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Abstract

These proceedings are a compilation of the papers that were presented at the regional meetings of the forest and conservation nursery associations in the United States and Canada in 2007. The Northeastern Forest and Conservation Nursery Association meeting was held July 16 to 19 at the Grappone Conference Center in Concord, NH. The meeting was hosted by the New Hampshire State Forest Nursery. Subject matter for the technical sessions included seed collection, handling, and storage, soil management, seedling nutrition, disease management, and fumigation alternatives. Field trips included an afternoon tour of the New Hampshire State Forest Nursery in Boscawen, NH, and a full day tour of the White Mountain National Forest, including timber management and wilderness projects across the Kancamagus Highway, and Hubbard Brook Experimental Forest. The combined meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Association was held at the Mary Winspear Centre in Sidney, BC, on September 17 to 19. The meeting was hosted by the Forest Nursery Association of British Columbia. Subject matter for the technical sessions included global climate change, business practices and marketing, forest nursery practices, nursery technology, disease management, and labor management. An afternoon field trip included tours of the Arbutus Grove Nursery Limited, Eurosa Gardens Limited, and Church and State Wines on the Saanich Peninsula.

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

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2007

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

Northeastern Forest and Conservation Nursery Association

July 16 to 19, 2007

The Grappone Conference Center | Concord, New Hampshire

Forest Nursery Association of British Columbia
Western Forest and Conservation Nursery Association
Combined Meeting

September 17 to 19, 2007

The Mary Winspear Centre | Sidney, British Columbia

Northeastern Forest and Conservation Nursery Association

July 16 to 19, 2007

The Grappone Conference Center | Concord, New Hampshire

Some Thoughts about Tree Planting and Nursery Culture in New Hampshire

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KEYWORDS
Norway spruce, eastern white pine, red pine, Douglas-fir, plantations

"He plants trees to benefit another generation"
— CICERO

As a forester one of the best and worse things about my work is the time involved to grow a forest. Most benefits of my work will be realized long after my last steps are taken on this earth. It's comforting to me to know that 3 generations from now, people will be harvesting trees that I have planted, or that have regenerated from cuttings that I have made in the forest.

On the other hand, I often wish that I could get in a time machine and zip ahead into the future to see how things will work out; which species will do best; how well they will grow; how well this forest will match up to the previous forest that I managed.

We owe so much to previous generations that have contributed their time, efforts, sweat, and careers to the forest. The State of New Hampshire has benefited from the planting stock that has been developed and provided by the State Forest Nursery and from the many cooperative programs over the years that helped improve the stock which it provided.

The Norway spruce (*Picea abies*) plantation (Figure 1) at the Caroline A Fox Research and Demonstration Forest in Hillsborough, New

Figure 1. Norway spruce (*Picea abies*) plantation on the Fox Research and Demonstration Forest in Hillsborough, New Hampshire.

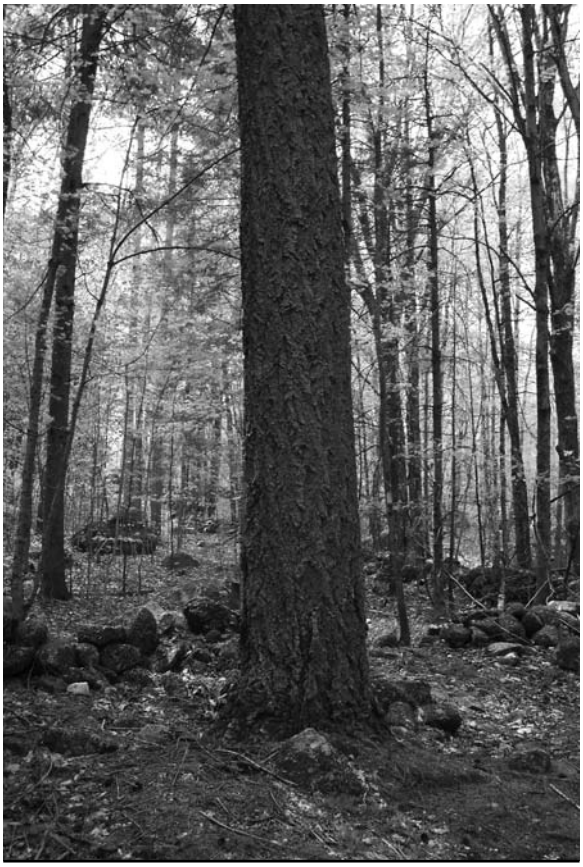

Figure 2. Douglas-fir (*Pseudotsuga menziesii*) groves on the Fox Forest.

Hampshire (43°08'15.86''N, 71°54'56.53''W) was planted in 1936. Seeds for this plantation were collected across much of Europe as part of a provenance trial to examine geographic variation of seed source, and to try and discern how local seed sources react to new climatic conditions. This stand, in particular, has been heavily studied by master and doctoral degree candidates and used to help decipher problems with Norway spruce in the Black Forest of Germany. These seeds were sent to Fox Forest and raised in a small nursery on site by Dr Henry I Baldwin, the forest's first research forester.

A similar plantation of Norway spruce was planted about 10 km (6 mi) east at the Vincent State Forest. This plantation was part of a huge provenance experiment by the International Union of Forest Research Organizations (IUFRO). New Hampshire has one of the best plantations from this experiment because most were situated in Europe and destroyed or severely damaged during World War II.

Also located at Fox Forest are several small groves of Douglas-fir (*Pseudotsuga menziesii*) (Figure 2). These seeds were collected from across the American west and planted in the Fox Forest nursery as part of a Christmas tree study to determine which local sources did best in New Hampshire. The Santa Fe New Mexico seed source eventually proved best for Christmas tree culture. Personally, Douglas-fir is my favorite Christmas tree. I usually need to grow my own, however, because New England growers prefer to grow balsam fir (*Abies balsamea*). Baldwin eventually elected to leave some of these groves instead of harvesting them all, providing us with this excellent specimen to enjoy. This grove currently has a dozen or so individuals growing in it, this stem being the largest at about 76 cm (30 in) diameter at breast height after about 70 years.

Just beyond the Douglas-fir grove is a small plantation of Carolina cottonwoods (*Populus deltoides*) (Figure 3). These trees were grown from cuttings provided by Oxford Paper Company in the 1930s. Their intention was to provide the cut-

tings to landowners who wished to grow pulpwood for their mill in northern Massachusetts. These trees are very fast growers.

Without question, Fox Forest has benefited greatly from nursery work across the world. It also contains several larch (*Larix* spp.) plantations, including specimens from the Duke of Atholl's Dunkeld home in Scotland. These amazing Dunkeld larch hybrids (*Larix marschlinsii*) are a cross between European (*L. decidua*) and Japanese (*L. kaempferi*) larch, and have been known to grow one third faster than either parent.

Other state reservations have also enjoyed the benefits of cultured forests. Figure 4 shows the red pine (*Pinus resinosa*) plantation located at Contoocook State Forest in the town of Hopkinton, New Hampshire. Like so many other red pine plantations in the state, this forest was planted by the Civilian Conservation Corps (CCC) during the Depression years of the 1930s. Red pine is a fast growing, straight-trunk tree that did not have the terrible weevil problem of our more common white pine (*P. strobus*). The CCC was geared up for planting, and the nursery was producing a lot of red pine seedlings during this time. In 1931, the production of red pine planting stock was only 58,932 seedlings. In 1934, however, the nursery produced 508,855 red pine seedlings. In 1935, it produced 361,329 red pine seedlings, with production dropping to 270,942 seedlings in 1936. During that same decade, much of Mast Yard State Forest was planted to red pine or white pine plantations. Some of the Mast Yard plantations have been thinned 6 times so far (Figure 5). In the fall of 1993, following a detailed forest inventory and review of past operations in the stands, some of the Mast Yard plantations had produced nearly 507 m^3 of wood/ha (57 cords of wood/ac) at about age 60.

White pine is probably the tree species most often planted in New Hampshire for reforestation. Over the years, we have improved and thinned many of these plantations with timber stand improvement treatments, biomass/whole tree harvests, and sawtimber thinnings, and we

Figure 3. Carolina cottonwood (*Populus deltoides*) plantation on the Fox Forest.

Figure 4. Red pine (*Pinus resinosa*) plantation on Contoocook State Forest in Hopkinton, New Hampshire.

Figure 5. Early thinning in a Mast Yard State forest red pine (*Pinus resinosa*) plantation.

are now regenerating many of these mature stands. White pine plantations carry a large amount of stocking, with volumes often between 115 to 175 m^3/ha (20,000 to 30,000 bd ft/ac). Many different loggers have harvested timber products from these stands and sent the logs to several sawmills, which process the lumber and send it out to a multitude of local industries in the state. A rule of thumb in New England is that US$ 1 of stumpage money yields another US$ 27 to the local economy. Just think of what the value-added potential is from seedling to sawlogs. The benefits from our white pine plantations have been a mainstay of the forest management program of the state.

What led to the formation of the New Hampshire State Forest Nursery in 1910? It was a reaction to the activities of the times. The science of forestry was in its infancy in the US. Much of the timber harvesting occurring in the state was very heavy-handed. Companies often would buy a woodlot, harvest everything of value on that particular land holding, and then sell the land. The widespread heavy cutting and the resulting forest fires inspired the adoption of the Weeks Act in 1911, which allowed the USDA Forest Service to purchase and protect land along navigable rivers. This was the beginning of our own White Mountain National Forest in New Hampshire and Maine.

So much land was cutover and bare that the state of New Hampshire instituted a program where landowners could deed cutover land to the state, and the state would replant the land and sell it back to the original owner for planting costs plus 4% interest. These were called "Ten Year Tracts" because the owners had up to 10 years to purchase them back. Old biennial reports for the New Hampshire Forestry Commission reveal that planting costs in 1914 were US$ 27/ha (US$ 11/ac), which included the cost of seedlings and all labor. In 1915, crews planted 93 ha (231 ac) of private lands under the program, and an additional 73 ha (190 ac) of state reservations. Even at these low costs, much land was never purchased back by the owners. Many of these tracts make up the current 201 individual properties that form the state reservation system in New Hampshire today.

The state wanted to achieve 3 goals with the formation of a state forest nursery: 1) supply the increasing demand for seedling stock to reforest cutover woodlands; 2) begin a cooperative planting program with towns and private lands; and 3) supply native trees grown locally to prevent the importing of diseased seedlings, especially from Europe.

In 1910, the state leased some land in the town of Pembroke and began a small nursery. The nursery planted transplants and offered them for resale to residents for US$ 3/thousand seedlings. The first year had great success, with 50,000 transplant seedlings sold. The estimated demand, however, was 200,000 seedlings. The following year, the state leased a larger tract of land in the town of Boscawen and produced 250,000 seedlings. This operation was considered so successful that more land was leased at this location and the operation was expanded. In 1914, the nursery produced 717,000 seedlings. To date, we estimate the New Hampshire nursery has sold approximately 76,500,000 seedlings.

Today, the New Hampshire State Forest Nursery continues to produce seedlings for reforestation, Christmas tree culture, wildlife habitat improvement, and ecological restoration. It is the only state nursery still in operation in New England. Its future will be decided by how innovative the staff can be and by how successful it can be at providing important services to residents of the state.

Diagnosing Plant Problems

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ABSTRACT
Diagnosing Christmas tree problems can be a challenge, requiring a basic knowledge of plant culture and physiology, the effect of environmental influences on plant health, and the ability to identify the possible causes of plant problems. Developing a solution or remedy to the problem depends on a proper diagnosis, a process that requires recognition of a problem and determination of the cause or causes.

KEYWORDS
disease symptoms, biotic, abiotic

Symptom Recognition

Before a grower can recognize symptoms, he or she must be familiar with the "normal" characteristics for the particular tree species and varieties grown. Characteristics such as unusual growth habit or needle color may be mistaken for disease symptoms unless the grower knows these characteristics are typical for that variety.

The first step in diagnosing a plant health problem is the recognition of *symptoms*. A symptom is any visible, "abnormal" condition of a plant caused by living organisms, such as fungi, insects, bacteria, or viruses, or non-living agents, such as environmental factors, chemical damage, or physical injury. Common symptoms of tree diseases and disorders include needle blights, chlorosis (yellowing), necrosis (browning), stunting, tip dieback, distorted growth, galls, needle drop, stem cankers (dark, usually sunken areas), wilt, and root rot, to name just a few.

Is There a Pattern to the Symptoms?

After noting the symptoms, make a general assessment of the affected tree(s) and nearby healthy trees. A series of questions may be helpful in assessing the problem: Is more than one plant affected and is more than one type of plant (genera or species) affected? Where on the tree(s) did the symptoms first appear? Is the problem limited to the interior or exterior portions of the tree (or plantation)? Are the symptoms localized or widespread? Are several types of symptoms pres-

ent? Is there a pattern of the affected plants in the plantation (rows, low areas, every nth plant)?

After making a general assessment, take a close look at the symptoms. Are fruiting bodies (fungal structures) visible in the discolored needles or cankered areas? What does the transition zone (border between healthy and "diseased" tissue) look like? Is a sharp line of discoloration between healthy and "diseased" tissue visible? Is there evidence of insect feeding? Is pitch evident? Whenever possible, check the roots. Tip dieback, needle browning, wilting, and what appear to be nutrient deficiencies are symptoms often associated with root rots.

If more than one genera of tree is affected, the cause is often due to an *abiotic* (non-living) agent. If the symptoms are limited to a single tree type, the problem is more likely to be caused by a *biotic* (living) agent—a pathogen or insect. Bear in mind, however, that a particular species of tree may be more or less sensitive to chemical or environmental problems, such as inappropriate use of fertilizers and pesticides, or drought.

Non-living agents are the most likely cause of symptoms appearing on only one side of a tree or planting, or in a repeated pattern, such as every 2 plants or every other row. Symptoms caused by living agents are more likely to be random in occurrence or pattern.

Are Signs Visible?

Signs are the actual visible evidence of fungi and/or insect pests. A 12X to 15X magnifying lens is helpful for viewing fungal structures and insects or mites. Examine the symptomatic plants for fungal fruiting bodies (black or brown pinpoint-size structures) embedded in the tissues (in leaf spots or cankered areas) or fungal growth (molds or strand-like growths).

Signs of insects include the insect itself in any of its life stages, cast exoskeletons (skins), webbing (spider mites), droppings (frass or honeydew), or sooty mold. Finding evidence of a pathogen or insect may not lead directly to the cause of the problem, however. Sometimes non-living factors can weaken a plant and predispose it to attack by pathogens and/or insects. For example, drought can predispose plants to root rot fungi, such as *Armillaria* spp., to fungal tip blights, such as those caused by *Diplodia* spp., or insect attack.

How Quickly Did the Symptoms Appear?

Another important perspective to consider is the time frame during which symptoms appeared. A record of the environmental conditions just prior to and during the time symptoms appeared may also be useful in determining the cause or causes of the problem.

When did the symptoms first occur? This is often a difficult question, as many problems seem to appear overnight. Symptoms caused by most living agents take several days, weeks, or longer to develop. Non-living agents are usually the cause of symptoms that appear suddenly (1 or 2 days).

Record-keeping

Keeping weekly records of general plant health will help pinpoint the appearance and track the progression of symptoms. Records are also helpful when trying to determine if particular management or cultural practices themselves may have caused the symptoms. It is important to keep records of the dates and rates of fertilizer and pesticide applications, along with notes about environmental conditions when the applications were made. In addition, keep records of new plantings (dates and source), as new trees may serve as the source of pathogens or insects.

Note any changes in the surrounding environment: Are the problem trees located in a windy or frost-prone site? Have there been extremes of moisture (drought/flood)? Questions such as these will help determine if the problem is caused by environmental factors.

Diagnostic Testing

Once you've noted a problem and identified potential causes, you may want to submit plants to a diagnostic laboratory for confirmation or further identification. When sending samples to a

lab for diagnostic testing, follow these steps to ensure an accurate and timely diagnosis:

- Include as much information as possible about the history of the problem (when symptoms were first noted, rate of progression, and any visible pattern to the symptoms, percentage of crop or plants affected).
- Provide information on pesticide and fertilizer applications, and any changes in the growing environment.
- Be sure to include the name and variety of the trees. Be sure to include a healthy sample for comparison purposes. If sending samples by mail, package the sample with packing material to avoid shifting during shipment and mail the package early in the week or by overnight delivery to avoid spoilage.

Every state has a diagnostic lab affiliated with their Land Grant University (and usually cooperative Extension). To find the diagnostic lab in your state, go to URL: http://www.npdn.org.

Solutions to the Problems

The best approach is to prevent problems in the first place. This may seem obvious; but many simple practices that can prevent plant health problems are often overlooked.

- Good sanitation is the best prevention and control for problems resulting from disease-causing microorganisms and many insects.
- Inspect all plant material before planting. Don't plant trees that show symptoms of any kind. You don't want to inherit a problem.
- Sanitize cutting, planting, and pruning tools before each use. Any plant tissues infected with disease-causing microorganisms can serve as a source of infection for nearby plants. Infected shoots or stems should be pruned and destroyed. If a large portion of the plant is infected, it is better to remove the entire plant. Prune infected trees last. When pruning infected trees and shrubs, sanitize the cutting tools between each cut (10% bleach or 70% alcohol) and destroy the prunings.
- Be sure plants are planted properly. J-rooted plants are more susceptible to Armillaria root rot and canker diseases.
- Seedling trays and pots should be sanitized before reusing. (It is best to use new).

Water, light, and proper nutrition are also key factors to monitor and adjust when necessary to avoid stressing plants. Remember, plants under stress are usually more susceptible to attack by both disease-causing organisms and insects.

Diagnosing plant problems can be both frustrating and rewarding. It is helpful and, at times, necessary to have a collection of reference sources, including plant disease and insect identification guides, to aid in the diagnosis. As with any other skill, the more you practice, the better refined your skills will become. Remember, you can always send samples to a diagnostic lab for confirmation before choosing a management practice, so don't be afraid to hone your own diagnostic skills.

Comments on Alternatives to Methyl Bromide for Quarantine Purposes in Forest Nurseries

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ABSTRACT
Viewpoints will vary in regards to the best alternative to methyl bromide (CH_3Br) fumigation. In some cases, crop value will determine the best alternative. As the value of the crop increases, the rate (and cost) of the best treatment might increase as well. In addition, the recommendation will depend on if the individual has a vested interest in the production of high quality seedlings. An individual with no economic incentive might recommend an uneconomical, impractical, or unreliable alternative. In contrast, an individual who intends to make a profit might recommend an alternative that would cause minimal impact on costs and revenue. According to tests in both the southern and western US, chloropicrin applied under a tarp at 336 kg/ha (300 lb/ac) will cause a minimal disruption to a well-managed forest nursery. If nematodes are present, a fumigant like 1,3-D may be applied at time of treatment. Although chloropicrin is not as effective as CH_3Br on certain perennial weeds, sanitation and the effective use of herbicides can minimize the population of troublesome weeds.

KEYWORDS
fumigation, chloropicrin, herbicides, nematodes, disease, weed control

Introduction

Methyl bromide (CH_3Br) is a natural compound that is produced by phytoplankton in oceans, by forest fires, by certain plants, and by ectomycorrhiza. The amount produced by natural events in the southern hemisphere troposphere might amount to 6 ppt of CH_3Br (which is enough to affect the stratospheric ozone layer) (Montzka and others 2003). Of the total amount of CH_3Br in the stratosphere (about 8.2 ppt), natural sources amount to 81%, while manufactured sources account for 19% (Fahey 2006). However, attempts to separate natural and anthropogenic components has generated considerable scientific and regulatory controversy. In the 1990s, oceans were thought to be a net source of CH_3Br. In 2007, oceans are viewed as a net sink (Yvon-Lewis and Butler 1997). Some assume that all "unknown" sources of methyl bromide are the result of human activity (Saltzman and others 2004), while others assume some of the "unknown" sources could be from natural sources. Some believe the 8.2 ppt level detected in 1997 (in the southern hemisphere) is about 1.6 ppt higher

than it should be (Figure 1). As a result, an international agreement (the Montreal Protocol) put limits on the manufacture of CH_3Br and other ozone depleting substances (Parker and others 2005). Due to countries adhering to the Montreal Protocol, production of ozone depleting substances was reduced from 1.8 million weighted tonnes/year (2.0 million tons/year) in 1987 to about 83,000 tonnes/year (91,500 tons/year) in 2005 (EPA 2007). Therefore, annual production (by humans) of ozone depleting substances has been reduced by more than 95%. The global consumption of CH_3Br was about 71,764 tonnes/year (79,100 tons/year) in 1991. By 2005, it was reduced to about 20,752 tonnes/year (22,875 tons/year) (MEBTOC 2006).

From 1998 to 2003, the bromide levels in the troposphere decreased by about 0.8 ppt (Montzka and others 2003). Dr Ian Porter of Australia (a co-chair of the United Nations MB Technical Options Committee) purportedly said that, due partly to the reduction in use of manufactured methyl bromide, "the hole in the ozone layer (Figure 2) should begin to decrease in size over Australia within the next few years" (Dowler 2007). Due to the phase-out, the price of CH_3Br has increased, and some managers are now seeking alternative treatments.

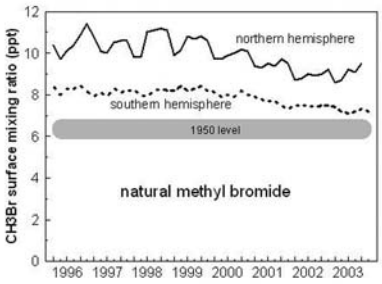

Figure 1. The amount of methyl bromide (CH_3Br) has declined in the stratosphere above the northern and southern hemispheres (Butler and others 2004; Fahey 2006). Higher levels of CH_3Br in the northern stratosphere are due, in part, to a greater amount of vegetation combined with more biomass burning (CH_3Br sources) in the northern hemisphere, and more oceans (CH_3Br sink) in the southern hemisphere. In 1950, the amount of CH_3Br in the stratosphere may have averaged 7 ppt (Fahey 2006).

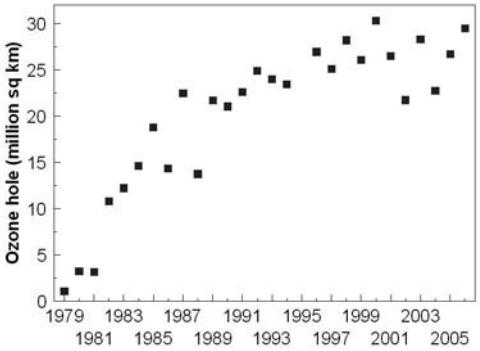

Figure 2. The ozone hole above Antarctica has increased from less than 5 million km^2 (1.9 million mi^2) in 1980 to more than 25 million km^2 (9.6 million mi^2) in 2006. On September 25, 2006, the size was 29.5 million km^2 (11.4 million mi^2). If computer models prove to be accurate, the recovery of the ozone hole should take place around 2060.

Quarantine and Pre-shipment

Paragraph 6 of Article 2H of the Montreal Protocol exempts the use of CH_3Br used for quarantine and pre-shipment (QPS). The Montreal Protocol provided no limitation to the production and consumption of CH_3Br when used for QPS purposes. When CH_3Br is used for this purpose, it is referred to as "QPS gas." Some nursery managers fumigate with QPS gas to help ensure that seedlings shipped are "free of injurious pests." A phytosanitary certificate is typically required before seedlings can be shipped over state or international borders. For example, in 2004, nursery stock and Christmas trees were shipped from Oregon to over 70 foreign countries. Soil fumigation (for example, QPS gas) is a tool used

The following is from the 2006 Report of the Methyl Bromide Technical Options Committee (MEBTOC 2006).

The Seventh Meeting of the Parties decided in Dec. VII/5 that:

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

(b) Pre-shipment applications are those treatments applied directly preceding and in relation to export, to meet the phytosanitary or sanitary requirements of the importing country or existing phytosanitary or sanitary requirements of the exporting country;

(c) In applying these definitions, all countries are urged to refrain from use of methyl bromide and to use non-ozone-depleting technologies wherever possible. Where methyl bromide is used, Parties are urged to minimize emissions and use of methyl bromide through containment and recovery and recycling methodologies to the extent possible.

to reduce the risk of spreading invasive diseases and pests on nursery stock.

The use of QPS gas is increasing in response to the International Standard for Phytosanitary Measures (ISPM 15), which is encouraging CH_3Br use on wooden packaging materials (FAO 2002). Ajavon and others (2007) believe, however, this increased use of QPS gas is offsetting the reductions which have occurred in soil fumigation and other non-QPS uses. They say that that "technical alternatives" exist for almost all controlled uses of CH_3Br. However, "technical alternatives" such as methyl iodide (a soil fumigant) and halosulfuron-methyl (an herbicide with activity on nutsedge), may not be used legally unless registered by the US Environmental Protection Agency (EPA). Thus far, we know of no herbicide or fumigant that EPA has approved as an alternative to CH_3Br fumigation in forest tree nurseries. Because EPA has not approved use of halosulfuron-methyl, MSMA, methyl iodide, or sodium azide, nursery managers will continue to use chemicals that have been approved by EPA.

Silvicultural Alternatives to QPS Gas

Landowners who wish to regenerate a stand after harvest have several options. Some landowners may choose to conduct a prescribed burn and then allow natural regeneration to occur. This option will result in some CH_3Br being released into the atmosphere during the burn. Global emissions of methyl bromide from biomass burning are estimated to be in the range of 10,000 to 50,000 tonnes/year (11,000 to 55,000 tons/year), which is comparable to the amount produced by ocean emission and pesticide use, and represents a major contribution (an estimated 30%) to the stratospheric bromine budget.

Direct seeding does not rely on the use of QPS gas and is another silvicultural option that some landowners have employed. The cost of site preparation, seeds, labor, and herbicides may range from US$ 615 to 1230/ha (US$ 250 to 500/ac) and the risk of failure can be high.

Some landowners may decide to purchase and plant container stock. In some locations, the price of container stock is similar to that of bareroot stock. For example, in the Pacific Northwest, container stock may cost US$ 0.34 per seedling, while bareroot stock (produced after fumigating soil with QPS gas) might cost US$ 0.30 per seedling. When container seedlings cost more than bareroot stock, one option is to plant fewer container seedlings to offset the higher cost. When container stock is 33% more expensive than bareroot stock, the cost to the landowner could be offset by reducing stocking by 25%. For example, if bareroot seedlings are sold for US$ 0.30 each, 1,000 trees would cost US$ 300. In comparison, if container seedlings are sold for US$ 0.40 each, 750 trees would cost US$ 300. Typically, hand-planting costs will also be reduced when stocking is reduced by 25%.

Bareroot nurseries in the Netherlands once relied on methyl bromide, but they increased the use of metham sodium and increased the use of container plants (MBTOC 2006). In British Columbia, the use of container stock gradually increased (van Eerden 1996). Recently, the International Forest Company (based in Georgia) decided to close 4 bareroot nurseries and to expand the production of container stock. The capital required, however, to convert from a bareroot nursery to a container nursery can be a limiting factor. Many state-owned nurseries operate under funding constraints and many privately-owned bareroot nurseries have no incentive to convert to container production. Applying alternative chemical fumigants is cheaper than investing in container equipment and facilities.

Chemical Alternatives to QPS Gas

A number of chemical fumigants have been tested in forest nurseries. Some are not registered and some have not proved to be effective. The following comments pertain to the practical alternatives to QPS gas.

Chloropicrin Under a Tarp

Chloropicrin has been tested in forest nurseries for more than 60 years. For example, chloropicrin was applied to conifer seedbeds in Nisqually, Washington (Breakey and others 1945). This treatment has shown promise in the Lake States, Pacific Northwest, and in the South (Enebak and others 1990a; Rose and Haase 1999; South 2007). New formulations that include "solvents" have also proven effective. Rates of 336 to 400 kg/ha (300 to 360 lb/ac) have been effective in forest nurseries.

Chloropicrin Plus 1,3-D Under a Tarp

At some nurseries, nematodes can be a problem and can reduce both yield and seedling quality. Therefore, monitoring of soil for nematodes will likely increase as the use of methyl bromide decreases. In cases where injurious populations are confirmed, nursery managers may decide to include 1,3-D when fumigating with chloropicrin. The rate may vary with nursery, but some managers have applied a rate of 269 kg/ha (240 lb/ac) chloropicrin plus 180 kg/ha (160 lb/ac) 1,3-D.

1,3-D without a Tarp

The rotation commonly used at a nursery may affect the timing of application. In some regions, there is one seedling crop per fumigation. Soil may be fumigated with QPS gas in the autumn; the following spring, seeds are sown to produce a 2+0 or 3+0 crop. This is often followed by a cover crop, and then the sequence is repeated. In the southern US, 2 or sometimes 3 seedling crops may follow the initial QPS fumigation. If the nematode population reaches a high level during the first rotation, an untarped treatment of 1,3-D may be applied in the spring (prior to sowing the second crop). In this case, a rate of 127 kg/ha (113 lb/ac) may be applied followed by pressing the soil with a roller (sealing) and then applying 1 to 2 cm (0.4 to 0.8 in) irrigation. When 1,3-D is applied more than once in 3 years, a buffer zone of 31 m (102 ft) may be required.

MITC Compounds

Methyl-isothiocyanate (MITC) is an active compound that is produced by several fumigants (dazomet, potassium methyldithiocarbamate, sodium methyldithiocarbamate). The MITC compound is produced when these compounds react with water. Most labels indicated sealing the soil by either a "water seal" or plastic tarp will increase efficacy. In addition, a water seal may reduce the amount of MITC that is released into the atmosphere (Wang and others 2006). In some cases, however, the soil has been sealed by a roller to compress the soil surface. Labels typically indicate that activity will be increased when the soil is covered with a plastic tarp.

Dazomet has been used in forest nurseries for more than 60 years. In 1956, Wilson and Bailey (1958) applied 156 kg/ha (139 lb/ac) at a nursery in Ohio; the following year, trial samples were sent to 70 forest nurseries. In 1963, a rate of 325 kg/ha (290 lb/ac) was tested (Iyer 1964). Three decades later, a rate of 140 kg/ha (125 lb/ac) was applied (Enebak and others 1990b). Recently, researchers applied 448 kg/ha (400 lb/ac) dazomet in Wisconsin (Wang and others 2006) and up to 560 kg/ha (500 lb/ac) have been applied in Georgia (Fraedrich and Dwinell 2003). The "recommended" application rate has increased by about 60 kg/decade (132 lb/decade). One possible explanation of the increase in rates is due to inconsistent results from lower rates (Enebak and others 1990b).

Several problems have been reported when using MITC fumigants. Most problems occurred when not using a tarp and when an inversion layer occurred soon after treatment. In some cases, the evolution of MITC has damaged pines (that is, bleached out needles) that were growing 120 m (400 ft) from the treated area (Buzzo 2003). Injury of this type has occurred at nurseries in Arkansas, Texas, Oregon, and Washington. At some nurseries, a negative effect of fumigation on soil fertility has persisted for years. In a Georgia nursery, corn (*Zea mays*) was stunted 2 years after treatment with dazomet and, at another nursery, *Trichoderma* levels remained depressed for more than a year.

Herbicides

QPS gas can be used to reduce the risk of spreading noxious weeds such as cogongrass (*Imperata cylindrica*), because methyl bromide will likely kill the seeds. Many nurseries rely on fumigation with QPS gas to control perennial weeds such as nutsedge (*Cyperus* spp.). At some nurseries, herbicides can be an economical alternative to controlling annual weeds (South and Gjerstad 1980).

Many predict herbicide use will increase as use of methyl bromide declines. This is based on reports where weed populations were higher when certain alternative fumigants were tested (but where herbicide treatments were absent). Several researchers believe that most weed populations can be kept low by applying sanitation in combination with judicial use of herbicides. The ability to maintain low weed populations, however, depends on both a sound knowledge of herbicide efficacy and an adequate number of legal herbicides. There can be several reasons why the number of herbicides available to nursery managers is small and may decrease in the future.

NOT A MAJOR FOOD CROP

A number of effective herbicides might be used in forest nurseries. However, the list of herbicides that are legal for use in conifer seedbeds is shorter than the list that the Environmental Protection Agency (EPA) has approved for use on major food crops. Prior to 1972, a nursery manager could legally apply any herbicide to control weeds, but managers can now only use an herbicide that is "registered for the site." For example, if a nursery manager wishes to control nutsedge with halosulfuron-methyl, *Zea mays* (a food crop) could be treated with an aerial application of 70 g/ha (1 oz/ac), but EPA would not permit hardwood seedlings (a non-food crop) to be treated with a ground application of 7 g/ha (0.1 oz/ac) of halosulfuron-methyl. To be legal,

research would be required, and then a chemical company would need to file a special local need (24-C) label. In some states, the 24-C label might be approved while rejected in other states. Therefore, it is easy to understand why many farmers (who do not fumigate soil with methyl bromide) have relatively weed-free corn fields, while nursery managers (who fumigate soil prior to sowing hardwoods) may require 500 hours of weeding/ha (200 hours/ac). If managers could legally apply any food-crop herbicide to suppress weeds in hardwood seedbeds, hand weeding times might be less than 50 hours/ha (20 hours/ac) and the need for fumigation to suppress troublesome weeds would be minimized.

LAWSUITS

Pesticide use in forest nurseries has evolved from relying on just 1 or 2 pesticides before 1940, to relying on a number of pest control products (some even with activity only on certain genera). This evolution was accomplished through cooperation and trust among nursery managers and researchers. This cooperation is essential if knowledge is to be increased in this important management area. It is important that knowledge obtained by nursery managers be shared with researchers, and that researchers share results from their trials with nursery managers. However, this cooperation was weakened by several lawsuits during the 1990s. For example, the EI Dupont Company withdrew the fungicide benomyl after numerous lawsuits and claims originated from horticultural nurseries. In one case, a forest nursery in North Carolina claimed that poor germination resulted after pine seeds were treated with benomyl. In New York, a manager applied oryzalin to young tree seedlings and then filed suit against the chemical company. As a result, nursery managers throughout the US may now no longer apply this herbicide to either seedbeds or seedling transplant beds. In addition, all the researchers' time and effort testing oryzalin in forest nurseries were wasted by one lawsuit. One should therefore not be surprised when some researchers are reluctant to share information with managers who might later sue a chemical company for monetary gains. "The actions of one individual can erase the potential benefit of many research years" (South 2002).

FOREST STEWARDSHIP COUNSEL

The Forest Stewardship Council (FSC) is an international non-profit organization created to support environmentally appropriate, socially beneficial, and economically viable management of the world's forests and plantations. FSC has developed a list of herbicides that may not be used in forest nurseries (FSC 2005). Plantation owners seeking FSC certification might not be allowed to obtain seedlings from nurseries that use herbicides such as atrazine, fluazifop-butyl, metalochlor, oxyfluorfen, and pendimethalin. Therefore, nursery managers who sell seedlings to customers who desire FSC certification for their plantations may have a very short list of permitted herbicides. In addition, FSC does not permit FSC seedlings to be treated with metam sodium or QPS gas (without special permission from FSC).

LIMITED HERBICIDE RESEARCH

At one time, a number of researchers were conducting herbicide studies in forest nurseries. Trials were conducted in Alabama, Connecticut, Idaho, Indiana, New York, and Oregon. Trials were initially funded by the USDA Forest Service, and then several forest companies sponsored research at universities. The interest in funding herbicide research declined and some researchers moved to other, better funded areas of forestry. Herbicide screening is now limited mainly to nurseries who are members of nursery cooperatives in the South and Pacific Northwest. If research on nursery weed control continues to decline (due in part to company mergers, a decline in artificial regeneration research at universities, and forest industry owning less land), nursery managers may have fewer weed control tools in the future.

Table 1. Estimates of prices of various fumigation treatments and the increase in seedling production required to justify the cost of fumigation (at a price of US$ 0.10/seedling).

Fumigant	Active ingredient/ha[z]	Price/ha (US$)[y]	Yield increase required per ha[x]
Methyl bromide (98%)—QPS	448 kg	3953	39,530
Chloropicrin under a tarp	336 kg	4200	42,000
Chloropicrin plus 1, 3-D under a tarp	270 kg + 180 kg	4448	44,480
1,3-D with no tarp	127 kg	482	4,820

[z] Active ingredient/ac is 1 kg/ha = 0.9 lb/ac
[y] Price per ac is US$ 100/ha = US$ 40.50/ac
[x] Yield increase per ac is 1000/ha = 405/ac

Economics

Opinions on the best alternative to QPS gas will vary depending on both economics and the individual's job. In some cases, crop value will determine the best alternative (South and Enebak 2006). As the value of the crop (per ha) increases, the rate (and cost) of the "best" treatment will increase. Therefore, a manager who routinely makes a profit of US$ 8000/ha (US$ 3,240/ac) will likely use a more expensive fumigant than someone who makes a profit of only US$ 800/ha (US$ 324/ac). Likewise, a manager that is required by law to "break-even" (that is, no profit) will likely be told (by a financial officer or lawyer) to select a low-cost soil fumigation treatment. At some nurseries, the cost of soil fumigation may exceed US$ 4000/ha (US$ 1620/ac) (Table 1).

Some nurseries produce more than 20 million seedlings annually and can afford to have a contractor apply fumigants that are classified as "restricted use pesticides." Fumigants in this category include methyl bromide, chloropicrin, and 1,3-D. In contrast, when the annual production at some nurseries is less than 2 million seedlings, the managers might not be able to afford to have professional applicators treat only 1 or 2 ha (2.5 or 5 ac) of seedbeds. Therefore, some managers may decide to apply fumigants that are not classified as restricted (for example, dazomet, potassium-N-methydithiocarbamate, sodium methyl dithiocarbamate). These fumigants may be applied by personnel that do not have a restricted pesticide license.

Summary

Some managers will use QPS gas to reduce or prevent the shipment of noxious pests from bareroot nurseries. Others might reduce their use of CH_3Br by ceasing the production of bareroot stock and by producing only container stock. Some managers will continue to produce less expensive bareroot stock by switching to alternative fumigants and increasing the use of herbicides and nematicides. Some managers who want to make a profit may decide to fumigate with chloropicrin (336 kg/ha [300 lb/ac]) under a tarp. These managers will likely treat soil with 1,3-D if the population of pathogenic nematodes exceeds acceptable levels. Troublesome weeds (for example, *Cyperus* spp.) will be controlled using effective herbicides on fallow ground, in cover crops, and in seedbeds.

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Relating Seed Treatments to Nursery Performance: Experience with Southern Pines

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ABSTRACT
Producing good quality seeds that perform well in the nursery continues to be challenging. High quality conifer seeds are obtained by optimizing collecting, processing, storing, and treating methodologies, and such quality is needed to consistently produce uniform nursery crops. Although new technologies are becoming available to evaluate seed quality, they have not been developed to the extent that they replace the more traditional methodologies developed over decades of trial and error. The most reliable approaches to predict nursery performance rely on obtaining high seed quality, applying appropriate treatments, and conducting germination evaluations that follow established practices.

KEYWORDS
seed collection, seed processing, seed pathogens, seed testing

Introduction

High quality seeds are essential for successfully producing nursery crops that meet management goals and perform well in the field. Uniformity in the production of pine (*Pinus* spp.) seedlings primarily depends on prompt and uniform germination, early seedling development, and a variety of cultural practices that are applied as seedlings develop. Most container nursery managers should be able to maintain 85% or higher germination, and 90% or higher survival after the emergence of the seedlings. Otherwise, oversowing will be necessary, and the subsequent waste of seeds will jeopardize efforts to produce high quality crops consistently and economically.

Meeting the goal of high levels of seed germination and seedling establishment requires considerable care in collecting, processing, and storing seeds, and in applying appropriate pregermination treatments. Seed maturity and dormancy, as measured by speed of germination, vary by species in southern pines. Collecting, handling, and processing affects seed quality (Barnett and McLemore 1970; Barnett 1976a). Dormancy can influence the germination pattern by slowing the initiation rate of germination, particularly in bareroot nursery beds where temperatures and photoperiods are often considerably

less than optimal (McLemore 1969). It is necessary, then, to understand the biology of the species to be produced in the nursery.

The ability to correlate seed parameters to nursery establishment and planting success has been sought by managers of forest nurseries for many decades. In recent years, new technologies to evaluate the physiological conditions of seeds have been developed and evaluated.

The objectives of this paper are to review practices needed to produce high quality, viable seeds and to evaluate current and new technologies that may increase our ability to predict seed performance in the nursery. The overall goal is to be able to consistently produce nursery crops that meet management targets.

Producing Seeds of High Quality

There is a demand for large quantities of consistently high quality seeds to meet the continuing emphasis on reforestation. If seeds are not properly collected, handled, and stored, the result can be poor quality. Viability may be reduced by 20% to 30% by improper handling, making nursery management difficult.

Numerous factors during cone collection and seed processing can affect quality. The most important of these are cone maturity and storage, cone and seed processing, seed moisture content, and storage temperatures. Unfavorable conditions in any one of these areas can cause secondary seed dormancy, the reduction of storability, or the immediate loss of viability.

Collecting and Processing Seeds

The initial germination of most conifer seeds is directly related to cone maturity at the time of extraction (McLemore 1959, 1975; Barnett 1976a; Tanaka 1984). Cones are considered mature when they can be easily opened at the time of collection (McLemore 1959). In some species, however, seed maturity may occur at a somewhat different time from cone maturity. An after-ripening period may be required to improve seed yields, but after-ripening may not improve viability (Barnett 1976a). Extended cone storage may also reduce seed quality.

After extraction, seeds must be dewinged, cleaned, and dried. These operations result in frequent injury to seeds, and particular care should be used in processing (Tanaka 1984). Precision sowers, such as vacuum seeders, generally require that there be fewer wings and less trash than conventional sowing machines, so extra care should be used in seed cleaning processes, or only seeds of the highest quality should be purchased.

All empty seeds should be removed from lots before use. This is the easiest means of upgrading a seedlot. Normally, empty seeds are removed by mechanical cleaning equipment, including scalpers and gravity, or pneumatic seed cleaning equipment. When seedlots are small, however, as in lots used for progeny tests, it is often convenient to use flotation in water or organic solvents to separate unfilled seeds (Baldwin 1932; Barton 1961; Barnett and McLemore 1970). Flotation in most organic solvents should be delayed until just before use. If the solvent is not thoroughly removed in drying, seeds so treated may rapidly lose viability in storage (Barnett 1971a).

Storing Seeds

Careful control of seed moisture content and storage temperatures is essential to maintain viability (Barton 1961; Jones 1966; Barnett and McLemore 1970). General recommendations for long-term storage of conifer seeds are to dry seeds to 10% or less moisture content, and hold at subfreezing temperatures. Seeds that are damaged or are known to have low vigor can be preserved by drying to a moisture content of 8% to 10%, and lowering the storage temperatures to about -18 °C (0 °F) (Kamra 1967).

Seed Sizing

The reported effects of seed size on germination and early seedling growth are conflicting. The operational objective of sizing is to produce a uniform crop of seedlings (Owston 1972). Medium to medium-large seeds have been

reported to produce larger and more uniform seedlings than smaller seeds (Ghosh and others 1976). Larson (1963) reported that, although seed size can influence subsequent seedling size when seedlings are grown under uniform conditions, as in greenhouses, seed size has a more pronounced effect on germination. Uniform speed of germination may therefore be the most important consideration in sizing. Recent tests under laboratory conditions of minimal environmental stress have shown that germinant size after 28 days of growth was strongly correlated with seed size (Dunlap and Barnett 1983). The faster germinating seeds in each size class produced larger germinants after 28 days of incubation (Figure 1). All seeds reached a maximum germination rate by the sixth day, but smaller seeds were slower to initiate germination (Figure 2). These results are in agreement with Venator (1973), who found that faster growing Caribbean pine (*P. caribaea* var. *hondurensis*) seedlings tend to develop from early germinating seeds. Consequently, seedling size, and possibly uniformity of growth, are primarily functions of germination patterns, which are partially determined by seed size.

