trees had notable resin exudations on their boles.

To determine if walnut allelopathy was the causal agent of the observed pine damage, 29 black walnut trees and 97 affected loblolly pines around them were located and measured. Because the black walnut trees were randomly scattered throughout the stand, a considerable amount of searching along transects was required to locate trees for inclusion in the study. Pine trees along the transect lines were carefully examined to determine if symptoms around the walnut trees also occurred away from the influence of those trees.

Walnut trees were measured for diameter at breast height (DBH) determined in 2-in (5.1-cm) diameter classes, tree height, and distance to the nearest and farthest affected pine trees. Pine trees were measured for DBH, distance to the nearest walnut tree, number of resin exudations, and height of resin exudations from ground level.

During initial sampling, it appeared that most resin exudation occurred on the side of the pine tree that faced the nearest black walnut tree, which may further indicate that the walnuts are the damage source. Because of this observation, a subsample of 9 walnut trees, each associated with 10 living pine trees, was measured for total number of resin exudations versus number of resin exudations facing the nearest walnut.

For statistical analyses, individual tree data were entered into a curve-fitting program (The MathWorks, Inc. 2014) to determine relationships among variables measured. Linear and second-order polynomial curves at 95-percent confidence level ($\alpha < 0.05$) were evaluated, and the curves with the best r^2 values are presented.

Results and Discussion

Resin exudation on the lower trunks of pines, thinning crowns, and mortality was found only in the vicinity of black walnut trees. Extensive searches in the pine stand found no similar symptoms on trees that were not associated with walnut trees. This finding indicates that the damage was related to the presence of the walnut trees and was likely caused by walnut allelopathy.

In the subsample, to determine whether resin exudation faced black walnut trees, the affected pines had a total of 45 points of resin exudation, with 43 of these (95.6 percent) on the side of the pine trunks that were facing the walnut tree at the center of the plot. This observation further confirms the black walnut trees as the focal point of the pine tree damage.

Based on our observations, we determined that the general progression of damage to loblolly pine trees begins with a few small areas of resin exudation on the lower bole (figure 1). As damage progresses, more areas of resin exudation appear higher on the bole and the phloem and sapwood beneath die. This progressive damage is followed by termite invasion into the dead wood, with accompanying termite soil tubes and bird (probably woodpecker) predation on the termites (figure 2). Advanced damage continues upward on the bole and may extend to 10 ft (3 m) or more. In the advanced stage, damage appears similar to lightning damage on the side of a tree. In our study, height of resin exudation on the bole was negatively correlated with the number of living, affected trees (figure 3), indicating that fewer trees survive as damage increases upward on the bole.

The damage pattern within a pine may be explained by pine roots in close proximity to walnut roots absorbing juglone along with water and nutrients. As water and nutrients

Figure 1. Resin exudation evident on the lower bole of a live 28-year-old loblolly pine tree. (Photo by Coleman Doggett, 2015)

travel through the pine roots and upward in the bole, the juglone could cause the observed damage. It would be interesting to test the needles of damaged pines to determine if juglone is present in the needles and may contribute to further damage after needle fall.

Positive correlations existed between black walnut tree DBH and distance to the nearest and farthest damaged pine trees (figure 4) and also the number of trees affected (figure 5). These data indicate that larger black walnut trees have a greater and farther allelopathic effect than smaller black walnut trees. From a practical standpoint, the forest manager is probably most interested in the maximum distance from a black walnut tree that allelopathic damage may occur.

Although black walnut is classified as a shade-intolerant species, small black walnut trees were present in the understory of our study and were adversely affecting pines. This understory presence may be due to the thinning operations, which are normally done in pine stands one to three times before stands are harvested. Each time a stand is thinned, light is admitted to the understory, which may allow for the small walnut trees to survive.

Figure 2. This recently dead loblolly pine tree has advanced symptoms of damage from walnut allelopathy, still visible on the side of the bole facing the walnut tree. The decayed area on the lower bole is riddled by termites. Above this area are holes through the bark caused by bird predation on the termites. Resin exudation is visible near the top of the photograph. (Photo by Coleman Doggett, 2014)3)

Recommendations for Plantation Establishment

When establishing loblolly pine plantations in areas where black walnut trees grow, we recommend surveying the plantation area for the presence of walnut trees before planting. The survey area should include not only the plantation area

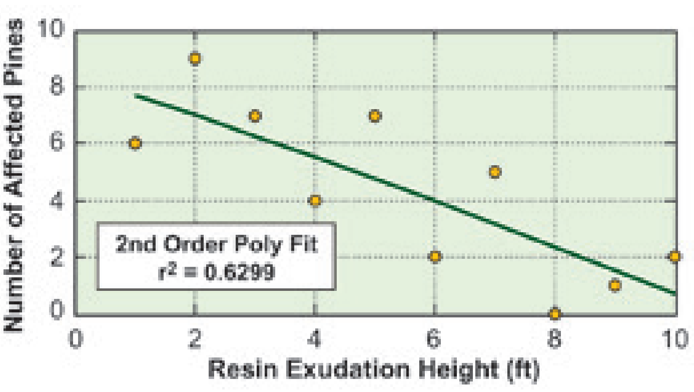

Figure 3. Height of resin exudation on the pine bole was negatively correlated with the number of living, affected trees.)

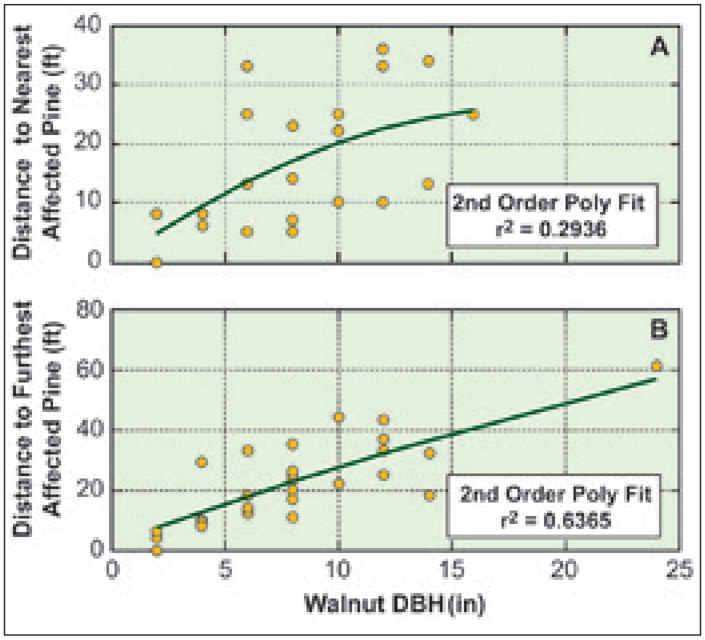

Figure 4. Black walnut tree diameter at breast height was positively correlated with the distance to the (A) nearest and (B) farthest affected pine trees.

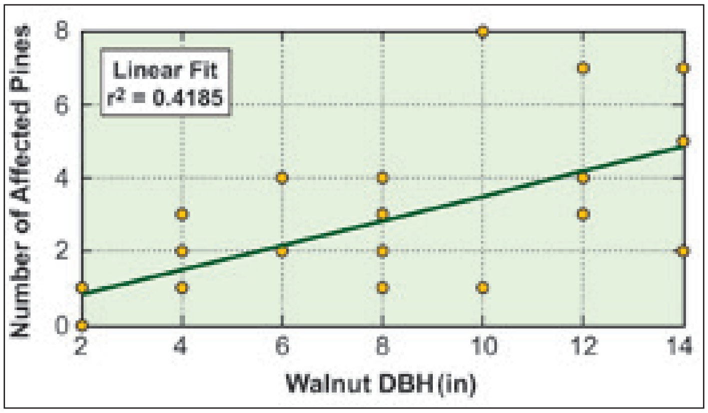

Figure 5. The number of pine trees affected around a black walnut tree was positively correlated with the walnut's diameter at breast height.

itself, but also areas along nearby streams, because the deep, moist soils along streams often support walnut tree growth. Any old homesites in or near pine plantation should also be carefully surveyed, since walnut trees have traditionally been planted near homes for both their nut production and lumber. If walnut trees are found, the forest manager must decide whether to remove or leave the walnut trees. If walnut trees are left in the vicinity of pine plantations, pines planted near established walnut trees will be adversely affected. To be conservative, no pines should be planted within 35 ft (10 m) of the driplines of existing black walnut trees. Although pine trees may survive for a period of time when planted near walnut trees, the roots of the pines and walnut trees will grow together prior to the pines' harvest age, resulting in mortality or degraded wood values.

After planting, the pine plantation should be surveyed at least every 5 years for the presence of black walnut seedlings established by nuts falling from trees or being carried and buried by squirrels. Any walnut seedlings found should be removed.

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Acknowledgments

This article is dedicated to Mike Burke, who is retiring from Duke Forest, the 7,060 acre research and educational forest that adjoins the Duke University campus. Mike Burke's keen understanding and knowledge of Duke Forest will be missed by everyone who has known him, especially the Duke faculty, staff, and students.

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Relationships Among Chilling Hours, Photoperiod, Calendar Date, Cold Hardiness, Seed Source, and Storage of Douglas-Fir Seedlings

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Abstract

Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings from three nurseries in the Pacific Northwest United States were lifted on five dates from mid-October through mid-December 2006. Each nursery provided seedlings from a low- and a high-elevation seed lot. Photoperiod and accumulated chilling hours (calculated using two methods) were evaluated throughout the lifting period. Seedlings had typical patterns of fall cold hardiness development, with some indication that the high-elevation lot at each nursery was hardier than the low-elevation lot. Photosynthetic yield measured on seedlings from one of the nurseries decreased with decreasing temperatures, thereby corresponding well to levels of tissue damage at each freezing test temperature over time. Seedlings were either cold- or freezer-stored until February 2007, then tested for physiological quality and planted into a garden plot. Overall, seedlings from earlier lift dates tended to perform poorly in all attributes compared with those from later lift dates. Low-elevation seedlings tended to have lower root growth potential after storage and also reduced survival and longer bud break in the garden plot compared with high-elevation seedlings, although low-elevation seedlings tended to have more height and stem-diameter growth. Freezer-stored seedlings tended to have greater survival compared with cold-stored seedlings, although storage type did not influence growth. This study exemplifies the many influencing factors that growers must consider when determining lift dates. This paper was presented at a joint meeting of the Western Forest and Conservation Nursery Association, the Intermountain Container Seedling Growers Association, and the Intertribal Nursery Council (Boise, ID, September 9–11, 2014).

Introduction

In temperate conifer species, the growth and dormancy cycle is an adaptation to prevent shoot growth during winter, when freezing temperatures would injure such growth. These phenological patterns are influenced by species, genetics, plant vigor, and environment. As winter approaches, plants respond to cues of decreasing photoperiod (daylength) and temperature by ceasing growth, setting buds (for determinant species), and developing the ability to withstand subfreezing temperatures with little or no damage (Bigras et al. 2001, Haase 2011). This development of cold hardiness involves several physical and chemical changes within the plant tissues that enable plants to resist freezing damage (Öquist et al. 2001).

Cold hardiness is defined as a minimum temperature at which a certain percentage of a random plant population will survive or will sustain a given level of damage (Ritchie 1984a). Hardiness is most commonly quantified as LT_{50} (lethal temperature for 50 percent of a population). Seedling cold hardiness in the nursery is also an indicator of overall resistance to stresses such as those associated with lifting, packing, storing, and outplanting (Burr et al. 1990, Faulconer 1988, Ritchie 2000). Cold hardiness has also been linked to subsequent survival and growth (Simpson 1990, van den Driessche 1977) and is therefore a useful seedling-quality test (Haase 2008).

In the northern hemisphere, temperate conifer seedlings typically achieve peak dormancy in October or November. Dormancy is quantified as the length of time before plants will resume growth in the spring; it is not the same thing as cold hardiness, which commonly peaks in January (Haase 2011, Timmis et al. 1994). Seedlings require a period of chilling to complete their dormancy cycle before they will resume growth in response to longer photoperiods and favorable spring temperatures. The chilling requirement for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) is 1,200 to 2,000 hours (Bailey and Harrington 2006, van den Driessche 1975). If not totally fulfilled by the time of lifting from the nursery, the chilling requirement may be met in cold storage (van den Driessche 1977); temperatures in freezer storage, however, are below optimum for accumulation of chilling to meet dormancy release requirements (Ritchie 1984b).

While the chilling requirement for bud break in Douglas-fir has been well documented, this information does little to assist with the more practical application of using chilling accumulation to determine the optimum timing for lifting, storing, and outplanting. For Pacific Northwest nursery applications, the typical target for Douglas-fir is a minimum of 300 to 400 chilling hours before lift and storage for optimum stress resistance. Very little research has been done, however, to verify the relationship between chilling hours and subsequent seedling quality and vigor, nor is adequate information available regarding other influencing and confounding factors. Similar questions have arisen regarding which factors are most useful for determining when to lift southern pine species (South 2013).

Various methods can be used to quantify chilling. The most common method used in forestry nurseries is the number of hours below 5 °C (41 °F). Another method, used in the fruit-tree industry, is the Richardson method (Richardson et al. 1974), which is more complex because it includes relative chilling effectiveness and variable chilling accumulation depending on temperature.

In a preliminary trial (fall 2005) to examine the relationship between shoot cold hardiness and accumulated chilling hours, Douglas-fir seedlings from six seed lots were frozen and evaluated for tissue damage and mortality every 2 weeks from mid-October to mid-December. As chilling hours accumulated from approximately 35 hours in mid-October to more than 150 hours in mid-November, the LT_{50} for all lots decreased (i.e., the seedlings became more cold hardy). When a rapid rise in chilling from 150 to more than 400 hours occurred during the 2-week period from November 17 to December 1, however, no corresponding rise in cold hardiness was observed for any of the lots. A model of the preliminary data showed that calendar date was the most significant factor related to seedling cold hardiness—more so than either chilling hours or seed lot (NTC 2006). Based on the results of that preliminary trial, this study was conducted in 2006 with the objective to further examine relationships among seed lot, chilling hours, daylength, lift date, and storage and their subsequent influence on cold hardiness, bud break, growth, and survival. Understanding these relationships can assist with nursery lifting and storage decisions to optimize seedlings' stress resistance and outplanting performance.

Materials and Methods

Seedlings, Sampling, and Storage

Three nurseries (A, B, and C) in Washington, United States, participated in the study; each chose two Douglas-fir seed lots (low and high elevations) to include in the study based on expected differences in cold hardiness (table 1). Seedlings from all nurseries were lifted every 2 weeks from mid-October through mid-December 2006 on the following five dates:

1. **October 16**
2. **October 30**
3. **November 13**
4. **November 27**
5. **December 11**

Table 1. Three nurseries participated in the study, each providing seedlings from two Douglas-fir seed lots (low- and high-elevation sources).

Seed lot	Stocktype	Seed zone[a, b] (State)	Elevation[c] (ft)
		Nursery A	
Low	2+0 bareroot	042 (WA)	1,000
High		631 (WA)	3,500
		Nursery B	
Low	Outside-grown container, plug-to-plug transplant, 21 in^3 (344 cm^3) plug	051 (OR)	1,000
High		452 (OR)	2,200
		Nursery C	
Low	1+0 bareroot (for transplant)	262 (OR)	500
High		262 (OR)	2,000

[a] Washington seed zones from Randall and Berrang (2002). [b] Oregon seed zones from Randall (1996). [c] 1,000 ft = 305 m.

Figure 1. Seedlings were lifted from mid-October through mid-December. This photograph was taken just before lifting at Nursery A for the mid-November lift date. (Photo by Nabil Khadduri, 2006)

On each lift date at each nursery, 260 seedlings from each seed lot were lifted (figure 1). A sample of 60 seedlings was designated for cold hardiness assessment and the rest were placed in storage at Nursery A's facility. Samples of 100 seedlings of each lot were placed in cold storage (1 to 3 °C [34 to 37 °F]) and in freezer storage (-1 to 0 °C [30 to 32 °F]).

Environmental Factors

Temperature sensors were installed at each nursery to monitor soil and air temperatures until all seedlings had been lifted. Data from these sensors were used to calculate chill hours over time, using both the standard method (total hours below 5 °C [41 °F]) and the Richardson method (table 2). Photoperiod (daylength) was determined using an online calculator (http://herbert.wikispaces.com/length+of+day), using each nursery's latitude coordinates.

Table 2. Quantification of chilling hours using the Richardson method.

	°C	°F		Chill hour
1 hour at:	< 2	< 35.6	=	0.0
	2.0 to 3.0	35.6 to 37.4	=	0.5
	3.0 to 9.0	37.4 to 48.2	=	1.0
	9.0 to 12.0	48.2 to 53.6	=	0.5
	12.0 to 15.0	53.6 to 59.0	=	0.0
	15.0 to 18.0	59.0 to 64.4	=	– 0.5
	> 18.0	> 64.4	=	– 1.0

Source: Richardson et al. (1974).

Seedling Physiology at the Time of Lifting

At each lift date, cold hardiness was evaluated using the whole plant freezer test (WPFT) (Haase 2011, Tanaka et al. 1997). A sample of 60 seedlings from each nursery/seed lot was randomly divided into four groups of 15 seedlings each and randomly assigned a target freeze temperature. Four target temperatures were chosen at each lift date based on their expected ability to bracket the LT_{50}. Each group was placed into a programmable chest freezer in which the temperature was lowered from room temperature to 0 °C (32 °F) at a rate of 20 °C (36 °F) per hour, then decreased to the target temperature at a rate of 5 °C (9 °F) per hour, held at the target temperature for 2 hours, then raised back to 0 °C (32 °F) at a rate of 20 °C (36 °F) per hour (figure 2a). Due to resource limitations, each WPFT freezing

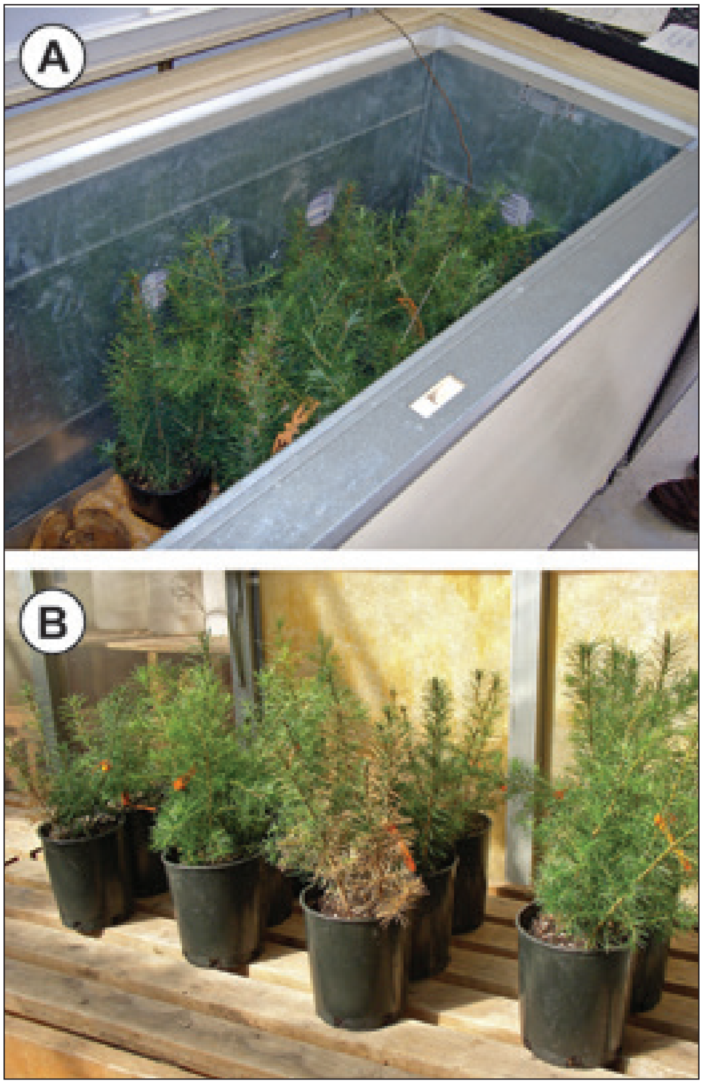

Figure 2. (a) A programmable freezer was used to subject seedlings to the whole plant freeze test. After freezing, seedlings were kept in (b) ambient conditions before assessing for freeze damage. (Photos by Diane L. Haase, 2006)

Figure 3. Six days after freezing, seedlings were evaluated for damage by examining (a) bud and (b and c) cambium tissues for browning. (Photos by Diane L. Haase, 2006)

temperature was run only once per sample date in the programmable freezer; because seedling response to freezing stress is well documented and reproducible, however, we expected that the resulting analyses would be very similar if additional freezers had been available.