Reducing Seed Coat Pathogens

Seeds of some conifers carry large quantities of microorganisms that may be pathogenic when seed vigor is low. For example, the seeds of longleaf pine (*Pinus palustris*) are large and have fibrous coats, and are often populated with pathogenic spores (Pawuk 1978; Fraedrich and Dwinell 1996). Results have shown that longleaf seedcoats carry pathogenic fungi that not only reduce germination, but also result in significant seedling mortality (Barnett and others 1999). Study results have shown that treating seeds with a sterilant or fungicide prior to sowing can improve both germination and seedling establishment (Barnett 1976b; Barnett and Pesacreta 1993; Littke and others 1997).

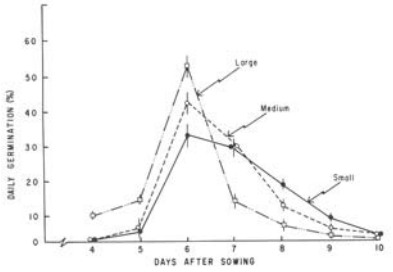

Figure 1. Mean daily germination of loblolly pine seeds from the large, medium, and small size classes (Dunlap and Barnett 1983).

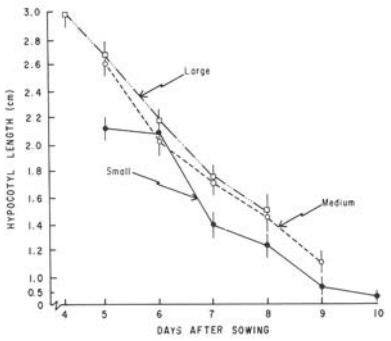

Figure 2. Mean hypocotyl length of loblolly pine germinants of different germination dates 28 days after germination (Dunlap and Barnett 1983).

Presowing Treatments

Conventional Seed Treatments

After seeds of high quality have been obtained and stored, they must be properly prepared before sowing. Overcoming seed dormancy is one of the major steps to ensure prompt and uniform germination. Presowing treatments to speed germination are discussed in detail by several authors (Allen and Bientjes 1954; Bonner and others 1974; Tanaka 1984). Typically, moist chilling (stratification) is done after an 8- to 24-hour period of moisture imbibition. Fully imbibed seeds are placed in polyethylene bags and held at temperatures of 1 to 5 °C (34 to 41 °F). Temperatures below freezing may injure imbibed seeds (Barnett and Hall 1977), while those above 5 °C

(41 °F) may result in germination during stratification.

The duration of stratification varies by the extent of dormancy present in the seeds. The recommendation for most species is a period of 30 days or less (Krugman and Jenkinson 1974). However, chilling of some pine species beyond 30 days markedly increases the speed and uniformity of germination (Allen 1960; McLemore and Czabator 1961; Boyer and others 1985). Longer chilling periods have been resisted by many nursery managers because some seeds may begin to germinate before the chilling needs of others are met. This precocious germination can be minimized by carefully controlling the treatment temperature. Under proper conditions, germination of more dormant pine species will not occur during 45 to 60 days of moist chilling, and the longer treatments can markedly increase the speed and uniformity of germination.

Uniformity in the production of seedlings is determined primarily by prompt and uniform seed germination and early establishment. Seeds germinating over an extended period have greater mortality during establishment and result in lower grade seedlings (Table 1).

Less Common Presowing Treatments

Through the years, techniques other than stratification have been investigated in an attempt to accelerate the dormancy-breaking process or obtain desirable germination patterns in a more efficient manner. Many of these techniques have shown little practical application. Three methods that have been used to meet particular needs will be discussed.

Aerated Water Soaks

Soaking seeds in aerated water is a technique that is frequently used for overcoming dormancy. Soaking pine seeds in continuously aerated water at 5 °C (41 °F) has increased the speed of germination as well as conventional moist chilling (Barnett 1971b). Aerating seeds at 10 °C (50 °F) stimulated germination as much as colder soaks, and did so in less time. Although very dormant seeds can be soaked at low temperatures for nearly 5 months without harm, periods of up to 60 days are usually sufficient. With less dormant seeds and higher soaking temperatures, it may be necessary to shorten periods to 2 to 3 weeks to prevent premature germination or induction of secondary dormancy. The water must be aerated continuously to keep the oxygen content near saturation.

Table 1. Mortality and seedling size of shortleaf pine (*Pinus echinata*) seedlings at lifting, as related to time of germination, averaged across 6 half-sib lots and stratification treatments (Barnett 1993).

Time after sowing		Germination per week	Mortality of germinants	Seeding size[z]	
Days	*Week*	*Percentage*	*Percentage*	*Height (mm)*	*Diameter (mm)*
8-10	1	6	12	157	4.1
13-17	2	52	13	160	4.0
20-24	3	30	18	144	3.5
27-31	4	10	27	118	3.1
34-38	5	2	56	115	2.7

[z]25.4 mm = 1 in

Although lengthy aerated soaks are feasible, they are not usually used because there is less confidence in the system. Lengthy treatments are best applied by moist chilling techniques. Aerated soaks are used in instances where insufficient time is available to stratify seeds.

Priming

Another technique that may result in more prompt and uniform germination is priming. In priming, seeds are incubated at optimum germination temperatures, but prevented from germinating by limiting the amount of moisture imbibed. Osmotic solutions, particularly polyethylene glycol (PEG), have been widely used to prime agricultural seeds (Bodsworth and Bewley 1981). Seeds are allowed to absorb moisture, but not to the level required for germination. Although Simak (1976) found that an 11-day treatment of Scots pine (*P. sylvestris*) seeds with a PEG solution (-800 kPa) improved germination, similar experiments by Fleming and Lister (1984) and Haridi (1985) provided variable results depending upon species and seed sources. Malek (1992) found that priming of black spruce (*Picea mariana*) seeds using PEG solutions was less effective than soaking in aerated water.

Solid matrix priming (SMP) uses another approach to limit moisture imbibition by seeds. In SMP, seeds are typically exposed at low temperatures to solid matrices with different water-holding capacities, such as sphagnum, cat litter, peat moss, or combinations of fine grades of sand. The results are again mixed, depending on species and seed source. Wu and others (2001) found that SMP treatments to loblolly pine (*Pinus taeda*) seeds after moist chilling improved the rapidity, synchrony, and completeness of germination. Ma and others (2003) reported that 4 fir species (Pacific silver fir [*Abies amabilis*], subalpine fir [*A. lasiocarpa*], grand fir [*A. grandis*], and noble fir [*A. procera*]) responded positively to SMP treatments. Feurtado and others (2003), however, found that western white pine (*Pinus monticola*) seeds did not respond to SMP as much as to conventional cold-water soaking. Consequently, due to inconsistent results, priming has not become a widely accepted presowing treatment for forest tree seeds.

Germinant Sowing

The principles involved with presowing treatments to speed germination are carried farther in the concept of germinant sowing. Managers of most container nurseries attempt to produce one tree per container cell. Sowing a germinant into each cell significantly improves the efficiency of the operation. Unless seed quality is very high, the traditional approach is to sow multiple seeds per cell, and thin cells that have more than one seedling. Germinating seeds prior to sowing and sowing only germinated seeds is one approach to increasing uniformity of seedling establishment. Barnett (1983, 1985) adapted the concept of fluid drilling (sowing germinants in a viscous gel) to southern pine species. The purpose of the gel is to protect the radicles of seedlings during sowing.

The key to success of germinant sowing is the capability to sort germinants from dead or non-germinating seeds. Normally, germinants will have a lower specific gravity than non-germinants, and separation can be accomplished by using a sugar solution (Taylor and others 1977). South and Young (1995) report that sowing germinants to improve seed efficiency is used operationally in South Africa. With their *Eucalyptus* and *Pinus* spp., separation is achieved by adding sugar to water until the germinants float to the top of the solution. Equipment has been developed in South Africa to mechanically sow these germinants into container cells.

A limitation of the application of germinant sowing to southern pines relates to the difficulty in sorting germinants from non-germinants. Barnett (1985) found that density sorting of seeds from species such as loblolly (*P. taeda*) and longleaf pines did not work, due either to seed density or seedcoat nature.

Although germinant sowing of coniferous seeds has not been widely accepted in the US, sowing germinants of oak (*Quercus* spp.) or walnut (*Juglans* spp.) species has been recommended and used in small scale operations (USFS 1948; Davis and others 2004). Germinating acorns and walnuts before sowing can be a beneficial practice for small-scale nursery culture and direct seeding operations.

Methods to Upgrade Quality of Seed Lots

The ultimate goal of techniques to improve seed quality, such as winnowing (commonly used for millennia), is the capability to separate filled dead from live seeds. For decades, the most effective means of improving pine seeds has been to remove all unfilled seeds, and then separate damaged or poorly developed seeds from filled seeds. Technology to accomplish these tasks has been based on seed flotation or the use of mechanical equipment. Aspirator and specific gravity table techniques work well for many tree species, but the most effective quality improvements have been achieved by density separation processing.

Technology to improve seedlot performance is particularly needed for species like longleaf pine that typically have poor viability. Because container nursery production has been widely adopted for this and other species, newer technologies are being sought to assure that one live seed can be sown in each container cell.

Three different approaches have been evaluated to achieve the goal of 100% germination (Barnett and Dumroese 2006). But, is it possible to determine which of 2 seemingly identical filled seeds is dead, and which is alive?

Incubating-Drying-Separating (IDS) Technology

The IDS process is based on the principle that water imbibed by live seeds is lost at a slower rate than water imbibed by dead filled seeds when both are subjected to uniform drying conditions. Ideally, seeds can then be separated in a liquid medium into a nonviable floating fraction and a viable sinking fraction based on the resulting density differences between the 2 fractions.

Certain IDS procedures have been used for a number of years, and IDS can help separate nonviable from viable seedlots of a number of northern conifer species (Simak 1984; Bergsten 1987; Downie and Wang 1992). This methodology has shown limited success with southern pine species. Karrfalt (1996) reported that his attempts using IDS to remove fungus-damaged seeds of slash pine (*P. elliottii*) failed completely. Donald (1985) achieved positive results with slash pine seeds in South Africa, but that technique was of little value for low-viability lots. McRae and others (1994) were able to separate dead from live seeds of loblolly and slash pine, but questioned whether an economic advantage could be expected from the treatment. Both McRae and others (1994) and Creasey (2003) found that the wing stub of longleaf pine seeds created flotation problems that prevented successful application of IDS techniques.

Chlorophyll Fluorescence

Chlorophyll fluorescence (CF) is a nondestructive and instantaneous method to measure differences in plant function by assessing the magnitude of CF signals. When chlorophyll molecules absorb light during photosynthesis, a small portion of that light is re-emitted, or fluoresced. Numerous studies have used CF to measure photosynthesis efficiency (Adams and others 1990). The same principle was used to estimate seed maturation (Ward and others 1995) and germination (Steckel and others 1989; Jalink and others 1998).

Because longleaf pine seeds have large embryos with considerable amounts of chlorophyll, we decided to evaluate CF as a method of sorting for viability improvement. Chlorophyll fluorescence was evaluated using SeedScan™ technology (Satake Corporation, Houston, TX). The SeedScan™ is a tabletop seed-by-seed maturity sorter that is designed to separate seeds based on their germination potential (Satake Corporation 2002). Although CF is related to germination in some species, no such relationship could be demonstrated when scanning longleaf pine seeds (Barnett and Dumroese 2006).

Near Infrared Spectroscopy

Near infrared radiation (NIR) is in the wavelength range of 780 to 2,500 nm; 400 to 780 nm is visible light, and above 2,500 nm is infrared. A commercial breakthrough for NIR spectroscopy came when it was shown that this technology could be used to determine the protein content in whole grains (Williams and others 1985).

Today, NIR technology is widely used not only in chemical, pharmaceutical, and food industries, but also in agriculture and wood technology (Downy 1985). The main use of NIR spectroscopy within the field of seed science is quantifying seed moisture content and chemical constituents like proteins and oils (Norris 1988). It is now being used as a quantitative tool that relies on chemometrics to develop calibrations relating reference analysis of the seeds or plant material to that of the NIR optical spectrum. In other words, germination data have to be correlated to the measured spectrum on the same seeds.

Lestander (2003) has demonstrated the potential of using multivariate NIR spectroscopy for conifer seed classification. He found that filled viable and nonviable Scots pine seeds could be separated with an accuracy of >95%.

We evaluated NIR technology in both informal tests with USDA Forest Service forest products scientists and, more formally, with Seed Meister™ technology (Brimrose Corporation, Baltimore, Maryland). The Seed Meister™ AOTF-NIR spectrometer is specially designed for high-speed discrimination, quantification, and sorting of hybrid agricultural seeds (Brimrose Corporation 2002a). The scanning technology can determine oleic and linoleic acid content in sunflower (*Helianthus* spp.), and protein and oil content of soybean (*Glycine* spp.) (Brimrose Corporation 2002b). However, our tests with longleaf pine seeds show no relationships among scanning spectra and germination potential (Barnett and Dumroese 2006).

Relating Results of Seed Tests to Performance

For decades, nursery managers and seed physiologists have sought techniques, generally with little success, that would more accurately predict seed performance in the nursery. Nursery germination is often poorly related to germination in laboratory tests. This is probably due to less than ideal environmental conditions and soil pathogens. Efforts have been made to develop vigor or stress tests that would enable the nursery manager to predict performance more accurately. Germination percentages, however, have remained the accepted means of estimating performance.

In an evaluation of the problem, Barnett and McLemore (1984) found that laboratory germination tests performed on stratified seedlots provided the best predictors of nursery-tree yield for dormant-seeded southern pines. To date, no consistently reliable methodology has replaced laboratory germination testing. The Association of Official Seed Analysts (AOSA) has established standardized germination testing by conducting them under optimum light and temperature conditions. These tests do not reflect germination of dormant seeds on nursery beds where temperatures and photoperiods are often considerably less than optimal (Table 2). A technique to improve prediction of seed performance is to determine chilling needs under stress conditions that relate to the nursery conditions where the seeds are to be sown (Barnett 1993). Extension of the prechilling period will minimize the effect of the less than optimal nursery conditions on initial seedling development.

The results of the Barnett and McLemore (1984) study indicate that germination percentages provide a good prediction of nursery performance for non-dormant seeds. Other germination related values, such as Czabator's germination value (Czabator 1962) that provides an estimate of speed as well as completeness of germination, did not improve the predictability of performance.

Conclusions

The ultimate goal of techniques to improve seed quality is the capability to sow filled, live seeds in

Table 2. Effect of length and method of stratification on a mixed loblolly pine seedlot in 2 testing environments (McLemore 1969).

Days of stratification	Stratified in refrigerator at 1 °C (34 °F)		Stratified outdoors	
	Percentage	*Value*[z]	*Percentage*	*Value*[z]
Tested at 16 °C (60 °F) with 11-hour photoperiod				
0	<1	0.0	<1	0.0
30	68	7.1	59	6.0
60	95	17.3	91	11.4
113	99	24.0	98	19.6
Tested at 22 °C (72 °F) with 16-hour photoperiod				
0	96	20.8	96	20.8
30	99	37.6	98	41.8
60	99	47.1	99	47.0
113	100	50.3	99	56.3

[z] Germination values represent the speed and completeness of germination (Czabator 1962).

a consistently uniform manner. Once seeds of the highest quality are produced, stored, and treated, the ability to predict nursery and field performance is greatly improved. Unfortunately, nursery managers often must use seeds of less than optimum quality. It then becomes important that nursery managers understand the biology of the tree species with which they work.

Through the last 6 or 7 decades, seed specialists and nursery managers have developed methodologies that have been proven to produce good nursery crops. Frequently, however, nursery production is adversely affected by poor seed quality. In the past decade, a number of new technologies have become available to seed specialists. Although a number of these have potential, none have replaced the basic technologies of seed processing known for decades.

It is important that the state-of-the-art seed processing information on species of interest is made available to nursery managers. It is challenging to inform and train managers responsible for growing numerous conifer and hardwood species because of the scope of knowledge needed.

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Methyl Isothiocyanate and Chloropicrin Concentrations in Bareroot Forest Nursery Soils and Above Soil Surface Treatment Following Fumigation

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ABSTRACT
Concentrations of methyl isothiocyanate (MITC) and chloropicrin (CP) in air spaces of nursery soil and in air at the soil surface following fumigation were determined in field trials in a Wisconsin and a Georgia nursery. MITC was measured in plots receiving either dazomet or co-application of metam sodium and chloropicrin; CP was measured in the latter plots. Soil surfaces were sealed with either a high density polyethylene tarp or by surface compaction and maintenance of a water seal following fumigation. Findings from the Wisconsin trial are highlighted. A very small percentage (< 3%) of the applied MITC was lost to the atmosphere, either through the plastic or water-sealed surfaces during the 14 days following treatment. For CP, 10% and 22% of the applied equivalent were calculated to have been lost to the atmosphere in the plastic-covered and water-sealed plots, respectively. Low MITC air emissions and MITC presence deep in the soil profile in the dazomet, water-sealed plots are partly attributed to over-irrigation on 5 of the 7 days following fumigation in Wisconsin. However, slightly higher, but still low, emissions in similar plots without excess watering in the Georgia nursery show that either surface containment method can be used in nurseries to reduce risk of MITC emissions to nearby seedling crops or harm to humans in nearby fields. The use of plastic tarps increases death rates of *Fusarium* propagules by maintaining higher chemical levels in the soil profile over time; however, dazomet has been used with water-seal treatment to give consistent, satisfactory control of soilborne pests in nurseries in the northern US.

KEYWORDS
dazomet, metam sodium, methyl bromide alternatives, soilborne pests, disease control

Introduction

The production of methyl bromide (MB) for routine soil fumigation is being phased out in anticipation of a full ban on its use for this purpose in the future. Recently, bareroot soils in some forest nurseries have been fumigated with MB by meeting the criteria for Critical Use Exemption and/or Quarantine and Pre-Shipment Uses. With the impending loss of MB, nurseries require economical, effective, and practical alternatives to the chemical in order to produce needed quantities of high quality tree seedlings.

Two promising chemical alternatives are dazomet for northern US nurseries (Borkenhagen 1994), and co-application of metam sodium and chloropicrin (CMS) for southern US nurseries (Carey 2000). Because methyl isothiocyanate (MITC) is the primary breakdown product from both chemicals, however, seedling damage and mortality in adjacent fields has been attributed to these alternative fumigants (Scholtes 1989; Carey 2000; Buzzo 2003). Collateral seedling damage was observed with CMS in an east Texas location when a temperature inversion occurred within 24 hours of the fumigation. The observed damage and subsequent attribution of the damage to "out-gassing" of MITC also raised concerns about possible toxicity to nursery workers in nearby fields. However, the surface containment methods associated with the reported "out-gassing" events appeared to be less than the minimum recommended for the products used based on available, published information. For example, manufacturer guidelines for dazomet fumigation state that the soil moisture at time of fumigation should be between 60% and 80% of maximum water-holding capacity. Immediately following fumigant incorporation, the soil surface is to be compacted with a roller and irrigation water applied daily during the first week in the amounts specified for Basamid® by Pennington (1995).

Amounts of MITC and CP that escape into the atmosphere after fumigation of forest nursery soils have not been published. Knowledge of gas movement through the soil profile to the surface is also lacking. Field studies were therefore initiated in a Wisconsin and a Georgia nursery by a team of soil scientists and plant pathologists to evaluate the potential for phytotoxicity and human toxicity in nearby fields following fumigation with either dazomet or CMS. The specific objectives were to measure: 1) soil gas concentrations and distribution patterns of MITC and CP; 2) emission flux and total emission loss of MITC and CP from treated nursery soil plots; and 3) levels of soilborne pathogens before and after treatment. Full results of these studies have been published in scientific journals (Wang and others 2005; Wang and others 2006) and readers are referred to these papers for in-depth findings. A synthesis and the author's interpretation of the key findings of these studies are presented in this paper. Although the focus is on results from the Wisconsin nursery, selected findings from the Georgia nursery are useful in the interpretation of the Wisconsin results.

Methods

Study Sites

Field studies were conducted in 2002 at the Hayward Nursery, Hayward, Wisconsin, and in 2003 at the Flint River Nursery, Byromville, Georgia. Soils in the fumigated fields at each nursery were loamy sands (average 86% and 88% sand in the upper 30.5 cm [12 in] of soil, Georgia and Wisconsin nurseries, respectively) with low organic carbon (< 1.1% and 1.4%, Georgia and Wisconsin, respectively).

Experimental Design

Four treatment combinations were used, including combinations of dazomet or co-application of chloropicrin and metam sodium, and 2 surface containment treatments (high density polyethylene tarps or surface compaction and maintenance of water seal per Pennington [1995]). A randomized complete block design with 4 replications was used in each of 2 field sections, that is, water-sealed versus plastic-sealed sections.

Chemical Treatment Specifications

Dazomet (Basamid®)

In Wisconsin, both 336 kg/ha (300 lb/ac) (incorporated with rotary tiller for maximum depth of incorporation at 13 to 15 cm [5 to 6 in]) and 448 kg/ha (400 lb/ac) (incorporated with a spading machine with maximum depth of incorporation at 20 to 23 cm [8 to 9 in]) rates of the product were applied. In Georgia, 560 kg/ha (500 lb/ac) product was applied and rototilled to a 20 cm (8 in) depth.

Metam Sodium (Vapam®)

In Georgia and Wisconsin, 700 l/ha (75 gal/ac) rate of metam sodium was sprayed on the soil surface and incorporated by tillers to the maximum depth possible (13 cm [5 in] in Wisconsin; 20 cm [8 in] in Georgia).

Chloropicrin (Chloro-O-Pic®)

The chloropicrin treatment occurred no later than 2 hours after application of the metam sodium. Shank injection of 112 kg/ha (100 lb/ac) of chloropicrin was done to a 20 to 25 cm (8 to 10 in) depth in both nurseries.

Sampling and Measurements

Rain gauges were installed inside and outside the water-sealed plots to measure water delivered via irrigation and rain at each location. Soil water content was measured in the plastic-covered and water-sealed plots at multiple depths using time domain reflectrometry (Wang and others 1998). Air and soil temperatures were measured in tarped and water-sealed plots using an automated thermocouple system (Wang and others 1998).

In both locations, atmospheric emissions of MITC and CP were measured with passive flux chambers modified from heavy-duty aluminum trays (25 x 48 x 15 cm [10 x 19 x 6 in]). To capture volatilized MITC and CP, air was drawn from the chambers through activated charcoal (for MITC) and polymer-based XAD tubes (for CP) at various times following fumigation.

Multiple port soil air sampling probes were used to sample MITC and CP concentrations at various depths in the soils of the treated plots. The probes were made with 127-cm (50-in) aluminum pipes fitted with 10 PTFE tubes. A 10-port air sampler was used to simultaneously withdraw air samples from each of the 10 tubes on the sampling probes.

Soil samples for fungal assay were taken from all plots the day before and 4 weeks after fumigation using a lined, tube sampler. Ten cores per plot were collected, each core sectioned sequentially into 5-cm (2-in) increments, and increment cores composited by depth for a plot.

Sample Analyses

Analyses of air emission and soil gas samples were made using a gas chromatograph with an electron capture detector and a nitrogen-phosphorous detector connected to a headspace auto-sampler. Separate capillary columns were used for MITC and CP. The amount of each chemical captured from each sample tube was quantified in the capture detector. Calibration standards were routinely run for each chemical. Collected soil samples were assayed for *Fusarium* spp. using the technique of soil dilution plating onto selective media in Petri dishes (Juzwik and others 1999).

Results

Chemical Levels in Soil Air

The highest concentrations of all 3 chemicals in the top 20 to 36 cm (8 to 14 in) of soil occurred during the first 3 days after soil fumigation in the plots with plastic-sealed surfaces (Table 1). In the rolled and water-sealed field section, peak concentrations of MITC from dazomet were found at a greater soil depth (20 to 51 cm [8 to 20 in]) than the depths for the peak MITC levels in the CMS plots (0 to 36 cm [0 to 14 in]). The time periods during which peak chemical concentrations were found were quite variable with the longer time periods associated with the metam sodium+ chloropicrin treatment in the water-sealed plots.

Table 1. Summary of methyl isothiocyanate (MITC) or chloropicrin (CP) detected at various depths below soil surface and over time (days) in plots receiving fumigation x soil surface treatments in the Wisconsin nursery.

Treatment combination[z]	Maximum Chemical Concentrations Detected			
	Chemical measured	Soil depth (in)	Amount (mg/m^3)	Time period (after fumigation)
Dazomet/spade/plastic	MITC	2 to 8	150 to 550	2 hr to 3 days
Dazomet/spade/water	MITC	8 to 18	50 to 300	6 hr to 6 days
Dazomet/rotary tiller/water	MITC	8 to 20	50 to 150	6 hr to 3 days
Metam sodium/rotarty tiller/plastic	MITC	2 to 12	120 to 420	< 30 hr
Chloropicrin/rotary tiller/plastic	CP	0 to 14	1500 to 11,500	< 30 hr
Metam sodium/rotary tiller/water	MITC	0 to 14	50 to 350	6 hr to 7 days
Chloropicrin/rotary tiller/water	CP	4 to 14	1000 to 5000	3 hr to 7 days

[z] See methods section for full description of treatments
1 in = 2.5 cm

Chemical Levels in Atmospheric Emissions

The highest level of MITC emissions to the atmosphere (as measured by flux, that is, $\mu g/m^2$/second) occurred in the CMS plots (Table 2). These peak emissions occurred over a longer time period for the plastic-covered plots compared to the water-sealed plots. The maximum CP flux to the atmosphere occurred between day 1 and day 4 after fumigation, regardless of surface treatment. CP flux was highest in the water-sealed plots. MITC emissions from dazomet/water-sealed plots were very low (Table 2).

A very small percentage (< 3%) of the applied equivalent of MITC was lost to the atmosphere, either through the plastic or water-sealed surface, during the 2-week period following soil fumigation. For CP, 10% and 22% of the applied equivalent were calculated to have been lost to the atmosphere in the plastic-covered and the water-sealed plots, respectively.

Fusarium spp. Occurrence in Soil

Prior to soil fumigation, *Fusarium* spp. were commonly present in the 4 replicate soil samples of all study plots in the upper 21.5 cm (8.5 in) of soil (Table 3). Lower incidences of *Fusarium* spp. occurrence were found in soil from lower depths (between 21.5 and 42 cm [8.5 and 16.5 in]). *Fusarium* spp. levels in post-treatment samples from the top 21.5 cm (8.5 in) in the soil profile differed by treatment combination. Fungal occurrence was lowest and similar in the dazomet and the metam sodium+chloropicrin plots where plastic covered the soil surface ($P < 0.02$). Little reduction in *Fusarium* spp. occurrence between pre- and post-treatment samples was found for the dazomet/rototilled/water-sealed and the metam sodium+chloropicrin/rototilled/water-sealed plots.

Irrigation and Rain Water Levels

The amount of irrigation water delivered to the water-sealed plots daily exceeded the intended target amount on days 2 through 7 following soil

Table 2. Summary of emission flux of methyl isothiocyanate (MITC) and chloropicrin (CP) over time through 2 surface treatments following soil fumigation with dazomet and co-applied metam sodium+chloropicrin in the Wisconsin nursery.

Treatment combination[z]	Maximum chemical emission		
	Chemical measured	Flux rate ($\mu g/m^2/sec$)	Time period (after fumigation)
Dazomet/spade/plastic	MITC	1 to 3	1 to 5 days
Dazomet/spade/water	MITC	< 1	2 days
Dazomet/rotary tiller/water	MITC	negligible	6 hr to 3 days
Metam sodium/rotary tiller/plastic	MITC	2.5 to 25	1 to 3 days
Chloropicrin/rotary tiller/plastic	CP	5 to 15	1 to 4 days
Metam sodium/rotary tiller/water	MITC	< 2.5	1 to 2 days
Chloropicrin/rotary tiller/water	CP	4 to 64	1 to 4 days

[a] See methods section for full description of treatments

Table 3. Incidences of *Fusarium* spp. isolation from replicate soil samples by soil depth and treatment before and after soil fumigation in the Wisconsin nursery.

Soil Depth (inches)	Pre-fumigation assay by treatment[z]					Post-fumigation assay by treatment				
	D/SP/W	D/SP/P	D/RT/W	CMS/RT/W	CMS/RT/P	D/SP/W	D/SP/P	D/RT/W	CMS/RT/W	MSC/RT/P
0 to 3	++++[y]	++++	+++ -	++++	++++	+++ -	- - - -	++++	++++	++ - -
3 to 5.5	+++ -	++++	++++	++++	++++	++ - -	++ - -	++++	++++	- - - -
5.5 to 8.5	++++	++++	++++	++++	+++ -	+ - - -	+ - - -	++++	++ - -	- - - -
8.5 to 11	++ - -	+++ -	- - - -	+ - - -	+ - - -	+ - - -	+ - - -	+ - - -	+ - - -	- - - -
11 to 14	+ - - -	- - - -	+ - - -	+ - - -	- - - -	- - - -	- - - -	- - - -	+ - - -	- - - -
14 to 16.5	+ - - -	+ - - -	++ - -	++ - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -

[z] Treatment descriptions: D/SP/W = dazomet/spade/water-seal; D/SP/P = dazomet/spade/plastic-seal; D/RT/W = dazomet/rotary tilled/water-seal; CMS/RT/W = chloropicrin/rotary tilled/water-seal; CMS/RT/P = chloropicrin/rotary tilled/plastic-seal. See methods section for futher details.

[y] + = *Fusarium* spp. isolated; - = no *Fusarium* spp. isolated from a replicate soil sample.

1 in = 2.5 cm

fumigation (Table 4). Negligible rainfall was received on 2 of the first 7 days following fumigation.

Discussion

With both surface treatments used in the Wisconsin and Georgia studies, more than 70% of total cumulative emissions of either MITC or CP occurred within 1 week of fumigant application. The final cumulative MITC emission losses accounted for a very small percentage of the equivalent MITC applied as either dazomet or metam sodium. This may be partly due to accelerated rates of MITC degradation in the soil. The short duration and the negligible levels of MITC emission from water-sealed plots is likely due, in part, to accelerated transformation of product to MITC in the first case and percolation of product to greater depths resulting in lower emissions in the second case. The excessive irrigation in the water-sealed plots in the Wisconsin nursery, thus, must be considered in the interpretation of the air emissions results. The fungal presence data suggests that concentrations of MITC in the upper 21.5 cm (8.5 in) of soil in the water-sealed plots were not sufficient to kill most *Fusarium* propagules. In addition, the recorded emissions of MITC in the Georgia nursery study are helpful in interpreting the Wisconsin MITC emissions results. Specifically, the percentages of applied equivalent of MITC lost to the atmosphere in dazomet/water-sealed plots in Wisconsin were one-tenth that of emissions in similar plots in the Georgia nursery (Table 5), supporting the interpretation that excess irrigation water was partly responsible for the very low emissions. Regardless of the irrigation water issue in the Wisconsin study, the very small percentage of MITC lost to the atmosphere through either surface treatment is not likely to result in damage to crops in adjacent fields and is much below the level known to

Table 4. Amount of irrigation and rain water measured in treatment plots during the 7 days following soil fumigation in the Wisconsin nursery.

Number of days after fumigation	Amount (in) of Irrigation Water		Amount (in) of Rain Water
	Targeted[z]	Actual	
0	3/4 to 1	1/2	0
1	1/2 + 1/2[y]	1	0
2	1/4 + 1/4	1	0
3	1/8 + 1/8	1 2/3	0
4	1/8 + 1/8	1 1/3	0
5	1/8 + 1/8	1/2	< 1/10
6	0	4/5 + 1	0
7	0	2/3 + 1/2	< 1/10

[z] Based on recommended irrigation schedule for Basamid© as outlined in Pennington (1995).

[y] Indicates split irrigation (morning plus afternoon)

1 in = 2.5 cm

Table 5. Comparison of cumulative emission losses of methyl isothiocyanate following dazomet fumigation in water-sealed plots at the Wisconsin and Georgia nurseries.

Nursery Location	Treatment [z]	Final Cumulative Emission mg/m^2	as percentage [y]
Wisconsin	Dazomet / spading machine	48	0.3
Wisconsin	Dazomet / rotary tiller	8	0.1
Georgia	Dazomet / rotary tiller	727	3.2

[z] 450 kg/ac (400 lb/ac) dazomet incorporated to 20 to 23 cm (8 to 9 in) depth using a spading machine; 336 kg/ha (300 lb/ac) dazomet incorporated to 13 to 15 cm (5 to 6 in) depth using a rotary tiller; 560 kg/ha (500 lb/ac) dazomet incorporated to 20 cm (8 in) depth using a rotary tiller.

[y] Assumed on a molar basis 100% conversion from dazomet to metam sodium (Kim and others 1994).

be a lethal threat to humans near fumigated fields (Wang and others 2005).

Implications for Nursery Managers

Use of surface compaction plus a water seal or plastic tarps delays and/or reduces the emission of MITC to the atmosphere compared to situations where no water-seal or plastic is used when fumigating with MITC-generating products. Thus, either surface containment treatment can be used by nursery managers to reduce risk of MITC emissions that could cause damage to nearby seedling crops or harm to humans.

The use of either surface containment method in operational fumigation would also increase the concentration, over time, of MITC in the soil and result in reduced populations of soilborne pests. Careful attention, however, must be paid to irrigation water amounts used to maintain water seals for dazomet fumigation. Too much water can result in little to no efficacy in reducing fungal populations in the soil. Logically speaking, plastic tarp use eliminates concerns about excess irrigation water or significant rainfall events and would maintain the chemical concentration over a sufficient period of time to significantly reduce levels of soilborne pests. With careful oversight and planning, however, MITC generating products can be applied in such a manner to give consistent and satisfactory control for soilborne pests in nurseries in the northern US (Borkenhagen 1994; Juzwik and others 2002).

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Tree Seed Handling, Processing, Testing, and Storage at Hayward State Nursery, Wisconsin Department of Natural Resources

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KEYWORDS
hardwood seeds, conifer seeds, seed collection

The Hayward State Nursery, Wisconsin grows more than 40 species from seeds. Up to 6000 bushels of raw unprocessed tree and shrub seeds are collected each year, and all seeds are collected in Wisconsin or adjacent states. All white spruce (*Picea glauca*) and some white pine seeds (*Pinus strobus*) are collected from orchards containing genetically improved material.

Most seeds are purchased through a state seed purchasing program, which allows collection from almost 1000 citizen collectors statewide. This program has been a very good vehicle for generating public interest and knowledge in forestry.

Fall Hardwoods

Oaks

Acorns are collected as soon after falling as possible, and samples are cut to test for viability. Red oak (*Quercus rubra*) acorns are collected without caps and floated to separate bad acorns from good. White oak (*Quercus alba*) acorns are stored below 4 °C (40 °F) to retard radical growth, or sown as soon as possible.

All acorns are turned every few days in the cooler.

Sugar Maple

Sugar maple (*Acer saccharum*) are dried until wings are brittle, and wings are then removed. Seedlots are run through a crippen to remove trash and hollow seeds, and allowed to dry to 6% moisture content for storage up to 3 years. Seeds per pound are calculated, and a cut test is done to determine good seeds per pound.

Other Hardwoods

Aspen (*Populus* spp.), black cherry (*Prunus serotina*), walnut (*Juglans* spp.), butternut (*Juglans cinerea*), birch (*Betula* spp.), ash (*Fraxinus* spp.), and basswood (*Tilia americana*) are processed as needed for seeding or storage.

Spring Hardwoods

Red Maple

Red maple (*A. rubrum*) seeds are collected and dried until wings are brittle; wings are then removed. Seedlots are run through a crippen to remove trash and hollow seeds. Seeds per pound are calculated, and a cut test is done to determine good seeds per pound. Seeding by machine takes place as soon as possible.

River Birch, Silver Maple

Seeds per pound are calculated for river birch (*Betula nigra*) and silver maple (*A. saccharinum*), and a cut test is done to determine good seeds per pound. Machine or hand sowing is done as soon as possible.

Fall Conifers

Cones are stored dry, and turned every few days or spread out to prevent heating. Seed extraction begins in late November; only one species is extracted at a time. The cone kiln is run at approximately 59 °C (138 °F). The time in the kiln depends on species and results. Some species need to be re-wetted and rerun to extract most of the seeds.

Seeds and trash from the kiln are thrashed for a predetermined time to remove wings without damaging seeds. (Spent cones are offered for sale to the public following extraction.) A crippen fanning mill is used to separate seeds from trash.

Seeds are dried to 6% moisture content, with a sample taken just before storage for germination testing. Follow-up germination testing is done every other year until the seeds have all been used or seed viability declines.

Seeds are stored in plastic bags in 114-L (30-gal) foil-lined paper barrels at -7 °C (20 °F). All inventory records are updated at the end of the extraction process.

Seed Storage and Testing at Pennsylvania Department of Conservation and Natural Resources Penn Nursery and Wood Shop

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KEYWORDS
seed collection, seed testing, conifers, hardwoods

Seed Collection

Planting tree seeds at the Pennsylvania Department of Conservation and Natural Resources Penn Nursery, Spring Mills, Pennsylvania occurs in spring and fall. Seeds acquired for these plantings come from 3 sources. The first source is our own orchards, which were developed to provide "improved" seeds. Improved seeds are produced from scion material collected from trees selected for various characteristics and grafted onto rootstock. The second source is from general collections that are collected by forestry personnel from various forest districts. We have developed a barter system with these districts; they receive credit from our wooden and plastic sign shops for any seeds they bring in. The third source of seeds is from privately owned seed companies, which we use only when the other 2 sources do not meet our needs.

Record-keeping

All seeds, no matter the source, are given individual lot numbers upon arrival. A record is kept of the species, date, amount of seeds, county and township where collected, who made the collection, and the GPS coordinates. This is to ensure that the seedlot can be traced back to its source of origin if needed.

Seed Storage

Seeds are placed in our cold storage locker, which is kept between 1 and 2 °C (33 and 36 °F). Any excess seeds (not sown in the nursery beds) are kept in this cold storage for future use during years having poor seed crops. These cold temperatures reduce the rate that the seeds deteriorate over time, keeping their viability as long as possible. Some of our conifer seeds have retained a certain amount of their viability up to 30 years at these temperatures.

Various containers are used for storage, depending on species and size of seeds. Large seeds, such as walnut (*Juglans* spp.) and acorns (*Quercus* spp.), are kept in 70-L (2-bushel) tubs. These species do not store well, and are discarded after the required amounts are sown; new seeds are acquired yearly. Large quantities of smaller seeds are kept in plastic carboys and placed on shelves. Smaller quantities of seeds are placed in plastic bags and stored in metal containers. All bags, carboys, and metal containers are marked with the seedlot and container number. These are recorded in the seedlot record books to ensure easy identification.

The only seeds we currently store in a freezer are aspen (*Populus* spp.). They are kept in small plastic jars and placed in a chest freezer located inside the cold storage.

We do monitor the moisture content of our conifer seeds using an Ohaus™ moisture meter. A 10 g (0.35 oz) sample is placed on the pan and heated for 22 minutes, at which time a reading is taken. A moisture percentage between 6% and 9% is desired for storage.

Seed Testing

As previously stated, our sowing occurs in spring and fall. Certain information is needed in determining the amount of seeds needed to be sown in order to acquire the desired quantities and densities of seedlings in the nursery beds. This information includes purity, seeds per pound, and germination percentage.

The first step is to obtain a good sample of seeds for testing. For seedlots stored in carboys, a seed trier is used. The seed trier allows samples to be drawn from the top to bottom of the container, yielding a truly representative sample. For small lots of seeds or large-sized seeds, samples are taken by hand.

To determine purity and seeds per pound, seed size determines the size of the sample: 50 seeds are sampled for very large seeds, such as acorns and walnuts; 10 g (0.35 oz) are used for large seeds, such as white pine (*Pinus strobus*), sugar maple (*Acer saccharinum*), white ash (*Fraxinus americana*), and so on; a 5-g (0.18-oz) sample is taken for medium seeds like white spruce (*Picea glauca*); and a 1-g sample is used for small seeds.

Purity

To determine purity of a seedlot, a representative sample is weighed, and then seeds are separated from impurities. Total weight of the seeds, in grams, is recorded, and then divided by the total weight of seeds plus the weight of impurities, multiplied by 100.

Seeds Per Pound

To obtain the number of seeds per pound, the number of clean seeds in the sample is multiplied by 453.6 g and divided by the total weight of seeds plus impurities in grams.

Seed Viability

The last test, which can be done several ways, is to determine seed viability. The preferred method is a germination test. We use a Pfeiffer germinator (JP Pfeiffer and Son Inc, Baltimore, Maryland), which simulates the daily fluctuation of tempera-

ture and light found in nature. We use a 10-hour photoperiod with the temperature ranging from 20 to 28 °C (68 to 82 °F). For this test, we use 4 Petri dishes per seedlot. A piece of Kimpak™ is placed in the bottom of the dish to hold moisture. A piece of blotter paper is then placed on top with the seedlot and container number written using a china marker. One hundred seeds are counted out and spread out on the blotter paper, making sure none of the seeds touch each other. This is replicated 4 times for each seedlot, with a total of 400 seeds per test. Seeds are kept moist and checked once a week for 4 weeks. Each week, any seed with a radical emerged to one half the length of the seed coat is considered to be a viable seed. These seeds are then counted and discarded. At the end of the 4-week period, the 4 samples are averaged for the germination percentage of that seed lot.

Some species may require stratification before testing. A cover is placed over the Petri dish, and they are placed in a refrigerator for the required amount of time before being put in the germinator.

When new seeds are received and sowing needs to occur immediately, we can't wait a month for results. In this case, a cutting test or x-ray is performed on the seeds to determine the filled seed percentage in the lot. A sample of 50 to 100 seeds is taken, depending on seed size. Seeds are cut longitudinally or x-rayed to see if the cotyledon fills the seed and has a healthy embryo.

Throughout the process of receiving seeds, it is very important that accurate records are kept for the storage, testing, and planting in the nursery beds. Seedlots should never be mixed so their source can be tracked for future reference.

Seed Storage and Testing Procedures Used at Saratoga Tree Nursery, New York State Department of Environmental Conservation

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KEYWORDS
seed germination, conifer seeds, hardwood seeds

The New York State Department of Environmental Conservation Saratoga Tree Nursery maintains over 120 ha (300 ac) of seed orchard and seed production areas. With the help of New York State Corrections crews, cones and fruits of desired species are collected when ripe. Cones and fruits are transported back to the nursery, assigned a seedlot number according to species, stored on drying racks for about 3 months, and processed in the seed extractory located at the facility. All corresponding data is recorded for each seedlot; all germination tests are performed by nursery staff.

Seed Record Sheet

A Seed Record Sheet (Figure 1) is completed for each seedlot, recording information pertaining to species, origin of cones or seeds, extraction data, storage data, and seed test summary.