After freezing, seedlings were placed into a greenhouse with adequate moisture, ambient photoperiod, and an average temperature of 20 °C (68 °F) (figure 2b). Six days after freezing, bud damage was determined by sectioning 5 to 10 randomly selected buds from throughout each seedling shoot and examining for evidence of browning (figure 3a). If more than 50 percent of the buds were damaged, then the seedling was considered nonviable. Cambial damage was evaluated by scraping the bark along the stem (figure 3b) and examining for browning (figure 3c). If the cambium was brown in the lower half of the shoot, the seedling was considered nonviable. Percent foliar damage (visual estimate) was a determining factor only when cambium or bud damage was borderline. The LT_{10} and LT_{50} for each seedling group on each date were then determined by plotting percent survival against temperature and assuming a linear relationship.

Chlorophyll fluorescence and genetic markers were also measured on seedlings from Nursery A. Due to labor-intensive sampling, only one nursery could be included for these measurements; Nursery A was chosen because the two seed sources were expected to have the greatest difference in cold hardiness. Approximately 24 hours after freezing in the WPFT procedure, chlorophyll fluorescence was measured on a single needle collected from 8 seedlings from each seed source and freezing temperature. Needles were exposed to a 3-second pulse of saturating light using a fluorometer (Model OS5-FL, Opti-Sciences, Inc.) (figure 4). The steady state (Fs) and maximal (Fms) fluorescence were determined and used to calculate photosynthetic yield (Y).

Needle and bud tissue from Nursery A seedlings (not frozen in the WPFT) were collected and processed from both seed lots on each lift date (figure 5) and sent for NSure genetic marker assessment, a molecular test for assessing cold tolerance in conifer seedlings developed at the Wageningen University and Research Centre in the Netherlands where researchers found that gene expression may be correlated with cold hardiness (Balk et al. 2007a, Joosen et al. 2006, Landis and van Wordragen 2006). The test is based on measuring the activity level of a selected set of genes.

Seedling Physiology and Performance After Storage

From late January through mid-February, seedlings were removed from storage (all seedlings from one nursery at a time). One week before removal from storage, those in freezer storage were moved to cooler storage to allow for

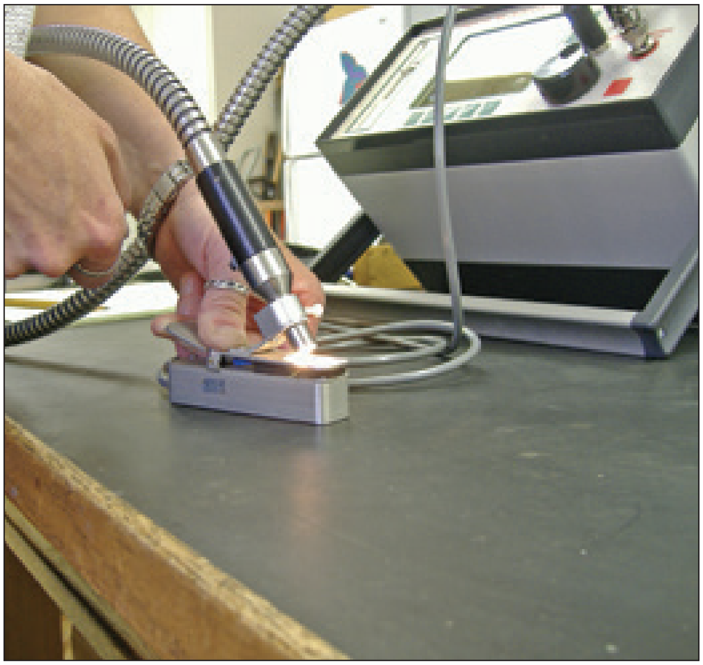

Figure 4. After freezing, chlorophyll fluorescence was measured on needles from Nursery A by exposing needles to a pulse of saturating light using a fluorometer. (Photo by Diane L. Haase, 2006)

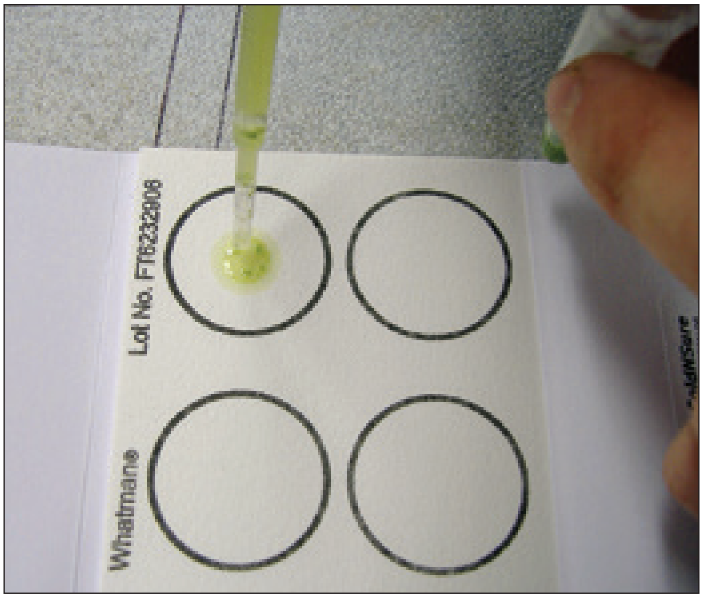

Figure 5. Needle and bud tissue from Nursery A seedlings (not frozen) were collected and processed from both seed lots on each lift date and assessed for genetic markers associated with cold hardiness. (Photo by Diane L. Haase, 2006)

thawing. A sample of 60 seedlings from each nursery/seed lot/lift date/storage treatment was immediately evaluated for cold hardiness using the WPFT. Because some groups sustained damage of more than 50 percent for all test temperatures, LT_{10} and LT_{50} could not be calculated. Thus, percent mortality at -9 °C (15.8 °F) is reported.

Root growth potential (RGP) was evaluated on a sample of 20 seedlings from each nursery/seed lot/lift date/storage treatment. Each sample was potted into 19-L (5-gal) pots (5 per pot) containing a peat-based growing medium and randomly placed in a warm greenhouse environment where they were kept well watered for 3 weeks. Seedlings were then removed from the pots and new root growth was quantified based on the following index (Burdett 1979).

RGP index	Description (1 cm = 0.4 in)
0	No new root growth
1	Some new roots but none longer than 2 cm
2	1–3 new roots longer than 1 cm
3	4–10 new roots longer than 1 cm
4	11–30 new roots longer than 1 cm
5	More than 30 new roots longer than 1 cm

The remaining 20 seedlings from each nursery/seed lot/lift date/storage treatment were randomly assigned to four replications (5 seedlings per treatment group) and planted into a garden plot at Nursery A for assessment of field vigor (figure 6). Seedling treatment groups were assessed weekly for percent bud break until late spring, when no further bud break was anticipated. In early March 2007 (before bud break), all seedlings were measured for initial height and stem diameter. In October 2007 (after bud set), seedlings were measured again for height and stem diameter and also for survival. Height and stem-diameter growth were calculated by subtracting initial values.

Statistical Analyses

Chlorophyll fluorescence yield data from Nursery A seedlings were analyzed for each sample date by analysis of variance (ANOVA) using the PROC GLM procedure of SAS software (SAS Institute Inc., Cary, NC) to determine significant differences between seed lots and among freezing temperatures.

RGP, bud break, height growth, stem-diameter growth, and survival data were all analyzed using ANOVA (PROC GLM, SAS Institute, Inc.) for a randomized complete block to determine differences among lift dates, seed lot, and storage type. Data from each nursery were analyzed separately. Fisher's Protected Least Significant Difference procedure was used to determine significant differences among treatment groups at the $\alpha \leq 0.05$ level. Tests for normality, linearity, and constant variance of the residuals were performed to ensure the validity of these assumptions on each dataset; no data transformations were deemed necessary.

In addition, probit regression was used to determine the predictive relationship of chilling hours (calculated using

Figure 6. After storage, samples of seedlings from each nursery, lift date, seed lot, and storage type were planted into a garden plot at Nursery A for evaluation of bud break, survival, and growth. (Photo by Nabil Khadduri, 2007)

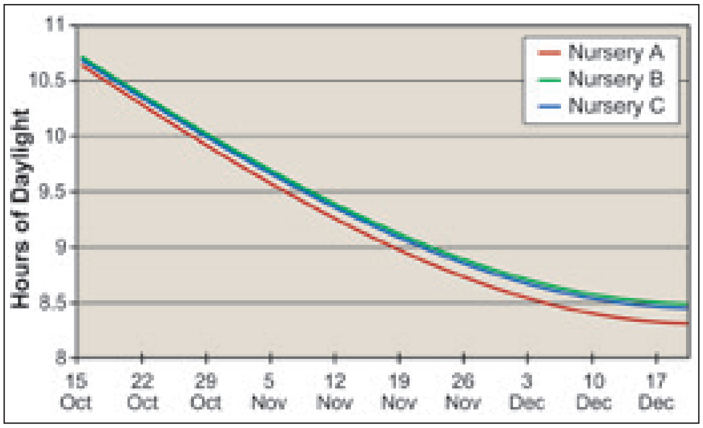

Figure 7. Photoperiod from mid-October through mid-December was similar among nursery locations.

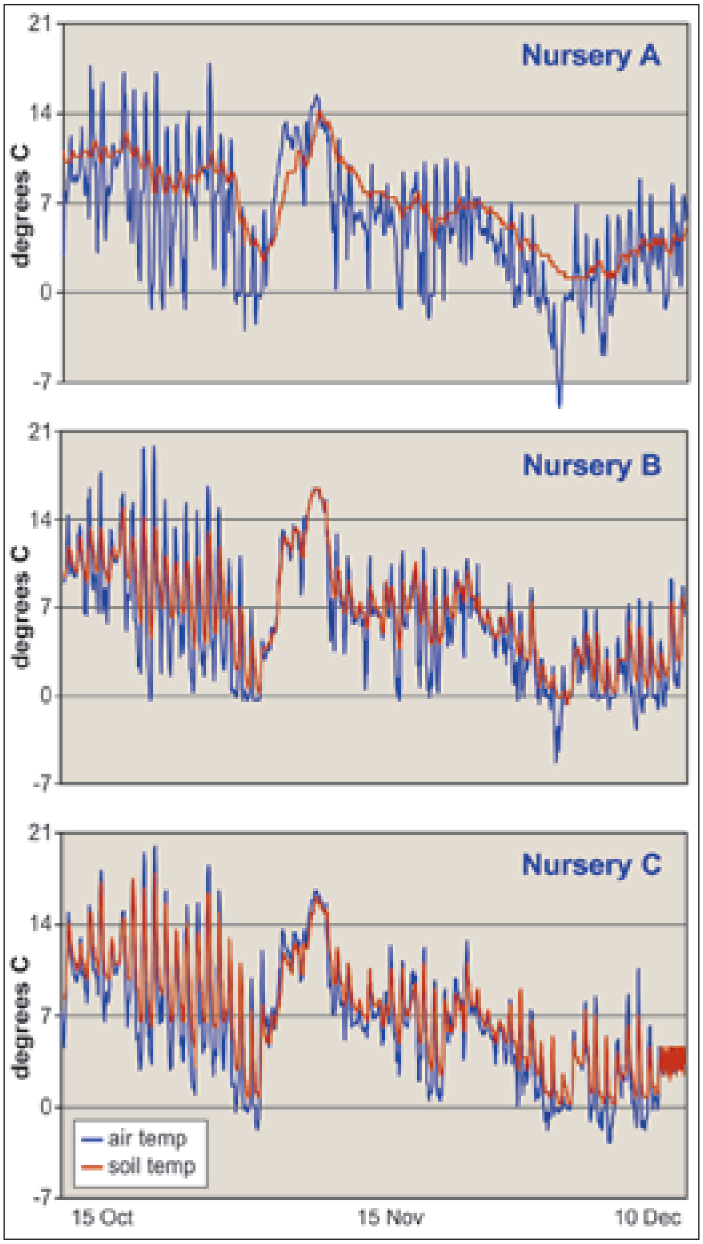

Figure 8. Air and soil temperatures were recorded during the fall 2006 lift dates at each nursery. Air temperature was used to calculate chilling hour accumulation.

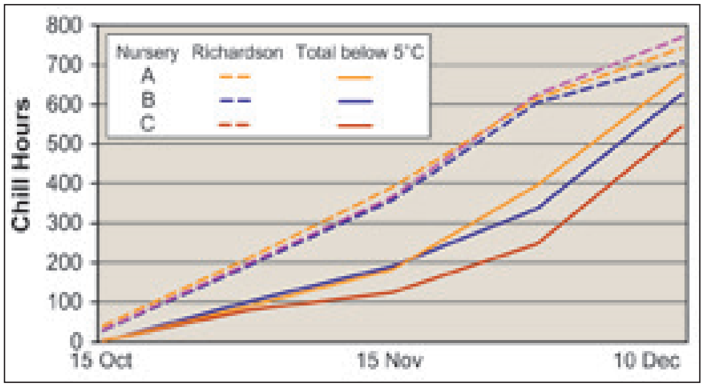

Figure 9. Chilling hours were calculated using the Richardson method or the conventional method (sum of all hours below 5 °C [41 °F]). The Richardson method resulted in a more rapid accumulation of chill hours from mid-October through mid-December 2006.

either the Richardson or conventional methods) and calendar date (quantified as number of days since October 15) on seedling mortality at various freezing temperatures.

Results

Environmental Factors

Photoperiod patterns were nearly identical for Nurseries B and C, which are located at similar latitudes. Nursery A is located approximately 129 km (80 mi) north of the other two nurseries and had slightly shorter photoperiods (by approximately 3 to 9 min) from October 15 through December 20 (figure 7). Based on air temperature readings at each nursery (figure 8), calculations using the Richardson method resulted in a more rapid accumulation of chill hours as compared with the conventional method (figure 9).

Seedling Physiology at the Time of Lifting

Seedlings had typical patterns of fall cold hardiness development with some indication that the seedlings in the high-elevation lot at each nursery were hardier than those in the low-elevation lot (figure 10).

The NSure assay on needle tissue from Nursery A did not correspond to data from the WPFT test. The NSure assay on bud tissue from Nursery A, however, distinguished three stages of cold hardiness, which correlated with WPFT values as previously reported (Balk et al. 2007b) and summarized on the following page.

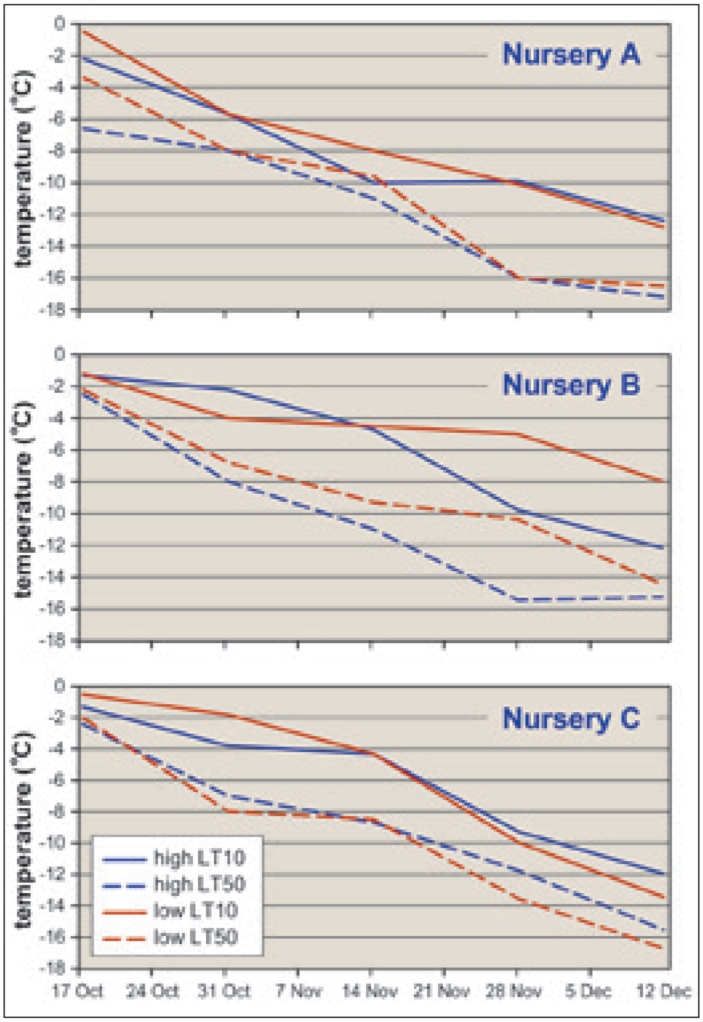

Figure 10. Cold hardiness was estimated for each seed lot from each nursery on each lift date.

NSure phase 0: No frost tolerance observed

NSure phase 1:

LT_{50} value between -5 and -10 °C (23 and 14 °F)

LT_{10} value between -1 and -5 °C (30 and 23 °F)

NSure phase 2:

LT_{50} value below -10 °C (14 °F)

LT_{10} value below -5 °C (23 °F)

Photosynthetic yield measured via chlorophyll fluorescence on Nursery A seedlings decreased with decreasing temperatures, thereby corresponding well to levels of damage from the WPFT at each freezing test temperature over time (figure 11). Seed lot affected photosynthetic yield on the October 17 sampling date (higher elevation lots had greater yield at all temperatures) and the November 28 sampling date (higher elevation lots had greater yield at all temperatures except -6.0 °C [21.2 °F]).