Conifer Seed Testing

Recommended procedures for seed testing at the Saratoga Tree Nursery are based on Heit and Eliason (1940) and have been modified slightly to

NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION

SEED RECORD

SPECIES _____ _____ _____
Common Name Scientific Name Variety or Strain

SEEDLOT NO. _____ S.O. FILE NO. _____ S.P.A. FILE NO. _____ S.C.A FILE NO. _____

Received From _____

Date Received _____

Total Cones in Lot _____ hls Total Seed in Lot _____ kg _____ gm
_____ bus _____ lb _____ oz

ORIGIN OF CONES OR SEEDS

Collected by _____ Date _____
Place Collected _____ _____ _____
Country State or Province Locality

Region, Area, Proposal _____
Altitude _____ meters _____ feet
How Collected: Standing Trees _____ Felled Trees _____ Ground _____
Squirrel Cache _____
Age of Seed Trees _____
Volume of Cones or Seeds in Lot as Stated by Vendor or Collector _____
Volume of Cones or Seeds in Lot as Measured by Nursery _____
Current Seed Crop: Light _____ Medium _____ Heavy _____
Remarks on Cones as to Insects, Disease, etc. _____
_____

EXTRACTION DATA

Dates Extracted _____ Extractory Operator _____
Air Dried _____ Kiln Dried _____ Kiln Temp. _____ No. Hours in Kiln _____
Remarks on Extraction _____

STORAGE DATA

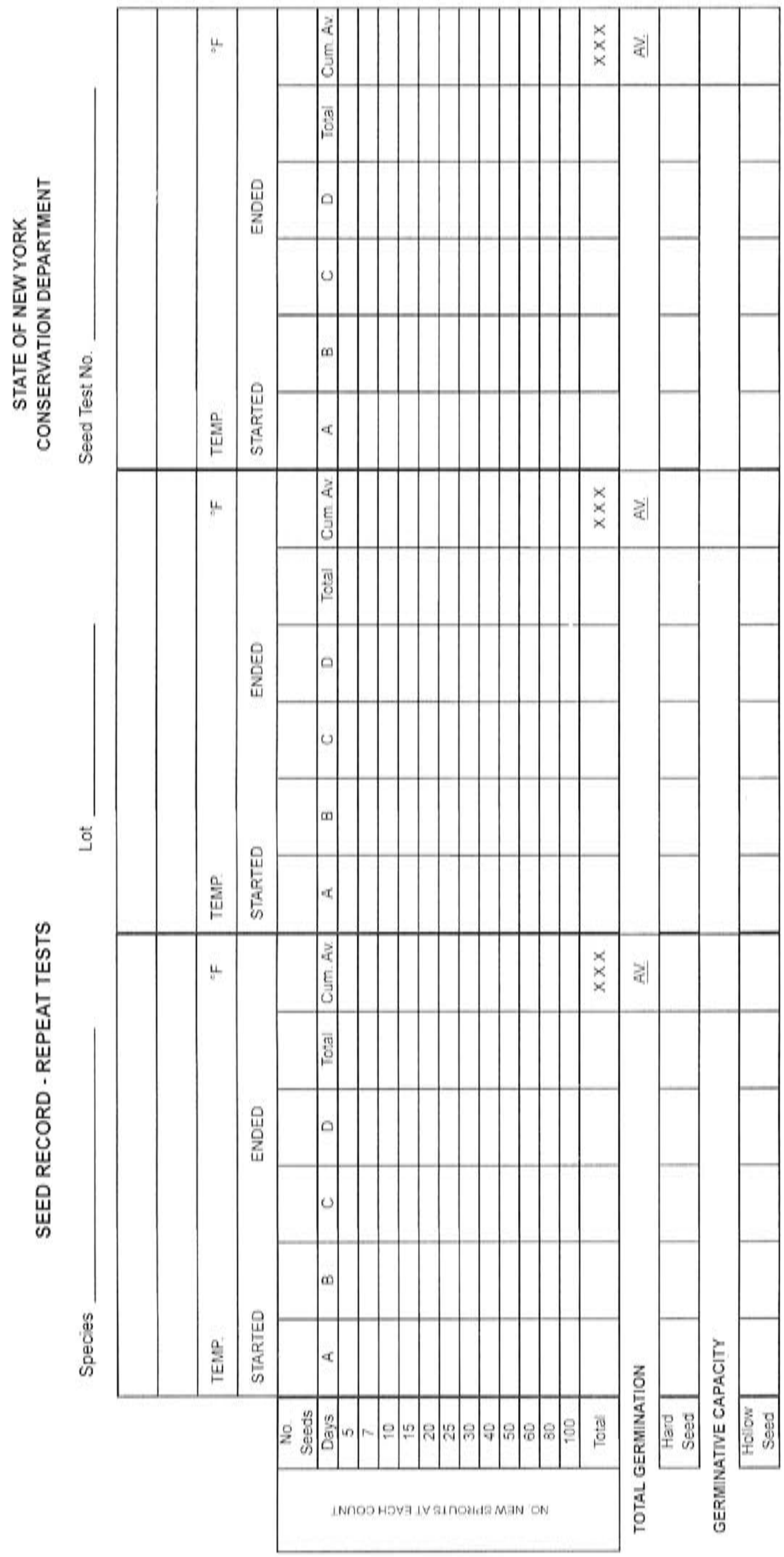
Date Seeds Bottled and Stored _____ Storage Temp _____
Moisture Content _____ Bottled and Stored by _____
Remarks on Storage _____
_____

SEED TEST SUMMARY

Total Germination _____ % in _____ Days Purity _____
No. Seeds per lb of Clean Seeds _____ Weight of Sample _____ gm
No. Seeds in Sample _____ Weight of 1000 Seeds _____ gm
Remarks _____
_____
Test Date _____ Tested By _____
Report Made by _____

LF-206 (2/75)

Figure 1. Seed record sheet for Saratoga Tree Nursery.



STATE OF NEW YORK
CONSERVATION DEPARTMENT

SEED RECORD - REPEAT TESTS

Species ______ Lot ______ Seed Test No. ______

NO. NEW SPROUTS AT EACH COUNT	A	B	C	D	Total	Cum. Av.	A	B	C	D	Total	Cum. Av.	A	B	C	D	Total	Cum. Av.
	TEMP.					°F	TEMP.					°F	TEMP.					°F
	STARTED		ENDED				STARTED		ENDED				STARTED		ENDED			
No. Seeds																		
Days 5																		
7																		
10																		
15																		
20																		
25																		
30																		
40																		
50																		
60																		
80																		
100																		
Total						X X X						X X X						X X X
TOTAL GERMINATION						AV.						AV.						AV.
Hard Seed																		
GERMINATIVE CAPACITY																		
Hollow Seed																		

Figure 2. Germination data sheet for Saratoga Tree Nursery.

meet the needs and equipment of the nursery seed extraction facilities. The recorded information is used when calculating sowing rates and referenced if productivity problems arise with seedlings produced from that seedlot.

Seed tests that are routinely conducted fall into 4 categories: 1) removal of a seed sample; 2) purity and number of seeds/kg (seeds/lb); 3) moisture percentage tested by either loss-upon-heating or a Dole moisture content meter; 4) germination percentage estimated by an unstratified (dry) test, a stratified (wet) test, or a cutting test.

Removal of a Seed Sample

When examining a population, it is necessary to conduct tests on a representative sample of that population. In testing seeds, a random sample is removed from the seedlot using a seed sampling tube and placed in a plastic tray. This sampling procedure may be repeated several times to obtain a true representative sample from a very large lot (90 kg [200 lb] or greater) or to obtain enough seeds for testing a small lot (less than 11 kg [25 lb]).

Purity and Number of Seeds/kg (Seeds/lb)

The determination of purity and the number of clean seeds/kg (seeds/lb) are done together in one operation. Initially, 10.0 g (0.35 oz) of seeds containing impurities are removed from the extracted seed sample. Impurities consist of small pieces of cones, bark, pitch, foliage, and so on. Seeds are counted into piles of 100, keeping all impurities in a separate pile. These seeds can be used later in germination tests. Once all seeds are counted and impurities separated, the impurities are weighed to the nearest 0.01 g. The purity is then calculated as follows:

% purity = (weight of sample — weight of impurity) x 10

For example:

(10 g sample — 0.39 g impurity) x 10 = 96.1% purity

Germination

The Saratoga Tree Nursery does germination tests on all conifer seedlots (Figure 2). Tests on hardwood and shrub seedlots are performed if time allows. A cut test is at least performed on these lots. For each seedlot being tested for germination capacity, a 30-day stratification test and a 30-day warm test is performed. Tests are performed in a germination chamber. At this time, it is our goal to test all lots (new lots or those in storage) once within a 4-year period. It is recommended that seedlots be tested at least 6 months prior to use. When performing seed testing it is imperative that set procedures are followed. A simple oversight, such as dirty utensils or contaminated testing trays, can affect results dramatically.

Seed Storage

After testing, unused seeds are stored in 19-L (5-gal) glass water bottles. These containers are corked and sealed with wax. The storage temperature is set at -2 °C (28 °F). Conifer and shrub species are stored for up to 10 years. Hardwood species are only stored for a maximum of 3 to 4 years. Currently, over 450 seedlots of various species are in storage at Saratoga.

Testing Data

Information obtained from germination testing before outplanting can mean the difference between a successful planting or failed crop. As the cost of soil treatment increases, nurseries can no longer afford to plant seeds for which potential viability is not known. As an example, the Saratoga Nursery spends over US$ 2,960/ha (US$ 1,200/ac) on soil fumigant/treatment materials alone. If your facility is unable to perform your own testing, it would be wise to have these tests done at a certified seed testing facility.

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Soils and Nutrition: A Forest Nursery Perspective

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ABSTRACT
A brief review of the published proceedings from meetings of nursery managers over the past 30 years reveals a high level of consistency with respect to topics of interest from year to year. Seedling quality, defined as seedling capacity to effectively compete after outplanting, has been the unifying theme. Production issues, including collection, storage, and sowing of seeds, planting density, irrigation, nutrition, pest control, and lifting, have been commonly featured. Soil management, with emphasis on organic matter and nutrition, occupies a prominent position among the individual titles. It seems that nursery production knowledge and practice have been maintained at a relatively high level; one would be hard pressed to identify the year of publication simply from a list of titles from any single proceedings. This paper focuses on soil and nutrition in nursery seedling production. One of the most recent additions is the topic of exponential nutrient loading. This practice is used to induce luxury consumption, producing loaded seedlings that are better able to compete in the outplanting environment. Performance of outplanted seedlings, followed for as long as 6 years, suggests that nutrient loading is an effective nursery practice; seedlings effectively compete in their outplanted environments. Nutrient loading papers are appearing with increasing frequency in the literature, reflecting a high degree of interest. Nutrient loading may become a standard practice as outplanted seedling success becomes more widely demonstrated.

KEYWORDS
analyses, fertility, management, organic matter

Introduction

I began preparing for this meeting by reviewing published proceedings of forest tree nursery workshops over the past 3 decades. A remarkable consistency among topics was readily apparent. The underlying theme of each meeting has been focused on production of disease-free, high quality seedlings. Although the specific morphological and physiological variables utilized to express seedling quality seem to be in a perpetual state of flux (Bunting 1980; South and Mexal 1984; Haase 2007), there appears to be general agreement that quality seedlings are those that will effectively compete in the outplanting environment.

Nursery production, at least in the northeast US, has gradually shifted emphasis from a primary focus on production of large numbers of conifer seedlings for planting by large industrial and government landowners to a more diffuse customer base with a wide array of smaller plantings. These plantings require a wide range in species for a variety of purposes, including stream bank stabilization, bioremediation of

mildly contaminated sites, bioenergy plantations, wetland restoration, and so on. Many of these seedlings will face competition from existing vegetation and, perhaps, will be planted on sites that are less than ideal. Consequently, the need for high quality seedlings remains strong.

Production of high quality seedlings is a complex process that begins with seed selection and sowing, culminating with lifting and shipping. The diversity of species produced at individual nurseries, each with their individual nuances for successful growth and development, further challenges nursery management. The production process has been described using a chain as an analogy. Each component is required to get to the end point; a break of any single link introduces a discontinuity that reduces quality and quantity of the final product.

This paper focuses on the soil system as an integral component of the production chain, with particular emphasis on nutrition. The underlying theme of this paper is that monitoring soil and plant conditions is an important component of nursery production. Soil testing and plant analysis were common components of nursery management in the northeast US in past decades. This type of activity apparently has recently dropped off as part of an effort to reduce costs. The lack of information reduces the capacity to address occasional problems in seedling quality. As the number and severity of production problems increase, soil and plant analyses will likely become more common in nurseries in the northeast US.

The Soil System

The root system plays a critical role in uptake of water, nutrients, and oxygen (O_2). (Removal of CO_2 is implicit.) Delivery of those components to the roots in hydroponic systems is totally controlled by management of technology. Production of plants in soil (bareroot systems) or containers (media) adds a layer of complexity in delivery of water, nutrients, and O_2; the soil (or media) system plays a critical role. Root growth and development attain their maxima when fluxes of nutrients, water, and gas meet plant demand for growing tissue. When those fluxes are constrained, growth and development are necessarily reduced, having a negative impact on seedling capacity to effectively compete in a new environment. Management of the soil and media in the nursery is critical for maintenance of those fluxes.

The impacts of nutrients and moisture on seedling quality can be graphically illustrated in the conceptualization by Stone (1984), substituting seedling quality for site quality (Figure 1). High quality seedlings require an effective supply of water and nutrients; adequate aeration is implied in an effective water supply. Ultimately, maximization of root growth and development can be thought of as minimization of constraints for nutrients, moisture, and aeration. Unlike hydroponic systems, where delivery of moisture, nutrients, and dissolved O_2 are strictly controlled by management and technology, those fluxes in the soil system are constrained primarily by distribution of pore sizes and, secondarily, by management (that is, irrigation and fertilization). It follows that managing seedling quality requires management of pore size distribution.

Pore space occupies approximately 50% of the volume of the "ideal" soil. The distribution of pore sizes is even more important than the total pore volume (porosity). Pore size regulates movement and storage of water, which directly impacts aeration. Macropores, defined as those pores that do not hold water against the force of gravity, allow for drainage of water and consequent exchange of O_2 and CO_2 between plant roots and the soil atmosphere. Ideally, the pore volume would be evenly split between macro- and micropores, simultaneously providing aeration and moisture retention.

Pore size distribution is a function of soil physical properties. In managing nursery soils, it may be useful to think of these soil properties in the context of time scales.

The Soil Matrix

Texture: Long-term (10^3-year) Management

Soil texture, the relative proportion of sand-, silt-, and clay-sized particles, is the primary control of pore size distribution. Soil texture is stable over thousands of years. Altering soil texture is not feasible on the scale of bareroot production in forest nurseries, but is feasible on the scale of container media where various mixtures of organic substrates and perlite/vermiculite are used.

The macropores between sand particles (2 to 0.05 mm effective diameter) drain freely and promote aeration. In contrast, the very small pores between clay particles (< 0.002 mm), termed micropores, hold water against the force of gravity and aeration tends to be poor. This effect is strongly modified by soil organic matter (SOM), which has high pore space, low density, and contributes to formation of water stable aggregates.

One of the major decision criteria utilized for siting forest nurseries is soil texture (Wilde 1958; Armson and Sadreika 1979). Sandy loam and loamy sand textures with minimal coarse fragments (rocks) are considered most desirable because they provide rapid drainage, leading to good aeration and early warming in the spring. These textures, although not immune, are less prone to frost heaving.

Soil Organic Matter (SOM): Mid-term (10^0- to 10^1-year) Management

A sandy loam texture, recommended for nursery sites, provides excellent physical conditions for plant roots, minimizing the constraints of poor drainage and aeration. Lack of clay, however, limits soil capacity to retain water and nutrients. Addition of organic matter, which plays a crucial role in retention of water and nutrients in coarse textured soils, compensates for this deficiency. Coarse textured soils are well aerated, promoting rapid oxidation and decomposition of soil organic matter (SOM). In addition, periodic lifting of seedlings removes a significant quantity of SOM from nursery beds on a regular basis. Davey (1980) referred to this removal as "mining." It is easy to understand why maintenance of SOM is a key concern for tree seedling nurseries.

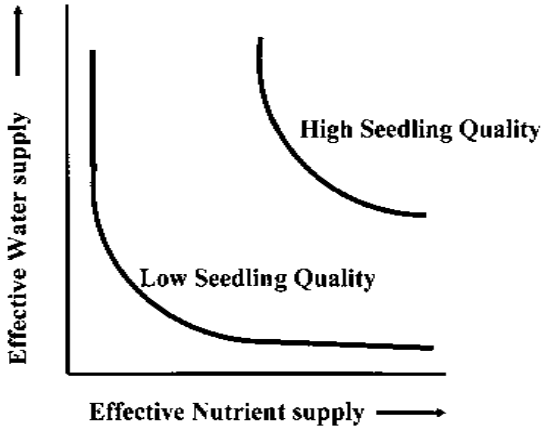

Figure 1. Seedling quality as a function of effective moisture and effective nutrient supply (after Stone 1984).

The topic of SOM in the context of forest nursery soil management has been treated extensively in the past decades. Papers dealing with SOM have been a prominent feature of nursery proceedings from 1980 (Abrahamson and Bickelhaupt 1980) to the present (Riley and others 2006). The topic's persistence over the past decades reflects the degree of attention and concern of nursery managers. The nature of that concern was articulately described by Davey (1984) in reviewing results from a nursery manager survey conducted by Oregon State University in the northwestern US. Eighty-six percent of 21 respondents intended to increase SOM levels; 62% listed SOM maintenance among their top 5 issues. A wide variety of amendments were applied to maintain or increase SOM, including cover crops or green manure (for example, peas, oats, lupines), peat, sawdust, bark, and sludge. The choice of amendment depends on a variety of local factors (not the least of which is availability). Davis and others (2007), summarizing a number of studies, pointed out that long-term application and continual monitoring are

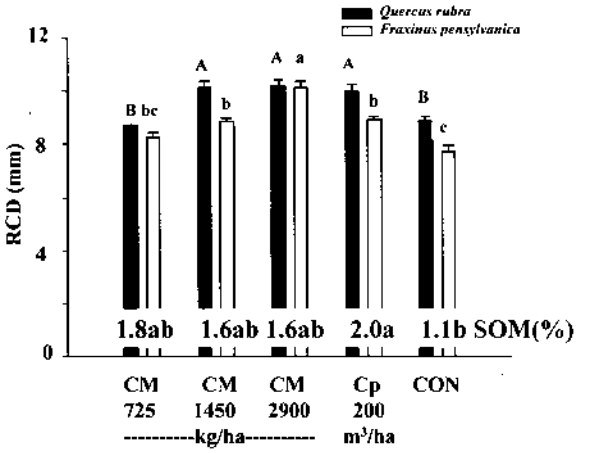

Figure 2. Influence of soil amendments (chicken manure [CM], compost [Cp]) relative to no amendments (CON), on soil organic matter (SOM) and root collar diameter for red oak and green ash seedlings prior to lifting. Different letters indicate significant differences at $P < 0.10$ (Davis and others 2006).

required in order to sustain SOM in forest nurseries.

Organic matter management practices continue to evolve, and research is ongoing. I selected 3 recently published papers to illustrate the nature of current scientific inquiry.

Davis and others (2006) incorporated the following organic amendments approximately 7.5 cm (3 in) deep in nursery beds at the Vallonia State Nursery, Indiana: chicken manure (following thermophilic decomposition) at 725, 1450, and 2900 kg/ha (650, 1300, 2590 lb/ac), and 2-year composted trimmings (leaf, tree, lawn) at 200 m^3/ha (106 yd^3/ac). Soil samples were collected for analysis immediately following amendment incorporation. Green ash (*Fraxinus pennsylvanica*) and red oak (*Quercus rubra*) seeds were sown in the beds. Seedlings were lifted and measured (root collar diameter [RCD], height, root volume) the following spring. All organic amendments increased SOM (Figure 2), as well as cation exchange capacity. All amendments increased seedling RCD above that of the control (no amendment) (Figure 2), with differences between the 2 species. Green ash seedling RCD attained the highest values on the highest level of chicken manure, while red oak seedlings attained equally high levels on all amendments except for chicken manure at 725 kg/ha (650 lb/ac), which did not differ from the control. Patterns of seedling root volume and height response were similar to those of RCD. Davis and others (2006) pointed out the need to continue this work to improve the integration of organic amendments with traditional inorganic fertilization practices.

Davis and others (2007) added an interesting twist by comparing the impacts of an array of organic amendments (incorporated as described in previous paragraph) on soil properties and sorghum (*Sorghum* spp.) cover crop production. Treatments evaluated were: a) 1450 kg/ha (1300 lb/ac) chicken manure (CM) alone; b) combination of 1200 kg/ha (1070 lb/ac) hardwood sawdust with 4350 kg/ha (3880 lb/ac) CM; c) combination of 1200 kg/ha (1070 lb/ac) hardwood sawdust with 8700 kg/ha (7770 lb/ac) CM; d) 2-year composted trimmings (leaf, tree, lawn) at 200 m^3/ha (2860 ft^3/ac); and e) 200m^3/ha (2860 ft^3/ac) uncomposted leaves. Sorghum was sown uniformly at 1.12 kg/ha (1 lb/ac), and soil samples were taken immediately after amendment incorporation. SOM and pH increased significantly above control levels (1.2% and 4.8%, respectively) for all treatments, with the exception of CM alone. All amendments had a positive effect on sorghum biomass production (Figure 3). The authors anticipate a long-term benefit from promotion of higher SOM from sorghum.

Riley and others (2006b) is an excellent illustration of the evolution of soil management practices, as well as many other aspects, for the J Herbert Stone Nursery in Central Point, Oregon. Their primary focus was on soil tilth and porosity rather than SOM *per se*. Addition of organic matter is one of several management practices used to maintain favorable soil tilth. In response to increasing costs of sawdust and N fertilizers, an alternative to sawdust was sought. Continuous

monitoring and use of a variety of treatments (deep subsoiling, wrenching, organic amendments, and pumice) ultimately resulted in favorable soil tilth, although their goal of 50% soil porosity has not yet been attained.

Fertilization

Fertigation: Short-term (10^{-1} to 10^{-3} year) Management

Given a configuration of pores resulting from the combined effects of soil texture and organic matter, the delivery of water and nutrients to root systems is managed on a daily/weekly basis through irrigation and fertilization. The presence of soil in bareroot systems, or medium in the case of container stock, adds a degree of complexity in supplying water and nutrients to the roots.

This complexity is managed by monitoring soil moisture, armed with knowledge of the soil moisture characteristic (relationship between soil water concentration and soil water potential). A system of tensiometers is often utilized for irrigation management. When soil moisture tension falls somewhat below field capacity, irrigation is applied and moisture stress is avoided.

The situation for nutrients is a bit more daunting and somewhat indirect. Soil testing provides a snapshot in time of nutrient solubility. Soil nutrients are conceptually identified in a system consisting of 4 components: 1) very slowly available (structural framework of primary minerals and organic matter); 2) slowly available colloidal fraction (structural framework of clay and humus); 3) adsorbed fraction (ions attracted to colloidal surfaces); and 4) readily available (soil solution fraction) (Brady and Weil 2002). Soil testing involves leaching soil samples with one of many extracting solutions varying in composition and ionic strength, extracting soluble nutrients (pool 4 described above) and a small portion of those in pool (3) and perhaps (2). The actual results are strongly dependent on the extractant and procedure used. In the past, these were often referred to as "available" nutrients, but there is no clear indication that those extracted fractions are equivalent to what plant roots absorb. Consequently, they are more appropriately referred to as extractable nutrients.

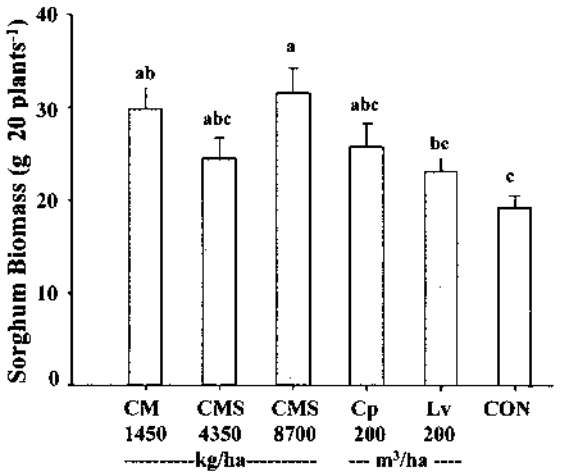

Figure 3. Influence of soil amendments (chicken manure alone at 1450 kg/ha [1300 lb/ac] [CM], chicken manure at 4350 and 8700 kg/ha [3880 and 7770 lb/ac] in combination with 1200 kg/ha [1070 lb/ac] sawdust [CMS], compost [Cp], and leaves [Lv]) relative to no amendments (CON) on sorghum cover crop biomass. Different letters indicate significant differences at $P < 0.05$ (Davis and others 2007).

Soil tests, in and of themselves, are only of use when a relationship has been established between some measure of seedling quality (that is, height, biomass, root volume, and so on) and soil test values. During the late 1970s and early 1980s, a considerable effort to develop such as relationship was expended by the Forest Soils Analytical Lab at the State University of New York College of Environmental Science and Forestry (SUNY ESF) in cooperation with 18 nurseries (Table 1) distributed across the US. Soil test recommendations developed on the basis of that work are summarized in Table 2. It is important to keep in mind that those recommendations are method specific. Armson and Sadreika (1979), for example, recommend minimum extractable P as 300 mg/kg, using the Bray-Kurtz (another common method) extraction.

The accuracy, hence utility, of soil test results depends on quality control in the laboratory.

Table 1. List of 18 nurseries (identified by location) involved with soil testing program at SUNY ESF (identified from MEMO to participating nurseries).

Carbondale, Colorado	Harmans, Maryland	Concord, New Hampshire
Magalia, California	Freesoil, Michigan	Saratoga Springs, New York
Coeur d'Alene, Idano	Manistique, Michigan	Bonanza, Oregon
Topeka, Illinois	Licking, Missouri	Swansea, South Carolina
Fryeburg, Maine	Bismark, North Dakota	Draper, Utah
Passadumkeag, Maine	Boscawen, New Hampshire	Lakin, West Virginia

Table 2. Recommended levels for chemical values for nursery soils based on research at SUNY ESF Forest Soils Analytical Lab.

Variable	**Conifer**	**Hardwood**
Organic matter[z]	2.5%	2.5%
pH[y]	5 to 6	6 to 7
P (mg/kg)[x]	100	150
K (mg/kg)[w]	100	150
Ca (mg/kg)[w]	500	1000
Mg (mg/kg)[w]	150	250
CEC ($cmol_c/kg$)[w]	8	11
EC (dS/m)[v]	< 2	< 2

[z] Organic matter determined by loss-on-ignition or Walkley-Black wet oxidation.
[y] Measured in1:2 soil:water slurry.
[x] P extracted with 0.002 *N* sulfuric acid (Truog procedure).
[w] Cations and CEC determined from extracts using neutral 1 *N* ammonium acetate.
[v] Electrical conductivity determined from saturated soil paste vacuum filtered 2 hours after mixing.

Credible labs have systems in place to insure accuracy and repeatability of their results. For example, our quality control program consists of several components:

1) Periodic analysis of "standard" soil and plant tissue samples (obtained from commercial sources or collected by us from a single location, homogenized, and stored);

2) Participation with US Geological Survey (USGS) round-robin analysis of water samples from a group of laboratories;

3) Inclusion of blank (distilled deionized water) and spiked (known concentrations) samples in every sample run.

Finally, dedication to a single laboratory is implicit. An excellent working example is provided by the Auburn Nursery Cooperative, which utilizes a single commercial lab for all of their analyses (Davey 1995). This allows comparison of results across nurseries, immensely increasing the

value of the program for all of the nurseries involved.

As government support for state and USDA Forest Service nurseries has diminished, so has routine soil testing. It has been almost a decade since the SUNY ESF Forest Soils Analytical Lab received a soil sample from a nursery. I suspect that this trend will be reversed. Monitoring soil conditions is an important component of nursery management and becomes more critical as problems with seedling quality develop. One of the most memorable examples of the utility of soil monitoring in identifying seedling quality problems is drawn from our cooperative work with the state nursery at Saratoga, New York. During the late 1970s, several conifer beds exhibited unusually high chlorosis and mortality. Laboratory analysis revealed soil pH well in excess of 7.0, which was traced to overzealous application of an organic amendment. The ample supply of horse manure (routinely treated with lime to control odor) from the adjacent harness track invited excessive application. The high buffering capacity of the added organic matter prevented rapid recovery; pH remained elevated for at least 4 years following application. Negative impacts on seedlings were documented by Bickelhaupt and others (1987). Amelioration required substantial applications of acid coupled with deep plowing over a lengthy time period.

In addition to its obvious utility as a tool for ensuring seedling quality, soil monitoring is becoming more important from an environmental perspective. Application of nutrients in excess of seedling uptake capacity is costly, both financially and environmentally. Excess nutrients dissolved in water move below the root zone and are delivered to surface water, degrading water quality. State and federal agencies are focusing more attention on water quality in efforts to control eutrophication. Eutrophication, a result of increased delivery of nitrogen (N) and phosphorus (P) to surface water systems, is a serious problem that has directly contributed to hypoxia and anoxia across the globe (Diaz 2001). Soil monitoring can be a useful tool in minimizing unnecessary or excessive nutrient amendments.

Nutrient Loading

One of the most exciting new areas of research and practice in seedling nursery management is nutrient loading, or the application of relative addition rate concept in nursery seedling production (Ingstead and Lund 1986). The efforts have been documented in a series of papers over the past 15 years.

Timmer (1996) provides a concise history of the development and application of exponential nutrient loading in greenhouse production. Seedlings are produced with nutrient reserves that promote maximum growth upon outplanting. Nutrients are concentrated (stored) in seedlings by inducing luxury consumption, matching nutrient supply with plant growth (biomass). Exponential loading is illustrated relative to conventional single dose and periodic application of a single rate in Figure 4, which also depicts an adjustment for incomplete root exploitation of the medium early in the seedling growth period.

Field performance of nutrient-loaded seedlings has been documented for a variety of species. Timmer (1999) found that 6-year biomass and height of nutrient-loaded black spruce (*Picea mariana*) seedlings outplanted on sites where competition had not been controlled were equivalent to conventionally fertilized seedlings planted on herbicide treated sites; nutrient loading compensated for lack of competition control (Figure 5). These results, which are based on the longest term data currently available, demonstrate the capacity of nutrient-loaded seedlings to overcome the negative effects of competition, a tremendous advantage as herbicide costs increase and application becomes more restricted.

Interest in exponential fertilization is increasing, and the technique is being expanded for application to hardwood species. Birge and others (2006) reported positive results of nutrient loading to induce luxury consumption of N for red

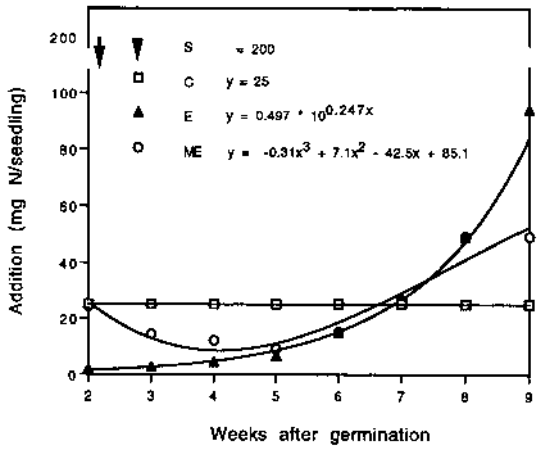

Figure 4. Scheduling of fertilizer additions by seedling age applied as single dose (S), constant top dressing (C), exponential (E), and modified exponential (ME) during greenhouse culture. (Initial exponential rate was reduced to account for incomplete root exploitation, but later balanced by increased exponential rate following complete root exploitation.) Note scale break in y-axis (from Timmer 1996).

(*Quercus rubra*) and white oak (*Q. alba*) at the Vallonia State Nursery, Indiana. Seedling biomass and tissue N concentrations of nutrient-loaded seedlings exceeded those of conventionally fertilized seedlings. As the exponential nutrient loading technique comes into greater use, additional long term data tracking outplanting performance will likely become more widely dissipated.

Slow-Release Fertilizer

Use of slow-release fertilizer (SRF) in nursery seedling production represents somewhat of a middle ground between exponential loading and conventional fertilization. Dissolution of polymer coatings over time allows nutrients to move outward over a period of months. Unlike exponential loading, where plant nutrient additions are estimated on a weekly or shorter term basis, nutrient release from SRF is not precisely quantified on such a fine time scale. Huett and Gogel (2000) showed that SRF nutrient release is uneven. Highest rates occur early in the release period, which is not well timed with steadily increasing seedling nutrient demand. Actual SRF nutrient-release periods were considerably shorter than nominal rates. In spite of these shortcomings, slow-release fertilizers have been used successfully in nursery production.

Haase and others (2006) supplemented conventional fertilization of container Douglas-fir (*Pseudotsuga menziesii*) seedlings with 4 SRF treatments: Apex 1®, Apex 2®, Forestcote®, and Osmocote®. Seedling growth and nutrient concentrations were monitored immediately after outplanting and over 4 growing seasons at 2 sites in Oregon. Only results from the Powers site in western Oregon are presented below. Luxury consumption was successfully induced by SRF. At the time of planting, seedlings grown with SRF in the medium treatment had higher concentrations of N and P relative to conventional fertilization; seedlings grown with Forestcote® were larger than those grown with Apex 1®, Apex 2®, or conventional fertilization (Table 3).

Height growth of all SRF-treated seedlings exceeded that of conventionally fertilized seedlings at the end of the first and second field seasons after planting (Table 3). Although no differences were observed in height growth among the 5 treatments following the third and fourth seasons after planting (data not shown), the initial SRF effect persisted, carrying through 4 years after planting. Height in 2001 (4 growing seasons after planting) of SRF-treated seedlings was significantly greater than conventionally fertilized seedlings.

Closing Comments

Seedling quality continues to be a unifying theme among nursery managers, as indicated in the papers that have appeared in proceedings published over the past 30 years. Soil management is critical to maintain production of high quality seedlings. Nursery soil management practices appear to be keeping pace with scientific knowledge through effective technology transfer; the contents of published proceedings provide ample evidence that this is the case. Soil and plant tissue analysis is an important tool that can be effective-

ly used in producing quality seedlings. Increasing pressure to reduce costs, while improving efficiency at many nurseries, seems to have reduced routine use of soil and plant analyses as a management tool for many nurseries in the northeast US. As seedling quality issues and environmental concerns increase, greater use of soil and plant tissue nutrient monitoring is likely.

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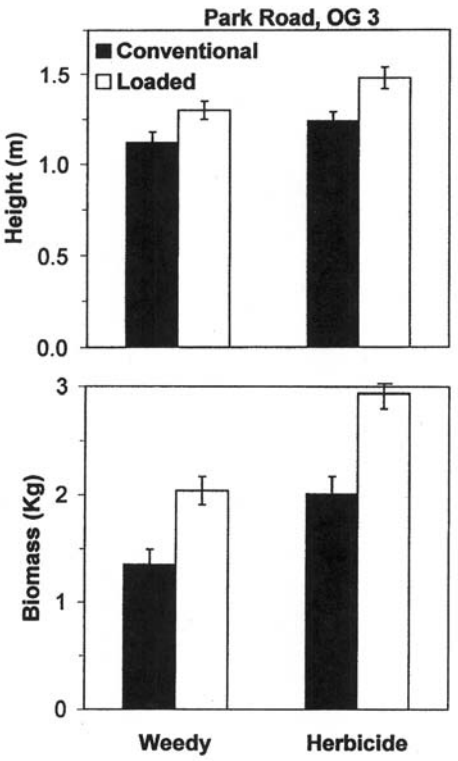

Figure 5. Comparison of 6-year height and biomass response of conventional and nutrient-loaded black spruce seedlings growing in untreated (Weedy) versus herbicide-treated (Herbicide) plots at time of planting (Timmer 1999).

Table 3. Initial height and annual growth (cm) for Douglas-fir seedlings produced in the nursery with conventional fertilization alone and supplemented with slow-release fertilizers prior to outplanting at the Powers site (after Haase and others 2006).

Treatment	Initial Height [x]	1998 Height Growth [x]	1999 Height Growth [x]	Σ4-year Growth [x]	2001 Height [x]
Conventional [z]	33.5 bc	9.3 b	18.1 b	128	162 b
Apex 1® [y]	33.5 bc	13.5 a	20.6 ab	144	177 a
Apex 2® [y]	32.1 c	14.1 a	21.8 a	136	168 ab
Forestcote® [y]	36.9 a	13.4 a	22.2 a	136	174 a
Osmocote® [y]	34.3 b	14.2 a	22.3 a	141	176 a

[z] Conventional fertilization consisted of regular application of soluble nutrients via overhead irrigation system.
[y] SRF treatments applied at 30 kg/m^3 (1.9 lb/ft^3) in addition to conventional fertilization.
[x] Different letters associated with means within a column denote statistically significant differences at $P < 0.05$.
(1 cm = 0.39 in)

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Performance of Nutrient-Loaded Red Oak and White Oak Seedlings on Mine Lands in Southern Indiana

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ABSTRACT
Exponential nutrient loading was used to build nutrient reserves in northern red oak (*Quercus rubra*) and white oak (*Q. alba*) seedlings during standard bareroot nursery culture at the Vallonia State Nursery, Indiana. Nursery grown seedlings were outplanted the following year onto a mine reclamation site in southern Indiana to evaluate effects of prior nursery treatments on field performance. At the nursery stage, exponential nutrient loading improved plant dry mass production. Nutrient loading increased nitrogen uptake 40% in red oak and 35% in white oak when compared to controls. When outplanted, exponential nutrient loading enhanced shoot height and root collar diameter response in the studied species. White oak seedling survival was 92%, compared with 83% for red oak in year 1. Survival decreased to about 74% for white oak and 65% for red oak in year 2. Results suggest exponential nutrient loading has potential to promote seedling performance on harsh site conditions, and are significant for reclamation efforts in Indiana and across the US.

KEYWORDS
biodiversity, forest restoration, nitrogen, *Quercus alba, Quercus rubra*, reclamation, seedling quality

Introduction

Successful forest restoration on abandoned surface-mined lands can yield multiple benefits, such as provision of timber, high quality water, wildlife habitats, and aesthetic landscapes. However, poor site fertility (Bussler and others 1984; Walker 2002), soil compaction (Unger and Cassel 1991), competition from herbaceous plants (Andersen and others 1989; Casselman and others 2006), animal browse (Tripler and others 2002), and poor seedling quality (Clark and others 2000; Ward and others 2000; Jacobs and others 2004) are key factors that may limit early establishment success of newly outplanted hardwood seedlings on degraded landscapes in the Central Hardwood Forest Region of the US.

Fertilization at planting, in combination with weed control with herbicides, can alleviate poor site fertility, reduce competition, and enhance seedling field survival and growth (Jacobs and others 2005). However, there is public sentiment against herbicide use in forests owing to potential negative impacts on the environment and on biodiversity (Thompson and Pitt 2003). Thus, storing nutrients in seedlings during nursery culture to reduce competitive effects provides a better rationale than field fertilization that may stimulate growth of competing vegetation. Moreover, studies have shown that the nutritional status of a plant is a better indicator of quality and field success (Timmer and Munson 1991; Malik and Timmer 1996). Therefore, appropriate nursery nutrient management can lead to the production of high quality seedlings with greater internal nutrient reserves to reduce competitive effects and enhance field performance (Malik and Timmer 1996; Thompson and Pitt 2003).

Several fertilization methods, such as conventional, exponential, or nutrient-loading models, can be used to nutritionally pre-condition plants at the nursery stage for outplanting. Conventional fertilization involves application of equal fertilizer doses at regularly spaced intervals throughout the growing season. This approach creates a surplus of nutrients to young seedlings early in the growth phase, but may cause a deficiency in larger seedlings by the end of the growing season (Imo and Timmer 1992). This occurs because nutrient requirements vary at different stages of seedling development throughout the growing season. Exponential fertilization closely matches nutrient supply with seedling growth demand during the exponential growth phase (Ingestad and Lund 1986; Timmer and Aidelbaum 1996), and enhances fertilizer uptake efficiency and minimizes potential leaching losses. Nutrient loading involves extra high fertilization to induce luxury uptake and build reserves in seedlings to benefit outplanting (Figure 1), and is more compatible with exponential fertilization. Thus, with exponential nutrient loading, nutrients are applied at exponentially increasing rates in excess of growth demand to induce luxury uptake, which builds seedling nutrient reserves to reduce competitive effects and benefit outplanting.

Although the above fertilization techniques may have universal application, protocols to determine optimal target rates associated with maximum growth and seedling nutrient storage are not well defined. There is apparent need to quantify target rates for each species and cultural system owing to differences in species demand for nutrients and variations in cultural conditions. An innovative approach that can help rationalize and quantify fertility targets in tree seedling culture is illustrated in Figure 1. The figure shows that the relationship between plant growth, nutrient status, and increased fertilization is curvilinear, but sectioned here into 3 phases to demonstrate points of nutrient deficiency, luxury consumption, and toxicity with increased fertility (Salifu and Timmer 2003; Salifu and Jacobs 2006). Generally, seedling growth is maximized at sufficiency. Optimum response is obtained when growth and nutrient uptake are maximized, which occurs during luxury uptake. Excessive fertilization induces toxicity, which is associated with increased tissue nutrient concentration but diminished growth (Figure 1).

Nutrient-loaded seedlings exhibit greater survival, growth, and competitiveness over non-loaded plants on a variety of habitats (Timmer and Munson 1991; Malik and Timmer 1996). The approach provides a new and useful technique to build plant nutrient reserve at the nursery stage, which has specific significance to reducing competitive effects and enhancing seedling performance on harsh outplanting environments. Birge and others (2006) tested application of exponential nutrient loading in standard bareroot red oak (*Quercus rubra*) and white oak (*Q. alba*) seedling culture to build nutrient reserves to benefit field outplanting. These nursery grown seedlings were then outplanted onto a mine reclamation site in southern Indiana to examine effects of prior nursery treatments on seedling field performance. Our objectives were to evaluate: 1) growth and nitrogen storage in exponentially fertilized seedlings compared with conventional cohorts at the end of nursery culture; and 2) survival and growth of exponentially nutrient-loaded seedlings compared with conventional cohorts planted on mined land in southern Indiana. These species were selected for the study because of their great importance to the local hardwood industry, wildlife habitat, and increased use in environmental plantings (Jacobs and others 2004).

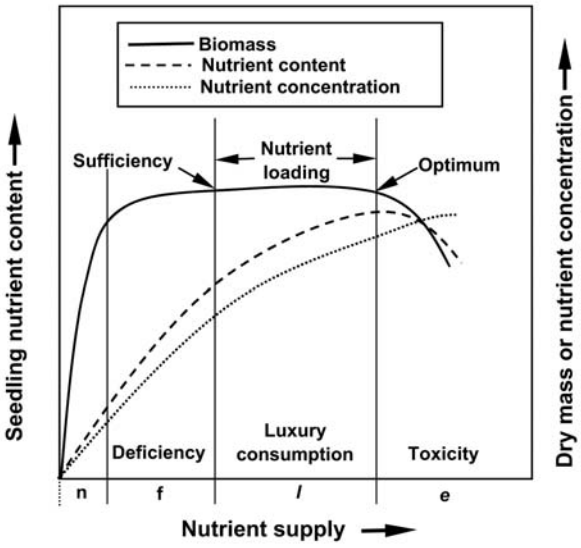

Figure 1. Relationships between nutrient supply with plant growth and nutrient status. Fertilizer (*f*) is added to supplement native fertility (*n*) to prevent nutrient deficiency and maximize growth to the sufficiency level. Optimum nutrient loading is achieved by adding fertilizer (*l*) that induces luxury consumption to build up plant nutrient reserves for outplanting. Excess fertilization (*e*) inhibits growth because of toxicity (adapted from Salifu and Timmer 2003).

Materials and Methods

Nursery Phase

Bareroot northern red oak and white oak seedlings were grown from seeds germinated in spring 2004 at Vallonia State Nursery, Indiana (38°85'N, 86°10'W). Seedlings were fertilized conventionally or exponentially using 10 fertility treatments that ranged from deficiency to toxicity (0 to 3.35 g N/plant/season). Higher fertility treatments were intended to build nutrient reserves in plants for later utilization when outplanted. Nitrogen was applied bi-weekly as ammonium nitrate in crystal form ($34N:0P_2O_5:0K_2O$). Further details on the nursery study can be found in Birge and others (2006). Seedlings were mechanically lifted in December 2004 and processed for overwinter storage in coolers (3 °C [37 °F]) at Purdue University, West Lafayette, Indiana. Plants were removed from storage in late April 2005, and sorted for the field experiment.