Seedling Physiology and Performance After Storage

Statistical analyses indicated multiple interactions among seed lot, lift date, and storage type at each nursery. In general, however, lift date had the greatest influence (based on magnitude of the F-value) on all variables for seedlings from Nursery B and Nursery C. Lift date also had the greatest influence on growth and RGP for Nursery A, but elevation had an even greater influence on survival and bud break. Overall, earlier lift dates tended to perform poorly in all attributes compared with those from later lift dates (table 3, figure 12). Seedlings

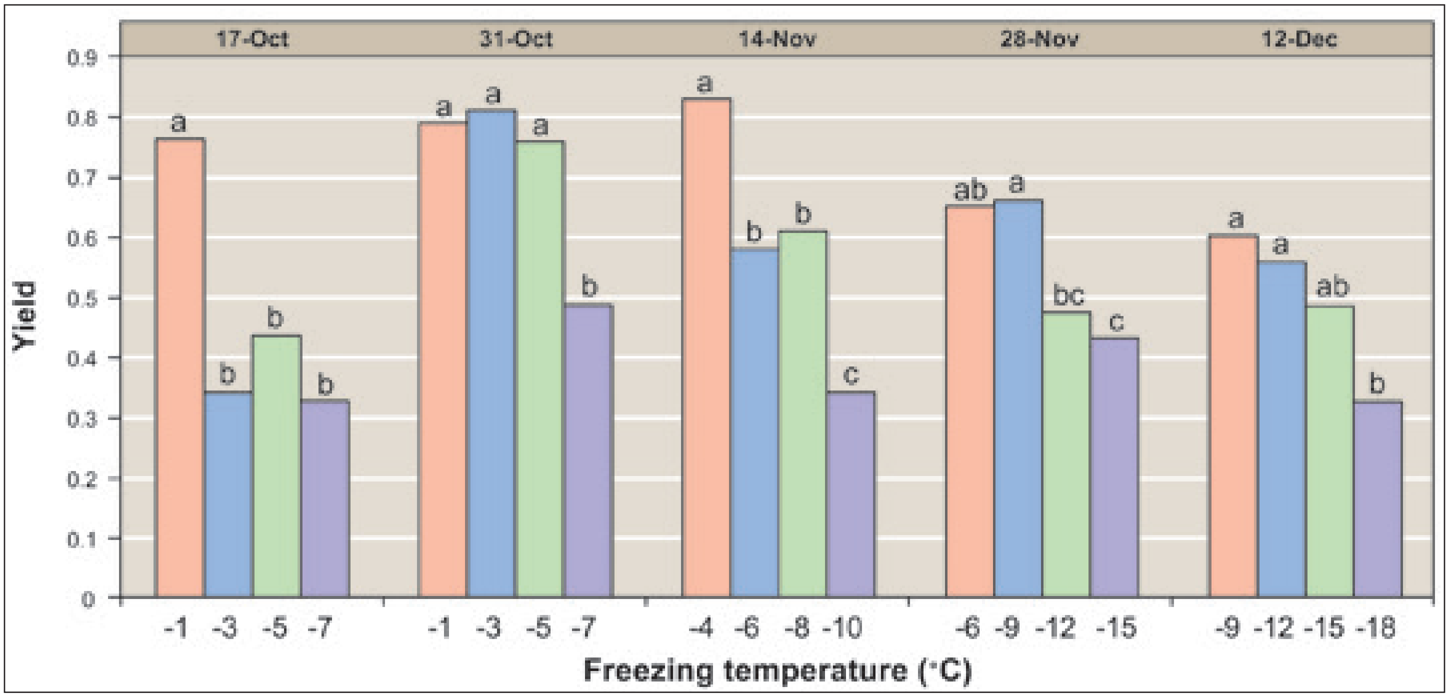

Figure 11. Yield was calculated from chlorophyll fluorescence measurements of needles from Nursery A seedlings frozen to different temperatures on each lift date. Note: A significant interaction between temperature and seed lot was observed on November 28 (needles from the high-elevation lot had greater yield than those from the low-elevation lot at all temperatures except -6.0 °C [21.2 °F]).

Table 3. Poststorage physiology for seedlings from each nursery group. Because, for all three nurseries, seed lot, lift date, and/or storage type significantly interacted, only means are presented here.

	Index of root growth potential				**Percent mortality at –9.0 °C (15.8 °F)**			
Seed lot elevation	**Low**		**High**		**Low**		**High**	
Storage Type	**Cooler**	**Freezer**	**Cooler**	**Freezer**	**Cooler**	**Freezer**	**Cooler**	**Freezer**
Lift Date	**Nursery A (2+0)**							
Oct. 16	0.65	0.60	2.75	1.45	73.3	83.3	16.7	30.0
Oct. 30	3.25	2.44	3.70	2.95	40.0	73.3	33.3	16.7
Nov. 13	3.10	2.95	3.25	2.90	13.3	63.3	20.0	20.0
Nov. 27	3.25	2.40	3.40	3.20	0.0	50.0	16.7	26.7
Dec. 11	2.83	3.00	3.55	3.10	10.0	70.0	16.7	6.7
Lift Date	**Nursery B (large plugs)**							
Oct. 16	0.05	0.20	0.20	1.21	100	96.7	100.0	93.3
Oct. 30	0.33	2.11	1.37	1.05	83.3	56.7	86.7	56.7
Nov. 13	1.42	2.45	1.50	2.65	73.3	50.0	43.3	46.7
Nov. 27	2.35	3.35	3.00	2.40	23.3	40.0	6.7	10.0
Dec. 11	3.10	3.74	3.35	3.10	16.7	3.3	20.0	20.0
Lift Date	**Nursery C (1+0)**							
Oct. 16	0	0	0	0.45	100	100	100	56.7
Oct. 30	0.25	0.17	1.15	1.85	93.3	90.0	53.3	33.3
Nov. 13	2.45	2.25	1.30	2.26	92.9	53.3	100	80.0
Nov. 27	2.60	2.56	2.95	2.95	43.3	46.7	23.3	43.3
Dec. 11	3.15	3.10	2.55	2.53	10.0	16.7	39.3	80.0

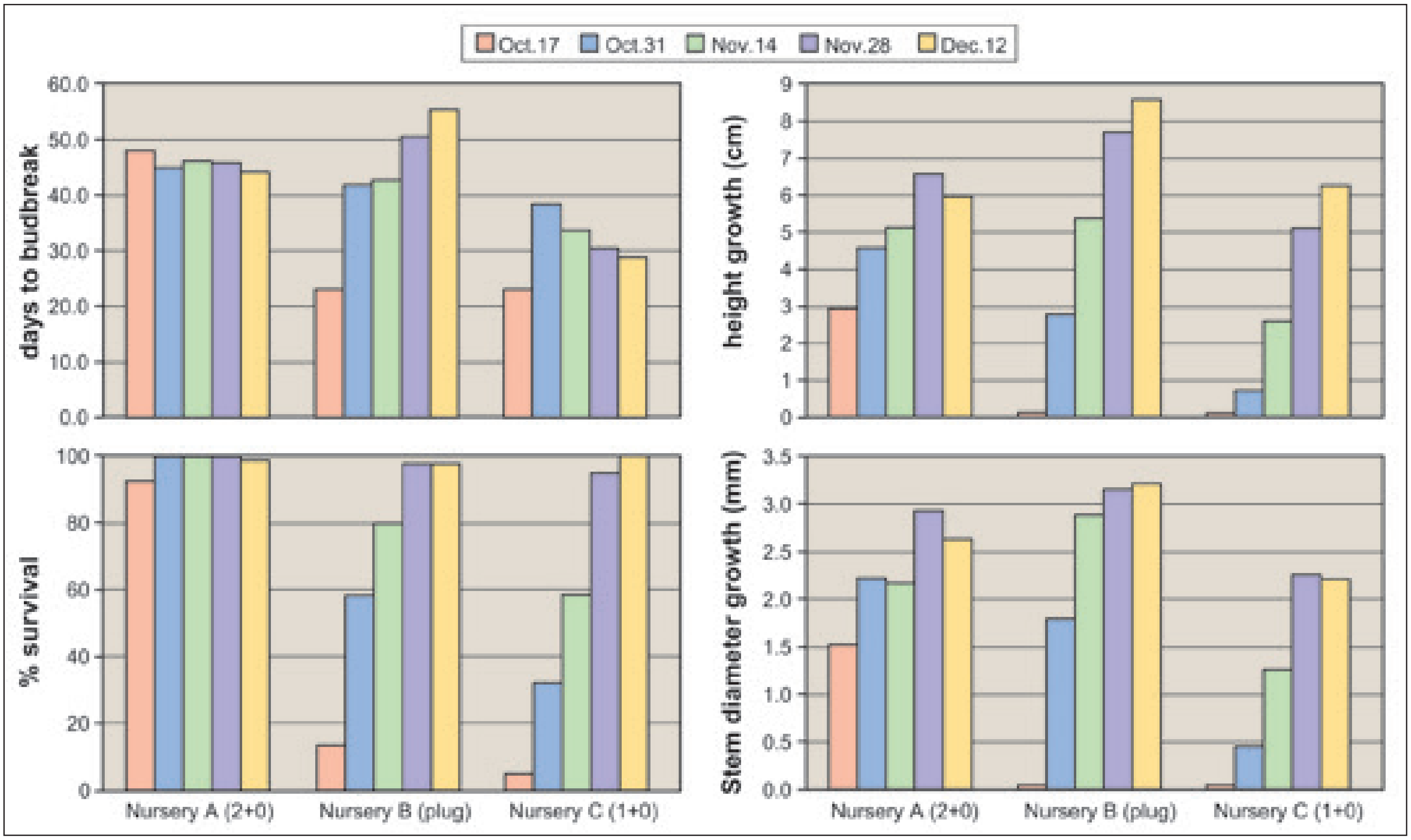

Figure 12. Lift date had a significant influence on poststorage seedling performance in the garden plot.

from the low-elevation seed lots tended to have lower RGP following storage (table 3) and lower survival and longer bud break in the garden plot compared with seedlings from the high-elevation seed lots, although seedlings from the low-elevation lots also tended to have more height and stem-diameter growth in the garden plot than those from the high-elevation lots (data not shown). Freezer-stored seedlings from Nursery B and Nursery C tended to have greater RGP compared with cold-stored seedlings, whereas the reverse was true for Nursery A seedlings (table 3). Freezer-stored seedlings also tended to have greater survival for all nurseries and seed lots in the garden plot compared with cold-stored seedlings, although storage type did not influence growth (data not shown).

Environmental Influences on Seedling Physiology

Probit analyses determined that chilling hours calculated with the Richardson method had the best fit for predicting mortality by freezing temperature (figure 13). Richardson chill hours were only a slightly better predictor of mortality than days since October 15 (data not shown); the two models did not differ significantly. Chill hours calculated with the conventional method (hours below 5 °C [41 ºF]), however, provided a significantly worse fit compared with Richardson chill hours or days since October 15.

Discussion

For the 2006–07 fall–winter season, Douglas-fir seedlings from the lots studied followed typical hardening and dehardening patterns (Haase 2011, Timmis et al. 1994). Photosynthetic yield also reflected damage levels seen in the cold hardiness test. Conifer species in northern latitudes, such as white spruce (*Picea glauca* [Moench] Voss), must achieve complete photosynthetic inactivation for protection against winter cold (Binder and Fielder 1996). Because Douglas-fir's relatively milder geographic range does not require a complete shutdown of photosynthesis, chlorophyll fluorescence is not well correlated with cold hardiness in nonfrozen seedlings (Rose and Haase 2002). Similar to results in this study, however, chlorophyll fluorescence has been shown to be well correlated with foliar damage following freeze stressing (Adams and Perkins 1993, Fisker et al. 1995), thereby serving as a quantitative and objective tool for rapid assessment of seedling vigor following freezing, although variations among tissues in freezing damage susceptibilities during the winter must be considered (Rose and Haase 2002).

Chilling hours and calendar date (days since October 15) had the strongest relationship with freeze damage at the time of lifting. Understanding the relative contributions of each factor to Douglas-fir seedling phenology, however, is nearly impossible, given that daylight, chilling hour accumulation, and calendar date are intrinsically confounded (Campbell and Sugano 1975, Fuchigami and Nee 1987). Furthermore, chilling hour accumulation varies with annual temperature patterns and by calculation method, and seedling phenology is influenced by stocktype, seed source, and nursery cultural practices. Faulconer (1988) noted several disadvantages for relying solely on chilling hour accumulation for determining seedling condition, including variations in hardiness among seedling lots, temperature changes from year to year, and uncertainties regarding the best method to quantify chilling. In an early study with several Douglas-fir seed sources, Campbell and Sugano (1975) noted that the effects of chilling, photoperiod, and temperature on subsequent bud break were highly interdependent. South (2013) also commented on confounding among multiple factors associated with chilling hours and seedling quality. While it may be possible to separate the varying factors in controlled laboratory studies, such an endeavor would not be representative of actual nursery and field conditions and would therefore be problematic to apply operationally (Haase 2014). Rather, as demonstrated by this study, it is important to consider all influences when determining lift date.

Similar to cold hardiness at lifting, poststorage RGP, cold hardiness, bud break, growth, and survival were strongly influenced by lift date (which, as described previously, is confounded with chilling hours and photoperiod). Some studies indicate that chill hours can be partially satisfied in cold storage (Carlson 1985, Ritchie 1989, van den Driessche 1977). Our study found, however, that those seedlings lifted on the earliest lift date performed poorly after outplanting. This finding indicates that seedlings require adequate time in ambient conditions to reach a certain chilling and accompanying photoperiod threshold, along with diurnal and nocturnal fluctuations, before lifting and storage, after which seedlings are less susceptible to handling stresses.

In addition to being influenced by lift date, seedling attributes were influenced by seed source. RGP after storage and also survival, bud break, height growth, and stem-diameter growth tended to differ between the low-elevation and high-elevation seedlings from each nursery. St. Clair et al. (2005) evaluated Douglas-fir seedlings from more than 1,000 locations in western Oregon and Washington and found that populations differed considerably for adaptive traits; bud phenology, in particular, was strongly influenced by elevation and temperature. Freezer-stored seedlings tended to have greater survival compared with cold-stored seedlings. Carbohydrate reserves tend to decrease in cold storage more rapidly than in freezer storage (Ritchie 1982), which may have been a contributing factor.

In the Pacific Northwest, Douglas-fir bareroot and container seedling growers have established annual lifting and storage schedules based on factors specific to their nursery environment, weather patterns, and customer demands and also on each crop's stocktype and genetics. These decisions are based on science and experience. As temperatures increase due to

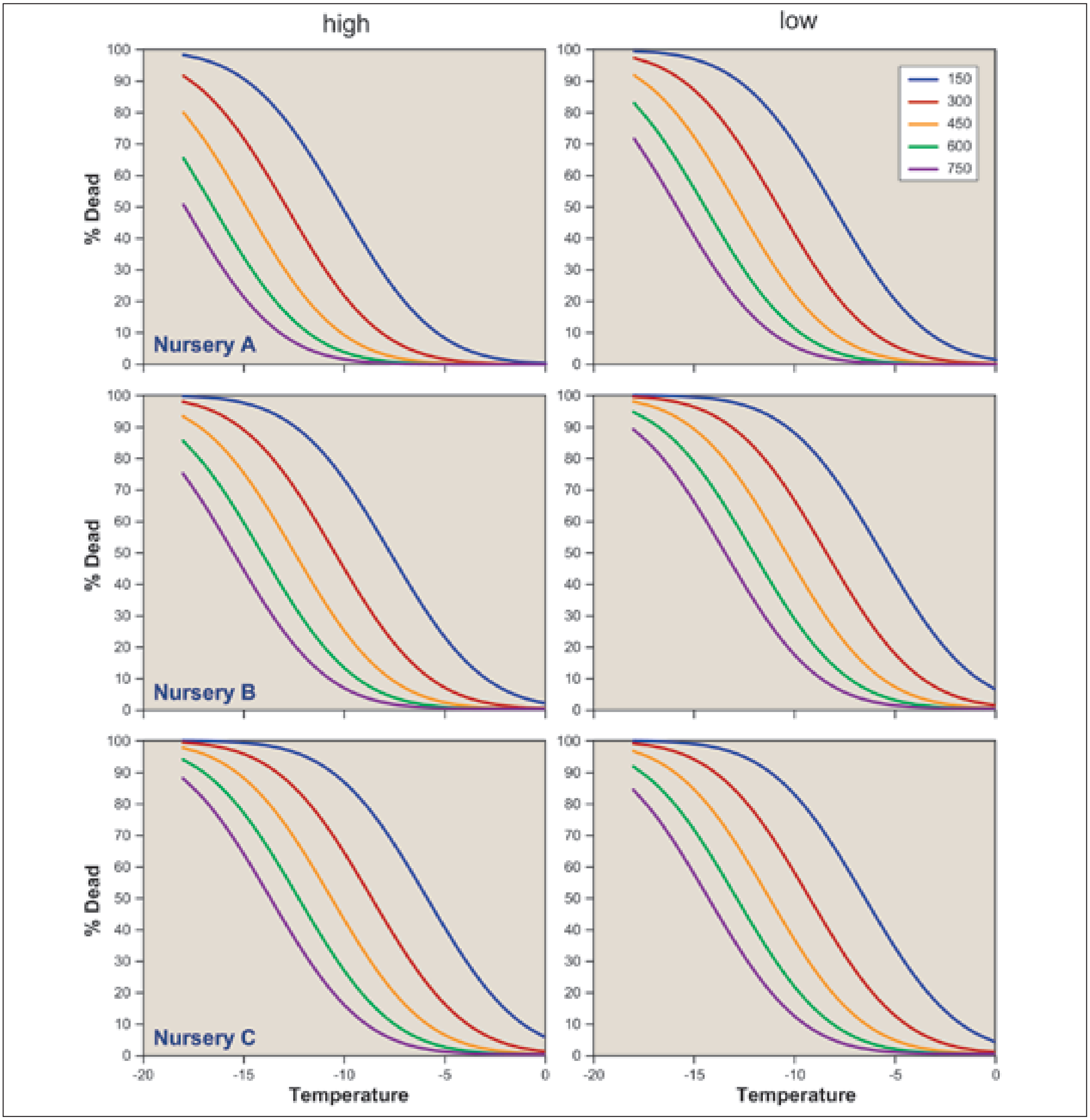

Figure 13. Probit analyses determined that chilling hours calculated with the Richardson method had the best fit for predicting mortality by freezing temperature. Days since October 15 (data not shown) also had a strong predictive fit, whereas the conventional method for calculating chill hours (hours below 5 ºC [41 ºF]) provided a significantly worse fit.

expected climate changes, however, winter temperature patterns will provide fewer annual chilling hours in temperate latitudes. This warming could affect Douglas-fir bud development and bud break. Douglas-fir seedlings grown in elevated temperature conditions had delayed cold hardening in the autumn and slowed dehardening in the spring and also had reduced maximum cold hardiness, reduced bud break, and reduced growth compared with those grown in ambient temperatures (Guak et al. 1998). In the near future, nursery managers may need to adjust their cultural practices, target species and seed sources, and lifting and storage schedules as they strive to maintain optimum seedling phenology (Tepe and Meretsky 2011, Williams and Dumroese 2014).

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Current Practices for Growing Whitebark Pine Seedlings at the U.S. Department of Agriculture, Forest Service, Coeur d'Alene Nursery

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Abstract

The USDA Forest Service, Coeur d'Alene Nursery has produced whitebark pine (*Pinus albicaulus* Engelm.) seedlings for more than 25 years, with 2014 production numbers reaching 214,464 seedlings. Germination remains the largest obstacle in whitebark pine production, due to both the physical and physiological dormancy of the seed. The protocol described in this article outlines the current steps the Coeur d'Alene Nursery uses to break seed dormancy and the growing regime for seedling production. This protocol, which is constantly evolving, serves as a guideline for the production of high-quality plant material for the reforestation and restoration of this important foundation species. This paper was presented at a joint meeting of the Western Forest and Conservation Nursery Association, the Intermountain Container Seedling Growers Association, and the Intertribal Nursery Council (Boise, ID, September 9–11, 2014).

Introduction

Whitebark pine (*Pinus albicaulus* Engelm.) is a high-elevation species found throughout the Western United States and western Canada. It is a foundation species that provides habitat and food for wildlife, including grizzly bear (*Ursus arctos* Linnaeus) and Clark's nutcracker (*Nucifraga columbiana* A. Wilson). Populations of whitebark pine are decreasing due to a nonnative blister rust pathogen (*Cronartium ribicola* A. Dietr.), fire suppression and exclusion practices, mountain pine beetle (*Dendroctonus ponderosae* Hopkins), and wildland fire (Mahalovich and Hipkins 2011). Because of these pressures and the species' importance on the landscape, whitebark pine seedlings are currently grown in nurseries to aid in the reforestation of this species.