Field Study

Nursery-reared seedlings were outplanted in April 2005 into an abandoned mine reclamation site in southern Indiana. The field design was a split-plot design with a 2 x 10 factorial treatment structure, and was replicated in 5 blocks. The main plot treatments were species at 2 levels and the sub-plot treatments were the 10 nursery fertility treatments. Each block measured 21 x 42 m (69 x 138 ft) and was separated from the next by 2-m (6.5-ft) buffers. Species were randomly allocated within blocks, and fertility treatments were randomly allocated within species. Each treatment consisted of 20 trees in one row within a block, and each block contained 20 rows. A total of 2500 seedlings were planted and monitored in this

experiment (10 treatments x 20 trees per treatment x 2 species x 5 replications). Trees were planted 1 x 1 m (3.3 x 3.3 ft) within rows and 2 x 2 m (6.5 x 6.5 ft) between rows. Sampling and quantification of seedling morphological and nutritional responses followed standard protocols (Salifu and Timmer 2003; Jacobs and others 2005). Growth and nutritional parameters were evaluated by analysis of variance using SAS (SAS Institute Inc, Cary, North Carolina), and treatments were declared significant at $P < 0.05$. Significant treatment means were ranked according to Waller-Duncan's multiple range test at $\alpha = 0.05$.

Results and Discussion

Nursery Phase

Growth and nutritional data sampled at the end of nursery culture (Birge and others 2006) conformed closely to trends shown in Figure 1, which demonstrates suitability of the model for application in hardwood culture. Exponential nutrient loading increased plant biomass 113% to 260% for red oak, and 49% to 144% for white oak when compared with nonfertilized plants. Similarly, nutrient loading stimulated N uptake 40% in red oak and 35% in white oak relative to controls. The greater nutrient reserves in loaded seedlings may serve as critical nutrient resources to reduce competitive effects and promote rapid growth under field conditions (Crow 1988; Dickson 1989; Tagliavini and others 1998). We recommend the use of balanced fertilizers to avoid potential antagonistic interactions associated with only N-based fertilizers in the nutrient loading process. Frequent fertilizer additions will make nutrients readily available to plants, which increases uptake efficiency and minimizes potential leaching losses associated with heavy and less frequent applications.

Field Response

Generally, field survival was high in year 1 (Figure 2), but decreased by year 2 (Figure 3). First year field survival for exponentially cultured seedlings ranged from 83% to 92%, which is higher than the 66% (Figure 3) observed for conservation tree plantings in Indiana (Jacobs and others 2004). Year 1 survival for white oak was 92%, which was higher than survival of red oak at 83% (Figure 2). Survival decreased to 74% for white oak and 65% for red oak in year 2. Exponential nutrient loading promoted seedling height and root collar diameter growth for red oak (Figure 4, top) and white oak (Figure 4, bottom). High survival and rapid early growth may allow the plants to reach free-to-grow status sooner, which may minimize animal browse and/or the need for vegetation control with herbicides. Results of our current study suggest that exponential nutrient loading provides a new and useful management technique which has specific significance to improving seedling performance on mine reclamation sites and reclamation success in Indiana. We anticipate improved performance in subsequent years because of the potential to exploit greater nutrient resources stored in roots to support ongoing growth demand. We suggest further testing of exponential nutrient loading to build nutrient reserves in plants at the nursery stage to improve performance on harsh site conditions. Other innovative approaches, such as the use of controlled-release fertilizers at planting (Jacobs and others 2005), different stocktypes (Dixon and others 1983; Davis and Jacobs 2004), and mycorrhizal inoculation (Zhou and Sharik 1997) have been found to promote seedling field performance. The potential of these new approaches to improve seedling field response needs further testing and refinement.

Conclusions

Storing nutrients in seedlings at the nursery stage provides a better rationale to promote seedling field performance. Higher pre-plant nutrient reserves have potential to reduce competitive effects and enhance internal redistribution to support new growth soon after outplanting. High survival and growth of competitive nutrient-loaded seedlings will accelerate forest restoration success on degraded landscapes, which will help to conserve soil resources as well as provide habi-

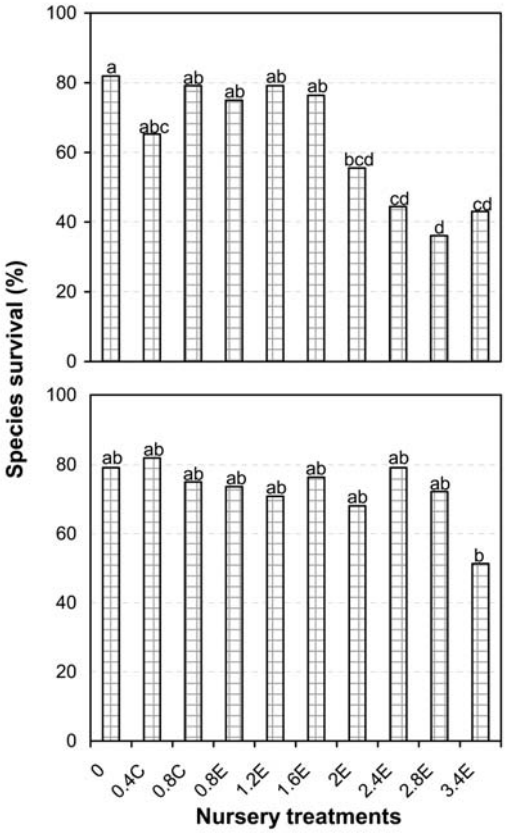

Figure 2. Field survival of red oak (top) and white oak (bottom) seedlings fertilized conventionally (C) or exponentially (E) during standard bareroot nursery culture for 8 months, and then outplanted for 4 months on a mine reclamation site in southwestern Indiana.

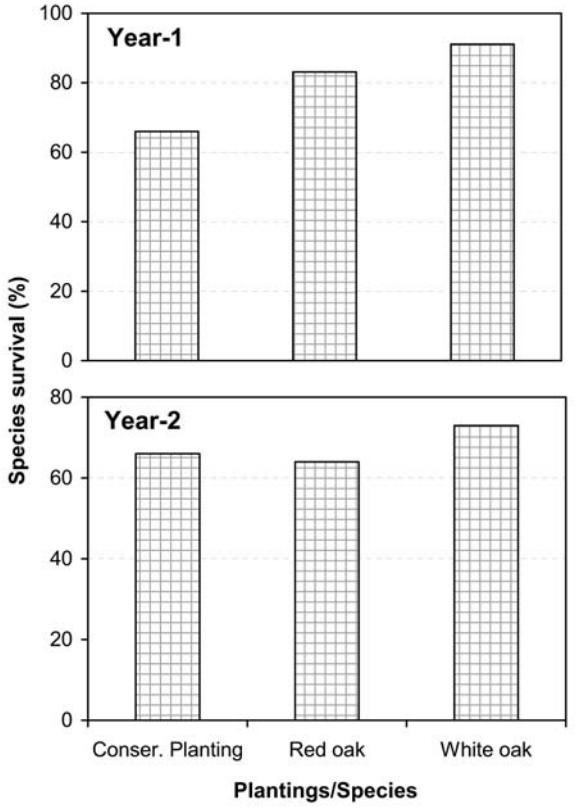

Figure 3. Survival of conservation tree plantations compared with overall field survival of red oak and white oak seedlings fertilized conventionally (C) or exponentially (E) during standard bareroot nursery culture for 8 months, and then outplanted for 4 months on a mine reclamation site in southwestern Indiana.

tat for wildlife. Early rapid growth will allow plants to reach free-to-grow status sooner and minimize potential for animal browse. Use of competitive nutrient-loaded seedlings will accelerate early growth and minimize the need to control competing vegetation with herbicides.

The use of balanced fertilizer is key to successful nutrient loading. Frequent and light applications will make nutrients readily available to plants. This increases uptake efficiency and minimizes potential leaching losses associated with heavy and less frequent applications.

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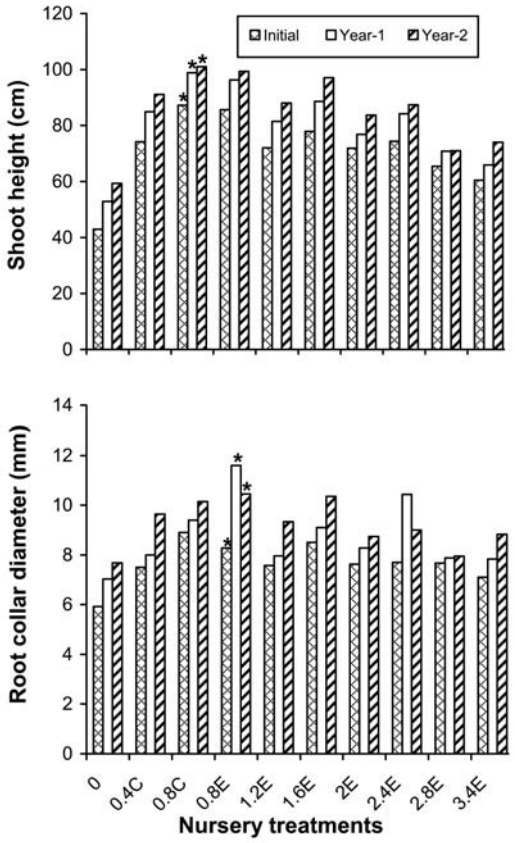

Figure 4. Shoot height and root collar diameter of red oak seedlings fertilized conventionally (C) or exponentially (E) during standard bareroot nursery culture for 8 months, and then outplanted for 4 months on a mine reclamation site in southwestern Indiana.

and R Winks during field or laboratory phase of this study.

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Effects of Fall Fertilization on Morphology and Cold Hardiness of Red Pine (*Pinus resinosa* Ait.) Seedlings

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ABSTRACT
Red pine (*Pinus resinosa* Ait.) seedlings were topdress-fertilized with granular ammonium nitrate (NH_4NO_3) at the rate of 0, 11, 22, 44, or 89 kg/ha (0, 10, 20, 40, or 80 lb N/ac) during fall of 2005 in Badoura State Forest Nursery, Akeley, Minnesota. Seedlings received either a single (September 16) or double (September 16 and 23) application of fall fertilizer. Seedling morphology and cold hardiness were evaluated in November of 2005

(1+0s) and 2006 (2+0s). Seedling morphological attributes were the same regardless of application method (single versus double). Seedling height and number of needle primordia increased significantly with increased fertilizer rate at the end of both growing seasons. In general, cold hardiness (measured by freeze-induced electrolyte leakage [FIEL] test) increased at the end of the 1+0 season in seedlings that received fall fertilization as either a single or double application. At the end of the 2+0 season, however, cold hardiness decreased (7% to 30%) with increased fertilizer rate in seedlings that received a single application of fall fertilization, but increased (15% to 50%) with fertilizer rate in seedlings that received double applications compared to controls. We are following these seedlings after outplanting to verify potential benefits of fall fertilization on seedling field performance.

KEYWORDS
electrolyte leakage, height, nitrogen fertilization, needle primordiaseed collection, seed testing, conifers, hardwoods

Introduction

Proper and regular fertilization is an integral component during nursery culture for the production of high quality seedlings for afforestation and reforestation programs. Fertilization during nursery culture can enhance plant growth, nutrient storage reserves, and resistance to stresses and diseases (Landis 1985; Rook 1991; van den Driessche 1991). In addition to conventional spring and summer fertilization, fall fertilization has been successfully used to improve overall seedling quality in loblolly pine (*Pinus taeda*) (Sung and others 1997; VanderScaaf and McNabb 2004), slash pine (*P. elliottii*) (Irwin and others 1998), and Douglas-fir (*Pseudotsuga menziesii*) (Margolis and Waring 1986; van den Driessche 1985, 1988). Fall fertilization allows roots to exploit nutrients for subsequent use while the terminal buds become dormant. Application of fall fertilization has significantly increased foliar nitrogen (N) levels (Simpson 1985; van den Driessche 1985, 1988). Additionally, fall fertilization resulted in significant increases in root growth potential (Simpson 1985; van den Driessche 1988), earlier bud break (Thompson 1983; van den Driessche 1985; Margolis and Waring 1986), and greater survival and growth rates than conventionally fertilized seedlings (Anderson and Gessel 1966; van den Driessche 1988).

Seedling cold hardiness in response to N fertilization in general, and fall fertilization in particular, showed contrasting results. For example, an excess of N decreased frost hardiness in young and adult Scots pines (*Pinus sylvestris*) and in adult Norway spruce (*Picea abies*) (Aronsson 1980; Soikkeli and Karenlampi 1984). In contrast, N addition enhanced cold hardiness in red spruce (*Picea rubens*) (DeHayes and others 1989). Additionally, N applied during fall increased cold hardiness in Douglas-fir whereas phosphorus (P) applied without N decreased hardiness (Thompson 1983). Furthermore, 2-year-old ponderosa pine (*Pinus ponderosa*) seedlings showed enhanced cold hardiness with increasing N concentrations (Gleason and others 1990). Cold hardiness was unaffected by 3 different rates of N and P application in Douglas-fir and western redcedar (*Thuja plicata*) seedlings (Hawkins and others 1995). Therefore, it appears that the cold hardiness responses to fall fertilization are species specific, and amount of fertilizer might have played an important role because too much application of N may delay onset of dormancy.

At Badoura State Forest Nursery, 2+0 red pine (*Pinus resinosa* Ait.) seedlings grown under conventional nursery culture often do not attain target height growth in their second year. This study was undertaken primarily to augment their second year growth. Our study objectives were to evaluate if fall fertilization applied at the end of the 1+0 growing season would: 1) increase the number of needle primordia, resulting in greater shoot elongation and biomass in the following season; and 2) affect cold hardiness as measured by the freeze-induced electrolyte leakage (FIEL) test.

Study Procedure

Plant Material and Fertilizer Treatments

Seeds of red pine were collected from north-central Minnesota during fall 2000, and subsequent seedlings were grown in Badoura State Forest Nursery (46°56'N, 94°43'W) in Akeley, Minnesota. Seeds were sown in October 2004, allowed to stratify naturally, and germinated in May 2005. The nursery bed had sandy loam soil, which was altered by incorporating peat to increase organic matter content and moisture retention. The soil pH was 5.5, with a buffer index of 6.5, and 3% organic matter content. Soil tests conducted in September 2005 showed a nitrate NO_3^-(N) ppm of 0.9, a Bray 1 P ppm of 30, and potassium (K) ppm of 74.

The nursery beds were watered 2 to 3 times every week (May through August) in 2005 and 1 to 2 times every week (May through September) in 2006. Fertilizer application in 2005 (the 1+0 year) was as following: 6 topdress applications of granular ammonium nitrate (NH_4NO_3; 34N:0P_2O_5:0K_2O) at 33 kg N/ha (30 lb N/ac) on June 27, July 5, 11, 18, 25, and August 1; 3 foliar applications of liquid fertilizer (20N:20P_2O_5:20K_2O) at 5.5 kg N/ha (5 lb N/ac) on June 20, 27, and July 5; and 3 foliar applications of liquid fertilizer (20N:20P_2O_5:20K_2O) at 9 kg N/ha (8 lb N/ac) on July 20, 28, and August 12. For the experiment, we topdressed 2 beds with granular NH_4NO_3 (34N:0P_2O_5:0K_2O) at 5 levels of N: 0 (control), 11, 22, 44, and 89 kg/ha (0, 10, 20, 40, and 80 lb/ac). These applications were applied systematically down each bed; each rate was replicated 4 times within each bed. One bed received all of the fertilizer on September 16 (single application). The other bed received one application of fall fertilizer (same dose as applied to the first bed) on September 16 and a second dose (double application) on September 23. The fertilizer application schedule for summer 2006 (the 2+0 year) was as following: 1 topdress application of granular NH_4NO_3 (34N:0P_2O_5:0K_2O) at 67 kg N/ha (60 lb N/ac) on June 5; and 5 topdress applications of granular fertilizer (10N:10P_2O_5:10K_2O) at 30.8 kg N/ha (28 lb N/ac) on June 26, July 10, 17, 24, and 31. Weeds were controlled with Fusilade® and hand-weeding.

Seedlings were randomly hand-dug from the nursery beds during the first week of November 2005 and 2006 and shipped to Purdue University. Seedling morphological attributes (height [root collar to tip of terminal bud]; root collar diameter; shoot, root, and total plant dry weight after oven drying for 72 hours at 68 °C [154 °F]; needle primordia [described below]); and a physiological attribute (cold hardiness [described below]) were measured.

Needle Primordia

Number of needle primordia was determined following Tampleton and others (1993). The shoot (at least 10 cm [4 in]) was excised from the seedling and needles were carefully removed. The excised shoot was observed at a magnification of about 10X using a dissecting microscope. Bud scales were carefully removed to expose the needle primordia by making an incision using a hypodermic needle at a depth of 0.5 mm through the bark at the base of the lower-most bud scales. The tip of the hypodermic needle was used to cut around the circumference of the shoot, which loosened the cap of bud scales. Once the bud scales were removed, the embryonic shoot was viewed under the bright-field microscope, and the needle primordia were counted using the average number in a row multiplied by the number of columns.

Cold Hardiness

We used the freeze-induced electrolyte leakage (FIEL) test (Burr and others 1990) to determine seedling cold hardiness. Needles (randomly from the crown) were carefully detached from seedlings and rinsed with distilled water to remove ions from the surface. For each fertilizer treatment and replication, a sufficient number of needles from 5 seedlings were obtained and cut into 1-cm (0.4-in) segments. From each group, 7 segments were placed into 7 separate vials (each vial for corresponding test temperatures; that is, 2

[control], -5, -10, -20, -25, -30, and -40 °C [36, 23, 14, -4, -13, -22, and -40 °F]). Each vial contained 1 ml of deionized water. To avoid excessive super-cooling during freezing, only 1 ml of deionized water was added to the vial prior to the freezing tests.

The vials were placed in a programmable freezer for approximately 1.5 hours at 2 °C (36 °F), after which the control treatment vials were removed. The temperature was then decreased at a rate of approximately -5 °C/hour (-9 °F/hour). Upon reaching each successive test temperature, the temperature was held for 30 minutes before decreasing again. Once all the vials were removed and thawed, which was usually done on the next day, 9 ml of deionized water were added to each vial to aid in measurement of electrical conductivity. After the initial measurements were taken, the vials and their contents were autoclaved at 121 °C (250 °F) for 20 minutes. After cooling overnight at room temperature, the second measurements were taken, which represent total electrolytes. Electrolyte leakage is expressed as percentage of the total electrolytes.

Data Analysis

Seedling height, needle primordia, and cold hardiness data underwent analysis of variance using SAS (SAS Institute Inc, Cary, North Carolina), and an alpha level of 0.05.

Results and Discussion

We found no significant effects between single or double application of fall fertilization on any seedling morphological attributes, so we will discuss those results based on the total amount of N applied. Fall fertilization had a significant effect on seedling height after the 1+0 and 2+0 growing seasons (Table 1). Seedlings that were fall-fertilized with 44 and 89 kg/ha (40 and 80 lb N/ac) exhibited 8% and 21% more height growth compared to the control (0 kg N/ha) at the end of the 1+0 season, and 23% and 20% more height growth after the 2+0 year. Irwin and others (1998) found no significant differences in heights of slash pine seedlings fall-fertilized with zero, low (57 kg N/ha [50 lb N/ac), or high (171 kg N/ha delivered in 3 applications of 57 kg/ha [3X at 50 lb N/ac]) rates. Red pine seedlings reached target height due to the extra height growth attained by providing the highest fertilizer rate (89 kg/ha [80 lb/ac]). All seedlings were subsequently sold (VanSickle 2006). The relative increment in height with increased fertilizer application suggests that even in the month of October, when ground temperature was declining and low, red pine seedlings growing in nursery beds were physiologically active (VanderSchaaf and McNabb 2004). All other morphological attributes were similar across fertilizer treatments (data not shown).

Fall fertilization also had a significant effect on numbers of needle primordia after the 1+0 and 2+0 growing seasons (Table 1). In each year, more needle primordia were formed as fertilizer rate increased, resulting in longer buds (Figure 1). This is possibly due to the continued assimilation and partition of carbon (VanderSchaaf and McNabb 2004). Seedlings that were fall-fertilized with 44 and 89 kg N/ha (40 and 80 lb N/ac) had 41% and 76% more primordia compared to the control (0 kg N/ha) at the end of the 1+0 season, and 29% and 73% more primordia after the 2+0 year.

Fall fertilization rates significantly affected cold hardiness. At the end of the 1+0 season, cold hardiness (determined by FIEL; lower EL values indicate enhanced cold hardiness; all values presented represent those collected at -40 °C [-40 °F]) was similar between seedlings that received either a single or double application of fall fertilizer. However, fertilized seedlings were more cold hardy than control seedlings (Table 1). At the end of the 2+0 season, however, cold hardiness decreased with increased fertilizer rate in seedlings that received the single application, but increased with increased fertilizer rate in seedlings that received the double application.

Although Timmis (1974) found that Douglas-fir seedlings that received more fertilizer (N concentration was 1.6%) were much more cold hardy (-30 °C [-22 °F] versus -13 °C [9 °F] than those

Table 1. Heights, numbers of needle primordia, and electrolyte leakage percentages of fall-fertilized red pine seedlings.

		Fertilizer rate in kg N/ha (lb N/ac)				
		0	**11 (10)**	**22 (20)**	**44 (40)**	**89 (80)**
Height[z]	1+0	6.2 (2.4)[y]	6.0 (2.4)	5.9 (2.4)	6.7 (2.6)	7.5 (3.0)
	2+0	13.4 (5.3)	14.2 (5.6)	14.9 (5.9)	16.4 (6.4)	16.0 (6.3)
Primordia[x]	1+0	17	19	18	24	30
	2+0	96	92	114	124	166
FIEL—single dose	1+0	31[w]	27	24	21	21
FIEL—double dose	1+0	28	20	19	22	19
FIEL—single dose	2+0	27	29	35	39	24
FIEL—double dose	2+0	39	26	35	30	26

[z] Average heights for single and double dose seedlings combined.

[y] cm (in)

[x] Average number for single and double dose seedlings combined.

[w] The percentage leakage. Lower values indicate higher levels of cold hardiness.

receiving less fertilizer (N concentration was 0.8%), Birchler and others (2001) found that fall fertilization did not affect cold hardiness in Douglas-fir seedlings. While single or double application of fall fertilizer did not significantly affect morphological parameters, it appeared that double application significantly increased cold hardiness in 2+0 seedlings. No visible winter damage, however, was observed on control 2+0 seedlings. Therefore, from a grower's perspective, a single application of fall fertilizer would probably suffice to ensure the extra, desired height growth.

Our fall fertilizer rates were relatively low considering the rates Birchler and others (2001) and Irwin and others (1998) have used. Our low rates, coupled with rapidly declining day length and the short duration of time before the ground froze in northern Minnesota, may be why we observed only a modest height increase in red pine seedlings in response to fall fertilization. We feel that 2 possible options may further augment height growth in red pine seedlings. First, the current summer fertilizer application pattern can be modified to an exponential application regime, as demonstrated by Birge and others (2006) with bareroot red and white oak seedlings. Exponential application of fertilizer would increase uptake efficiency of the seedlings (Dumroese and others 2005) and mini-

mize leaching losses because sandy soil has low retention of nutrients. Second, application of significantly more fall fertilizer than used in our current study may provide additional benefit.

Conclusions

We were able to increase the heights of 2+0 red pine approximately 20% by applying 44 or 89 kg N/ha (40 or 80 lb N/ac) during the fall of the 1+0 season. Although fall fertilization did not affect some seedling morphological attributes, increasing amounts of fertilizer yielded longer buds with more needle primordia. More needle primordia after the 1+0 season may be the cause for increased height growth during the second season. The effect of fall fertilization continued through the second year of growth, with the 44 or 89 kg N/ha (40 and 80 lb N/ac) treatments yielding longer buds and more needle primordia at the end of the 2+0 season as well. The extra growth provided by fall fertilization helped 2+0 red pine at Badoura State Forest Nursery better meet target specifications without reducing cold hardiness. We have outplanted these seedlings and will evaluate them to see if the effect carries over to field performance.

Figure 1. Red pine bud size increases with fall fertilizer rate, indicating potential for shoot growth the following season. Buds represent (from left to right) 0 and 89 kg N/ha (0 and 80 lb N/ac) fertilizer treatments, respectively. Buds shown are from 2+0 seedlings.

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Climate Change, Forests, and the Forest Nursery Industry

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KEYWORDS
ancient carbon, living carbon, mountain pine beetle, Dothistroma needle blight

Introduction

The devastating consequences of Hurricane Katrina demonstrate how ill-prepared people are when it comes to extreme weather events and potential changes in climate. The hurricane itself cannot be directly ascribed to climate change, but the likelihood of stronger hurricanes can be. The more energy the atmosphere has as it warms because of increasing concentrations of greenhouse gasses, the more energy it needs to shuffle around. Hurricanes are one way of doing just that. The potential risks from just such an event had been described in the region's major daily paper, yet the response to the hurricane seems to indicate that little action had been taken to get ready. The lessons of the event must be taken seriously by all sectors of society because climate change is a certainty, is now well underway, and will impact us all (Fischlin and others 2007).

The fourth series of reports issued in 2007 by the Intergovernmental Panel on Climate Change (IPCC IV), in what is a conservative account of climate change and its impacts, warns us clearly that major effects on forests must be expected (Fischlin and others 2007; Nabuurs and others 2007). In northwestern North America, the climate has already changed and is continuing to change, and those changes are having serious impacts on regional forests. Furthermore, in one of the IPCC IV reports, Fischlin and others

(2007) identify northwest North American forests as especially likely to be impacted by climate change. The devastating mountain pine beetle (*Dendroctonus ponderosae*) outbreak in the interior of British Columbia and adjacent regions has single-handedly altered the character of forests over a huge area in less than decade (Carroll and others 2006). Increases in Dothistroma needle blight on lodgepole pines (*Pinus contorta*) are also attributed to changes in climate (Woods and others 2005). In the coastal temperate rainforests of British Columbia, western redcedars (*Thuja plicata*) are showing excessive autumn branchlet drop and top die-back, likely as a result of increased summer moisture deficits (Hebda 2006).

Lessons on Climate Change from the Past

Studies of sub-fossil pollen and other plant remains from lake and wetland sediments provide insight into what the region might be like under warmer than present climates. Between 10,000 and 7000 years ago, the earth received slightly more solar energy than it does today because of normal variations in the earth's orbit and angle of spin. In southern British Columbia, the climate was warmer than today by about 2 to 4 °C (3.5 to 7 °F), and summers were drier (Hebda 1995). On the coast, dry conifer forests dominated by Douglas-fir (*Pseudotsuga menziesii*) extended well into the zone occupied by moist western hemlock (*Tsuga heterophylla*) and western redcedar forests. In the dry rain shadow of Vancouver Island, open meadows and, later, Garry oak (*Quercus garryana*) savannah or possibly forest predominated where Douglas-fir forests occur now (Pellatt and others 2001). East of the Coast and Cascade mountains, grassland occupied a much greater area than today in the place of mid-slope and valley-bottom conifer forests of Douglas-fir and pine (Hebda 2007). In the southern part of the province, today's montane and high elevation spruce-fir forests were home to pine forests where fires were more active than today (Hebda 1995). Inland rainforests of cedar and hemlock were absent (Hebda 1995; Rosenberg and others 2003) and, to the north, pine forests occupied regions where spruce-dominated Cordilleran boreal forest occur today (Hebda 1995).

Today's geographic pattern of forest ecosystems and forest composition is only 4000 or so years old. It arose as a result of the development of relatively moist mild climate at this time. Major features included: widespread expansion of the range of western redcedar and moist conifer forests; shrinking of interior grasslands; spread of dry interior conifer forests down-slope; and development and expansion of high elevation spruce forests, especially in the south (Hebda 1995; Heinrichs and others 2002). With cooling and moistening climate, fire activity declined and wetlands, especially bogs, spread. The tree ring sequence from fossil Douglas-fir logs recovered from Heal Lake sediments, near Victoria, suggest that the climatic change at this time may have occurred rather rapidly, ushering in 4000 years of relative climatic stability (Zhang and Hebda 2005).

Considering what the fossil record reveals and the trends already evident, we can expect climate change now underway to be of large amplitude, to take place rapidly, and to include extreme events. Unlike many past natural major climatic changes, the effects will be felt world-wide, and unfold upon a landscape much disturbed by human activity. The net effect will be widespread ecological change, which obviously poses a challenge to forest managers and those working to sustain forest values.

Climate Impacts from Models

Clearly, a business-as-usual approach to managing and sustaining forests is risky when climatic conditions within the next few decades are uncertain. Growing and planting nursery stock depends on matching sites, seedlings, and treatment strategies. Global, and now regional, climate model output can be used to gain insight into what future forest conditions may be (Hamann and Wang 2006), and what changes in

reforestation, including seedling stock choices, might be appropriate.

For British Columbia, on average, widespread warming of about 5 °C (9 °F) in the mean daily minimum and maximum temperatures must be expected by about 2080; in the extreme, as much as 10 °C (18 °F) warming is not outside the range of possibility (PCIC 2007b). Climate models do not represent future precipitation as reliably as temperature, but a trend to a generally wetter climate is likely. However, summers in parts of the region will effectively be drier because of the increased temperatures.

Using a Canadian climate model, Hamman and Wang (2006) showed that future climatic conditions will lead to dramatic changes for the potential distribution of forest ecosystems in the region, with obvious consequences to tree growth and forest management. For example, the climate of the Ponderosa Pine Biogeoclimatic zone in British Columbia (see Meidinger and Pojar 1991 for description of modern forest zones) that is representative of the dry climates of the Okanagan valley of southern interior British Columbia might occur in the Peace River region of northeast British Columbia and reach into the Northwest Territories by 2080. A climate change impact model for western redcedar, on exhibit at the Royal British Columbia Museum (Victoria, British Columbia), shows suitable climate for this species disappearing in lowlands of southern British Columbia, but spreading into northern British Columbia by 2080 (PCIC 2007a). Western redcedar is a good indicator for the productive rainforests of northwest North America. The likelihood of changes in cedar distribution signal major changes in the character and distribution of this globally important biome in the near future. As mentioned earlier, declining cedar health is evident now, so we must take these projected changes seriously indeed. Replanting schemes involving this species need careful reconsideration.

Whereas we must be cautious about the way we use and replant moisture-needing tree species in our forests, the situation for warm climate, drought-tolerant species is less acute. For example, the climatically suitable zone for warmth-loving Garry oak will expand greatly. Areas of suitable climate could even appear on islands of the Alaska panhandle and on the adjacent mainland coast by mid century. The enormous increase in area of climate suitable for oak by 2080 east of coastal mountain ranges (PCIC 2007c) poses critical questions about what the nature of future forests in that zone might be. The results of climate impact models are consistent with some observed trends and with past forest ecosystem responses to warmer-than-present climates, a clear indication that models realistically portray the direction and nature of change.

Impacts models for ecosystems, however, have limitations, because they only address the question of where suitable climate may occur. At the predicted rate of change, loss of some tree species, such as cedar, will considerably exceed range expansion into newly suitable regions. Long-lived species, such as dominant trees, often have limited dispersal rates, long intergeneration times, and require centuries to occupy suitable climatic zones. This "big squeeze" of the geographic range means that natural ecological equilibrium will not be achieved in our forest ecosystems for centuries. Furthermore, stable forest ecosystems require the development of appropriate soils, with characteristic organic matter—also a lengthy process.

Whatever way we look at the future character of forest ecosystems in our region and around the world, major changes must be expected. For that reason alone, forestry management and practices will have to change. Along with this, we need to consider that the role of forests, based on the values we want from them (and even for them), will shift, too. The objectives of timber and fiber production may be replaced by carbon accumulation and water yield. In general, the cut and re-grow cycle typical of forest stewardship until recently will likely have to move to one of continuous growth and maintenance of resilience (the ability

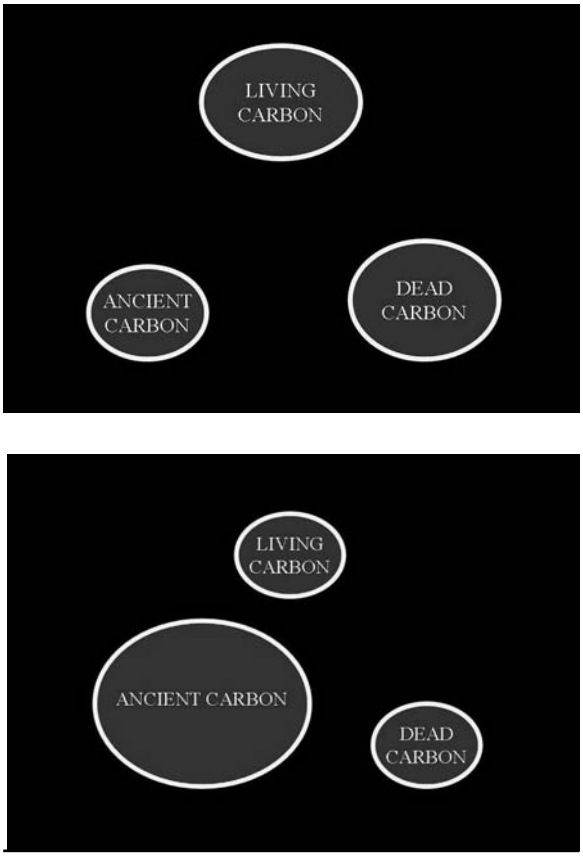

Figure 1. A key role for the forest nursery industry is re-balancing the emphasis in the battle with climate change from one focused on dependence on Ancient Carbon (reducing emissions and use of fossil fuels) (A) to a focus on supporting Living Carbon (ecosystems and species) and the Dead Carbon (organic matter) that supports it (B).

to withstand stress without catastrophic transformation). Indeed, the economic pay back of maintaining healthy carbon-scrubbing forests and avoiding release of carbon into the atmosphere through disturbance may be much greater than widespread removal of forest products.

Carbon stewardship is a new integrated way of looking at the issue of human response to climate change. It combines actions aimed at the reduction of carbon dioxide emissions with those aimed at adapting to the inevitable changes ahead. In so doing, the approach shifts the focus from simply finding alternate sources of energy and reducing energy consumption (dependence on Ancient Carbon) to sustaining and restoring Living Carbon (Figures 1a and b). We cannot exist without the Living Carbon of terrestrial ecosystems, agricultural fields and aquatic environments. Living Carbon feeds us, provides jobs and many other values, and depends on organic matter in the soil or sediments, which I call Dead Carbon. The Living Carbon also plays a central role in removing carbon dioxide through photosynthesis and primary production. Good Carbon Stewardship considers the impacts of activities on all aspects of carbon, not just the reduction of emissions. For example, if production of biofuels, such as converting forest wastes to stove pellets, jeopardizes Living Carbon systems and the Dead Carbon in the soil that supports them, then it may not be as effective a strategy as we may think to deal with climate change.

The forest nursery industry has a particularly important role in supporting Living Carbon by providing the appropriate raw materials for terrestrial ecosystems to return to a healthy condition, either by replanting on non-forested sites or in-planting on sites whose resilience has been jeopardized. This new role was explicitly recognized in the theme and title of this 2007 nursery meeting in Sidney, British Columbia, "Growing and Planting More Trees: A Common Goal and Responsibility." Restoring, rebuilding, or regenerating forest ecosystems around the globe is the most effective mechanism we have at this time for beginning to take carbon dioxide out of the atmosphere today. At this point, it is important to note that the species that will be used for this purpose in the future may not be the same, or be limited to, those that are traditionally supplied to the forest industry for timber or fiber production.

Strategies for the Future

Climate change poses many challenges and provides opportunities for the forest nursery industry. Wide-scale ecological and, likely, economic change is certain to occur; however, the degree of change and the path that change will take is not well understood yet. For sure, there will be alterations in the structure and composition of

forests, and, in some places, forest ecosystems will simply not be supported by future climatic conditions. In northern and high elevation portions of our region, forests are likely to expand their range (Hamann and Wang 2006) and trees will grow more rapidly and to a larger size than today. Considering the enormous and likely impacts of the climate change challenge ahead, we must begin taking action now, both at the strategic and practical levels.

General Strategies

At the strategic level, the focus needs to shift from growing trees to growing and sustaining forests. These two are certainly mutually compatible goals, but they are not the same. Society will almost certainly expect many roles from our forest ecosystems, especially those related to mitigating carbon dioxide levels in the atmosphere and providing a reliable supply of water.

We also need to prepare for extremes and surprises. The mountain pine beetle outbreak in central British Columbia has come as an unpleasant surprise (Carroll and others 2006). The blowdown of many large trees during an intense windstorm in Vancouver's Stanley Park was another surprise. Exceptional weather events are almost certain as climate moves toward a new set of norms.

Bold forest and landscape management experiments will prove valuable as an adaptation strategy for the future. Following a status quo approach brings with it high risks of failure, especially because decisions made now commit us to outcomes many decades down the line. We need to establish practices that broaden our options and spread the responsibility for risk of failure in the future. Diversified replanting schemes involving several species is one mechanism to reducing overall risk.

The uncertainty in terms of the character and location of future forest ecosystems and the expectation of multiple values requires landscape-level planning of forest ecosystems and, especially, forest management and use. Establishing the sensitivity of geographic regions, species, and genetic stocks to climate change will be part of the landscape approach. There is little point in planting standard species or stocks in regions highly sensitive to climate change. In British Columbia, most biogeoclimatic zones (macro-ecosystems) are expected to change by at least one type (to a warmer one) (Hebda 1994).

Our knowledge of the ecology of species and ecosystems remains poor. We have an excellent descriptive knowledge of the distribution and character of our forests but we have only a rudimentary understanding of the processes that shape them (mycorrizhae for example). An understanding of the climatic controls on species distributions and key processes (bio-climate profiles) is especially needed for planning future forests. With a sound knowledge of species-climate relations, and especially of the responses of pests to climatic change, decision tables could be constructed to assist in forest management at specific sites.

Operational Strategies

With respect to timber and fiber production, it may be necessary to develop intensively managed ecological plantations for high value yield. In such plantations, comprehensive monitoring of growth, pests, and diseases might allow risks to be minimized or avoided through practices such as pest control and moisture management. Under such conditions, there might also be interest in species and stocks with short rotation times to reduce exposure of the stand to unacceptable climate variability.

Other on-ground adaptive approaches might include:

:: Minimizing soil disturbance when replanting to limit sites for invasive species, and to limit exposure of organic matter to decomposition;
:: Use of plantings of mixed species and genetic composition, perhaps at high initial densities to optimize survival rates;

:: Regular monitoring for impacts and immediate repeat planting where poor growth or seedling and sapling death is noted;
:: Saving and propagating genetic stocks from hot and dry extremes of a species range, because growing trees for survival may become as important as growing perfectly formed trees quickly.

One caution, at least for the time being, is to avoid the inclination to propagate and use species foreign to the region just because they grow well. Invasion of native ecosystems by alien species will almost certainly be a major battle for decades to come. The use of species that might naturally migrate into a region is acceptable. But introducing exotic species from other continents or across major natural barriers is not recommended at this time. These species have the capacity to weaken the natural resilience of the forest landscape at a time when it needs to be as strong as possible.

As far as the forest nursery industry is concerned, it will be hard to anticipate when the demand for a diversity of seedlings will arise. Nurseries should be prepared, however, for the potential demand by broadening their capacity, by increasing expertise, and beginning experimentation with different species. Opportunities to do this may arise through experimental trials in collaboration with a range of land tenure holders, such as watershed agencies, land trusts, First Nations, and various levels of government. Such experiments might well be supported through grants, providing an opportunity for pre-adaptive development by the industry.

In general, the rate of climate change is going to increase; the longer we wait to return ecological integrity to our forest ecosystems, the more difficult it will be to do so. Widespread replanting and in-planting to establish healthy stands now is vital to prepare our forested landscape before major changes really set in. It would seem that the forest nursery industry is likely to have bright future ahead. And the knowledge and skills gained here in the Northwest may have broad application around the globe.

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Building Trust—Business Essentials

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ABSTRACT
Trust is particularly vital in the leadership of organizations. Trust is built by working through joy, fear, and vulnerability, especially as it relates to trust in others and in teams. Key is learning to trust the right people in the right way in the right circumstances. In addition to proven competence and confidence in a fellow employee s or teammate s ability to get a job done, creating a trust space requires 4 people practices: being truly open; really listening; developing common passion and/or purpose; and collaboratively sharing responsibility. Continually working at those 4 practices makes it more likely that people will be honest and forthright with one another, be able to rely on one another to get a job done well, keep confidences, fulfill expected roles, and so on. The ultimate trust test is when someone takes a leap of faith with another person or with an entire team of people. Whether that leap of faith involves thoughts on how the group could improve, radical or creative ideas that would normally be withheld, feelings about progress or lack of same in a group or job, or something else, the embrace of the leap by the other person(s) is essential to achieving high-performance, bonding, and organizational success.

Enhancing Forest Nursery Education: Input from the 2007 Joint Meeting of the Western Forest and Conservation Nursery Association and Forest Nursery Association of British Columbia

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KEYWORDS
seedling quality, native plant regeneration, silviculture

Introduction

Concern has been noted over the lack of qualified applicants for vacancies in forest nursery positions. The University of Idaho Center for Forest Nursery and Seedling Research is uniquely qualified to address the issue of training given its faculty, staff, and resources. The keystone resource in this regard is the Franklin H Pitkin Forest Nursery, a seedling production facility that operationally grows up to 500,000 plants per year for forestry, conservation, and restoration use in the Inland Northwest. This facility has an on-site research laboratory for conducting seedling quality assessment, and is located near the main campus of the University of Idaho.

Faculty expertise in silviculture, ecology, nutrition, pathology, and entomology will allow for

inclusion of a broad set of courses with appropriate personnel directing them. The degree to which these courses should be included, however, is unknown. Thus, it was decided that, for a program of this nature to be successful, a survey was needed to draw input from current nursery professionals into the fold. By addressing the breadth of nursery professionals, in terms of organizational structure, ownership, geographic distribution, and species produced, it is possible to identify the common threads across the profession to provide adequate educational opportunities needed to prepare students for this field. Development of a program that involves stakeholder input to meet the needs of the forest and conservation nursery profession will help to avoid the potential dichotomy that currently exists between training needs and what is provided, as described by Sample and others (1999) pertaining to forestry education in the US.

Methods

Perusal of current course offerings and identification of potential shortcomings resulted in a list of subject areas that pertain to nursery production. This information was used to collate a survey. Following a short presentation highlighting the current program at the University of Idaho Center for Forest Nursery and Seedling Research, attendees of the Joint Meeting of the Western Forest and Conservation Nursery Association and Forest Nursery Associations of British Columbia in Sidney, British Columbia, Canada (17–19 September 2007) were asked to complete the survey. A list of courses currently required for the Bachelor of Science in Forest Resources was provided to the attendees. Results are presented as interim findings only and will be collated with data collected from other meetings as well.

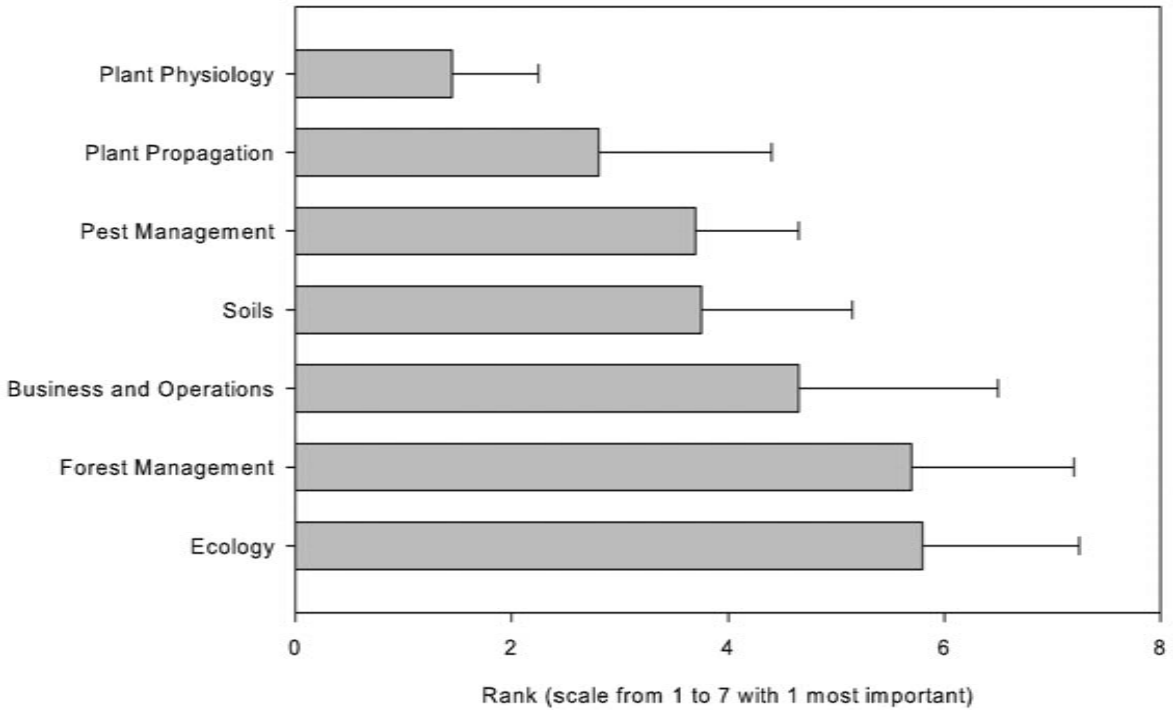

Figure 1. Responses to being asked to rank broad subjects in order of importance (1 is most important; bar represents standard deviation). N = 78.