The mission of the USDA Forest Service, Coeur d'Alene Nursery is to provide high-quality plant material for restoration and reforestation to the USDA Forest Service's National Forest System, and to other public land management agencies. The Coeur d'Alene Nursery has produced whitebark pine seedlings since 1988, with production numbers increasing to 214,464 seedlings for the 2014–15 planting season. Adequate germination is the largest obstacle in whitebark pine seedling production at the Coeur d'Alene Nursery due, in part, to the complex dormancy of the seeds. Whitebark pine seeds exhibit both physical and physiological dormancy. In 2014, the average germination of nine seed lots was approximately 61 percent, with individual seed lot germination rates ranging from 28 to 90 percent.

The following sections describe the Coeur d'Alene Nursery's current seed preparation and seedling production protocols for whitebark pine. This protocol is an update to the procedures outlined by Burr et al. (2001) and Robertson et al. (2013), but it is a working protocol, meaning that nursery staff are constantly evaluating protocols and changing procedures to incorporate new technologies and streamline production.

Seed Processing

Cleaning and Storage

High-quality seedling production begins with high-quality seed. Whitebark pine cones are hand cleaned in the seed extractory at the Coeur d'Alene Nursery. Throughout the cleaning process, digital x-rays are used to determine percent seed fill. Seed lots are cleaned to a minimum of 95 percent fill and 99 percent purity (figure 1). Seeds are stored at 5.5 to 8 percent moisture and kept in walk-in freezers maintained at 29 ºF (-1 ºC). On average, 2,867 seeds/lb (632 seeds/100 g) are in the 123 seed lots of whitebark pine that are currently stored in the Coeur d'Alene Nursery's seedbank.

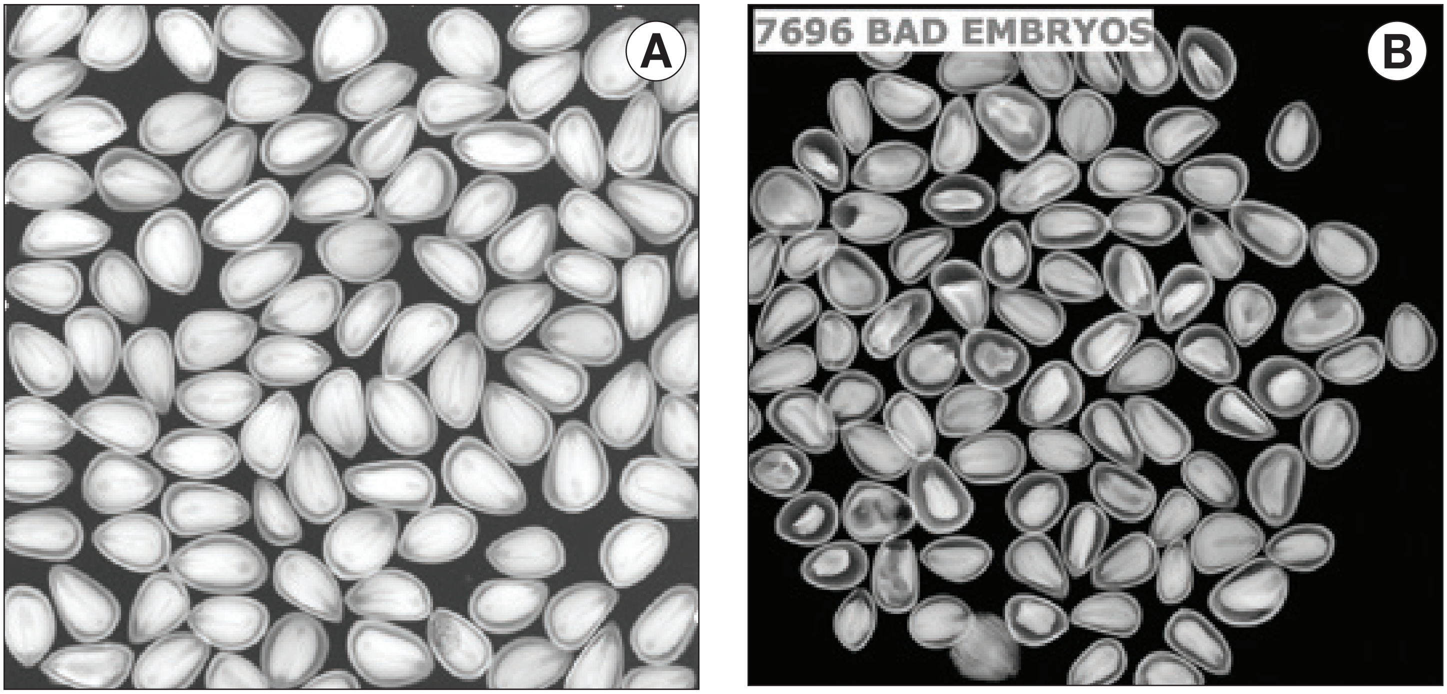


Figure 1. Digital x-rays are a tool used throughout the seed-cleaning process to ensure high-quality seed is stored in the Coeur d'Alene Nursery's seedbank. (a) An x-ray of cleaned seed with at least 95 percent filled embryos and more than 95 percent purity. (b) An x-ray of seed with damaged and underdeveloped embryos that will not be stored. (Photos by Jerri Park)

Treatments

To overcome whitebark pine's physical and physiological seed dormancy mechanisms, seeds are subjected to a 120-day stratification followed by scarification (Burr et al. 2001, Robertson et al. 2013). The stratification process starts with a 48-hour running water treatment in nylon mesh bags. The mesh bags are then placed inside plastic bags that are 1 mil (1000 mil = 1 in, 2.54 cm) in thickness to prevent seed moisture loss and allow for respiration during stratification. The bagged seeds are put into a 30-day warm stratification in germinators set to 86 ºF/68 ºF (30 ºC/20 ºC) day/night, with 12-hour days, and no light. Seeds are taken out every week and rinsed in cold water for 1 hour. Following warm stratification, the plastic bag is changed, and the seeds are moved into cold stratification for 90 days. Cold stratification takes place in a walkin stratification room set to 36 ºF (2.2 ºC) with no light. Every week, the seeds are rinsed for 1 hour in cold water, and every month the plastic bag is changed.

When the 120-day stratification is completed, the seeds are surface dried and placed in a specially designed, rotary-drum sander (USDA Forest Service, Missoula Technology and Development Center, Missoula, MT; Gasvota et al. 2002), consisting of four coffee cans lined with 50-grit sandpaper. The sander spins at 70 revolutions per minute, and the seeds are scarified for 3 hours (figure 2). Following scarification, the seeds are rinsed to remove dust and are placed in a plastic

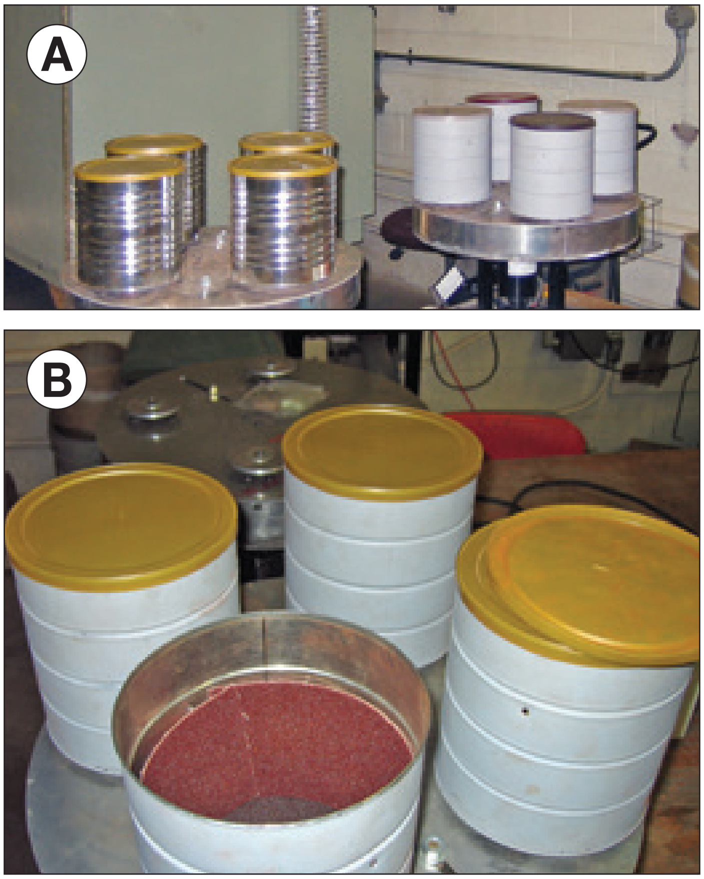
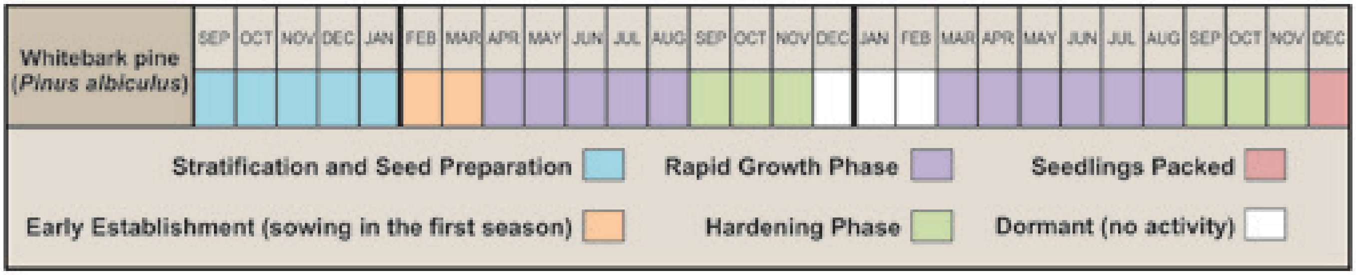


Figure 2. The USDA Forest Service, Missoula Technology and Development Center developed this rotary drum sander to scarify seed (Gasvota et al. 2002). (a) Seed is placed in each coffee can lined with 50-grit sandpaper and (b) seeds are scarified for 3 hours at 70 revolutions per minute. (Photos by Nathan Robertson and Emily Overton)



Whitebark pine (*Pinus albiculus*)	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
	🟦	🟦	🟦	🟦	🟦	🟧	🟧	🟪	🟪	🟪	🟪	🟪	🟩	🟩	🟩	⬜	⬜	⬜	🟪	🟪	🟪	🟪	🟪	🟪	🟩	🟩	🟩	🟥

Stratification and Seed Preparation 🟦
Rapid Growth Phase 🟪
Seedlings Packed 🟥
Early Establishment (sowing in the first season) 🟧
Hardening Phase 🟩
Dormant (no activity) ⬜

Figure 3. The production schedule for whitebark pine seedlings at the Coeur d'Alene Nursery shows that this species requires a 4-month seed stratification and two complete growing seasons to make packing specifications of 3 in (7.6 cm) heights and 3 mm (0.12 in) root collar diameters.

bag. A recent addition to the Coeur d'Alene Nursery seed preparation protocol includes placing the scarified seeds in a germinator set to 86 ºF (30 ºC) with 24 hours of light. Seeds are kept in the germinator for a minimum of 22 hours until sowing (figure 3).

Seedling Production

The full production cycle for whitebark pine from seed to harvest at the Coeur d'Alene Nursery is shown in figure 3 and is described in detail in the following sections.

Sowing

Whitebark pine seedlings are grown as a 2-year container stocktype at the Coeur d'Alene Nursery. Depending on the depth of soils at the planting site, seeds are hand sown into 10 in^3 (164 ml) or 7 in3 (107 ml) Ray Leach Cone-tainers™ (Steuwe & Sons, Tangent, OR) (figure 4). This container system works well because it allows for consolidation into less growing space in the event of poor germination, has potential to prevent disease issues during production, and prevents root damage at seedling extraction (figure 5). Seeds are planted into a custom blend of 70:30 peat:finely screened composted Douglas-fir bark (Phillips Soil, Canby, OR). The medium is watered to field capacity at the time of sowing. Whitebark pine seed is very valuable because of the difficult and costly cone collection and the lengthy and labor-intensive seed-cleaning and stratification processes. As a result, only one or two seeds are sown per cell (figure 6). Seeds are planted into predibbled holes that are just slightly deeper than the seed, usually about 0.25 in (6.35 mm). Planting depth seems to play an important role in germination success; seeds that are planted too deep often fail to emerge, possibly due to a lack of energy in the seed. The surface of the container is covered with nonporous Styrogrit® (figure 7) (Beaver Plastics, Alberta, Canada) to prevent moisture loss at the medium surface and to discourage growth of weeds and algae. During germination, greenhouse temperatures are maintained at 86 ºF (30 ºC)

Figure 4. Whitebark pine seed that has been stratified, scarified and placed in germinator at 86 ºF (30 ºC) for 24 hours. This seed is now ready to be hand sown into the greenhouses at the USDA Forest Service, Coeur d'Alene Nursery. (Photo by Nathan Robertson, 2010)

Figure 5. The seedlings on the left were hand sown 8 weeks before the seedlings on the right. After full germination, the Ray Leach Cone-tainers™ (Steuwe & Sons, Tangent, OR) were consolidated to reduce greenhouse space due to poor germination. (Photo by Emily Overton, 2014)

Figure 6. Whitebark pine seeds are hand sown into 10 in^3 (164 ml) Ray Leach Cone-tainers™ (Steuwe & Sons, Tangent, OR) at the USDA Forest Service, Coeur d'Alene Nursery. Because of the expense of the seed, only one or two seeds are sown per cell. (Photo by Nathan Robertson, 2012)

Figure 7. The USDA Forest Service, Coeur d'Alene Nursery uses a recycled non-porous Styrogrit® product to cover all of our whitebark pine seedlings. This photo compares traditional rock grit (on the left) to two examples (in the middle and on the right) of Styrogrit® products. (Photo by Nathan Robertson, 2012)

maximum and 68 ºF (20 ºC) minimum. The containers are also covered with plastic to maintain humidity and trap heat until germination begins (figure 8).

Figure 8. Early germination of whitebark pine seedlings at the USDA Forest Service, Coeur d'Alene Nursery. (Photo by Nathan Robertson, 2012)

Fertilization and Irrigation

In the first year of growth, whitebark pine fertilization with a low rate (120 ppm N) of Wil-Sol® Pro-Grower ($20N:7P_2O_5:19K_2O$, Wilbur-Ellis, Yakima, WA) begins when one-half of the seedcoats are off the cotyledons (figure 8). Approximately 3 weeks later, seedlings are switched to a higher rate (200 ppm N total) of Wil-Sol® Pro-Grower ($20N:7P_2O_5:19K_2O$, Wilbur-Ellis, Yakima, WA) supplemented with CAN-17 (17N:8.8Ca, Wilbur-Ellis, Yakima, WA) for approximately 20 weeks. High rates of nitrogen are used to promote apical growth in this slow-growing species. Irrigation timing is determined by gravimetric weights (Dumroese et al. 2015) and visual assessment of the root plug. During rapid growth, irrigation occurs when trays reach 75 percent of their field capacity weights. Seedlings are switched to Wil-Sol® Pro-Finisher (4N:25P2O5:35K2O, Wilbur-Ellis, Yakima, WA) at a rate of 40 ppm N during hardening, at which point irrigation schedules are also shifted to 55 to 60 percent of field capacity weights before watering. Supplemental light is added to extend the natural day length to 16 to 18 hours from the time of germination until the end of the rapid-growth phase. After seedling establishment and during rapid growth, greenhouse temperatures are maintained at a minimum of 60 °F (15.5 °C). Seedling mortality increases when daytime temperatures are more than 95 °F (35 °C), so 47 percent shadecloth is added to the greenhouses during the summer months to reduce heat loads.

In the second year of growth, fertilizer and irrigation schedules are similar to those of the first year. Greenhouse systems are turned on between early March and mid-March, with minimum temperatures of 60 °F (15.5 °C), and day lengths extended to 16 to 18 hours using supplemental light. Seedlings are immediately put on 200 ppm N of Wil-Sol® Pro-Grower (20N:7P2O5:19K2O) and CAN-17 (17N: 8.8Ca, Wilbur-Ellis, Yakima, WA) to promote apical growth, and they are irrigated when weights are 75 to 80 percent of field capacity. This regime continues for 20 to 25 weeks. Following the rapid growth phase, supplemental light is removed, seedlings are switched to a hardening fertilizer regime of

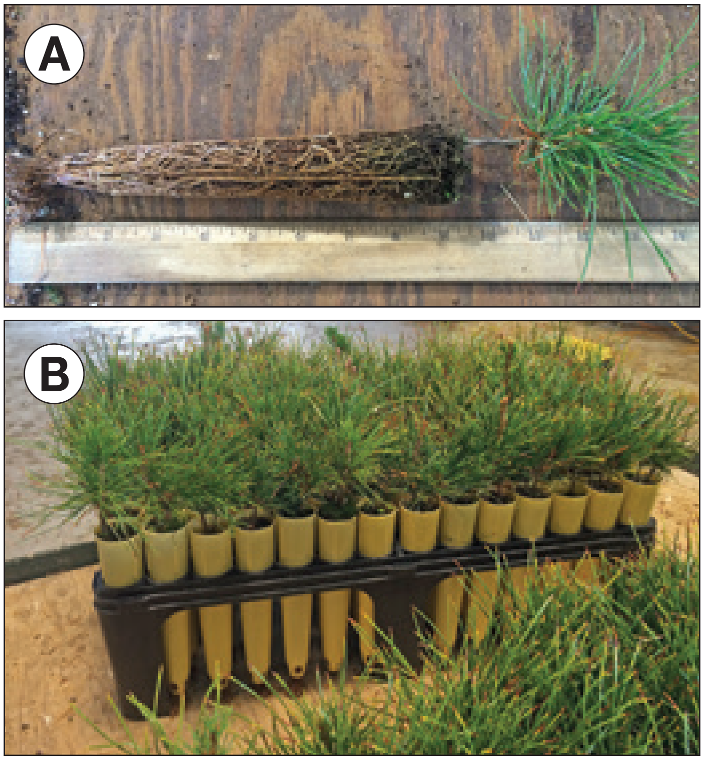


Figure 9. Whitebark pine seedlings ready for extraction and packing in the fall. Seedlings are graded on minimum height (3 in [7.6 cm]) and root collar diameter (0.098 in [2.5 mm]) specifications before shipment. (a) A single seedling extracted from a Ray Leach Cone-tainers™ (Steuwe & Sons, Tangent, OR). (b) A tray of 98 whitebark pine seedlings. (Photos by Emily Overton, 2015)

Wil-Sol® Pro-Finisher (4N:25P2O5:35K2O, 40 ppm N), and irrigation frequency is decreased to watering at 60 to 65 percent of field capacity. Seedling specifications at packing are 3 in (7.63 cm) height minimum and 2.5 mm (0.098 in) root collar diameter (figure 9).

Future Outlook

The Coeur d'Alene Nursery continues to refine and modify the whitebark pine seed and seedling production protocols by participating in small- and large-scale research opportunities. Small studies were conducted in the spring of 2015 to monitor germination rates, following the newest addition to the seed preparation protocol, which involves placing the seeds in a germinator set to 86 °F (30 °C) postscarification. Trials were also set up to explore if germination would improve when using a lightweight germination fabric on newly sown seeds. A large-scale coordinated research effort among the Coeur d'Alene Nursery, USDA Forest Service, Lucky Peak Nursery, USDA Forest Service, Dorena Genetic Resource Center, and USDA Forest Service, National Seed Laboratory is also under way, which seeks to improve germination by further investigating seed imbibition, stratification lengths, and alternate scarification methods. The results from this study and the trials conducted at the Coeur d'Alene Nursery will help to further streamline the production of this important restoration species.

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