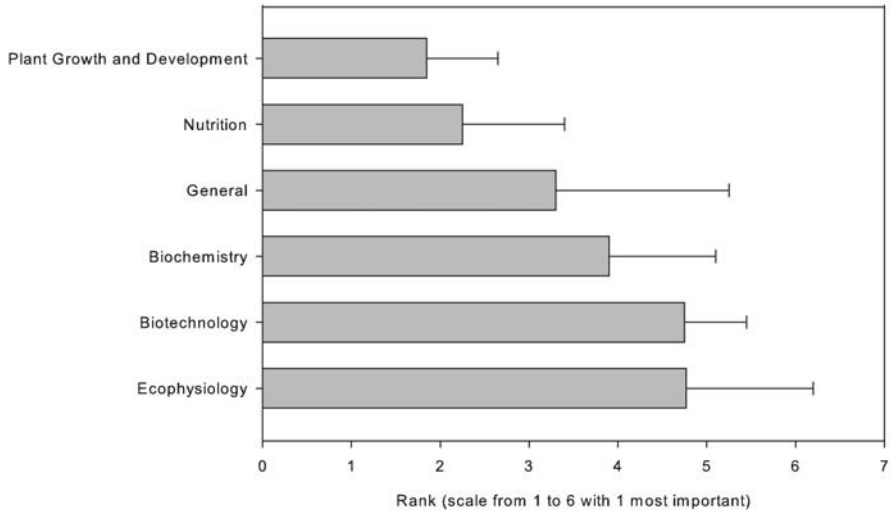

Figure 2. Ranking of potential courses in the field of plant physiology in order of importance (1 is most important; bar represents standard deviation).

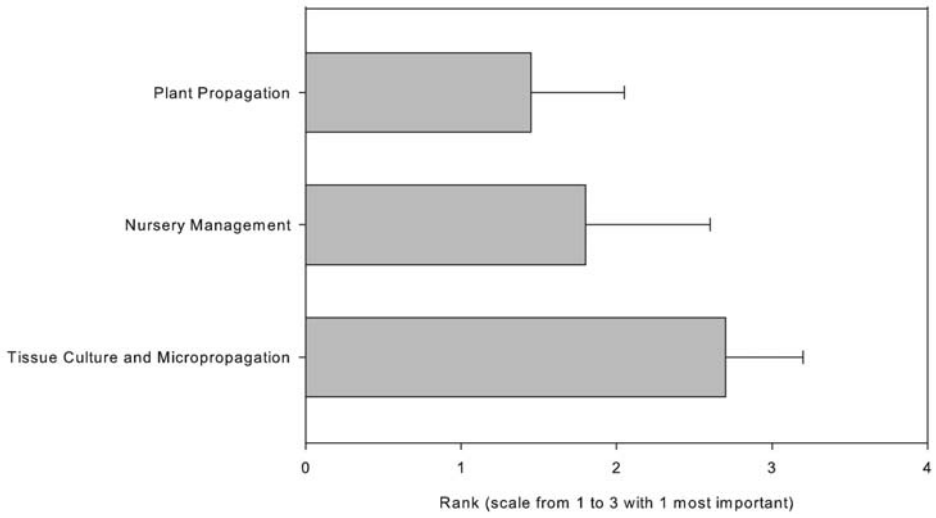

Figure 3. Ranking of potential courses in the field of plant propagation in order of importance (1 is most important; bar represents standard deviation).

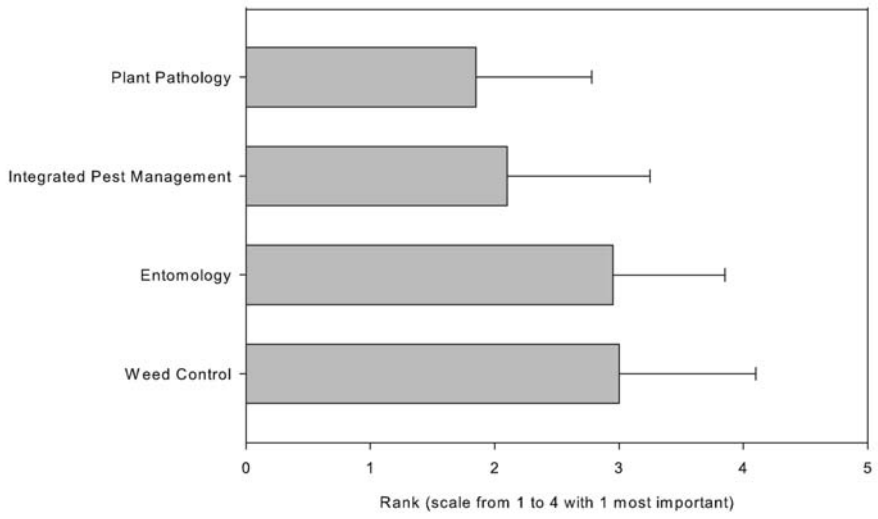

Figure 4. Ranking of potential courses in the field of pest management in order of importance (1 is most important; bar represents standard deviation).

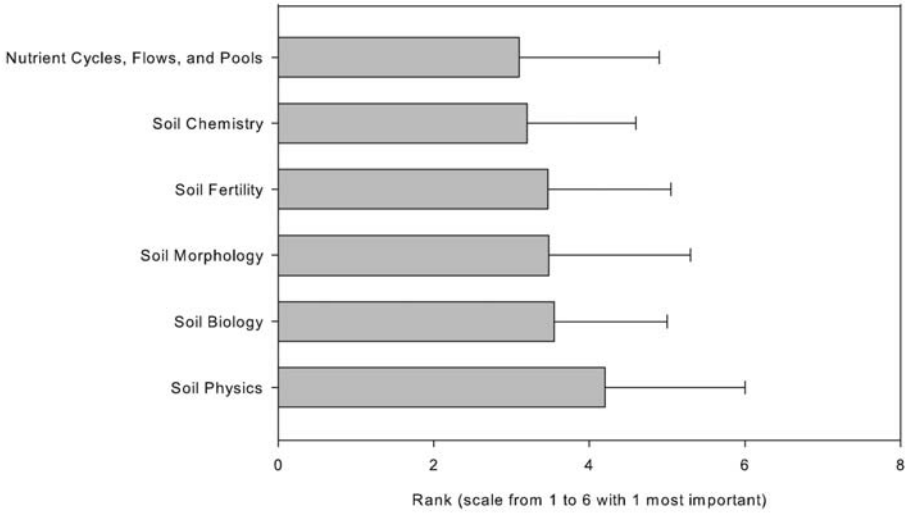

Figure 5. Ranking of potential courses in the field of soils in order of importance (1 is most important; bar represents standard deviation).

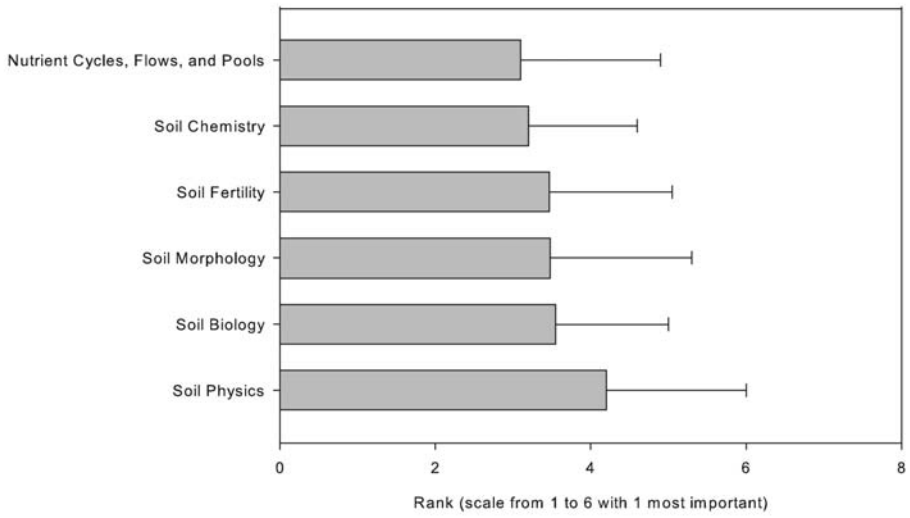

Figure 6. Ranking of potential courses in the field of business and operations in order of importance (1 is most important; bar represents standard deviation).

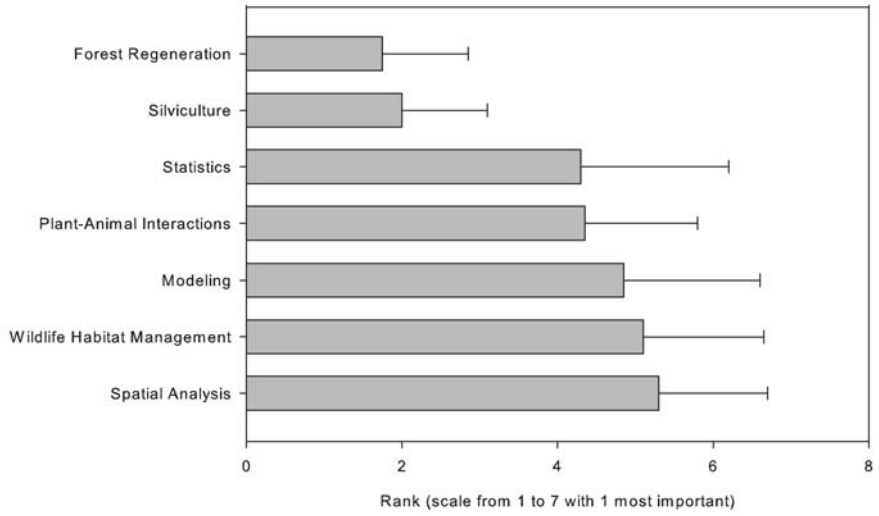

Figure 7. Ranking of potential courses in the field of forest management in order of importance (1 is most important; bar represents standard deviation).

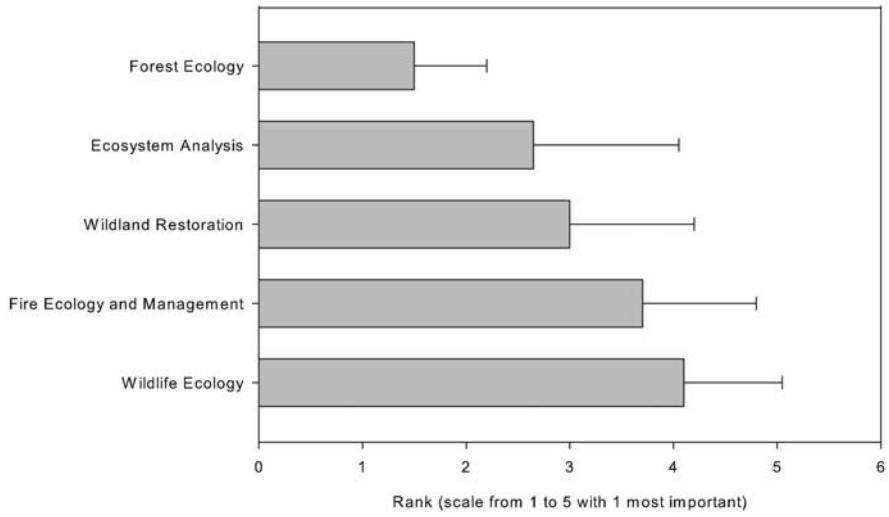

Figure 8. Ranking of potential courses in the field of forest ecology in order of importance (1 is most important; bar represents standard deviation).

Results

Demographics

A total of 77 surveys were returned. Of those, 34% of respondents are growers, 49% are managers, 14% are researchers, and 1% are students. The employment base was 65% in the private sector, 29% in government, and 4% in academia. For 8% of respondents, the highest level of education completed is high school; 31% have some college education; 35% have a bachelor's degree; and 23% have a graduate degree. Finally, respondents have worked an average of 19 ± 9 years in the nursery trade.

Response to the Need for Nursery Education

A total of 86% of respondents reported that educational opportunities were currently inadequate for students interested in forest nursery production. The other 14% did not indicate a yes or no answer, but did complete the remainder of the survey. As well, 86% of respondents reported that a nursery minor would be an effective way to address this problem. Respondents reported the most important subject area component (Figure 1) of such a nursery minor program was plant physiology, followed by plant propagation, pest management, soils, business and operations, forest management, and ecology. Potential specific subjects were ranked for each of those options (Figures 2 through 8).

A total of 94% of respondents reported that a nursery minor should include practical work experience; 3% felt that it should not. A total of 68% felt it should occur during the summer months, 52% during the fall semester, 56% during the spring semester. When broken down further, 42% felt it should be administered by a university; 31% reported that such a practicum should occur at a government nursery; and 71% reported that it should occur at a private nursery.

A total of 49% of respondents felt that nursery minor students should be required to complete a senior research project associated with nursery production.

Discussion and Future Directions

From the demographic data, we obtained a relatively good idea of the composition of those who

attended the meeting. As one might expect, most participants are professionals. Of particular relevance to this paper, however, is the low student attendance, which could be symbolic of the greater problem of within-profession recruitment and replacement. Without student attendees, are we missing an opportunity to engage new people in our profession? Without a professional organization dedicated to recruitment of future nursery production employees, it is incumbent on professionals to work together in this regard.

In terms of course work, the high emphasis on plant physiology identifies this subject area as one of key importance. Within plant physiology, 2 courses, plant growth and development and nutrition, received consistently high grades. The general subject area of plant propagation ranked second and, not unexpectedly, highlights the importance of this subject matter in the forest nursery production field. Forest management and ecology scored relatively low, likely indicating that these subject areas are adequately covered within the existing Forest Resources degree.

It is clear that practical work experience is viewed as valuable, with 94% of respondents identifying that this should be part of a minor program. This is an area that, again, can be achieved through partnership. Ensuring the availability of professional development opportunities for students will help them from both an educational perspective and a career development perspective. Sample and others (1999) identified that paid summer internships were a strong opportunity to recruit into forestry. We can refine this mentality to the nursery perspective, and work together to develop learning objectives for any student intern.

In conclusion, current needs in nursery education may be addressed through a program such as a minor to accompany an undergraduate degree. Stakeholder input has highlighted subject areas such as plant physiology and plant propagation as being of high importance in this education. As more data points are added to the pool from additional surveys, results will be analyzed in detail and used to inform decision-making as we work towards new educational programs.

Acknowledgments

Thank you to RK Dumroese, who provided valuable insight into the formation of the survey; AL Ross-Davis, who contributed extensively to survey design and data analysis; and the many individuals who completed the survey.

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What's in your Douglas-fir Bark?

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ABSTRACT
Douglas-fir bark is a common waste product of forest industry, and has potential use as a substrate in container nurseries. Douglas-fir bark (DFB) is strongly acidic and contains amounts of phosphorus, potassium, iron, copper and manganese within or above the levels recommended for growing container crops. As the pH of DFB decreases, electrical conductivity and amounts of extractable phosphorus, calcium, magnesium, boron, and iron increase. Although liming unfertilized DFB with calcium carbonate up to 3 kg/m^3 (5 lb/yd^3) is effective at raising pH, the resulting pH is higher than desired for container plants after 6 incubation weeks. Native phosphorus in aged DFB leaches quickly under typical nursery conditions, but it may still be a reliable phosphorus source for plant growth for at least a month, providing pH is kept low.

Bark screen size seems to have a stronger effect on the uniformity of DFB chemical properties throughout the year than does bark age. Non-amended fresh and aged DFB provided sufficient micronutrients for the growth of annual vinca for up to 2 months when pH was kept low (< 5.7).

KEYWORDS
Pseudotsuga menziesii, fresh bark, aged bark, container growing media, macronutrients, micronutrients, phosphorus

Introduction

Douglas-fir (*Pseudotsuga menziesii*) bark (DFB) is considered by many nursery managers to be an excellent growing medium (substrate) for container production, hence its widespread use in Oregon and other regions where DFB constitutes a significant portion of the forest products industry. DFB is sold fresh or aged for use in container nurseries. Fresh DFB is sold soon after it is removed from the tree, ground to smaller particle sizes, and screened. Aged DFB goes through the same process, then sits undisturbed in large piles (7 to 12 m [23 to 39 ft] tall) for an average of 7 months before use. Although nursery managers are equally divided in their preference for fresh or aged bark (Hoeck 2006), some who prefer fresh material do so believing it has more consistent properties throughout the year. No research supports this statement.

Despite widespread use, little information is available on the chemical properties of either fresh or aged DFB as a growing medium for use in containers, whereas pine bark-based substrates commonly used in the southeastern US and sphagnum peat-based substrates have been more thoroughly studied. In general, the pH of pine bark is strongly acidic, ranging from 3.4 to 4.8 (Odgen and others 1987; Tucker 1995). The pH of sphagnum peat-based substrates can range between 3.5 and 5.5 (Williams and others 1988). Liming pine bark or peat with either calcitic ($CaCO_3$) or dolomitic ($CaCO_3$ - $MgCO_3$) lime is a common practice. The typical range of lime added to pine bark is 3.0 to 15.0 kg/m^3 (5 to 25 lb/yd^3), depending on the pH correction required (Prasad 1979; Nelson 1998). Williams and others (1988) showed that pH of sphagnum peat increased quadratically with increasing lime rates up to 7.0 kg/m^3 (11.6 lb/yd^3).

Based on Warncke's (1998) water-extractable macronutrient standards for container crops, non-amended pine bark has low ammonium (NH_4-N) (0.3 ppm) and nitrate (NO_3-N) (0.7 ppm) (Pokorny 1979), high phosphorus (P) (11 to 23 ppm) and potassium (K) (134 to 215 ppm) (Tucker 1995), and low calcium (Ca) (21 to 39 ppm) and magnesium (Mg) (7 ppm) (Starr and Wright 1984). In general, non-amended pine bark contains sufficient micronutrients for woody plant production (Niemiera 1992; Svenson and Witte 1992; Thomas and Latimer 1995; Rose and Wang 1999; Wright and others 1999).

Bollen (1969) showed that pH and nutrient content of DFB differs from other conifers, therefore research conducted on nursery use of pine bark cannot be extrapolated to DFB. Lacking information on DFB chemistry, we conducted a one-year survey of fresh and aged DFB from the 2 primary suppliers (bark sources) for Oregon nurseries; for practical purposes, we will present the 25% to 75% quartiles of the data collected for each chemical variable measured. We conducted experiments to discern: 1) the effect of liming on DFB pH; 2) how aging affects DFB chemistry; and 3) how those changes affect growth of annual vinca (*Catharanthus roseus* 'Peppermint cooler'). We used vinca because it is responsive to variable micronutrient nutrition. Although the complete results are presented in detail in Buamscha and others (2007a,b) and Altland and Buamscha (2008), here we present a summary of our important findings that may be used as a starting point for managers of forest and conservation nurseries interested in trying DFB in their production system.

DFB pH and Liming

Throughout the year, we found that pH of DFB was about 4.0 to 4.7 when fresh, and 3.5 to 4.5 after aging 7 months. These values were below the recommended range (5.0 to 6.0) for container crops (Yeager and others 2000), but similar to the range of pH reported for non-amended pine bark (Odgen and others 1987). This low pH is why most ornamental nurseries in Oregon amend DFB with lime. We conducted a separate study to understand the effect of liming DFB with increasing rates of calcitic lime ($CaCO_3$) (Altland and Buamscha 2008). We found that, after 6 weeks, non-amended aged DFB had a pH of 5.7, whereas amending it with 0.6, 1.5, 3.0, and 4.5 kg $CaCO_3$/m^3 (1.0, 2.5, 5.0, and 7.5 lb $CaCO_3$/yd^3) raised pH to 6.1, 6.7, 7.0, and 7.1 respectively. Although some Oregon growers use rates up to 20 lb/yd^3 (personal observations), we found that pH did not increase appreciably above the 5 lb rate; pH was 7.2 when adding 12kg/m^3 (20 lb/yd^3). Our results agree to similar research conducted in peat (Williams and others 1988). Most ornamental species grow well in containers with pH between 5 and 6. Our work with DFB indicates that even low (1.5 kg/m^3 [2.5 lb/yd^3]) rates of $CaCO_3$ after 6 weeks of production seem to raise pH values above the recommended range in the absence of other fertilizer additions. Growers may still wish to add lime, particularly if they use fertilizers with a strong acid reaction. Our results suggest that adding 3.0 to 4.5 kg $CaCO_3$/m^3 (5.0 to 7.5 lb/yd^3) would be sufficient for amending

DFB pH to near neutral, but the short duration of our study failed to provide much insight on the question of how the pH of container media changes over the course of an entire growing season in Oregon nurseries. More research is needed to quantify this aspect of DFB pH.

Macronutrients and Salts

Throughout our one-year survey, we found that electrical conductivity (EC) and sodium (Na) levels were low (data not shown) in fresh and aged DFB. As expected, NH_4-N and NO_3-N levels were also consistently low and similar between bark ages (average 1.3 and 0.3 ppm, respectively), concurring with Bollen (1969) for DFB and Pokorny (1979) for pine bark. We found extractable P levels in fresh and aged DFB were several times higher than those recommended by Warncke (1998) and higher than a pine/hardwood bark amended with a P fertilizer (Rose and Wang 1999) (Table 1). Potassium levels in DFB were generally within Warncke's (1998) recommendation. Unlike NO_3-N and P, K is not considered a pollutant or a cause of surface water eutrophication (Mengel and Kirkby 2001), but these significant K levels should be considered when developing fertility programs. Further research should address the availability and fate of K in DFB substrates throughout a growing season.

For the secondary macronutrients Ca, Mg, and SO_4^-, we found that the values in fresh and aged DFB (Table 1) were below those suggested by Warncke (1998). Our low levels of native Ca and Mg were similar to those found by Yeager and others (1983) and Starr and Wright (1984) in pine bark. In addition, Ca and Mg salts dissolved in irrigation water can be a significant source of these nutrients for plant growth (Whitcomb 1984) and should be accounted for when designing fertility programs. The low native secondary macronutrients we found in DFB are probably not much of a concern because growers typically amend DFB bark with dolomitic lime and S fertilizers.

Micronutrients

The extractable amount of the micronutrient iron (Fe) was adequate in fresh DFB and high in

Table 1. Parts per million (ppm) of macro- and micronutrients found in fresh and aged DFB and optimum amounts recommended by Warncke (1998) for container plants.

Nutrient	Fresh	Aged	Recommended (Warncke 1998)
	ppm		
P	8 to 17	12 to 35	3 to 5
K	64 to 131	40 to 157	60 to 149
Ca	12 to 28	8 to 64	80 to 199
Mg	5 to 14	3 to 34	30 to 69
SO_4^-	11 to 16	6 to 23	30 to 150
Fe	19 to 29	56 to 103	15 to 40
Cu	0.4 to 0.5	0.3 to 0.4	0 to 0.4
Mn	7 to 13	8 to 13	5 to 30
B	0.2 to 0.3	0.3 to 0.6	0.7 to 2.5

aged DFB compared to recommendations (Warncke 1998); these values are much higher than the trace amounts found in pine bark (Pokorny 1979; Odgen 1982). Extractable copper (Cu) in our DFB was at the high end of recommendations (Warncke 1998) and higher than those reported for pine bark (Pokorny 1979; Odgen 1982). Extractable manganese (Mn) was at the low end, but within recommendations (Warncke 1998), and much higher than that reported for pine bark (Pokorny 1979; Odgen 1982). Extractable boron (B) was well below recommendations (Warncke 1998); pine bark levels have been reported as either lower (Pokorny 1979) or higher (Neal and Wagner 1983) than those measured in DFB.

Although both fresh and aged DFB contain significant amounts of micronutrients, 2 new questions arose: 1) whether these micronutrients are available for plant growth; and 2) whether DFB age has an effect on micronutrient availability. To answer these questions, we grew annual vinca in fresh or aged DFB amended with one of these 3 micronutrient sources: 1) a standard micronutrient fertilizer (Micromax®, The Scotts Company, Marysville, Ohio); 2) yard debris compost; and 3) a non-amended control (Buamscha and others 2007a). We repeated the experiment twice; both times all plants looked healthy and none developed growth or foliar color symptoms that could be related to micronutrient deficiency or toxicity.

The first experiment lasted 6 weeks and we detected no differences in growth (shoot dry weight) between fresh or aged DFB or among the 3 micronutrient sources (Figure 1). The second experiment lasted 8 weeks (Figure 2); plants in non-amended fresh DFB were smaller than plants growing in fresh DFB amended with Micromax® or compost. Nevertheless, micronutrient nutrition failed to explain these growth differences because: 1) compost and non-amended plants had similar foliar nutrient levels with the exception of B (data not presented); and 2) Micromax®-amended plants had higher Ca, Mg, S, Mn, Cu, and zinc than non-amended plants. The same trend occurred in aged DFB and did not affect plant growth. In addition, plants growing in aged DFB tended to be larger than those in fresh bark. These growth differences could be attributable to higher foliar N in plants growing in aged compared to fresh bark (4.7% versus 3.2%, respectively). Differences in physical properties between fresh and aged bark might be another contributing factor in the differential plant growth between fresh and aged DFB; we documented a consistently higher water-holding capacity in aged compared to fresh DFB (Buamscha and others 2007b).

Based on our results, we cannot state which DFB age provided greater micronutrient nutrition, but it appears that both fresh and aged, non-amended DFB provided sufficient micronutrients for annual vinca grown for up to 8 weeks. Research in pine bark substrates has found similar results (Niemeira 1992; Svenson and Witte 1992; Rose and Wang 1999). We do not necessarily recommend that growers remove micronutrient amendments from their fertility programs, but rather we suggest that native micronutrients in DFB should be considered when developing a fertilizer management plan.

Effects of Aging

Aged DFB, especially bark with larger particles sizes (2.2 cm [≤ 0.87 in]), tended to have a lower pH (3.5 to 4.1) than fresh DFB (4.1 to 4.4). These results are unlike those of Pokorny (1979) and Harrelson and others (2004) who found no effect of pine bark age on substrate pH, and Cobb and Keever (1984) who found a higher pH in aged compared to fresh pine bark. This again confirms that pine bark research cannot be directly extrapolated to DFB.

There is a relationship between DFB pH, age, and extractable nutrients; as pH decreases, EC and amounts of extractable P, Ca, Mg, B, and Fe increase. Not surprising, aged DFB, which had lower pH than fresh DFB, also had higher levels of each of these parameters. It may be that during

Figure 1. Annual vinca growth (shoot dry weight) resulting from 2 bark ages and 3 micronutrient sources at 6 weeks after planting (Experiment 1). No significant differences in growth between fresh and aged bark, or the 3 micronutrient sources.

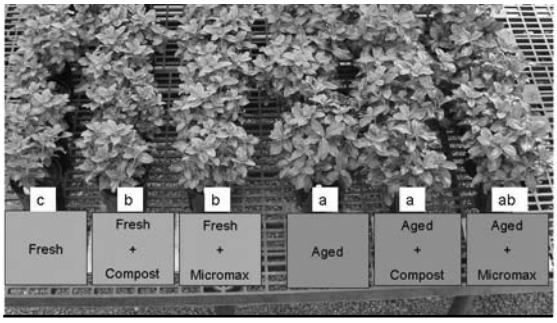

Figure 2. Annual vinca growth (shoot dry weight) resulting from 2 bark ages and 3 micronutrient sources at 8 weeks after planting (Experiment 2). Means with different letters within a treatment are significantly different, separated by LSD test ($\alpha = 0.05$).

the aging process, bark particles break down, bacterial activity increases, and, as a consequence, nutrients and H^+ ions are released. As the H^+ concentration increases, pH decreases, and the availability of key plant macro- and micro-nutrients increases (Lucas and Davis 1961; Odgen and others 1987).

Douglas-fir Bark Consistency Through 1 Year

In our work (Buamscha and others 2007b), we tested the growers' hypothesis that fresh DFB had more uniform chemical properties than aged DFB. We calculated coefficients of variation for each nutritional parameter measured in fresh and aged DFB to estimate data consistency over time. Our results indicated that bark age seemed to be less important in terms of consistency than the source from which it was collected. In general, we found nutritional parameters of the coarser bark source tended to have less variation than the finer source, and this was true for both fresh and aged DFB. Considering the primary difference in bark sources is the screening size (0.9 cm [≤0.37 in] for fine; 2.2 cm[≤0.87 in] for coarse), this implies that chemical properties of DFB might be more uniform or consistent throughout the year in coarser bark grades.

Phosphorus Leaching from DFB Substrates

Phosphorus leaching from DFB substrates may be of concern because high levels of P discharged from the nursery can lead to serious water quality issues, including surface water eutrophication. Unlike mineral soils, P in soilless container substrates leaches readily under standard nursery overhead irrigation practices (Marconi and Nelson 1984). Because we found high levels of P in fresh and aged DFB (Buamscha and others 2007b), we examined the fate of this native P during the course of a 24-week growing season and assessed the effect of pH on P availability and leaching from the containers. We monitored water-extractable P levels in 1-gal containers filled with fresh and aged DFB, amended with either 0 or 6 kg/m^3 (0 or 10 lb/yd^3) dolomitic

lime, that were placed under typical nursery conditions with daily overhead irrigation. Adding lime increased fresh DFB pH from 4.0 to 7.5, whereas it increased aged DFB pH from 3.4 to 6.5, similar to results reported in Altland and Buamscha (2008).

Throughout the experiment and regardless of lime rate, we measured P levels in fresh DFB below 3 ppm; these seemed unusually low considering we detected 8 to 17 ppm P in fresh DFB (Buamscha and other 2007b). Further, we failed to see any relationship between fresh DFB pH and extractable P, perhaps because our P levels were so low. Overall, P in fresh DFB declined slowly over time, and was less than 2 ppm after 24 weeks.

Phosphorus in aged DFB was initially high (9 to 17 ppm). When amended with dolomitic lime, P was reduced by roughly 50% compared to the non-amended control. A similar relationship between increased substrate pH and reduced availability of P has been reported for a peat/pine bark substrate (Peterson 1980) and non-fertilized DFB (Altland and Buamscha 2008). After 7 weeks in a typical nursery environment, P was reduced 60% and 53% in non-amended and amended aged bark, respectively, from their original values. After 12 weeks, even non-amended aged bark had only 3 ppm P, which is close to the lower limit of what is recommended for container fertility by Warncke (1998).

After 18 weeks, P in fresh and aged DFB was less than 2 ppm, concurring with Yeager and Wright (1982) who, after amending pine bark with superphosphate fertilizer, measured a decline in water extractable P from 248 ppm to less than 10 ppm (96% reduction) in only 5 weeks. This is not too surprising as Marconi and Nelson (1984) reported P leaches readily from container substrates under typical nursery irrigation management.

Although aged DFB contains relatively high levels of water-extractable P at the onset of the growth cycle, irrigation rates and leaching events typical of container nurseries reduce those levels below that necessary to sustain plant growth. Lime additions appear to contribute to reducing P availability in aged DFB. Based on this work, it appears that aged DFB without a lime amendment has sufficient P to support plant growth for a month, suggesting that the industry-wide practice of incorporating high levels of water-soluble P as a "starter" fertilizer may not be necessary, assuming lime rates and substrate pH are kept low. Further research should be conducted to increase P retention in DFB substrates to maximize plant nutrient use efficiency and minimize environmental impact from P in nursery water runoff. Amending container substrates with clay minerals may have a promising future; Owen (2006) reported increased water buffering capacity, buffer substrate solution pH, and P retention when amending pine bark with a clay mineral.

Summary

Douglas-fir bark appears to be a reliable container substrate with fairly consistent chemical properties throughout the year. DFB is strongly acidic. Without additional fertilizer, increasing lime rates above 3 kg $CaCO_3/m^3$ (5 lb/yd^3)in DFB does not translate into appreciably increasing pH values during the first 6 weeks of the growing season. DFB contains significant amounts of plant nutrients, in particular P, K, Fe, Cu, and Mg. As DFB ages, pH tends to decrease and EC and extract-ability of P, Ca, Mg, B, and Fe increases. Coarser DFB has more uniform chemical properties over the course of a year than the finer source. Our results do not support the belief of some growers bark consistency is a factor of bark age throughout the year. When fresh and aged DFB pH is kept low (< 5.7), micronutrient nutrition was adequate for annual vinca for up to 2 months, indicating that growers should consider native micronutrients when developing fertilizer management plans.

Native P leaches quickly from DFB substrates under typical overhead irrigation regimes. Nevertheless, native P in aged DFB bark may be a reliable source of P for plant growth for at least a

month, providing substrate pH is kept low. Under these conditions, growers should re-evaluate the practice of incorporating high levels of water-soluble P as a "starter" fertilizer. Although the average DFB screen size offered by Oregon bark companies (approximately 1.6 cm [0.62 in]) is too large for the small volume cells used in forest nurseries for reforestation, this product may have utility for conservation and native plants grown in larger volume containers. Moreover, bark companies could grind and sieve DFB to smaller screen sizes that would fit containers typical of reforestation stock.

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Effects of Container Size and Copper Treatment on Western White Pine Seedling Growth and Survival

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KEYWORDS
seedling quality, stocktype selection, stocktype, inland northwest

Introduction

Dry, south-facing slopes, such as those commonly found in north-central Idaho, often need to be planted more than once due to poor seedling survival. The Idaho State Forest Practices Act requires minimum stocking levels to be achieved within 5 years following harvest. However, this requirement can be difficult to meet based on seedling mortality due to moisture stress, vegetative competition, and animal browse. As container seedlings are useful in revegetating harsh sites (Walker and Kane 1997), a strong industry interest exists in determining optimal stocktypes for regenerating inland northwest forests.

Container size can be manipulated to meet certain management objectives, such as site conditions, cost constraints, and the silvics of the species of interest. Larger container size is well correlated with larger seedling size at the end of the nursery production cycle (Pinto 2005; Dominguez-Lerena and others 2006). Typically, larger seedlings cost more initially (Davis and others 2007), but can outperform smaller seedlings in the field (Dominguez-Lerena and others 2006).

Copper coating of containers is done to enhance early root growth of container seedlings.

Copper is toxic to roots, and roots will self-prune on contact with the copper-treated cell wall. After outplanting, these root tips will grow more lateral roots, particularly in the upper profile of the root plug (Burdett and others 1983; Wenny and Woollen 1989; Dumroese 2000; Campbell and others 2006). This improved lateral root development can result in altered, and potentially improved, seedling water and nutrient acquisition (Burdett and others 1983).

Our study objective was to compare survival and growth of outplanted western white pine (*Pinus monticola*) seedlings, grown in 2 sizes of containers, with and without copper coating. We hypothesized that seedlings grown with copper treatment would outperform non-copper seedlings based on better root penetration, which facilitates water and nutrient uptake, and that seedlings that were larger at outplanting would maintain their size difference over time.

Methods

Western white pine seedlings were grown in 2004 under operational practices at the University of Idaho Franklin H Pitkin Forest Nursery (Moscow, Idaho) in 2 sizes (120/80 and 91/130) of Copperblock™ and Styroblock™ containers (Beaver Plastics, Edmonton, Alberta, Canada). Copperblock™ containers are coated with copper oxychloride on the inside walls, serving as a root-pruning mechanism. The 120/80 containers have a growing density of 120 seedlings per container and a cavity volume of 80 ml (5 in^3), while 91/130 containers have a growing density of 91 seedlings per container and a cavity volume of 130 ml (8 in^3). At lifting (December 2004), a subsample of seedlings was oven-dried for biomass assessment. The remaining seedlings were placed in refrigerated storage until spring, at which time they were outplanted.

An experimental site was chosen near Elk River, Idaho. Four replications were established as a randomized complete block design with 20 seedlings per treatment replication. The study was a 2 x 2 factorial (copper coating x container size). One replication was dropped due to excessive damage by ungulates. Survival, height, and root collar diameter were measured after outplanting, and at the end of each growing season (2005 and 2006). Data were analyzed with 2-way analysis of variance using SAS software (SAS Institute Incorporated, Cary, North Carolina), and significant differences at $\alpha = 0.05$ highlighted using LSMEANS.

Results

Container size had a significant influence on initial seedling root biomass ($P = 0.0422$), shoot biomass ($P = 0.0026$), and total biomass ($P = 0.0091$), with seedlings from larger cavities having greater biomass than those from smaller cavities (Table 1). Similarly, seedlings grown in the

Table 1. Initial biomass of seedlings. Letters indicate significant differences ($\alpha = 0.05$).

Container Size	Type	Dry weight (g) Root ± SE	Shoot ± SE	Seedling ± SE
120/80	Styroblock®	1.27 ± 0.16 a	1.34 ± 0.13 a	2.61 ± 0.27 a
	Copperblock®	1.21 ± 0.16 a	1.37 ± 0.20 a	2.80 ± 0.35 a
91/130	Styroblock®	1.80 ± 0.27 b	2.60 ± 0.37 b	4.40 ± 0.63 b
	Copperblock®	2.00 ± 0.17 b	2.88 ± 0.22 b	4.88 ± 0.38 b

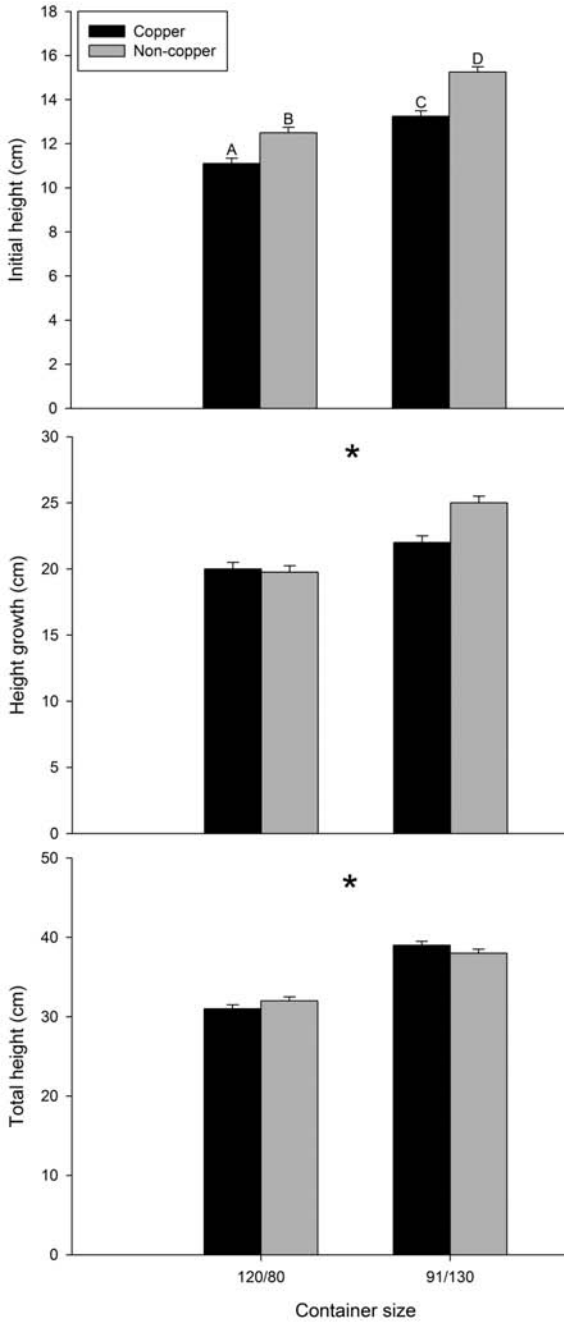

Figure 1. Seedling height: initial (top), 2-year growth (middle), and total (bottom). Main effect of container size was significant for all 3, and copper treatment significant for only initial height. Letters and * indicate significant differences ($\alpha = 0.05$).

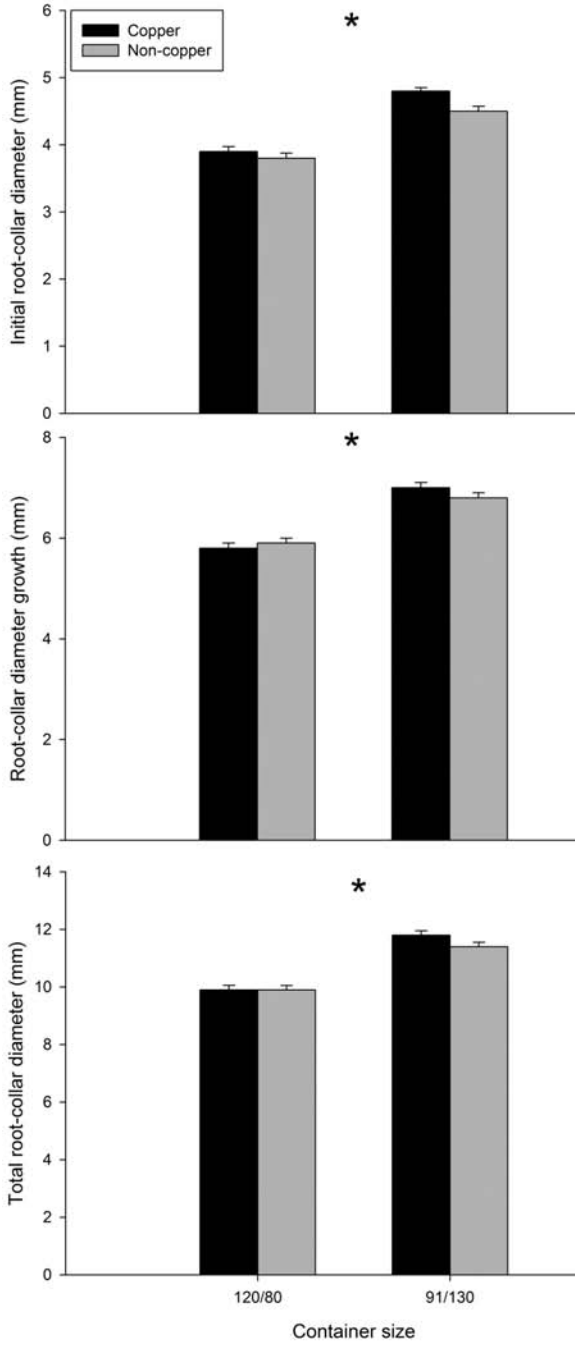

Figure 2. Seedling root collar diameter: initial (top), 2-year growth (middle), and total (bottom). Main effect of container size was significant for all 3. * indicates significant difference ($\alpha = 0.05$).

Table 2. Initial shoot-to-root ratios of seedlings. Letters indicate significant differences ($\alpha = 0.05$).

Container Size	Type	Shoot:root ± SE
120/80	Styroblock®	1.10 ± 0.07 a
	Copperblock®	1.14 ± 0.08 a
91/130	Styroblock®	1.48 ± 0.06 b
	Copperblock®	1.45 ± 0.05 b

larger (91/130) cavities had greater shoot-to-root ratios ($P = 0.0008$, Table 2) than those grown in 120/80 containers. Treatment with copper had no significant effect on initial biomass ($P = 0.5900$). At outplanting, seedlings grown in copper-treated containers were shorter ($P = 0.0164$) than those grown in regular containers, but no difference was identified for initial root collar diameter ($P = 0.1354$). Cavity size had a significant effect on both initial height ($P = 0.0018$) and root collar diameter ($P = 0.0004$), with seedlings grown in 91/130 containers being larger than those in 120/80 containers (Figures 1 and 2).

Seedling survival was not influenced by treatments ($P = 0.9636$), and was approximately 90% across all treatments. Height growth (Figure 1) and root collar diameter growth (Figure 2) were significantly greater in larger cavities ($P = 0.0215$ and $P = 0.0012$, respectively), but not influenced by copper treatment ($P = 0.3095$ and 0.8359, respectively). At the end of 2 growing seasons, height ($P = 0.0069$; Figure 1) and root collar diameter ($P = 0.0003$; Figure 2) were significantly greater in 91/130 containers than in 120/80 containers.

Discussion and Future Directions

Seedlings grown in larger containers grew more in height and root collar diameter than seedlings in smaller containers after 2 growing seasons, in addition to being larger initially. This corresponds to expected behavior, given knowledge of plant use of growing space and resource acquisition, as well as existing evidence (Dominguez-Lerena and others 2006). However, nursery cultural practices and early stand silviculture must be treated as dynamic processes to ensure proper seedling production. Furthermore, increasing shoot size may not be without detriment to root morphology, as identified in a study of longleaf pine (*Pinus palustris*) by South and others (2005).

Copper treatment had no effect on seedling growth after 2 growing seasons, despite some initial differences in seedling height. Therefore, treatment of container seedling cavity walls with copper was not effective at addressing initial concerns over seedling performance on this site. This corresponds with the findings of Wenny and others (1988) investigating western white pine. However, given known variability in growth in other species, such as lodgepole pine (*Pinus contorta*), where results have found both improvement (Burdett and others 1983) and no difference (Campbell and others 2006) in seedling growth after outplanting, attention must be paid to species, site, and environmental conditions at time of establishment. The potential for added mechanical stability (Burdett and others 1983) further highlights a possible long-term benefit of copper-coated containers. Finally, longer term assessment may be warranted, as height growth was not significantly impacted by copper treatment until year 4 of the study described by Burdett and others (1983). However, Wenny (1988) did not find any difference in Douglas-fir (*Pseudotsuga menziesii*), western white pine, and ponderosa pine (*Pinus ponderosa*) seedling growth after 3 field growing seasons.

Our intention is to continue to maintain and monitor these plots. Thus, they may be thinned to minimize within-treatment competition. Furthermore, in addition to continued assessment of seedling morphology, it is intended that economic analysis of treatments will be performed after seedlings reach free-to-grow status.

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Fusarium Species—a British Columbia Perspective in Forest Seedling Production

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ABSTRACT
This review provides a brief biological outline of some species in the genus *Fusarium* and how these can be implicated as seedborne organisms leading to conifer seed and seedling losses in British Columbia. *Fusarium* spp. are implicated with pre- and post-emergence damping-off, seedling wilt, late damping-off, root rot, and seedling mortality after outplanting. Current understanding of *Fusarium* spp., with regard to cone and seed pest management in British Columbia, is outlined. Shortfalls that still exist and how these might be addressed with the development of a vision for better understanding this group of fungi and a mission statement of how this might be achieved are presented.

KEYWORDS
diseases, pest management,
seedborne diseases, damping-off

The Genus *Fusarium*: an Overview

Members of the genus *Fusarium* are among the most important plant pathogens in the world. *Fusarium* spp. are a widespread cosmopolitan group of fungi that commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders. Fungi in this genus cause a huge range of diseases on a wide range of host plants. The fungus can be soil-, air-, and waterborne, or carried in or on plant residue or seeds, and can be recovered from any part of a plant: roots, shoots, flowers, fruits, cones, and seeds (Summerell and others 2003).

Summerell and others (2003) point out that *Fusarium* taxonomy has been plagued by changing species concepts, with as few as 9 to over 1,000 species being recognized by different taxonomists during the past 100 years. Differing opinions on species identification has stabilized since the 1980s following publications by Gerlach and Nirenberg (1982) and Nelson and others (1983) who defined widely accepted morphological species concepts. Since that time, however, the application of biological (Leslie 2001) and phylogenetic (Nirenberg and O'Donnell 1998) species concepts to new, as well as existing, strain collections has resulted in further splitting of many of the previously described species. If changing

these taxonomic designations were only rare, or of limited economic importance, they could be viewed as being merely pedantic. However, many of these species can be important. For example, *F. andiyazi* and *F. thapsinum* are major pathogens of sorghum that differ from one another but had previously been grouped as *F. moniliforme* (Leslie 2001; Marasas and others 2001). Further description of taxonomy is well beyond the scope of this note. However, its complexity and the recognized difficulty of rapidly identifying cultures to species (Summerell and others 2003) has meant that research and development of cone and seed pest management associated with *Fusarium* spp. in British Columbia (BC) has generally been limited to genus.

A taxonomic treatment for *Fusarium* is presented for completeness:

KINGDOM: Mycetae (fungi); DIVISION: Eumycota; SUBDIVISION: Deuteromycotina (the imperfect fungi); CLASS: Hyphomycetes; ORDER: Hyphales (Moniliales); GENUS: *Fusarium.*

Fusarium spp. are grouped in the subdivision Deuteromycotina, which encompasses the imperfect (asexual) fungi. Nelson and others (1983) point out that the perfect (sexual) states of *Fusarium* are generally unfamiliar to many people working with these fungi. Plant pathologists most often deal with the imperfect states, as the perfect states often have little to do with the disease problem under study. Some of the most successful *Fusarium*, for example, *F. oxysporum* and *F. culmorum*, appear to have lost their sexual ability and have adopted other methods of facilitating genetic adaptations (Booth 1981).

General Characteristics

Due to the great variability within this genus, it is one of the most difficult of all fungal groups to distinguish taxonomically (Alexopoulus and Mims 1979). Conidia (asexual spores) are hyaline and can be divided into 3 groups: macroconidia, microconidia, and chlamydospores. Macroconidia are several-celled, crescent or canoe-shaped spores. Their ends vary in that some species produce sharply pointed macroconidia, while others produce spores with rounder ends. The shapes of these spores are used to differentiate morphologically between species (Toussoun and Nelson 1968). Most *Fusarium* produce their macroconidia on sporodochia, which are cushion-shaped fruiting structures covered with conidiophores (simple or branched hyphae bearing conidia) (Figures 1 and 2). Macro-conidia can also be found, however, throughout the aerial mycelium. Microconidia are 1- or 2-celled, ovoid or oblong, and borne singly or in chains. These spores are found scattered throughout the aerial mycelium. The 1- or 2-celled microconidia are usually smaller than the macroconidia. Macroconida and microconidia are produced from phialides (a type of conidiogenous cell). Chlamydospores are round, 1- or 2-celled, thick-walled spores produced terminally or intercalary on older mycelium (Agrios 1988). Chlamydospores generally function as resting spores, having the ability to survive adverse conditions and enable the fungus to regenerate when favorable conditions for growth are reencountered. This is illustrated by a disease triangle (Figure 3). In the presence of a suitable host (for example, seedling) and pathogen (for example, *Fusarium* chlamydospore), disease of the host will progress when the environment favors spore germination and infection over time.

Disease Cycle

Fusarium are soil inhabitants that overwinter between crops in infected plant debris as mycelia and in 3 spore forms. As chlamydospores, *Fusarium* can remain in the soil for long time periods. Mycelium can infect healthy plant tissue in the same manner as spores do. Healthy plants can become infected through their root tips; either directly, through wounds, or at the point of formation of lateral roots (Agrios 1988). The fungus can grow as mycelium through the root cortex intercellularly, ultimately advancing to the vascular tissue. As the mycelium continues to grow, usually up toward, and into the stem, it branches

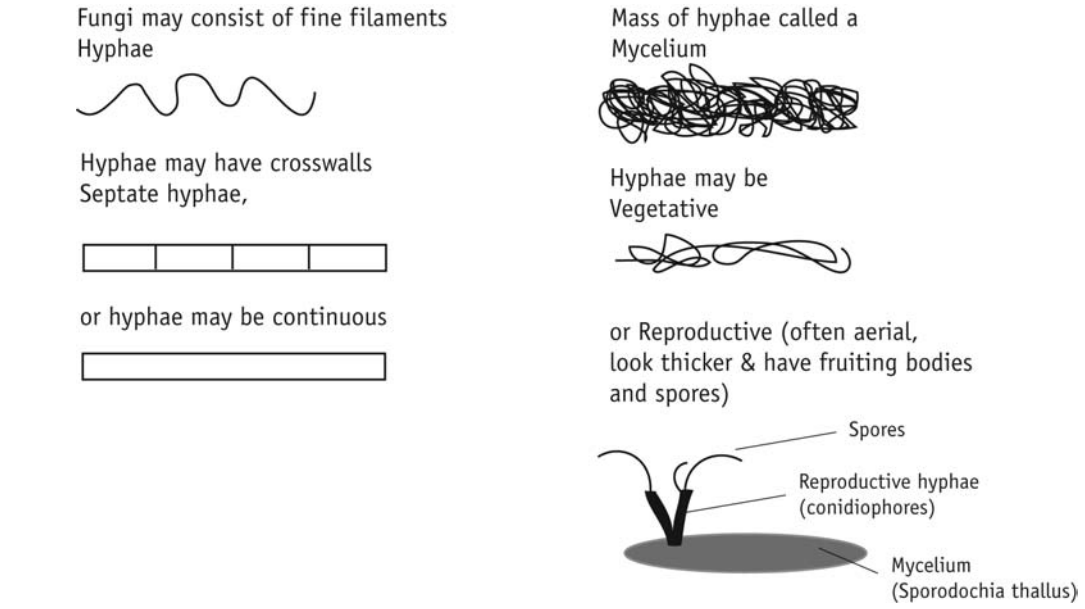

Figure 1. Structure of some fungi associated with disease (fungi imperfecti).

and produces microconida. The proliferation of fungal growth in a plant's vascular tissue can eventually cause the plant to wilt and die. Conifer seedlings are especially susceptible to this pathogenic modality when subjected to drought stress and high transpirational demands. The fungus can continue to grow on the decaying tissue, where it can sporulate profusely, visibly presenting salmon to coral-pink colored sporo-dochia on the lower portion of seedling stems. At this point, the spores can be spread to other plants or areas by wind, water, or through the movement of seedlings themselves (Agrios 1988).

Types of Disease

In addition to vascular wilting, *Fusarium* can infect other plant parts close to the soil to induce root and stem rots. When seeds become contaminated or seedlings are infected with *Fusarium*, damping-off may occur. The *Fusarium* that cause vascular wilts can be spread in soil, dust, and irrigation water. Wind, rain, nursery equipment, and decaying plant tissue can also help to spread the fungus. Additionally, *Fusarium* can enter nurseries as seed contaminants, be carried over from previous years within surface cracks on dirty growing containers, or within attached extraneous root fragments.

Forest seedling nurseries represent artificial growing environments. In BC, seeds are sown in soilless, peat-based growing media in Styrofoam™ (Styroblock™) containers. Styro-block™ containers generally reside on benches over concrete or gravel. Seed germination and the early part of the growing cycle take place in polyethylene covered greenhouses where temperature, light, and moisture are closely controlled, and nutrients are applied through overhead irrigation.

Fusarium are considered natural soil inhabitants and readily isolated from agricultural and forest soils. Understanding *Fusarium*-caused diseases in forest seedling container nurseries, however, requires the recognition that in this growing environment, *Fusarium* are *introduced* pests. They are introduced to the container nursery via seeds, water, and wind, or on old containers or dirty equipment. *Fusarium* can lead to seed and seedling losses in several ways:

1) seedborne contamination;
2) pre- and post-emergence damping-off;
3) seedling wilt;

4) late damping-off;
5) seedling root rot;
6) seedling mortality after outplanting.

Seedborne Contamination

Seedborne fungi are defined as those "that are dispersed in association with some kind of dispersal units of the host (that is, seeds)" (Ingold 1953). This definition includes all seed types and all associated microfungi, and is the one adopted with reference to conifer seedborne fungi in BC. Some authors classify fungi as being either seedborne or seed-transmitted (Thomsen and Schmidt 1999). They define seedborne fungi to include all fungal types contaminating the surface of seeds or infecting seed tissues. Seed-transmitted fungi are those that cause no infection of a seed itself, but infect seedlings in the nursery or field (Neergaard 1979). It must be remembered that not all seedborne fungi are pathogenic, and they may include symbionts actually beneficial to the plant (Mallone and Muskett 1997). With regard to seed transmission of fusaria, we are more interested in it as a seedborne pathogen than a seedborne disease. Seedborne pathogens (as opposed to diseases) are defined here as organisms, whether on or in seeds, which may or may not cause infections and symptoms on the seeds. Some seedborne pathogens may actively infect seeds, and may or may not cause symptoms on the seeds. Seedborne pathogens associated with conifer seeds may inhabit the external or internal tissues of seeds. Seedborne diseases occur on seedlings as a result of pathogens carried in or on the seeds. Evidence shows that *Fusarium* rarely exist within conifer seeds (Peterson 2007).

Seeds harbouring fungi can be described as being either contaminated or infected. Contamination is used to denote the occurrence of a pathogen as either spores or mycelium on the surface of seeds. Contamination may be entirely superficial, where spores or mycelium are usually retained in small cracks or fissures in the seedcoat. Infection refers to the penetration of seeds

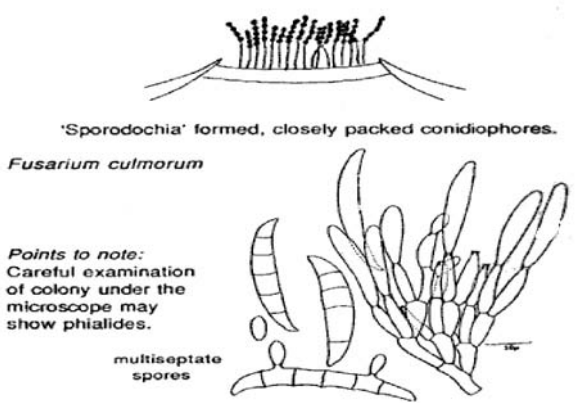

Figure 2. *Fusarium culmorum* showing sporodochia, macroconidia, and conidiophores.

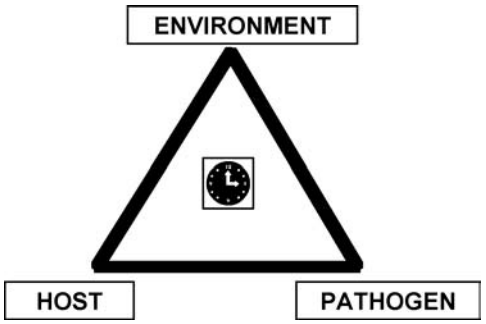

Figure 3. Disease triangle indicates 3 conditions that must be maintained over time for any disease to progress.

by an organism followed by the establishment of a relationship (that is, saprophytic or parasitic) within the seeds. Once established, such a relationship can give rise to outward hyphal growth from within the seeds, which becomes apparent upon penetrating the seed surface. While this hyphal growth can appear as a contaminant, it is indicative of the presence of an infection deeper within the seeds. In certain situations, it is possible to disinfest seeds that are only superficially contaminated. Surface disinfestations of infected seeds are of little value, as an internal relationship between the seeds and fungi will still exist. One of the easiest ways to eliminate or reduce seedborne contamination is through the use of running water during imbibition, followed by a post-strat-

ification rinse with running water (Kolotelo and others 2001). Disinfestation in this manner can reduce the incidence of seedborne *Fusarium* by reducing what has previously been observed as the tendency for contamination to actually increase during stratification.

Seedborne contamination may occur through indirect routes, such as via cone parts to the ovary and ovule tissues, or through direct routes when seeds contact contaminated soil and water. Dirty equipment in a processing facility may also contaminate seeds during interim storage, processing, or seed treatment for stratification. As spores can be released throughout the year, at almost any time in the general lifecycle of major BC conifer seedlings, seeds are exposed to contamination over a wide range of conditions. Examination of tree seed samples from over 2600 seedlots stored at the BC Ministry of Forests and Range (MoFR) Tree Seed Centre has indicated the frequency of seedborne *Fusarium* to be the same on seeds originating from seed orchards and those taken from natural stands (Peterson 2000). Spores freed from soil or grasses within and around seed orchards may be spread by irrigation sprinklers. This could be exacerbated by the use of sprinklers to control pollination in the spring. Indirect contamination through cone parts to the ovary and ovule tissues such as this could similarly occur in wild stands via rainfall. Fungal inoculum (for example, spores) reaching maturing cones on trees is thought to be one way that seeds can become contaminated and *Fusarium* become seedborne. Seeds and cone parts harbouring the fungi can contaminate processing facility equipment, contributing to further contamination of otherwise clean seeds. Regardless of the initial source, seedborne *Fusarium* can intensify throughout a contaminated seedlot during seed stratification.

Pre- and Post-Emergence Damping-off

Pre-emergence damping-off is characterized by seeds failing to germinate, or rotting of emerging shoots or radicals with the associated seedling losses. Damage and losses here are usually confined to individually contaminated seeds. Symptoms of post-emergence damping-off include stem rotting at the groundline and subsequent toppling of the seedling shoot. Post-emergence damping-off results in damage and loss of infected germinants after the stems rot. However, the disease can also spread by spores produced on the infected stems, which can then infect adjacent seedlings and cause further losses.

Seedling Wilt

Conifer seedlings, especially Douglas-fir (*Pseudotsuga menziesii*), are susceptible to seedling wilt caused by *Fusarium* when fungal growth proliferates in the plant's vascular tissue. This condition is often encountered when cool and overcast weather in the late spring or early summer is followed by a sudden clear warming trend. Seedlings that may otherwise have been tolerating a compromised vascular system are then subjected to drought stress induced by sudden high transpirational demands. The avoidance or reversal of these conditions (for example, increased irrigation) may either prevent or reverse the symptoms and minimize any subsequent damage and loss.

Late Damping-off

Late damping-off, also sometimes called Fusarium top blight, is often a progression from the intensification of seedling wilt. Symptoms include needle chlorosis, browning, and desiccation with a hook or crooked-shaped leader tip. Fusarium top blight following wilt damage will not necessarily lead to seedling losses if the trees are promptly treated with a systemic fungicide. When seedling death results, that is, late damping-off, the fungus may continue to grow on the decaying tissue where it can sporulate profusely, visibly presenting salmon to coral-pink colored sporodochia on the lower portion of seedling stems.

Seedling Root Rot

The symptoms of seedling wilt and late damping-off can also be indicative of Fusarium root rot, which is further characterized by blackened,

thin and wispy roots with little sign of actively growing root tips. The root cortex often easily strips away, leaving an exposed root stele. Fusarium root rot does not necessarily lead to seedling losses if the damage to the root system is limited. Root rot often occurs later in the growing season or can also occur if seedlings with infected roots are mishandled after leaving cold storage. Fusaria are natural rhizosphere inhabitants, and healthy, unstressed seedlings can survive well in their presence. The avoidance of stresses to the plants will limit damage and losses caused by Fusarium root rot.

Seedling Mortality after Outplanting

Fusarium are commonly found on conifer seedling roots and in the root zone throughout the growing media plug. In BC, the presence of *Fusarium* on seedling roots in the absence of any disease symptoms is generally not sufficient grounds to reject seedlings scheduled for outplanting. However, as seedlings commonly have *Fusarium* on or around their roots, it is important that proper handling care is taken so that any fungi present do not become aggressively pathogenic. Seedlings scheduled for outplanting must never be allowed to remain in boxes or in conditions where they can become overheated and the roots remain warm and moist for prolonged periods. Under such conditions, *Fusarium* can rapidly spread from seedling to seedling, as well as intensify within the roots of infected seedlings. When outplanted following these conditions, seedlings can quickly succumb to planting shock and, if exposed to a subsequent heat or drought stress, will often die.

Cone and Seed Pest Management: Research to Date

Tree species occurring in BC that are affected by seedborne *Fusarium*, in decreasing order of frequency as indicated from fungal assays, include: Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*), western larch (*Larix occidentalis*), western white pine (*Pinus monticola*), western redcedar (*Thuja plicata*), ponderosa pine (*Pinus ponderosa*), grand fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), subalpine fir (*Abies lasiocarpa*), sitka spruce (*Picea sitchensis*), yellow cedar (*Chamaecyparis nootkatensis*), noble fir (*Abies procera*), amabilis fir (*Abies amabilis*), interior spruce (*Picea glauca* and *P. engelmannii*, mountain hemlock (*Tsuga mertensiana*), and interior lodgepole pine (*Pinus contorta* var *latifolia*) (Kolotelo and others 2001).

It was indicated earlier that, within the context of the forest conifer container nursery system, *Fusarium* can be viewed as introduced pests. Species of *Fusarium* that are part of this disease complex can be introduced via air, water, on greenhouse equipment, on contaminated plant parts, as well as on seeds. Research and development of cone and seed pest management practices to reduce the negative effects of *Fusarium* on conifer seedling production have focused on all of the previously discussed aspects of this disease complex. Each of these areas of investigation is summarized here.

Seedborne Contamination

Initial Contamination

Several species of *Fusarium*, that is, *F. sporotrichoides*, *F. acuminatum, F. avenaceum* and *F. culmorum* have been isolated from Douglas-fir seeds (Mallams 2004). *Fusarium solani* and *F. oxysporum* are 2 other *Fusarium* species that Mallams (2004) notes have been isolated from diseased seedlings in fields at the J Herbert Stone Nursery, Central Point, Oregon. However, as she did not isolate these species from seeds, Mallams (2004) suggested that these infections occurred during or after sowing.

Although *F. acuminatum* and *F. avenaceum* commonly colonize conifer seeds, James (2000) found most *F. acuminatum* isolates he studied were not pathogenic to Douglas-fir. Other studies by James (1985a, 1993) found *F. acuminatum* and *F. avenaceum* both associated with pre- and post-emergence damping-off of conifers, and he suggests they were the result of seedborne inocula.

Several different species of *Fusarium* can cause root rot of container seedlings, with the major source of inocula being the seeds (Landis and

others 1990). Seedborne *Fusarium* are usually responsible for pre-emergence damping-off, but can also lead to post-emergence damping-off as well as *Fusarium* root rot and shoot blight, in this order of importance.

A sound understanding of 2 important seedborne fungi, *Caloscypha fulgens* and *Sirococcus conigenus*, has led to management guidelines to reduce their incidence on seeds, thus lowering outplant mortality attributable to their occurrence. A similar understanding of infection routes for seedborne *Fusarium* does not exist, and establishing this remains an essential first step to developing guidelines for reducing its incidence. As a seedborne contaminant (that is, carried on seeds) or seed infection, *Fusarium* can attack roots and be implicated as a wilt following outplanting. Observations during the winter of 2003/04 by Applied Forest Science Limited (AFS), as part of their seedling diagnostic and adjudication services for the BC MoFR and private forest companies, indicate that *Fusarium* have the ability to grow systemically within the vascular system of 2-year-old seedlings (Peterson 2004a). As a seedborne disease (that is, actively attacking seeds), *Fusarium* can be responsible for pre-emergence damping-off where seeds fail to germinate. To function as a seedborne contaminant as well as a disease suggests more than one infection route. Direct infection of angiosperm seeds occurs as systemic invasion via mother plant tissues to the seed embryo, whereas indirect infection and contamination can occur via the stigma to the seed embryo or via the flower/fruit to parts of the ovary and ovule tissues (Maude 1996). Direct infection via mother plant tissues is common for biotrophic fungi, which are parasitic in nature and dependent upon the survival of their host. Maude (1996) states, however, that this form of seed infection is less likely to occur within nectotrophic fungi which degrade tissues as they advance, with the exception of wilt fungi, including Fusarium, which invade vascular tissue. As a wilt fungus invading vascular tissues, *F. oxysporum* has been shown to infect seeds via the xylem of the mother plant (Baker 1948). *Fusarium moniliforme, F. oxysporum*, and *F. scirpi* have been isolated from vascular bundles from all parts of cotton plants, including bolls and seeds (Rudolph and Harris 1945). *Fusarium moniliforme* has been shown to invade corn seeds through vascular tissues of the stalk (Kingsland and Wernham 1962), while systemic infection in sweet corn plants by *F. moniliforme* and *F. oxysporum* has been shown to occur with hyphae of each species growing in intercellular spaces (Lawrence and others 1981). Mycelia of *F. oxysporum* f. sp. *carthami* have been observed in receptacles of safflower heads where hyphae traversed through the abscission zone of the cypsil and were associated with, but not limited to, the xylem (Klisiewicz 1963). Finally, mycelium has been observed to be inter- and intracellular, and also seen in the vascular elements of the seedcoat and cotyledons in seeds of Fabaceae (Sharma 1992).

Littke (1996) speculated that seed association with this pathogen originates from aerial deposition on developing cones. He deduced that likely routes of subsequent seed contamination exist as a physical transfer from exterior cone parts (bracts and scales) to seedcoat surfaces during seed development. AFS Limited routinely isolates *Fusarium* from seed surfaces during fungal assay testing for the BC MoFR Tree Seed Centre, confirming that *Fusarium* inoculum exists on the seedcoats of many conifer species. Littke's hypothesis requires airborne *Fusarium* spore inoculum to be present in the vicinity of receptive cones during pollination. Data collected by AFS Limited, as one of 24 locations across Canada over the past 10 years under the auspices of the Weather Network/MetroMedia and calculated by Aerobiology Research Laboratories, Ontario, showed airborne spore densities in the Victoria region to closely mimic those of conifer pollen density during the spring of 2003 (Peterson 2003). That airborne spore inoculum can occur during times of pollination in the vicinity of Douglas-fir seed orchards supports Littke and could explain the presence of inoculum on seed surfaces.

Research by Peterson (2007) indicates that seedborne *Fusarium* on several conifer species does not likely occur as internal infections, but is limited to seedcoat contamination. These observations appear to indicate that seedborne fungi in some conifer species do not occur systemically, and more likely occurs following Littke's (1996) hypothesis. How seeds become initially contaminated remains uncertain, and more research is needed to precisely define how and when this occurs prior to developing pest management guidelines to minimize this occurrence.

Spread and Intensification of Initial Seed Contamination

Regardless of the initial source, infested seedlots can potentially cross contaminate those that are uninfested, as well as intensify within themselves during imbibition and stratification. Cross-contamination between seedlots can occur wherever mutual seedlot contact exists through shared seed handling equipment. This can occur when unsanitized cone sacks are reused between cone harvests, or inoculum can potentially be transferred from seed handling equipment during various stages of the seed extraction process. Kolotelo and Peterson (2006) found some trends with regard to the presence of *Fusarium* on cones, debris, and seeds at various stages during cone and seed processing. They found processing stages incorporating agitation to indicate increases in contamination. Kilning appeared to decrease contamination on seed and cone scales despite peak kilning temperatures of 40 °C (104 °F) not totally eliminating the fungal contaminant. Their overall finding was that initial seed contamination levels may not be indicative of final seedlot contamination. This was emphasized by the fact that, despite BC interior Douglas-fir having a very low incidence of seedborne *Fusarium*, some of the associated debris had significant amounts of contamination. Also, although initial levels on cone scales, seeds, and debris in some seedlots were high to moderate, the final contamination levels were very low and below what are considered to be a concern. Their observations indicated trends only that were difficult to substantiate statistically, indicating this to be an area in need of further investigation.

The potential for conifer seeds to become contaminated with *Fusarium* makes testing for its presence a viable first step for managing it as a seedborne organism, thus allowing specific seedlots to be targeted for special treatment. The ability to spread or intensify within a seedlot, the fact that some tree species are more susceptible to the effects of the fungi, as well as the fact that some species represent a higher potential monetary loss, are also reasons that seeds are tested for contamination. A matrix established by the BC MoFR Tree Seed Centre outlining the seed fungal testing priorities for the 3 important seedborne fungi in BC has been developed. The priorities for *Fusarium* testing are such that subalpine fir, coastal Douglas-fir, western larch, western white pine, and ponderosa pine are all rated high; amabilis fir, grand fir, western redcedar, interior Douglas-fir, western hemlock, Sitka spruce, interior spruce, and Sitka x interior spruce hybrid are rated medium; and coastal and interior lodgepole pine, and yellow cedar are rated low priority (Kolotelo and others 2001).

Past sampling has indicated average levels of *Fusarium* on seeds to be typically less than 2.5%, with a moderate degree of variation within seedlots (Kolotelo and others 2001). Not all species of *Fusarium* are pathogenic; those that are pathogenic are often weakly so. In addition, past studies to detect seedborne *Fusarium* in BC were often limited to genus. Thus, when routine fungal assays of seeds in BC were adopted, it was elected to detect levels within any seedlot at a relatively conservative level of 5%. To detect levels of 5% with a 95% degree of confidence requires a sample size of 500 seeds per seedlot for each seedlot tested. Samples are not adjusted for seedlot size, but sampling intensity is adjusted according to the ISTA (1999) standards. The laboratory methods used to test seedborne *Fusarium* are outlined in Peterson (2007), and testing for its presence

provides useful information for nursery growers.

The results of fungal assays are available for each seedlot tested on the Seed Planning and Registry Information System (SPAR), in seedlot detail reports from SPAR, as well as on the sowing request label sent to growers with each batch of seeds. Knowing the percentage of contaminated seeds within a seedlot provides growers, as well as others handling the seeds, with the option of taking steps to minimize their impact on seedling germination and growth. Historical records indicate contamination levels of greater than 5% within any seedlot to be significant for disease potential, and growers target seedlots with levels higher than this. The main strategy for levels above this are aimed at minimizing its ability to spread within a seedlot.

Seed orchard seeds appear to be affected by *Fusarium* at the same rate as seeds collected from natural stands (Peterson 2000). Current knowledge still does not provide a clear understanding of how cones become contaminated. More control is available, however, when collecting in seed orchards compared to natural stands, and Kolotelo and others (2001) point out 3 things that can be done when making these collections to prevent further spread of the fungus. First, cones should be collected during dry weather whenever possible. Second, cones should be stored in new, or steam- or hot water-sterilized sacks to prevent contamination from previous year's collections. Informal investigations conducted by AFS Limited for the BC MoFR have shown that cone sacks can become contaminated, and the sacks themselves, especially when wet, will readily trap airborne inoculum (Peterson 2004b). Third, filled sacks should be stored following the general recommendations for all species described in Portlock (1996).

The ability of *Fusarium*-contaminated seeds to intensify within seedlots during imbibition and stratification, as well as the management practices to reduce this phenomenon, are extensively reviewed in Kolotelo and others (2001). Seedborne *Fusarium* primarily exist as contaminants and do not readily infect the seed interior during storage. Infection of an emerging shoot and/or radical may occur in the germination phase, but Kolotelo and others (2001) point out that early stages of seed colonization are primarily dependent on abiotic factors, such as environmental water availability and temperature, rather than seed moisture content. Strategies to reduce the exposure of contaminated seeds to environmental conditions conducive to fungal growth can help prevent any intensification of seedborne *Fusarium* within a seedlot.

Three strategies to minimize losses from seedborne pathogens are: 1) eliminating or reducing initial inoculum; 2) slowing the rate of pathogen spread; and 3) shortening the time seeds are exposed to the pathogen (Berger 1977). It is valuable to view these strategies in the context of a disease triangle with the seeds as host, *Fusarium* the *pathogen*, and seed handling (from cone collection, through extraction, storage, sowing, germination, and seedcoat loss) as the environment, representing each corner, respectively. Sanitation encompasses cone collection procedures, seed orchard management and seed processing, falling into Berger's (1977) first category. Kolotelo and others (2001) relate seed treatments and storage to the second, and stratification and germination procedures to the third category. The first category is quite well understood, as presented by Eremko and others (1989), Leadem and others (1990), and Portlock (1996). Some questions still remain, however, with regard to when *Fusarium* become seedborne on seeds produced in orchards (Peterson 2007). Kolotelo and others (2001) present a good summary of collection methods to minimize seedborne disease, as well as discussions of seed processing. Initial research toward the potential for seedborne *Fusarium* to spread during seed processing has been started, but some questions still remain in this area (Kolotelo and Peterson 2006). Long term seed storage in BC generally takes place at -18 °C (0 °F), and between 4.9% and 9.9% moisture content, neither measure being conducive to fungal growth. Therefore

storage itself does not present a significant threat to spread or intensification.

Research in BC with regard to cone and seed pest management and seedborne *Fusarium* within Berger's (1977) third category to minimize loss to seedborne disease, that is, stratification and germination, has concentrated on treating tested seedlots having significant contamination by cleaning seed surfaces. Most research in this area has aimed at reducing seedcoat infestations (James 1985b; Axelrood and others 1995). And the importance of this research is emphasized by the findings that infestation levels of can increase significantly during seed imbibition and stratification (Axelrood and others 1995; Hoefnagels and Linderman 1999). Likewise, dry seed levels (< 1%) of *Fusarium*, below what is considered a potential disease threat (> 5%), can substantially increase during stratification to as high as 10% (Neumann 1996). Neumann investigated potential external sources that may have contributed to these increases, for example, airborne inoculum in the drying room and the soaking mesh and/or tanks. It was ultimately deemed, however, that as the observed "bulking up" of seedborne *Fusarium* occurred during the first 6 hours of imbibition, a faster water flow over the seeds during this time might be a simple cultural control to prevent this escalation. Further studies by Neumann (1997) concluded that simple cleaning of soaking tanks between seedlots could reduce inoculum and the potential for cross-contamination.

Applying fungicides directly to seedcoats to control seedborne *Fusarium* has been investigated. However, it is difficult to find fungicides that meet the many requirements necessary for their safe and effective application (Bennett and others 1991). These range from being suitably efficacious under different climatic conditions, being non-phytotoxic, being residue-free, as well as non-toxic to humans and wildlife. Earlier research findings of the negative effects of fungicides on seed germination, as well as variable efficacy and handling difficulties have all led to their reduced usage (Lock and Sutherland 1975; Lamontagne and Wang 1976; Wenny and Dumroese 1987).

The use of running water to imbibe seeds followed by a post-stratification running water rinse is the simplest strategy to reduce the intensification of seedborne contamination (Kolotelo and others 2001). Running water treatments appear to reduce the incidence of post-stratification seedborne *Fusarium* (James 1985b; Dumroese and others 1988; Axelrood and others 1995). The method used at the BC MoFR Tree Seed Centre is to imbibe seeds in mesh bags in a tank of running water for 24 to 48 hours. However, this requires significant water resources. Kolotelo and others (2001) suggest complete water changes every 4 to 8 hours will have similar effects. This is likely due to the response noted by Neumann (1996), that the first 6 hours of imbibition is critical for what she termed "bulking up" of seedborne *Fusarium.* Seed imbibition at the BC MoFR Tree Seed Centre generally involves soaking several sowing requests of differing seedlots and conifer species in the same tanks for a running water soak. However, Neumann (1995) identified a potential for cross-contamination between low- and highly-infested Douglas-fir and western larch seedlots when soaked together, and these are now soaked in individual tanks at the BC MoFR Tree Seed Centre.

Very effective chemical seed sanitation can be achieved using hydrogen peroxide. Differences in concentration and treatment duration, stratification timing, and conifer species tolerance exist, and an excellent summary of hydrogen peroxide seed treatment is presented in tabular form in Kolotelo and others (2001). It is worth noting that for 12 conifer species, 4 hydrogen peroxide concentrations, up to 10 exposure durations, and for both pre- and post-stratification treatments, no reductions in germination are indicated and neither were any increases in fungal contamination. In BC, the recommended hydrogen peroxide technique is to treat post-stratification seeds by immersing them in a 3% hydrogen peroxide solution, at 3:1 solution to seed volume ratio, for 30 minutes to 4 hours followed by a running water

rinse. The potential for reducing fungal levels on seeds with hydrogen peroxide clearly exists. However, some *Abies* species do not respond consistently, and for this reason Kolotelo and others (2001) suggest more research and operational studies are needed to address this.

Pre- and Post-emergence Damping-off

Pre-emergence Damping-off

Seedborne *Fusarium* are most often responsible for pre-emergence damping-off, that is, seeds that become infected and fail to germinate. Technically, the seed contents do not become infected prior to their being exposed to the environmental conditions of moisture and temperature that allow seedcoat inoculum to germinate. If these conditions are present while actual seed germination is slow to initiate, the seed contents may become infected and rot. However, what commonly happens below ground is that the beginnings of a radical and shoot will emerge and can become infected by any seedcoat inoculum present, with the result that no sign of a germinating seedling will appear above the soil line. Pre-emergence damping-off refers to both of the above situations. Given the appropriate conditions, pre-emergence damping-off might still occur in the absence of seedborne *Fusarium*. This can happen if sufficient inoculum is present in the growing media, most often encountered when dirty growing containers carry over inoculum from the previous year. Neumann (1993) did not find planting mix or water to be a source of inoculum in a 2-year study of seedborne *Fusarium* and root colonization of container-grown Douglas-fir, but she did suggest that other sources of inoculum likely came from wooden pallets. Axelrood and Peters (1993) found 50% of the cavities in operationally sanitized Styro-block™ containers to contain infested root fragments. They also found 60% of the growing cavities to be contaminated with *Fusarium* on their surfaces. *Fusarium* have also been found on the wooden pallets used to support growing containers, as well as on plant debris beneath these pallets (Neumann and Axelrood 1992).

Greenhouse sanitation, including floors, benching, pallets, and Styroblock™ containers, will all reduce levels of inoculum in the immediate vicinity of germinating seeds. However, these strategies are targeted more to reduce risk to seedlings in the post-emergence environment. Aside from employing the strategies outlined in the previous section to reduce the ability for *Fusarium* to intensify on contaminated seeds during imbibition and stratification, some other methods can be employed to reduce pre-emergence damping-off. For seedlots with a greater than 5% incidence of contamination, it is recommended that greenhouse temperatures be optimized to encourage rapid germination. This will often reduce the incidence of pre-emergence damping-off and promote rapid shedding of the seedcoat.

Post-emergence Damping-off

Young germinant or seedling root infections, resulting from roots growing in close proximity to germinating chlamydospores, can lead to stem rotting at the groundline, which typifies post-emergence damping-off. Seedborne *Fusarium* can also be responsible for post-emergence damping-off when the seeds germinate. However, for reasons such as a slow-to-shed seedcoat, for example, inoculum contacting and infecting the emergent tissues will often cause the new shoot to rot at the goundline. Young germinants rotting at the goundline and breaking or falling over at this point typify symptoms of post-emergence damping-off. The strategy of encouraging rapid loss of the seedcoat will reduce the time any contamination on the seedcoat surface is likely to be in contact with the germinating needles and stem, and can reduce losses here. It is also important during this growth phase to irrigate early in the day to encourage rapid drying of seedling foliage, which will also help reduce the spread of spore inoculum.

Sanitation of older Styroblock™ containers can significantly extend the useful life of these growing containers by reducing pathogen inoculum associated with old rough surfaces and associated extra-

neous root material from past years use. Peterson (1990) achieved significant reductions in levels on old Styroblock™ containers using a variety of sanitation techniques, and developed these into a set of practical guidelines for the sanitation of nursery seedling containers using either heat or chemical methods (Peterson 1991). The adoption of many of these guidelines is commonly used to extend container life while reducing the presence of inoculum in the container seedling root zone.

Seedling Wilt

Following germination and subsequent seedling growth, it is important to reduce stress on the plants. Seedlings can tolerate low levels of *Fusarium* on their roots. However, heat or drought stress can impair the seedling's ability to transport water and nutrients, especially if fungi have entered the roots and xylem tissues. Seedlings that continue to grow and become infected by either chlamydospores, introduced air- or water-borne spore inoculum, or from infected root fragments or dirty container surfaces, can be influenced by heat or drought stress leading to top blight or wilting. Top blight or wilt, also sometimes called late damping-off, often shows symptoms of needle chlorosis, browning, and desiccation with a hook or crooked-shaped leader tip.

Top blight and wilt damage will not necessarily lead to seedling losses if the trees are promptly treated with a systemic fungicide. In Canada, Senator® 70WP is registered for use on container greenhouse conifers for controlling *Fusarium*, and can be applied at 14-day intervals to provide systemic control. Also, as this damage is often initiated by a sudden heat or drought stress in the presence of the pathogen, the avoidance or reversal of these conditions can either prevent or reverse the symptoms and minimize any subsequent damage and loss. Sutherland and others (1989) point out that *Fusarium* can enter seedling roots early in a growing season, with disease development being delayed until the seedlings become stressed for moisture and nutrients.

The potential for beneficial soil or growing media amendment should be mentioned here, as the use of artificial media is particularly suited to this. Suppressive growing media can be created by introducing beneficial organisms, or by using media components that suppress disease organisms. An excellent review of this technology is presented by Linderman (1986). One soil-inhabiting fungus, that is, *Trichoderma harzianum*, is actively antagonistic to *Fusarium*, as it competes with the pathogen for substrate. *T. harzianum* is the active ingredient in the biological fungicide RootShield®, and it is best applied as a greenhouse and nursery soil amendment early in the growing season.

Seedling wilt caused by *Fusarium* usually only affects young germinants. Resistance of conifers to wilt diseases develops with ageing, and plant resistance to wilt pathogens is known to depend on the synthesis rate of phenolics, with free and bound phenolics preventing or retarding disease development. Shein and others (2003) treated Scots pine (*Pinus sylvestris*) seedlings with virulent spore suspensions of *F. sporotrichiella* and deduced conifer seedling resistance to wilt diseases to be correlated with the synthesis rate and accumulation of insoluble phenolic polymers. The ability to resist wilt was higher in plants unable to synthesize these polymers as they accumulated with age.

Late Damping-off

If left unchecked, seedling wilt can progress as foliage becomes chlorotic to brown, severe needle necrosis occurs, needles drop, and the seedling dies. Seedlings at this point usually become crooked at the leader and appear to die from the tip down. Little can be done to reverse the disease at this point. It is important, however, to remove affected seedlings, as they can lead to increased spore inoculum in the greenhouse. Left in containers, infected seedlings often develop salmon-pink sporodocia that produce and release conidia that can be splashed from irrigation water to infect adjacent seedlings. Not only is it important

to remove any infected seedlings at this stage, but attention must be paid to seedling growth. Seedlings usually present the above described symptoms during their rapid growth phase. This is characterized by accelerated tissue growth and expansion, and is generally considered the time when seedlings are most succulent and susceptible to infection. Dennis and Trotter (1995) point out that environmental and cultural manipulation during the rapid growth phase must concentrate on providing the seedling with select growing conditions in order to accentuate its growth potential. They also emphasize that seedling environment and culture have a significant impact on whether disease develops or not, pointing out that disease-causing fungi can infect seedlings at an early stage of development, and then remain latent and cause disease later in the growing season when plants become stressed.

Seedling Root Rot

Fusarium root rot, characterized by blackened, thin and wispy roots with little sign of actively growing root tips, is often the final stage of a disease continuum that may have begun with the seeds, or at least at the time of sowing. Not all species of *Fusarium* are pathogenic (James and others 1989), and many of those that are pathogenic are weakly so. However, Neumann (1993) points out a very important adaptive characteristic that contributes to its persistent ability as a pathogen, and that is that *Fusarium* are often facultative parasites well adapted for survival in either dormant (chlamydospores) or saprophytic states (Bruehl 1987). Saprophytic survival in container nursery settings occurs when dead root fragments, carried over on old containers, have been colonized by saprophytic *Fusarium* following parasitic colonization during the previous year. Thus, although often a weak pathogen that is tolerated in a stress-free environment, as a facultative parasite it persists in seedling containers, alternating between saprophytic and more aggressively pathogenic phases while the environment corner of the disease triangle changes as seedlings develop. Root rot often occurs later in the growing season, or can also occur if seedlings with infected roots are mishandled after leaving cold storage. *Fusarium* root rot does not necessarily lead to seedling losses if the damage to the root system is limited.

Seedling Mortality after Outplanting

Seedlings with minor amounts of *Fusarium* on their root surfaces, or low levels of root infection, often readily survive being outplanted when handled properly and not exposed to severe planting shock. However, when infested seedlings remain in storage or shipping containers under warm, moist conditions for extended periods prior to planting, the disease can rapidly develop into root rot, severely jeopardizing seedling survival. In British Columbia, the presence of *Fusarium* on seedling roots in the absence of any disease symptoms is generally not sufficient grounds to reject seedlings scheduled for outplanting. In fact, Axelrood and others (1998) concluded that *Fusarium* are probably of little consequence with regard to the mortality of seedlings on reforestation sites after they were unable to find a significant difference between seedling infections and root colonization. However, the mean age of the outplanted and naturally regenerated seedlings examined was 5.6 and 4.7 years, respectively, and they did not take into account seedling mortality that may have arisen immediately after outplanting. Thus, their results perhaps speak more for the long-term than for what might happen in the short-term, when planting shock may have a role in initial survival.

Fusarium can be isolated from visually healthy nursery-grown conifer seedlings (Bloomberg 1966; James 1986; Kope and others 1996). Because of this, Axelrood and others (1998) point out that the recovery of *Fusarium* from the roots of nursery-grown conifers does not necessarily indicate a disease situation. Instead, they state that this can be indicative of a potential for disease to develop following outplanting if conducive environmental (refer to environment corner of disease triangle) conditions are present. Container seedlings commonly have *Fusarium* on

or around their roots (Landis 1976; Graham and Linderman 1983; James 1985a), and it is therefore important that care is taken so any fungi present does not become aggressively pathogenic. Seedlings scheduled for outplanting must never be allowed to remain in boxes, or in conditions where they can become overheated and the roots remain warm and moist for prolonged periods. Under such conditions, *Fusarium* can rapidly spread from seedling to seedling, as well as intensify within the roots of infected seedlings. When outplanted following these conditions, seedlings can quickly succumb to planting shock and, if exposed to a subsequent heat or drought stress, will often die.

Cone and Seed Pest Management: Vision for the Future

Understanding the disease biology of the major fungal pathogens of forest nursery conifer seedlings in BC has been an important step toward developing pest management plans to eliminate or minimize their impact on cone production and seed handling, as well as forest nursery seedling production and increased seedling survival after outplanting on reforestation sites.

Fungi in the genus *Fusarium* can negatively affect the reforestation value chain from the time of cone and seed production, through seed handling and processing, as well as during the course of nursery operations, to successful survival of outplanted seedlings. Increased understanding of *Fusarium* host-pathogen interactions throughout many aspects of conifer seedling production in BC is desirable.

A vision of increased seed and seedling survival from cone and seed production to outplanting at reforestation sites is attainable through better understanding of the disease mechanisms associated with these fungi, and will lead to the development of more effective cone and seed pest management plans.

Increased understanding is needed of the following *Fusarium* host-pathogen interactions of conifer seedling production in BC:

1) Seedborne contamination:
   a) How seeds become contaminated is still not clear;
   b) It remains unclear how seed contamination may be exacerbated during cone and seed processing.

2) Pre- and post-emergence damping-off:
   a) Develop better Standard Operating Procedures (SOP) to eliminate *Fusarium* as introduced greenhouse pests, that is, benching, pallets, and container sanitation;
   b) Improve seedcoat disinfestations procedures.

3) Seedling wilt:
   a) Improve SOP to reduce risks to sudden heat or drought stress induced transpirational demands.

4) Late damping-off:
   a) Improved understanding of 2 and 3 will help resolve this.

5) Seedling root rot:
   a) Improved understanding of all steps 1, 2, 3 and 4 will reduce losses to root rot;
   b) Improved handling practices during storage and especially post-storage will reduce losses to root rot.

6) Seedling mortality after outplanting:
   a) Plantation failure usually occurs when *Fusarium* have survived through all the components of the reforestation value chain described above, and a satisfactory environmental component of the disease triangle is met at the reforestation site. Improved understanding of value chain steps 1 through 5 could lower the incidence at the pathogen corner of the disease triangle to below what is necessary to cause significant losses at the reforestation site.

Cone and Seed Pest Management: Mission Statement

For the vision of increased seed and seedling survival from cone and seed production to outplanting at reforestation sites to be attainable, better understanding of the disease mechanisms associated with *Fusarium* and seedling production are needed. Many of these mechanisms are understood individually, perhaps the most important being the fact that the fungi are facultative parasites. As such, it has the ability to move in and out of a pathogenic or saprophytic relationship with its host, depending, in part, on the conditions at the environment corner of the disease triangle. The ability to survive as a saprophyte on tissues it has previously colonized as a parasite allows some fusaria to enter the reforestation value chain as a seedborne contaminant and still pose a threat to seedling survival many months later at the reforestation site. Better understanding of the key components of this value chain and the interactions between host, pathogen, and environment will allow the development of cone and seed pest management plans so that interventions can be made to break these disease triangle connections where possible.

To achieve the vision of increased seed and seedling survival from cone and seed production to outplanting at reforestation sites requires building on the current state of knowledge with regard to *Fusarium* as cone and seed pests. Specifically, better understanding is needed as to how seeds become contaminated; how seed contamination may be exacerbated during cone and seed processing needs to be examined; and what improvements if any, can be made to seedcoat disinfestation procedures.

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Physiological Responses of Planting Frozen and Thawed Douglas-Fir Seedlings

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ABSTRACT
We studied the short-term (7-day) physiological responses of planting thawed and frozen root plugs of Douglas-fir (*Pseudotsuga menziesii*) seedlings in 2 separate experiments under cool-moist and warm-dry growing conditions, respectively. Our results showed that shoot water potential, root hydraulic conductance, net photosynthesis (A), and transpiration (*E*) were significantly lower in frozen seedlings compared with thawed seedlings under both growing conditions. Chlorophyll fluorescence values in frozen and thawed seedlings were similar throughout the measurement

period at both growing conditions except at 0 hours. We detected no significant differences in electrolyte leakage and chlorophyll content between frozen and thawed seedlings under both environmental regimes. When planted under warm-dry conditions, thawed seedlings had more buds that began to elongate and had more new roots than frozen-planted seedlings at the end of the experiment. When planted in cool-moist conditions, however, neither frozen nor thawed seedlings had buds that resumed growth. Comparatively higher photosynthesis rates observed in thawed seedlings planted under warm-dry conditions might have contributed toward the production of more new roots, and could be advantageous for survival and early growth after outplanting.

KEYWORDS
chlorophyll fluorescence, gas exchange, *Pseudotsuga menziesii*, root hydraulic conductance, transplant stress, water relations

Introduction

Dormant conifer seedlings are often stored in freezers prior to spring outplanting in temperate climates (McKay 1997; Paterson and others 2001). Freezer storage mainly provides flexibility in scheduling seedling delivery to outplanting sites in spring (Rose and Haase 1997). Frozen seedlings, however, require thawing prior to outplanting to facilitate seedling separation because root plugs freeze together during storage (Kooistra 2004). Proper thawing of root plugs demands additional nursery resources, and can have potential negative impacts on seedling health and physiology (Hocking 1971; Puttonen 1986). These factors suggest that direct outplanting of frozen root plugs after removal from storage would streamline the seedling production process and improve logistics of seedling delivery (Kooistra and Bakker 2002).

Several studies have reported that outplanting frozen seedlings had no damaging effects on seedling performance compared with thawed seedlings (Silim and Guy 1998; Kooistra and Bakker 2002, 2005). When seedlings are outplanted into warm soil, (18 to 32 °C [64 to 90 °F]), thawing of frozen root plugs is unnecessary (Camm and others 1995). However, Helenius (2005) reported higher mortality and reduced growth in Norway spruce (*Picea abies* L. Karst) seedlings outplanted frozen compared with thawed cohorts.

Spring outplanting of trees usually starts when average ambient temperature is above freezing, and could continue at difficult-to-access sites until the average ambient temperature rises to 30 °C (86 °F). These conditions are likely to occur under certain circumstances, such as when snow limits access to high elevation sites until early summer. For example, frozen root plugs of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) outplanted in Colorado, and ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and western white pine (*Pinus monticola* Dougl. ex D. Don) outplanted in Idaho, under hot, sunny weather showed nearly 100% mortality (Jacobs, personal observation; Dum-roese, personal observation). Heikurinen (1981) reported similar plantation failure using frozen root plugs. Therefore, it is possible that the physiological dysfunctions may occur immediately, and be expressed in a short time interval after outplanting.

Although some studies have focused on the effects of thawing regime and long-term response of frozen-planted root plugs (Camm and others 1995; Kooistra and Bakker 2002; Helenius and others 2004; Helenius 2005), little is known about the short-term physiological changes occurring in seedlings planted with frozen root plugs under relatively high or low ambient air temperatures. In the present study, we examined short-term (7-day) responses of planting frozen and thawed Douglas-fir (*Pseudotsuga menziesii*) seedlings into 2 growing conditions—cool-moist at 10 °C (50 °F) and relative humidity (RH) of 75%; hot-dry at 30 °C (86 °F) and RH 50%—to better understand the response mechanisms that take place immediately after transplanting.

Study Procedure

Plant Material

Douglas-fir seeds were collected from the Flathead National Forest in western Montana (Hun-

gry Horse Ranger District; elevation 1675 m [5495 ft]) and were grown in 3 x 15 cm (1.2 x 6 in) containers (315B [160/90] Styroblock™, Beaver Plastics, Ltd, Edmonton, Alberta, Canada) filled with 1:1 (v:v) peat:vermiculite medium at the USDA Forest Service research facility in Moscow, Idaho (46.7°N, 117°W) for one season using standard operational methods. A total of 125 seedlings were placed in groups of 20, sealed in plastic bags, placed in boxes, and shipped to Purdue University in West Lafayette, Indiana in December 2006. Upon arrival at Purdue University, the root plug of each seedling was wrapped with Saran™ premium wrap and the group of 20 seedlings were placed into sealed plastic bags and stored in a freezer at approximately -2 °C (28 °F) until the experiment started.

Root Plug Treatments, Growing Conditions, and Experimental Design

At the beginning of the experiment, a sub-sample (n = 5) of seedlings had the following characteristics (mean ± SE): height (19.0 ± 0.9 cm [7.5 ± 0.35 in]); root collar diameter (1.90 ± 0.08 mm); shoot (0.66 ± 0.01 g) and root (0.62 ± 0.04 g) dry mass. For frozen root (FR) planting, seedlings remained in freezer storage until the time of planting for both experiments. For thawed root (TR) planting, seedlings were taken out of freezer storage and kept at room temperature for 24 hours to ensure proper thawing prior to the start of the experiment. Thawing was done in dark conditions. Seedlings were planted into Treepot™-Tall One (36 x 10 cm [14 x 4 in]; 2.83 L [0.75 gal]) pots (Stuewe and Sons, Inc, Corvallis, Oregon, USA) filled with 2:1 (v:v) peat:vermiculite and immediately transferred to the growth chamber.

Experiment 1— Cool-Moist Conditions

Seedlings were transferred to a controlled environment chamber with a day temperature of 10 °C (50 °F) and night temperature of 6 °C (43 °F), relative humidity of 75% ± 2.5 %, and an 18-hour photoperiod with photosynthetic photon flux density, measured at seedling top height, of 300 mmol/m^2/s, provided by fluorescent lamps and incandescent bulbs.

Two groups of root plug treatments (a total of 60 seedlings), frozen roots and thawed roots, were randomly distributed within the growth chamber. Chlorophyll fluorescence, gas exchange, and shoot water potential measurements were taken at 0, 6, 12, and 24 hours, and 3 and 7 days, while root hydraulic conductance, electrolyte leakage, chlorophyll content, and root respiration measurements were taken only at 0 hours, and 1, 3, and 7 days. At each measurement time, measurements were taken on 5 randomly selected seedlings from each treatment. The experimental design was completely randomized.

Experiment 2— Warm-Dry Conditions

For the second experiment, the conditions, experimental design, and sampling were the same as Experiment 1, except that the controlled environment chamber was set at a day temperature of 30 °C (86 °F) and a night temperature of 20 °C (68 °F), with a relative humidity of 50% ± 2.5 %.

Chlorophyll Fluorescence and Gas Exchange Measurements

Leaf photochemical efficiency was expressed as leaf chlorophyll fluorescence (F_v/F_m). Chlorophyll fluorescence (CF) was measured on the upper 3 cm (1.2 in) portion of the shoot using an integrated fluorescence chamber head, LI-6400-40 leaf chamber fluorometer (LI-COR, Inc, Nebraska), on 5 different seedlings from each treatment at 0, 6, 12, and 24 hours, and 3 and 7 days after planting.

The terminal shoots were allowed to dark-adapt by covering the shoots with Ultra-black film for 20 minutes before CF measurements. Maximum fluorescence (F_m) was determined following a red light saturating pulse (> 7000 µmol photons/m^2/s) and centered at wavelength 630 nm. The F_v/F_m ratio estimates maximal quantum yield of PS II photochemistry in dark-adapted needles.

Gas exchange measurements were performed after chlorophyll fluorescence measurements using a LI-6400 portable photosynthesis system and 6400-05 conifer chamber on the same 5 different seedlings from each treatment at 0, 6, 12, and 24 hours, and 3 and 7 days after planting. Shoot water potential was determined using a pressure chamber immediately following gas exchange measurements.

Root Hydraulic Conductance

Roots hydraulic conductance was measured in intact roots of the same seedlings used for the gas exchange measurements with a high pressure flow meter (HPFM) as described by Tyree and others (1995). The use of the HPFM allows for measurement of intact roots, because water is applied under increasing pressure through an excised stem (around root collar level) into the whole root system (Tyree and others 1995). Stems of both frozen and thawed seedlings were cut 2 cm (0.8 in) above the root collar, and flow rates of all seedlings were measured over a range of 0 to 2.75 MPa (0 to 27.5 bars) to obtain a linear pressure-flow relationship (Tyree and others 1995). Root hydraulic conductance of 5 root systems was measured for each treatment on each measurement period and expressed as kg/MPa/s.

Needle Electrolyte Leakage

Following the measurement of root hydraulic conductance, needle electrolyte leakage (a measure of cell integrity and cell membrane leakiness) was measured on the same seedlings with a Seven-Easy Conductivity meter as described by Zwiazek and Blake (1990). Approximately 100 mg (fresh weight) of needles were taken from 5 seedlings per treatment, washed with deionized water, and placed in separate vials, each containing 15 ml of deionized water. After incubation for 6 hours on an orbital shaker, electrical conductivity of each solution (initial conductivity) was measured. Total electrolytes of the samples were obtained by autoclaving the samples at 120 °C (248 °F) for 20 minutes. The autoclaved samples were allowed to cool, total electrolytes of the sample solutions were measured, and electrolyte leakage (EL) was calculated as initial conductivity as a percentage of the total electrolytes.

Chlorophyll Content

Needle chlorophyll was measured using the method of Arnon (1949) as modified to use dimethyl sulfoxide (DMSO) (Hiscox and Israelstam 1979). Needles (100 mg) were placed in test tubes with 7 ml of DMSO and transferred to an oven at 68 °C (154 °F) for 30 minutes, with a marble on top of each tube to prevent solvent evaporation. The sample was then removed from the oven and made up to a total volume of 10 ml with DMSO. A 3-ml aliquot was transferred to a cuvette to measure absorbance. A Perkin-Elmer LC-95 UV/Visible spectrophotometer was used to measure the absorbance of the solution at 645 and 663 nm. Chlorophyll *a* (C_a), chlorophyll *b* (C_b), and total chlorophyll (C_T) were determined from the absorbance at 645 (D_{645}) and 663 (D_{663}) according to Arnon's formulae (1949).

Root Respiration

Root respiration, which was measured as oxygen uptake using an oxygen electrode (Model 58, Yellow Springs Instruments, Inc, Ohio), was determined at 1, 3, and 7 days after planting. The oxygen probe and the root system were placed in a 1500 cm^3 (91.5 in^3) airtight cylinder filled with aerated distilled water that was continuously stirred with a magnetic stirrer. Root respiration measurements were made in respective growing temperatures and were monitored for 20 minutes by recording the oxygen uptake every 4 minutes. Root respiration rates were calculated as a mean of oxygen uptake over time and values were expressed in mmol $O_2/cm^3/min$.

Statistical Analysis

Analysis of variance (ANOVA) was performed using SAS (SAS Institute Inc, Cary, North Carolina). The means were compared using Tukey's pairwise multiple comparisons test, and were

considered significantly different at $P \leq 0.05$. Gas exchange, F_v/F_m, root hydraulic conductance, shoot water potential, needle electrolyte leakage, chlorophyll content, and root respiration were analyzed for each measurement period.

Results

Experiment 1— Cool-moist Conditions

F_v/F_m values for frozen and thawed seedlings were 0.69 and 0.72, respectively, at the beginning of the experiment (0 hours), but they were not significantly different. F_v/F_m values for frozen- and thawed-planted seedlings varied from 0.60 to 0.73 for the duration of the experiment.

Negative mean values for A were recorded in frozen seedlings at 0 hours, but photosynthesis rates continued to increase as the experiment progressed. Although thawed-planted seedlings exhibited significantly higher rates of photosynthesis than frozen-planted seedlings, rates dropped on day 3, and increased on day 7. Photosynthesis rates ranged from -0.5 to 2.16 mmol $CO_2/m^2/s$ in frozen seedlings, and from 0.77 to 2.7 mmol $CO_2/m^2/s$ in thawed seedlings during the experiment. Thawed-planted seedlings maintained higher rates of stomatal conductance during the measurement period compared to frozen-planted seedlings. Stomatal conductance ranged from 0.01 to 89.48 mmol $H_2O/m^2/s$ in frozen seedlings, and from 36.63 to 117.79 mmol $H_2O/m^2/s$ during the experiment. A similar trend in transpiration was observed for both thawed- and frozen-planted seedlings, where it ranged from -0.001 to 1.08 mmol $H_2O/m^2/s$ in frozen seedlings, and from 0.33 to 1.58 mmol $H_2O/m^2/s$ in thawed seedlings during the experiment.

The root treatments had significant effect on shoot water potential (y_w). Thawed-planted seedlings had significantly less negative y_w compared to frozen-planted seedlings at 12 hours, but they maintained a less negative water potential compared to frozen seedlings throughout the experiment. Although thawed-planted seedlings showed slightly higher rates of root hydraulic conductance than frozen-planted seedlings, they were not significantly different. Overall, root hydraulic conductance was also significantly higher in thawed-planted seedlings compared to frozen-planted seedlings. There were no significant differences in needle electrolyte leakage, root respiration rates, and chlorophyll content between frozen- and thawed-planted seedlings.

No terminal or lateral buds began to elongate either in frozen- or thawed-planted seedlings during the duration of the experiment.

Experiment 2—Warm-dry Conditions

At the beginning of the experiment (0 hours), F_v/F_m values for frozen and thawed seedlings were 0.64 and 0.74, respectively, and they were significantly different. Thereafter, the F_v/F_m values ranged from 0.72 to 0.75 in both frozen- and thawed-planted seedlings for the duration of the experiment. Although F_v/F_m values were higher for thawed than for frozen seedlings at 12 hours and 7 days, the differences were not statistically significant.

In general, thawed seedlings maintained significantly higher rates of photosynthesis than frozen-planted seedlings. Frozen-planted seedlings had a very low mean value for photosynthesis (0.110 µmol $CO_2/m^2/s$) at 0 hours compared to a significantly higher rate of A (3.049 µmol $CO_2/m^2/s$) in thawed seedlings. Photosynthesis increased gradually in frozen-planted seedlings from 0 hours to 3 days, but declined on day 7. Thawed seedlings had significantly higher rates of photosynthesis than frozen-planted seedlings at 12 hours, but their overall rates fluctuated throughout the measurement period. Photosynthesis rates ranged from 0.11 to 2.5 µmol $CO_2/m^2/s$ in frozen seedlings, and from 2.35 to 4.13 µmol $CO_2/m^2/s$ in thawed seedlings during the experiment. Stomatal conductance and transpiration measurements showed the same overall trend. The rates of g_s and E were higher in thawed-planted seedlings, except for day 1. Values for g_s and E were significantly lower in seedlings planted while root plugs were frozen compared with thawed root plugs at 0 hours. Thawed

seedlings maintained higher rates of stomatal conductance and transpiration after 7 days compared to frozen-planted seed-lings, but differences were not significant.

The root treatment had significant effects on shoot water potential (y_w). Over the course of the experiment, thawed-planted seedlings had significantly less negative y_w compared to frozen-planted seedlings at 0 hours and 1 day. Time zero y_w values were -1.34 ± 0.10 MPa (-13.4 ± 1.0 bar) and -1.03 ± 0.04 MPa (-10.3 ± 0.4 bar) for frozen and thawed seedlings, respectively. The frozen seedlings showed more negative y_W over the measurement period. Frozen seedlings had a gradual increase in y_w after 3 and 7 days. Overall, root hydraulic conductance was also significantly higher in thawed-planted seedlings compared to frozen-planted seedlings. Needle electrolyte leakage was significantly higher on day 3 in frozen-planted seedlings, although EL was not significantly different at any other measurement period. One day after planting, root respiration rates were 25% higher in frozen-planted seedlings (2.15 mmol $O_2/cm^3/min$) compared to thawed-planted seedlings (1.61 mmol $O_2/cm^3/min$). However, root respiration remained the same for frozen and thawed seedlings after 3 and 7 days.

Thawed-planted seedlings had a significantly higher number of new roots on day 7 (38 ± 5) compared to frozen-planted seedlings (20 ± 5) (Figure 1). Although thawed-planted seedlings had a higher mean number of broken terminal and lateral buds (3.6 ± 0.2) than frozen-planted seedlings (0.4 ± 0.2) at 7 days, differences were not significant.

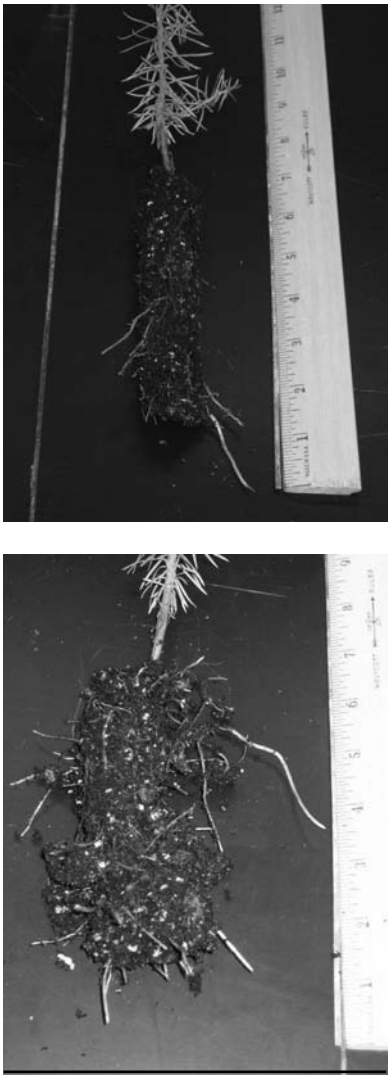

Figure 1. Development of new roots in frozen (left) and thawed (right) root plugs 7 days after planting.

Discussion

Chlorophyll fluorescence (F_v/F_m) value reflects the potential quantum efficiency of PS II and provides a sensitive indicator of plant photosynthetic performance (Björkman and Demmig 1987) and plant stress. F_v/F_m value close to 0.80 indicates a healthy seedling, while a decrease from this value indicates a stress (Fracheboud and others 1999). In our study, we observed high-

er ranges of F_v/F_m values for frozen and thawed seedlings, even at 0 hours. The lower initial (0 hours) F_v/F_m values in frozen and thawed seedlings may suggest that physiological processes (for example, photosynthetic apparatus of PS II) were not yet metabolically activated to resume normal growth. Both frozen and thawed root plugs showed an increase in F_v/F_m values 6 hours after planting under both environmental regimes. The increase in F_v/F_m values in both treatments after 6 hours may suggest that they began to become metabolically active.

In order to meet transpirational demands, plant roots must efficiently and continuously absorb and transport water from soil to shoot. An initial higher resistance to root water uptake soon after outplanting causes seedling water stress, and this may subsequently lead to outplanting failures if frozen root plugs are outplanted. In both experiments, we have shown that y_w was significantly higher under cool-moist and warm-dry conditions, respectively, in thawed-planted seedlings than in frozen-planted seedlings. This water deficit condition in frozen was possibly because of lower root plug water content in frozen seedlings compared with thawed seedlings, suggesting that the water stored in the frozen seedlings was not available to roots. This is reflected by relatively low root hydraulic conductance in frozen roots compared to thawed roots in both growing environment experiments. Because root plugs were still frozen at 0 hours, the lower y_w observed in frozen seedlings compared with thawed seedlings could be related to a reduction in root water uptake resulting in decreased g_s and E and, in turn, resulting in much lower A rates than where roots are thawed. Previous studies revealed that both g_s and root water uptake were reduced when plants were exposed to different environmental stresses (Wan and others 1999; Kamaluddin and Zwiazek 2001), suggesting that root water flow and overall plant water status are interrelated. In our present study, it is plausible that the decline in E and g_s in frozen-planted root plugs was partly due to the reduction in root hydraulic conductance.

Although no significant new root growth was observed in either frozen or thawed seedlings under cool-moist conditions, thawed seedlings grown under warm-dry conditions had significantly more new roots than frozen-planted seedlings (Figure 1). Thawed seedlings had a greater mean number of broken buds compared to frozen-planted seedlings under warm-dry conditions. Higher net photosynthesis rates probably contributed towards emergence of more new roots in thawed seedlings. This has been confirmed in a study by van den Driessche (1987), where new root growth in Douglas-fir and Sitka spruce (*Picea sitchensis*) seedlings was associated with current photosynthesis.

In our present study, we observed reduced levels of root hydraulic conductance in frozen-planted seedlings under both environmental regimes. Disruption of root plasma membrane functions could be a factor that would interfere with water uptake (Crane and Möller 1988). Apostol and Zwiazek (2003) have shown that an increase of tissue ion leakage indicates loss of membrane integrity and, consequently, leads to failure in root functions. The comparatively higher root respiration rates in frozen seedlings compared with thawed seedlings, as an initial transient response, might reflect increased respiratory substrates from damaged cells, which is commonly observed as a wounding response (Klotz and others 2003). On the contrary, the higher oxygen uptake by thawed roots planted under cool-moist environment could possibly be due to resumption of metabolic and cell repair processes. It is quite possible that frozen-planted roots planted under cool-moist conditions had not started the same processes because the soil temperature was not very conducive for higher oxygen uptake. We hypothesize that a similar mechanism affecting survival in frozen seedlings is partly due to membrane permeability, resulting in increased membrane electrolyte leakage. This requires further investigation.

Conclusions

In both cool and hot environments, net photosynthesis, transpiration, stomatal conductance, shoot water potential, and root hydraulic conductance (RHC) for thawed-planted seedlings were higher than frozen-planted seedlings. We conclude, however, that higher photosynthesis and water conductance rates in thawed seedlings planted at both cool-moist and warm-dry conditions would help them overcome initial outplanting stress, and the contribution of higher photosynthesis rates in thawed-planted seedlings may prove advantageous for survival and early growth. Our results, combined with those of other studies, suggest that field establishment success of frozen root plugs is dictated by environmental conditions to which they are exposed at outplanting. Under hot conditions with high vapor pressure deficit combined with cold and/or dry soils, thawing delay could lead to an imbalance between root water uptake and transpiration that can cause desiccation and may lead to mortality. Potential for frozen plugs to establish and survive is high, however, when outplanting under cloudy, cool conditions with high relative humidity and low vapor pressure deficit combined with warm and moist soils. On sites and at outplanting dates when these stressful environmental conditions are likely to prevail, it may be advisable to avoid outplanting frozen root plugs. Future studies (longer duration) are needed to find the link between more specific physiological mechanisms (for example, root hydraulic conductance, root membrane injury) that may dictate outplanting performances of frozen root plugs from a wider range of species and stocktypes.

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Somatic Embryogenesis Tissue Culture for Applying Varietal Forestry to Conifer Species

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

KEYWORDS
vegetative propagation, rooted cuttings, loblolly pine, southern yellow pine

Vegetative Propagation Systems

Two criteria are considered important for the successful implementation of vegetative propagation systems within an operational forestry program. First, the propagation system must have the ability to preserve superior candidate varieties. The ability to propagate elite varieties requires a capacity to maintain varieties in a form capable of regenerating after a minimum period of 5 to 10 years needed in order to test and select varieties in the field. Second, the propagation system has to be able to multiply selected varieties in large enough numbers at a reasonable cost. If these 2 criteria can be reasonably met, the selected vegetative propagation systems can be imple-

mented to incorporate varietal forestry within an operational forestry program.

There have been major advances over the past 50 years in the development of operational vegetative propagation systems for conifer species used in plantation forestry programs. These propagation systems provide a means of bringing new genetic material into forestry programs through the capture of a greater proportion of the genetic gain inherent within a selected tree species. Vegetative propagation systems also provide a method for multiplying superior varieties and/or families identified in tree improvement programs.

Rooted Cuttings

Currently, rooted cuttings are a propagation technique that is available on an operational level to multiply specific varieties that have desirable traits. The primary use of rooted cutting technology is for bulk production of genetically improved materials. This propagation technique is used world-wide to produce tens of millions of rooted cuttings for forest regeneration programs.

Micropropagation through Organogenesis Tissue Culture

Organogenesis is a tissue culture system that relies on the multiplication of shoots or the *de novo* formation of organs originating from either unorganized callus, preformed shoots, or induced buds. Propagules produced through this system are essentially treated as microcuttings. Thus, shoot propagules are placed in an optimal rooting environment and treated in a similar manner as cuttings. This propagation technique has been used in operational forestry programs in New Zealand on radiata pine (*Pinus radiata*).

Somatic Embryogenesis

Somatic embryogenesis (SE) is a tissue culture approach where proliferative embryo suspensor masses are established from non-meristematic cells and subsequently cultured to produce organized somatic embryos possessing shoot and root meristems. The term somatic refers to embryos developing asexually from vegetative (or somatic) tissue. This method has been used in horticulture and agriculture on a limited basis, and is now being used to a greater scale in forestry.

Somatic embryogenesis is the only vegetative propagation technology that provides long-term preservation of the selected genetic component of a conifer species that can be used for extended timeframes within an operational forestry program. Thus, somatic embryogenesis is now becoming a viable operational propagation technology for conifer species.

Basic Laboratory Protocols for Somatic Embryogenesis in Conifers

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

Culture Initiation

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

Proliferation

Maintenance of embryonal suspensor mass is characterized by the presence of early-stage somatic embryo structures that are analogous to those occurring during normal seed development. This is followed by a multiplication step when the tissue multiplies and develops as early-stage somatic embryos. Embryogenic cultures can be proliferated in a juvenile form for long periods of time to produce unlimited numbers of propagules from the same variety. At this point, tissue can be allowed to continue to grow or it can be placed into long term storage.

Cryopreservation

Cryopreservation is a means whereby germplasm can be stored. The embryogenic tissue is treated with cryoprotectants, frozen to -35 °C (-31 °F) under a controlled freezing rate, and then

Figure 1. Tubes of embryogenic tissue being removed from cryopreservation storage containers (Courtesy of CellFor Inc).

Figure 2. Nursery production of CellFor Inc bareroot loblolly pine (*Pinus taeda*) somatic seedlings growing at Plum Creek Jesup Nursery, Georgia.

subsequently stored in liquid nitrogen (-196 °C [-321 °F]) (Figure 1). Cryopreserved tissue can be stored indefinitely and then regenerated within a few weeks after a simple thawing process. This long term storage option offers a distinct advantage of somatic embryogenesis tissue culture over rooted cuttings and organogenesis tissue culture.

Maturation

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

In vitro Germination

The final lab step is *in vitro* germination, in which embryos are placed on a germination medium under controlled environmental conditions. *In vitro* germination occurs within a week and proceeds to the development of true needles. At this point, young somatic seedlings can be transferred to *ex vitro* nursery conditions.

Nursery and Field Performance of Conifer Somatic Seedling Stocktypes

From the early 1990s until the present, germinants from somatic embryogenesis technology have shown continued improvement in their development into high-quality somatic seedlings in the nursery. In addition, somatic seedling propagation technology has been successfully integrated into both the bareroot (Figure 2) and container seedling production systems. The initial response of germinants to the nursery environment will have a profound influence on subsequent morphological development. Recent nursery performance of somatic seedlings has shown that a proper nursery cultural environment (nutrients, temperature, and moisture) during the initial establishment stage will result in normal morphological development of seedlings. Thus, currently produced somatic seedlings consistently meet morphological standards required for production of operational bareroot or container seedlings. Reforestation site trials have tested the field performance of somatic seedlings and have found that somatic and zygotic seedlings have comparable field performance as a stocktype, indicating that somatic seedlings have all of the traits that are desired in seedlings for use in forest regeneration programs.

Integration into Tree Improvement Programs

There is an opportunity with somatic embryogenesis technology to capture value-added traits at the individual tree or family level. Testing of progeny from selected parents will capture additional gain for improved performance. The value-

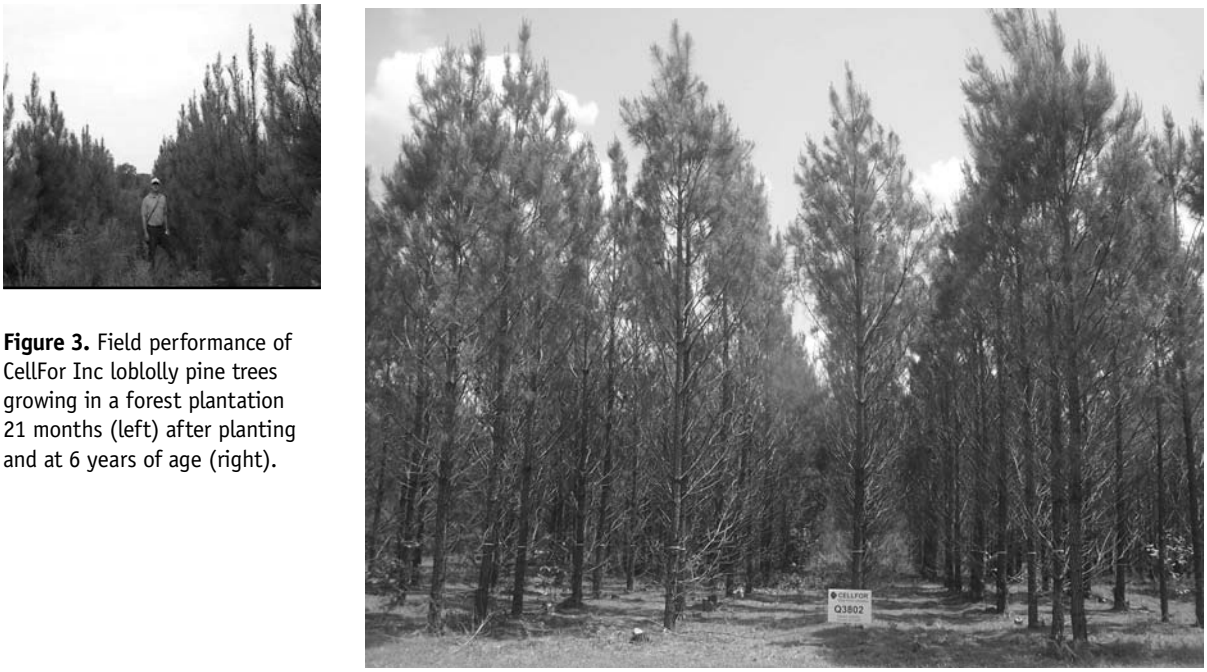

Figure 3. Field performance of CellFor Inc loblolly pine trees growing in a forest plantation 21 months (left) after planting and at 6 years of age (right).

added traits that can be captured and propagated through somatic embryogenesis are those that can be identified through any tree-breeding program, and include growth and yield, wood quality, plus stress, pest, and disease resistance. Thousands of varieties can be produced for field trials from selected families having desirable genetic traits. Embryogenic cultures from these varieties can be cryopreserved for long-term storage until field selections are made. Ultimately, a population of varieties, large enough to ensure genetic diversity, can be selected based on field performance criteria. From this type of selection program, CellFor Inc, among others, now commercially offers elite varieties of loblolly pine (*Pinus taeda*) seedlings with yield improvement averaging 42% (Figure 3). At Cellfor Inc, the selected varieties are removed from cryostorage and produced as somatic seedlings in the 10s of millions, and are then deployed operationally to reforestation sites as diverse genetic mixtures.

Operational Somatic Embryogenesis Production System

Commercial acceptance of a novel technology such as somatic embryogenesis requires the ability to develop and implement a successful operational use for the technology. The key components that must be addressed during this stage are as follows:

1) development of a cost-effective manufacturing process;
2) delivery of high-quality products that provide predictable and reproducible performance;
3) technology validation and promotion in the market place.

During the past decade, significant progress has been made towards developing reliable, high-volume, cost-effective somatic embryogenesis production systems. Organizations are working on commercialization programs for spruce (*Picea* spp.), Douglas-fir (*Pseudotsuga menziesii*), loblolly pine, and radiata pine.

Future of this Technology in Operational Forestry Programs

Forestry companies, advance seed production companies, and government organizations around the world are currently working at bringing this tissue culture technology to a point where it can produce conifer somatic seedlings, on a cost effective basis, with desired genetic characteristics because of the potential return on this investment. For example, in the southeastern US, the returns of planting elite varieties of loblolly pine seedlings produced from this tissue culture technology are evident in more tons per acre grown per year, with fewer diseased stems, higher quality, and straighter logs with small knots that will command the highest prices on the sawtimber market. A southeastern US landowner can expect to realize a 10% to 18% return from an investment in seedlings produced from these elite loblolly pine varieties, and harvest revenues that may be 75% greater in terms of 2006 dollars (net present value) than revenues from traditional orchard stock. With these types of potential economic returns, somatic embryogenesis is now a viable vegetative propagation system within operational forestry programs.

In the North American forestry market, CellFor Inc and Arborgen are currently the 2 companies using somatic embryogenesis tissue culture technology to apply varietal forestry in the production of conifer seedlings for the commercial market place. For the 2008 planting season, CellFor Inc will produce 10 million seedlings, while Arborgen will produce 500,000 to 1 million seedlings of southern yellow pines. In the southern US, the current market for southern yellow pine seedlings is 1.2 billion seedlings on an annual basis. It is projected that the market share of elite varieties of yellow pine will be 5% within the next 2 to 3 years. Thus, the application of somatic embryogenesis as a viable operational propagation technology for producing elite varieties of conifer species is just coming of age.

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Gene Activity Test Determines Cold Tolerance in Douglas-fir Seedlings

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KEYWORDS
cold hardiness testing, seedling dormancy, *Pseudotsuga menziesii*, whole plant freeze test, gene expression analysis

Forest tree nurseries rely on a tight scheduling of operations to be able to deliver vigorous seedlings to the planting site. Cooler or freezer storage is often used to maintain planting stock in an inactive condition and to ensure a plant supply for geographically diverse planting sites, which is a requirement for large-scale or internationally operating nurseries.

Nursery growers often wish to know the earliest possible time when they can lift and store seedlings, and optimum scheduling depends on the physiological condition of the seedlings. Lifting and storage of insufficiently hardened plants reduces their vitality and may lead to cold damage, dehydration, and fungal infection. To prevent this kind of damage and its adverse economic effects on nurseries and end-users, it is of vital importance to be able to accurately determine the peak physiological condition for lifting or transfer. Cold hardiness testing is one method to determine seedling physiological condition because it is strongly linked to the seedling dormancy cycle and stress resistance, and is influenced by seed source, nursery practices, and environment (Faulconer 1988; Burr 1990).

Cold hardiness is traditionally defined as a minimum temperature at which a certain per-

centage of a random seedling population will survive or will sustain a given level of damage (Ritchie 1984). The term LT_{50} (lethal temperature for 50% of a population) is commonly used to define the hardiness level. Simpson (1990) found that LT_{50} at lifting correlated well with first-year survival and shoot growth of conifer seedlings. Over the past several decades, cold hardiness has been measured using the whole plant freeze test (Tanaka and others 1997), freeze induced electrolyte leakage (Burr and others 1990), or chlorophyll fluorescence (L'Hirondelle and others 2006). This paper describes a new technology that has emerged for cold hardiness testing.

New Technology

In 2006, a new method to measure cold hardiness was introduced by NSure, a spin-off company from Wageningen University in The Netherlands. The test is based on measuring the activity level of a carefully selected set of genes. When a gene becomes active, it produces a molecule called messenger RNA, or mRNA. This molecule travels from the nucleus to the cytoplasm and triggers subsequent actions of the cell. The NSure test measures the relative amount of certain mRNA molecules and uses the results to calculate the activity of the corresponding genes. Because all physiological responses are started by, and directed by, genes switching on or off, this method can be highly accurate and reliable. The history and actual condition of any plant, animal, or microorganism is reflected in the activity profile of its genes. Gene expression analysis, or transcriptomics, is performed using microarrays or biochips. On these microarrays, several thousand copied genes are present on a glass slide of a few square centimeters. The array is used to simultaneously detect the level of activity of each of the represented genes. Thus, the responses of the plant to any environmental trigger can be followed in a direct way. The information is used to select those genes that are most involved in controlling the trait of interest, in this case cold hardiness. Because microarray analysis is very expensive and not suitable for use in practice, NSure translates the selected set of indicator genes into a reliable and robust assay. A comparable technology has been used in medical diagnostics for several years, predominantly for making complex diagnoses such as tumor typing (Landers and others 2005; Modlich and others 2005). The bottleneck for using mRNA as a diagnostic tool in production and trade of agroproducts was the fact that RNA molecules are highly unstable. Taking a reliable sample required skilled personnel and hazardous chemicals or liquid nitrogen. The sampling procedure developed by NSure is based on FTA technology from Whatmann (Roy and Nassuth 2005), and circumvents these problems.

In 2006, NSure cold-tolerance assays were made available for Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and European beech (*Fagus sylvatica*). The assay is based on the relative activity of 3 indicator genes that together provide enough information to give an estimate of the cold hardiness stage of the seedling. The corresponding genes dominate the process of hardiness development in all provenances studied and have a strong correlation with shoot electrolyte leakage (SEL) values (Joosen and others 2006; Balk and others 2007).

Douglas-fir Test

Because nurseries in British Columbia, Canada and the US Pacific Northwest region were interested in the technology, NSure developed a new assay aimed at one of the most economically important species in this region, Douglas-fir (*Pseudotsuga menziesii*). This new test was evaluated during the 2006 to 2007 season as part of a larger cold hardiness project with the Nursery Technology Cooperative (Oregon State University, Corvallis, Oregon). This project examined the relationships among cold hardiness development, thermoperiod (chilling hours), photoperiod, and calendar date in order to generate information to assist with nursery lifting and storage decisions. This paper will only discuss the results as they relate to the gene activity test. The full results will be published separately.

Material and Methods

Seedlings and Sampling Procedures

Douglas-fir seedlings from 3 stocktypes and 3 participating nurseries were used:

1) 2+0 bareroot (Webster Nursery, Olympia, Washington);
2) 1+0 bareroot for transplant (Lewis River Reforestation, Woodland, Washington);
3) PP21 outside-grown container (Microseed Nursery, Ridgefield, Washington).

Two seed sources (low/high elevation) within each stocktype were chosen for expected differences in hardiness:

1) 2+0 stocktype
   Low = lower Columbia (0 to 300 m [0 to 1000 ft])
   High = Yakama (900 to 1200 m [3000 to 4000 ft])
2) 1+0 stocktype
   Low = seed zone 262 (150 m [500 ft])
   High = seed zone 262 (610 m [2000 ft])
3) PP21 container stocktype
   Low = seed zone 051 (300 m [1000 ft]); coastal
   High = seed zone 452 (670 m [2200 ft]); Clackamas

Seedlings were lifted on 5 lift dates in 2006 (16 October, 30 October, 13 November, 27 November, 11 December) using standard nursery procedures. At each lifting date, a sample of 60 seedlings was assessed for cold hardiness by the whole plant freeze test (WPFT) and the NSure test.

Cold Tolerance Testing

Seedlings in the WPFT were divided into 4 groups, each frozen to a specific target temperature in a programmable chest freezer. After freezing, seedlings were placed into a greenhouse with optimal growth conditions for 6 days, and then assessed for foliar, bud, and cambial damage to estimate the LT_{10} or LT_{50} (lethal temperature to 10% or 50% of the seedlings, respectively). The NSure test was conducted on needles and buds collected from the same seedlings used for the WPFT test. The tissue was processed according to the sampling protocol provided with the NSure sampling kit. For each seedling group on each test date, buds and needles were sampled from 15 seedlings. Buds and needles were separately transferred to a vial containing extraction fluid, and a pestle was used to crush the tissue in the fluid for about 1 minute. Subsequently, 1 drop of extract was transferred to an FTA card and allowed to dry at room temperature. There were 3 replications for every sample date and seedling group. Samples were then sent to The Netherlands for analyses. NSure measured the level of expression of 3 genes for which the activity is expected to change in relation to cold hardiness development. Two should show increased activity, and 1 should show decreased activity. In addition, 3 control genes for which the activity was expected to remain constant were measured. Expression levels were measured using real-time RT PCR and specific primers designed by NSure, according to standard protocols. The corresponding hardiness status was calculated using models derived from Scots pine and Norway spruce. The Douglas-fir specific indicator genes were isolated based on their homology with the corresponding Scots pine genes.

Selection of Douglas-fir Specific Indicator Genes

Three of the most reliable indicator genes used in the Scots pine assay were selected for the Douglas-fir test. The corresponding Douglas-fir genes were identified through data mining in a public database (URL: http://www.ncbi.nlm.nih.gov). Because the provenance used in the database was not the same as the ones used in this trial, we decided to further maximize the homology by isolating the gene fragments specific for a common Oregon seed source. Therefore, specific PCR primers were constructed based on the sequence from the database, and the corresponding gene fragments were isolated from the cDNA of the

seedlings. Clones obtained were sequenced and subsequently identified using BLAST analysis. Specific primers were designed, based on the new specific sequence information, and those were used for expression analysis.

The differences in hardiness development are reflected in the development of gene expression of the 3 indicator genes. All gene expression values are relative to the expression of control genes that remain a constant activity over time. This method ensures that different samples can be compared even though the absolute levels of mRNA may not be the same, for example, due to differences in the extraction procedure.

Results

Cold Hardiness Development

As expected, the 6 provenances showed variations in cold tolerance development associated with the geographical source from which the seeds originated. Figure 1 shows the development of cold hardiness (LT_{50}) of each seedlot through the fall months of 2006 as determined with WPFT.

The results of the NSure cold tolerance test are shown in Table 1. The table gives an overview of all data and indicates the NSure hardiness stage that is commercially used. From the table, it is obvious that the patterns in cold tolerance that were detected through the WPFT correspond to that of the NSure assay on buds. The only exception was found to be the high elevation seed source grown at Webster Nursery (Yakama, 900 to 1200 m [3000 to 4000 ft]), which showed early entrance into NSure phase 3.

The expression pattern obtained from needles was less conclusive. This is partly due to the fact that, in some cases, no RNA could be extracted from the FTA Cards; in other cases, some of the indicators were not detectable at all. In cases where, technically, everything was correct, phase definition deviated regularly from that of the buds.

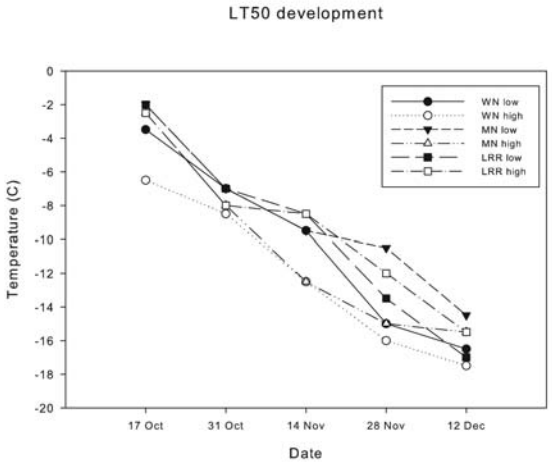

Figure 1. Development of LT_{50} temperatures over time for the 6 seedlots tested in the study. The graphs show the mean values of 3 measurements for each time point. WB = Webster Nursery; LRR = Lewis River Reforestation; MS = Microseed Nursery; high = high elevation seed source; low = low elevation seed source.

Discussion

Comparison of the gene expression profiles derived from bud tissue and the results of the WPFT showed a strong correlation between the 2 datasets. This indicates that the gene expression method is a good alternative for cold hardiness testing. Statistical analyses of the data allowed us to distinguish 3 stages of frost tolerance. These phases were shown to correlate with the LT values in the following way:

NSure phase 1: No frost tolerance observed.
NSure phase 2: LT_{50} value between -5 and -10 °C (23 to 14 °F); LT_{10} value between -1 and -5 °C (30 to 23 °F).
NSure phase 3: LT_{50} value below -10 °C (14 °F); LT_{10} value below -5 °C (23 °F).

This correlation remains true for all stocktypes and for seedlots from both high and low elevations, provided buds were used for the gene profiling. In the case of the high elevation seed source grown at Microseed Nursery (Clackamas), expression profiles seem to be even more consistent than LT profiles.

Table 1. Gene expression profiles and ColdNSure stages for *Pseudotsuga menziesii*. The table shows the gene expression levels relative to internal standards. The first portion gives the result for buds, and the second portion for needles, taken from the same seedlings. The right column indicates the corresponding ColdNSure phase: Phase 1 = no detectable sign of frost tolerance development; Phase 2 = start hardening; Phase 3 = fully hardened; ND = not detectable. WB = Webster Nursery; LRR = Lewis River Reforestation; MS = Microseed Nursery; HI = high elevation seed source; LO = low elevation seed source.

BUD TISSUE

Sampling date (2006)	Description	biological replicate	expression (relative to internal standard)				NSure phase
			PmD01	PmU01	PmU02	PmU01/PmD01	
12 October	LRR HI	1	nd	nd	nd		
		2	nd	nd	nd		
31 October	LRR HI	1	13.23	9.50	10.12	0.5	2
		2	68.56	10.86	6.90	2.7	2
14 November	LRR HI	1	14.04	7.06	9.01	1.0	2
		2	33.49	29.68	28.32	0.2	2
28 November	LRR HI	1	24.35	32.40	44.39	-0.4	3
		2	21.62	27.91	53.03	-0.4	3
12 December	LRR HI	1	26.43	115.43	90.89	-2.1	3
		2	20.46	98.29	150.51	-2.3	3
12 October	LRR LO	1	11.17	nd	3.01		1
		2	9.58	2.14	4.13	2.2	2
31 October	LRR LO	1	102.09	22.93	11.06	2.2	2
		2	79.56	78.98	53.44	0.0	2
14 November	LRR LO	1	91.51	68.34	34.38	0.4	2
		2	53.29	128.36	81.69	-1.3	3
28 November	LRR LO	1	41.14	111.69	96.81	-1.4	3
		2	108.95	566.49	253.23	-2.4	3
12 December	LRR LO	1	44.43	259.34	209.52	-2.5	3
		2	27.37	384.27	368.54	-3.8	3
12 October	MS HI	1	nd	9.22	3.27		1
		2	21.60	7.83	35.73	3.3	2
31 October	MS HI	1	49.06	42.24	12.85	0.6	2
		2	5.56	55.05	42.28	-1.2	2
14 November	MS HI	1	5.73	62.93	45.54	-1.3	2
		2	8.80	32.00	28.00	-0.4	2
28 November	MS HI	1	1.01	195.31	153.18	-3.7	3
		2	0.70	260.86	206.04	-4.2	3
12 December	MS HI	1	0.46	533.59	398.90	-4.9	3
		2	1.34	420.22	357.29	-3.1	3
12 October	MS LO	1	18.35	7.59	12.58	1.3	1
		2	17.70	6.73	10.31	1.4	1
31 October	MS LO	1	167.73	100.30	29.96	0.7	2
		2	110.75	61.88	28.64	0.8	2
14 November	MS LO	1	166.85	55.91	32.30	1.6	2
		2	135.26	108.72	68.80	0.3	2
28 November	MS LO	1	98.60	392.39	104.24	-2.0	3
		2	319.85	659.77	195.06	-1.0	3
12 December	MS LO	1	67.59	408.21	241.53	-2.6	3
		2	23.09	235.06	166.16	-3.3	3

BUD TISSUE

Sampling date (2006) phase	Description	biological replicate	expression (relative to internal standard)				NSure
			PmD01	PmU01	PmU02	PmU01/PmD01	
12 October	WB HI	1	10.58	11.00	2.84	-0.1	2
		2	4.64	11.35	1.07	-1.3	2
31 October	WB HI	1	126.51	691.73	397.56	-2.5	3
		2	554.41	2738.83	1241.91	-2.3	3
14 November	WB HI	1	179.05	2998.76	1498.32	-4.1	3
		2	335.24	3435.51	1629.01	-3.4	3
28 November	WB HI	1	57.87	2031.05	1279.30	-5.1	3
		2	38.41	1397.53	1070.07	-5.2	3
12 December	WB HI	1	16.55	438.24	399.44	-4.7	3
		2	31.42	937.10	1038.56	-4.9	3
12 October	WB LO	1	75.19	31.16	27.72	1.3	1
		2	64.50	15.01	14.62	2.1	1
31 October	WB LO	1	141.60	777.06	374.28	-2.5	2
		2	32.88	42.18	36.89	-0.4	2
14 November	WB LO	1	60.00	170.22	131.15	-1.5	2
		2	69.73	403.09	216.29	-2.5	2
28 November	WB LO	1	11.26	933.25	987.79	-6.4	3
		2	19.75	761.43	903.82	-5.3	3
12 December	WB LO	1	25.27	754.09	608.63	-4.9	3
		2	41.73	845.72	623.06	-4.3	3

NEEDLES

Sampling date phase	Description	biological replicate	expression (relative to internal standard)				NSure
			PmD01	PmU01	PmU02	PmU01/PmD01	
12 October	LRR HI	1	18.99	6.71	6.62	1.5	1
		2	109.88	28.03	45.29	2.0	1
31 October	LRR HI	1	12.21	40.69	21.96	-1.7	1
		2	35.73	135.04	82.90	-1.9	2
14 November	LRR HI	1	15.20	215.85	135.91	-3.8	2
		2	15.56	157.32	91.49	-3.3	2
28 November	LRR HI	1	47.11	1492.80	866.13	-5.0	3
		2	26.06	2621.27	960.06	-6.7	3
12 December	LRR HI	1	13.21	382.93	402.73	-4.9	3
		2	5.71	694.91	877.97	-6.9	3
12 October	LRR LO	1	17.19	30.48	30.97	-0.8	1
		2	32.88	10.07	nd	1.7	1

Table 1 [continued]. Gene expression profiles and ColdNSure stages for *Pseudotsuga menziesii*. The table shows the gene expression levels relative to internal standards. The first portion gives the result for buds, and the second portion for needles, taken from the same seedlings. The right column indicates the corresponding ColdNSure phase: Phase 1 = no detectable sign of frost tolerance development; Phase 2 = start hardening; Phase 3 = fully hardened; ND = not detectable. WB = Webster Nursery; LRR = Lewis River Reforestation; MS = Microseed Nursery; HI = high elevation seed source; LO = low elevation seed source.

NEEDLES

Sampling date	Description	biological replicate	expression (relative to internal standard)				NSure phase
			PmD01	PmU01	PmU02	PmU01/PmD01	
31 October	LRR LO	1					
		2	31.55	76.84	47.43	-1.3	2
14 November	LRR LO	1	2.77	25.15	19.67	-3.2	2
		2	6.50	17.48	18.92	-1.4	2
28 November	LRR LO	1	nd	356.83	253.40		
		2	nd	180.00	204.57		
12 December	LRR LO	1	nd	93.76	105.53		
		2	4.02	233.77	156.26	-5.9	3
12 October	MS HI	1					
		2	12.00	1.71	1.03	2.8	1
31 October	MS HI	1	7.58	61.16	48.81	-3.0	3
		2	8.55	100.43	37.97	-3.6	3
14 November	MS HI	1		208.86	73.59		
		2		250.06	123.04		
28 November	MS HI	1	6.03	76.08	50.85	-3.7	3
		2	3.92	185.55	104.08	-5.6	3
12 December	MS HI	1	21.58	448.85	204.75	-4.4	3
		2	41.14	515.26	329.41	-3.6	3
12 October	MS LO	1	2.58	38.19	34.54	-3.9	2
		2					
31 October	MS LO	1	4.64	332.30	166.03	-6.2	3
		2	33.57	983.82	597.40	-4.9	3
14 November	MS LO	1	nd	286.92	231.80		
		2	18.94	511.63	306.63	-4.8	3
28 November	MS LO	1	2.52	627.87	333.71	-8.0	3
		2					
12 December	MS LO	1	nd	946.59	440.43		
		2				-6.5	3
12 October	WB HI	1	4.35	11.55	12.66	-1.4	1
		2	9.64	10.13	12.14	-0.1	1
31 October	WB HI	1	7.46	57.06	35.47	-2.9	2
		2	14.30	125.31	96.61	-3.1	2
14 November	WB HI	1	0.70	60.85	31.40	-6.4	2
		2	13.25	567.25	340.06	-5.4	3
28 November	WB HI	1	8.83	1544.77	763.51	-7.5	3
		2	14.27	1148.79	553.57	-6.3	3
12 December	WB HI	1	1.83	134.92	74.30	-6.2	2
		2	17.67	272.88	186.83	-3.9	2

Sampling date phase	Description	biological replicate	expression (relative to internal standard)				NSure
			PmD01	PmU01	PmU02	PmU01/PmD01	
12 October	WB LO	1	1.55	22.37	41.59	-3.9	2
		2	6.44	69.22	87.83	-3.4	2
31 October	WB LO	1	5.83	280.79	320.62	-5.6	3
		2	36.10	182.81	79.50	-2.3	3
14 November	WB LO	1	41.50	299.60	237.98	-2.9	3
		2	64.88	791.65	nd	-3.6	3
28 November	WB LO	1	11.75	1193.79	1577.10	-6.7	3
		2	227.39	13272.08	7774.97	-5.9	3
12 December	WB LO	1	12.32	270.04	307.02	-4.5	3
		2	42.08	269.81	306.43	-2.7	3

When needles were used, however, the correlation was poor. In contrast to previous findings with Norway spruce and Scots pine, this study indicates that Douglas-fir needles may not be as reliable for test material. One possible technical reason could be in cases where no good quality RNA is present on the FTA Cards. The tissue may be much more rigid, or may contain more inhibiting components which hamper extraction. This can also be a reason for the fact that, in other cases, indicators simply couldn't be measured. However, there may be a physiological explanation in cases where extraction proceeds properly, but phase definition deviates from that found for buds from the same seedling (for example, when frost tolerance levels in the needles are subject to fluctuation; Stralbiski 2007). Further research is required for this situation.

The fact that the expression profile of the same set of genes is indicative for cold tolerance in such diverse species as *Pinus sylvestris*, *Fagus sylvatica*, and *Pseudotsuga menziesii* suggests that the NSure test measures a biological process that was highly conserved in evolution. Usually this indicates that the process concerned is essential to plant survival. In practical terms, it means that measuring the activity of these conserved genes will give a highly accurate indication of the cold hardiness development, since they are likely the basis of the physiological process which occurs.

The big advantage of the NSure test is that seedlings do not have to be transported to a test laboratory. The samples can be taken and stabilized on site. Furthermore, the result time of the test is short, just a few days, which makes it suitable for integration into nursery logistics.

Conclusions

The strong correlation between the 2 cold hardiness tests in Douglas-fir seedlings, combined with the short analysis time required, suggests that NSure is highly suitable for implementation as a nursery management decision support tool.

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Deployment of Deer-Resistant Western Redcedar (*Thuja plicata*)

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ABSTRACT
Protecting planted western redcedar (*Thuja plicata*) seedlings from deer browse in the Pacific Northwest and British Columbia is estimated to cost up to CAN$ 25 million annually. Recent studies linking deer browse and needle monoterpenes has resulted in the initiation of a breeding program for deer-resistant western redcedar at Cowichan Lake Research Station on Vancouver Island. Selections that are not preferred by deer are currently going through non-preferred rapid breeding and testing cycles for enhanced needle monoterpene concentrations. However, planting stock with increased monoterpene concentrations through genetic manipulation selection and breeding alone may not ensure that seedlings will be adequately protected from browse if deployment strategies are not carefully considered. To provide more robust protection to allow seedlings to reach the free-growing stage, iInteractions must be evaluated of between genetic selection for enhanced monoterpene concentrations,ally enhanced plants with developmental stage (ontogeny), and nutritional quality, as well as appropriate deployment strategies that allow deer some level offorage choice, could ensure adequate protection from browse.

KEYWORDS
needle monoterpenes, ontogenetic effects, plant nutrients

Introduction

Deer and elk browsing on newly planted western redcedar (*Thuja plicata*) seedlings in the Pacific Northwest (PNW) can result in delayed regeneration and potential plantation failure. As a result, CAN$ 20 to 25 million are spent annually in British Columbia to bring a plantation to free-to-grow status using individual tree guards (van Nienjenhuis 2007). Licensees who avoid planting western redcedar incur additional indirect costs, including maladapted or inappropriate species selection, and reduced manufacturing opportunities.

The role of plant secondary metabolites (PSM) in plant defenses has long been recognized (Coley and Barone 1996; Huber and others 2004). Monoterpenes, a group of PSM, have been shown to reduce herbivore predation, including ungulate feeding (for example, Duncan and others 1994; Vourc'h and others 2002a). Plants contain both beneficial and deleterious phytochemicals that impact palatability and diet selection for ungulates (Kimball and Provenza 2003).

Levels of monoterpenes in conifer needles can be impacted by genetics and environmental factors, as well as their interactions (for example, Baradat and others 1972; Vourc'h and others 2002b). Monoterpene concentrations also vary seasonally and with age (for example, Schonwitz and others 1990; Powell and Raffa 1999).

Recent studies linking deer browse and needle monoterpenes in western redcedar (Vourc'h and others 2002b; Russell and Kimball nd) have driven the initiation of a deer-resistance western redcedar breeding program at Cowichan Lake Research Station, Mesachie Lake, British Columbia (CLRS). Deer "non-preferred" selections are currently going through rapid breeding and testing cycles for enhanced needle monoterpene concentrations. However, planting stock bred for higher monoterpene levels alone may not ensure that seedlings will be adequately protected from browse. Interactions between a plant's genetic background, developmental stage (ontogeny), nutritional quality, and silviculture, particularly appropriate deployment strategies that allow deer some level of choice, must all be considered to provide robust protection from browse. This paper outlines current knowledge, the ongoing breeding program at CLRS, and future research directions in western redcedar deer resistance.

Linking Deer Browse and Needle Monoterpene Concentrations in Western Redcedar

A subsample of individuals from a western redcedar population study with family structure at Holt Creek (HC) on southern Vancouver Island indicated that trees that were heavily browsed tended to have low monoterpene content (Vourc'h and others 2002b). A subsequent complete survey of all 2200 trial trees at HC confirmed this relationship (Russell nd). Both of these studies involved seedlings planted in an uncontrolled environment. To obtain a more precise measure of the relationship between deer browse and needle monoterpenes, a study was designed using multiple copies of clones planted in deer enclosures and in small openings in the forest with heavy deer browsing pressure. Cuttings were rooted from 60 trees at HC that varied in monoterpene concentrations and planted at the USDA National Wildlife Research Center, Olympia Field Station, Olympia, Washington (Figure 1) and 2 reforestation sites on southern Vancouver Island. Consistent results were obtained at all 3 sites, demonstrating that browse preference is a function of total needle monoterpene content (Figure 2; Russell and Kimball nd).

Some Factors Affecting Needle Monoterpene Concentrations in Western Redcedar

Genetics

In the HC trial mentioned earlier, a complete sample of needles from all test trees indicated substantial genetic variation in monoterpenes. Narrow-sense heritabilities for 6 of the major needle monoterpenes varied from 0.25 to 0.44, and coefficients of additive genetic variation varied from 19% to 27% (Russell nd). There were significant seed source effects, that is, northern British Columbia populations had greater needle monoterpene concentrations than southern British Columbia sources. Thus, a base population with substantial genetic variation in needle monoterpenes for the initiation of a breeding program is available.

Ontogenetic and Age Effects

In a fertilization study with planted western redcedar in western Oregon, total needle monoterpenes increased linearly from age 1 to 3 (Kimball 2007). Similarly, 1-year-old rooted cuttings originating from 7-year-old western redcedar trees had greater total needle monoterpene concentrations and significantly reduced browse damage than genetically comparable 1-year-old seedlings (Figure 3; Russell nd). Utilizing either 2-year-old seedlings, or rooted cuttings from older hedged donors, can increase needle monoterpene levels and potentially minimize deer browsing.

Figure 1. Western redcedar feeding choice trial with Columbian black-tailed deer at the USDA National Wildlife Center, Olympia Field Station.

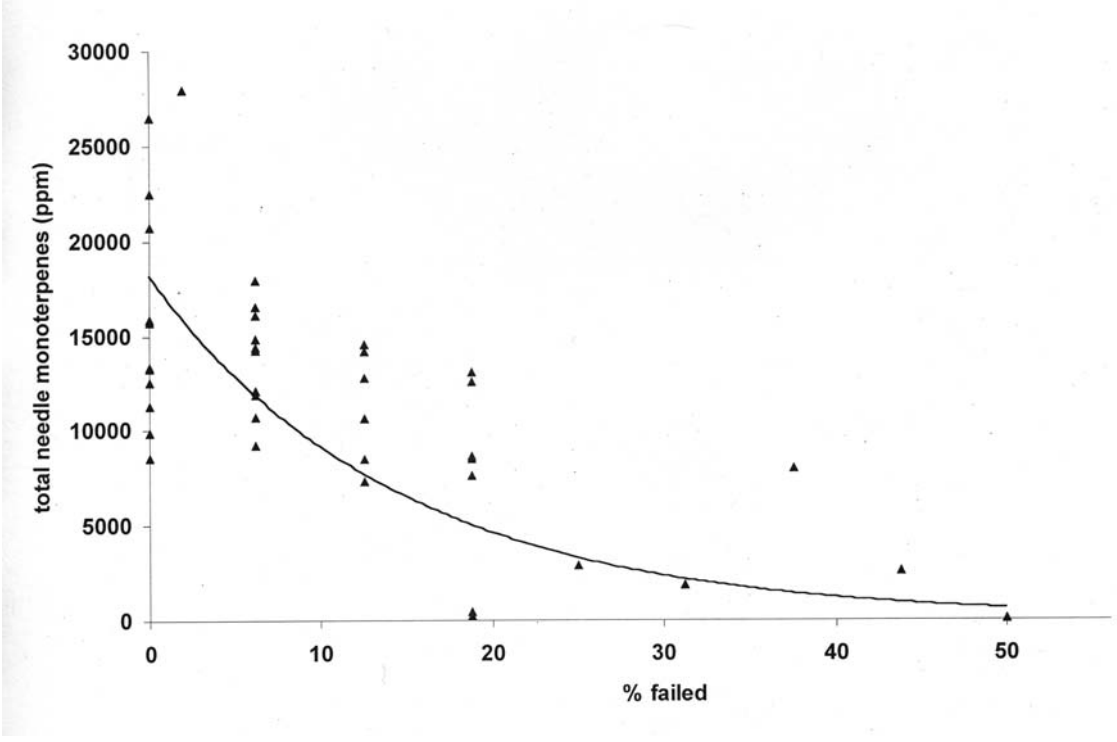

Figure 2. Relationship between total needle monoterpenes and mean failure by clone in western redcedar at the USDA National Wildlife Center, Olympia Field Station.

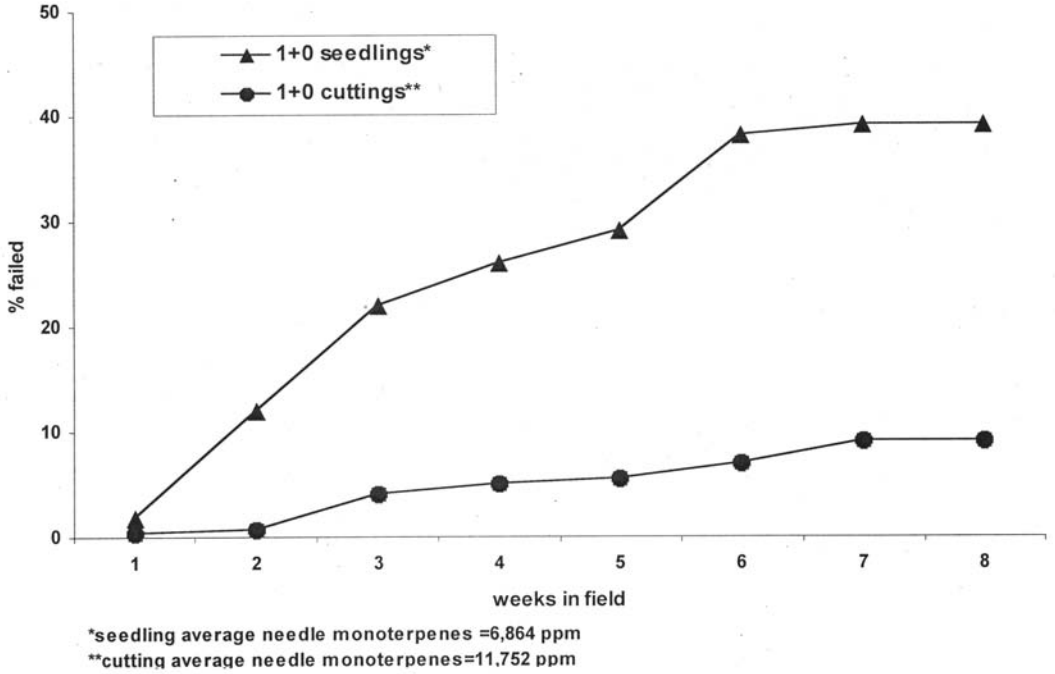

Figure 3. Relationship between deer browse and time since outplanting duration for western redcedar stocktypes.

Plant Nutrient Levels

Nutritional quality of seedlings at the time of planting can have a paradoxical effect. High nitrogen, for example, may make a seedling more palatable to deer, whereas it has been well documented for western redcedar that fertilization at planting stimulates early growth, and thus earlier free-to-grow status (Blevins and others 2006). A higher nitrogen level may also help seedlings recover following browse (see references in Jacobs 2005). Little research has explored the relationship between nutritional quality and monoterpene production. A current study is underway in the PNW examining the influence of mineral nutrition on susceptibility and recovery of planted western redcedar to animal browse (Jacobs 2005).

Breeding for Enhanced Needle Monoterpenes

A breeding program for enhanced needle monoterpene concentrations is currently underway at Cowichan Lake Research Station. Over 60 "not preferred" selections (minimal deer browse and enhanced needle monoterpenes) from a base population of approximately 2500 range-wide western redcedar trees have been selected for the breeding program. In addition, a number of "deer preferred" selections (high browse and low monoterpene concentrations) have also been included. Terpenes are expressed during a seedling's first year, and breeding can be done within 2 years (Figure 4; Russell and Ferguson forthcoming). Additional selections for deer resistance can be quickly incorporated into the program.

Figure 4. Breeding young western redcedar trees at Cowichan Lake Research Station.

Deployment of Deer-resistant Seedlots

Planting seedlings that are high in needle monoterpenes doesn't necessarily guarantee browse resistance. Plants contain beneficial and deleterious phytochemicals. If bitter, high monoterpene trees are the only available food source, deer will select based on the balance between energy input and cost of detoxification (Kimball and Provenza 2003). Results from a study deploying different mixtures of hybrid poplar clones in the Columbia Valley that were preferred and avoided by deer showed that a 2:1 mixture of "not preferred" to "preferred" trees was optimal to yield final free-to-grow stocking (Stanton 2004).

The next stage in developing deer-resistant western redcedar seedlots at CLRS is to outplant mixtures of trees with varying levels of needle monoterpenes in operational trials, including genetic selections, seedlings of varying age, and rooted cuttings. The ultimate goal is to develop custom-made seedlots with varying degrees of resistance tailored for specific sites.

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Marketing: the Roots of your Business

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ABSTRACT
Today s culture is in a constant state of flux. The ways we think, plan, and communicate are changing rapidly. Why should that matter to you? Because you have marketing and sales goals to meet. You need a successful sales process. Your methods of communication with colleagues are critical to the success of your day, projects, and professional enjoyment. Here I present the 13 key techniques for implementing a successful marketing strategy. What follows is practical information. If you take time to ponder the application of these techniques, you can improve your marketing and sales.

KEYWORDS
public relations, communication, advertising, branding

The Elements of Marketing

The elements of marketing you may be able to apply are:

:: Your Marketing Toolbox
:: Public Relations
:: Communications and How We Think
:: Customer Service
:: Word of Mouth Advertising
:: Catalogues
:: Website
:: Packaging
:: Direct Mail
:: E-mail
:: Trade Shows
:: Advertising
:: Branding

These tools will help you turn the features of your products and services into benefits. A feature is defined from your point of view; a benefit is defined from the customer's point of view. The potential customer has to be helped to understand why you are the right choice for him or her. In this way, you lead them to the decision you want them to make, that is, choosing your product or service.

Repositioning your descriptions and statements may require an attitude shift in your thinking—don't let yourself become stale and stuck in a rut. Effective marketing communication relies on content, a consistent company message and style, and a clear call to action.

Shifting your thinking requires some mental work. "It is imperative to frequently get your head out of the business and into something else. If not, you chance becoming one-dimensional and going stale" (Hatch 2007). Lee Iacocca learned from experience, "I can accomplish a year's work in eleven months, I cannot do it in twelve." Many of us assume, if we work harder and longer, we will do better. That is not always the case. The following tools are designed to help you be prepared to meet your marketing challenges and avoid some of the multitude of "scrambles."

Your Marketing Toolbox

Your basic tools include the company logo, tag phrase, consistent colors, and a consistent design style.

Whether you are selling business-to-business or retail, or you are working on your intra-organization communications, you must be able to answer the questions:

Who are my customers?
To whom is my message directed?
What is their expectation?

Finally, with the above tools in place, what are you going to do with them? Your Marketing Plan will answer that question. Remember, if you don't know where you're going, any road will take you there.

Having these tools cleaned and sharpened enables you to shape and implement your sales or communications plan.

Consider this illustrative story about the farmer who was located on a windy bit of ocean-front property. When the frequent storms hit, the animals often escaped as the wind loosened their gates and destroyed fences. Equipment was damaged when left outside or the barn doors slammed open. When his strong, young, hired hand moved on, the farmer was desperate to find a strong replacement, capable of battling the winds and preserving as much of the farm as possible. As it turned out, the only available hand was a rather elderly gentleman who said he was a good worker. After securing the job, the new hired hand mentioned, "By the way, I sleep through storms." Now, the farmer was truly at his wits' end, vowing to confidentially continue his search. His worst fears were borne out. The very next night, a violent storm boiled up. The farmer ran to his hired hand's house and pounded on the door and walls, all to no avail. Frantic, he dashed to check first on his livestock. They were securely peaceful, locked in their various sections of the barn. Next, he feared for his equipment; the doors were tightly fastened, the equipment without damage. The next morning the farmer expressed his amazement to his new worker. "I like my peaceful sleep, so I prepare before retiring at night."

This is the preparedness your well thought-out toolbox will provide for your marketing and communications needs.

Public Relations

Technically, public relations refers to non-paid methods of communicating your message. In your industry, one of the most important aspects of public relations is being ready to react in case a newsworthy event occurs on your site. You need to collect the hard questions potential customers or the media might ask. Bring together staff and outside resources to formulate answers. Go through the same process for potential disasters. Be ready to respond, rather than just hoping "it never comes up." Then, spread the answers company-wide. Are your staff and/or colleagues prepared with the same answers? A document filed away cannot go to work for you. Disseminate the information. You cannot count on the media's contacting only you or your public relations person. If an incident occurs, the first contact could be made with whomever is available.

Communications and How We Think

Communications

Content is king. Any copy, brochure, or text you write should reflect the consistency you identified as you formulated your marketing toolbox.

Ideally, frequent readers of your material should recognize the style and presentation. "The tone of a good direct mail letter is as direct and personal as the writer's skill can make it. Even though it may go to millions of people, it never orates to a crowd, but rather murmurs into a single ear. It's a message from one letter writer to one letter reader" (Harry B Walsh in Hatch 2007). Do you have a friend who tells you like it is? That's effective marketing—a conversation between you and one friend.

If your customers aren't listening to you, who is going to change, you and your communication, or their listening habits? Obviously, you need to speak to each individual in his or her language. Marketing and direct mail writer Denny Hatch (2007) summarizes the underlying key to effective communication this way: THINK.

How We Think

Effective communication depends on reaching the decision-making section of your audience's brain. Williams (2001) supposes:

:: You are an advertising message. Your hope is to arrive at the Emerald City, the prefrontal cortex of the human brain, that place where decisions are made in the mind.

:: But a journey to the Emerald City is both long and difficult. Located just behind the forehead, the prefrontal cortex is isolated from all the parts of the brain that gather information from the outside world.

:: The good news is there is a Yellow Brick Road, a highway that will take you directly there!

:: The bad news is the only entrance to the Yellow Brick Road is through a tollbooth called Broca's Area. Will you be able to pay the toll? "Interest me!" cries Broca. "Surprise me with something I didn't know. If you're not carrying new information or a new perspective, you'll not enter my Yellow Brick Road."

:: Although it's positioned next the ear, Broca's tollbooth screens not just auditory data, but most types of neural information. Sight, sound, taste, pain, pressure, position, movement, and temperature are gathered and processed in various outlying areas of the brain, but all must pass through Broca's tollbooth on their way to the prefrontal cortex. Sitting in his tollbooth, Broca attaches verbs to actions as he anticipates the predictable. Broca turns away everything that he "sees coming."

:: Broca hates the predictable.

Now that we have some vital information about the audience, we need to consider a couple of writing principles. Many new copywriters are too effusive. American author Ernest Hemingway described his self-editing as frameline magnetism, "I always try to write on the principle of the iceberg. There is seven-eights of it under water for every part that shows. Anything you know you can eliminate and it only strengthens your iceberg. It is the part that doesn't show" (Williams 2001).

Copywriting, marketing, and advertising experts have filled bookshelves with different ways to categorize copywriting approaches. It all comes down to what you want to say and how concisely can you do so, compellingly. One relevant description that will give you perspective is, "*You* copy, *me* copy, and *it* copy."

You Copy: found in a letter, a highly emotional, personal message from the writer to the reader that translates the features of the product or service into the benefits to *you, your family, your health, your wealth, and your standing in the community and the world.*

It Copy: the brochure, press release, or E-mail attachment that shows and describes "it"—the features of the product or service being offered.

Me Copy: found in the order or reply device. "Yes, send me the such-and-such under the terms

and conditions described elsewhere in the mailing. I understand that I have two full weeks to make up my mind" (Walter Weintz in Hatch 2007).

Another way to consider crafting your copy is described in the chapter "Principles of Being Perfectly (Robert) Frank" (Williams 2001).

1. Choose a revealing angle. Put the reader/listener/viewer on the scene.
2. Select your details sparingly. Include only what's interesting. And, barely that.
3. Put the known "under water." Never tell the reader/listener/viewer anything he already knows or can figure out for himself.

To write *Robert Frank* is to communicate in the fewest words and from the most interesting perspective. It's how to speak to the left brain with accuracy and clarity without being boring.

Both Hemingway and Frank underscore the effectiveness of your basic message being clearly presented. Ask yourself, "What do I want to say?" Then, say it. You can always add words if necessary. Remember, this information refers to technique; your established style must remain consistent to keep building your image.

To understand the power of reaching Broca's Area with a concise message, imagine yourself skiing down an intermediate hill. It's not unusual to come upon a young skier who's crashed, pieces of ski equipment scattered about his spot in the snow. Responsible skiers yell out, "You okay?" "Yeah," is the usual embarrassed reply. The shock of, "No," reaching your Broca's Area is enough to whip you around, sending you up the hill or into your own crash. In this particular case, the skier actually had a broken leg. Broca was surprised. Broca directed just what you want—action.

Consider the techniques required to communicate effectively. It is clear you will benefit if you buy out the time to plan. Don't operate on the basis that there's not time to fully accomplish a task, but there's always time to do it over because of a crisis.

Customer Service

Good customer service is the gold that keeps your cost-effective customers returning, rather than having to spend 4 to 8 times the cost to obtain a new customer. The biggest marketing mistake made by small businesses is not collecting contacts and customer information, then creating a database. Keep it current. The database gives you a handle on your market.

You can actually write your way to better customer service. Too often, business owners and their employees fail to recognize that the written communications you send can significantly affect how your customers feel, and thus act, about your business.

E-mail and letters that fail to answer your customers' questions or address their concerns are hard to read, are abrupt in tone, or contain errors all convey the message that you don't place much value on their business. Poor writing can also cause frustrating misunderstandings that waste valuable time and resources. Ensuring that every E-mail and letter your customers receive is friendly, helpful, and conveys a positive image that your business is a worthwhile investment of your time and money.

Whether you are a small, family company or a large organization, taking the time to establish good writing standards for your company pays off with clarity and consistency.

1. State the main point clearly and quickly. People often read only the first few lines of an E-mail message or letter before deciding whether it's worth their time. Pay special attention to the subject line, using about 5 words.
2. Respond clearly and directly to a customer's specific questions and concerns. A customer who has raised a concern isn't interested in hearing about "how much you value their business" until the concern has been addressed.
3. Use an appropriate tone. Most people are careful not to be rude or abrupt when

speaking to customers on the phone or face-to-face, but sometimes fail to understand their writing conveys a tone. That tone represents you, the company. Never use all capital letters. It's perceived as shouting.
4. Keep sentences and paragraphs short and concise. Proofread for errors in grammar, punctuation, and spelling. Watch out for spell check with words such as "their," and "there" (MelissaDATA 2007).

Keep in touch with current and past customers. It will be illuminating to survey them. First, separate current and past customers, so your information fits into a time frame. If customers are leaving, perhaps you will discover a point at which something changed in your business. Based on MelissaDATA (2007), a summary of questions could include:

1. Why did you buy my product or service?
2. Why did you buy at that specific time?
3. Why did you buy right away, or take your time?
4. What do you like best about my product or service?
5. What do you like least?
6. Would you refer others to me? If not, why not? If yes, why?
7. What specific benefits do you see in my product or service?
8. What specific need of yours does it satisfy?

Word of Mouth (WOM)

Word of Mouth marketing requires that you listen. It can be one of your most powerful tools, as it's the feedback loop that forces marketers—you—to join the conversation. It brings advertisers out of the woodwork and forces them to confront the impact their marketing has on real persons. It puts the consumer at the head of the boardroom table. A happy customer is the greatest advertisement (Sernovitz 2006). Every company can't accomplish this, but if you can become a buzzworthy company, your success is virtually locked in (Sernovitz 2006). The "buzz" may be about an attribute of your company's business practices, a new product, a marketing campaign, or some other element clearly linked to your company. For example, do you know what company released the iPhone? Of course you do. That's "buzz."

The WOM Manifesto

1. Happy customers are your best advertising. Make people happy.
2. Marketing is easy. Earn the respect and recommendation of your customers. They will do your marketing for you, for free.
3. Ethics and good service come first.
4. UR the UE: You are the user experience, not what your ads say you are.
5. Negative word of mouth is an opportunity. Listen and learn.
6. People are already talking. Your only option is to join the conversation.
7. Be interesting, or be invisible.
8. If it's not worth talking about, it's not worth doing.
9. Make the story of your company a good one. (Many native nurseries are hitching to the "ecologically responsible" theme; be sure to set yourself apart with enticing specific information.)
10. It is more fun to work at a company that people want to talk about.
11. Use the power of word of mouth to make business treat people better.
12. Honest marketing makes more money.

How Do You Implement WOM?

There are 5 basic techniques for implementing WOM (Sernovitz 2006):

1. **Talkers.** Find people who will talk about you (for example, fans, volunteers, customers, bloggers, influencers).

2. **Topics.** Give people a reason to talk (for example, a special offer, great service, cool product, silliness, neat ad, or a new feature).
3. **Tools.** Help the message spread faster and farther (for example, tell-a-friend form, viral E-mail, blogs, handouts, samples, message boards, online communities).
4. **Taking part.** Join the conversation (for example, let staff surf and reply to comments, post on blogs, join discussions, answer E-mail, and offer personal service).
5. **Tracking.** Measure and understand what people are saying (for example, search blogs, read message boards, listen to feedback, use advanced measurement tools).

Are your colleagues or employees your most powerful advocates? Are you asking them to be? Are you making it easy?

1. **Involve everyone.** Your most powerful evangelists aren't in your marketing or PR department. They are the receptionists, the IT folks, the warehouse crews, and the interns. Look for the person wearing the company shirt on weekends. Enthusiasm counts more than titles.
2. **Make it easy.** Arm your employees with everything they need to talk about you. They may effectively use text for the signature files of their E-mails, samples, copy, logos, and photos to paste into their blogs. If your business lends itself to coupons, every employee should have 100 coupons in hand at all times. You pay to put coupons in a newspaper, so why aren't you giving them to people who have a stake in the success of the company?
3. **Go where they already are.** Any method an employee uses to help promote the company is good. It doesn't matter if they want to use MySpace, Facebook, a personal blog, a shirt, or a bumper sticker. Don't make them do it your way. Support whatever method the employee wants to use instead of recreating or competing with it. You will be shocked at how many of your employees are already on these platforms every day. They are probably holding back and not mentioning work-related topics, because they are not sure how you will respond. Tell them that it's okay and unleash a torrent of advocates.
4. **Make them stars.** Internal rewards are nice, but public recognition is powerful. When employees are out there talking about you, thank them—big time. Put it on your website. Blog about them. Put up their pictures. Create a formal evangelism program so they feel part of the team. Give them hats, shirts, and other toys. For example, the Chicago Bagel Authority restaurant publicly thanks its employees by selling t-shirts, with proceeds going right into the employee tip jar. To make this technique powerful, the customer should see you put the funds into the jar. A sign describing the process is easily disregarded or unbelieved.
5. **Employees should be customers, if appropriate.** Nothing is more embarrassing than employees who don't use their company's products, or walk around with ratty, out-of-date versions. It's a double problem. Your most powerful advocates can't show off to their friends, and their friends instantly wonder what's wrong with your products if even the employees won't use it. That's why Apple gave iPhones to everyone who works there (GasPedal 2007).

Catalog

Your catalog must feel like your website, as much as it can while following the principles of effective print presentation. These 2 tools are the major presentations of your style, image, writing, logo, and identity phrases. If you incorporate direct mail into your marketing scheme, it also dramatically represents your image.

Inform yourself about the direction our eye follows on the page, which is from upper right,

curving through the hot spot at the bottom of the upper one-third of the page, and on to the bottom right. Other questions you need to be able to answer parallel those offered below in the website section.

Repeat your contact information at each point you are asking customers to buy or take other action. Make your expectations clear. What is your "call to action?"

Website

Picking up from the last comment about the catalog, be clear about what action you expect at each stopping point. Have firmly in mind the purpose of your website and specific pages. Is this an informational, motivational, and/or sales site?

When research was conducted about website users' being able to find information, the importance of the home page was clearly underlined. "It turned out that users were far more successful at finding their targets when the description words, which they told us before they saw the website, appeared on the home page. In the tasks where users successfully found their target content, the description words appeared on the home page 72% of the time. When users were unsuccessful, their words only appeared an average of 6% of the time on the home page (Williams 2001).

To increase sales, your homepage is 50% of the battle. If loading the page goes beyond 30 seconds, you can kiss most of your marketing budget, and all of your first time potential impulse-buyers, goodbye. Designers typically live in a world of T1 lines and broadband connections. The hard reality of the online sales world is that many customers still surf at 56K or slower. In concrete terms, this means your home page should be 35 to 40K.

Other critical website elements to consider include:

1. Is the look and feel professional?
2. Is the navigation obvious, simple, and consistent throughout the website?
3. Is your Unique Selling Proposition (USP) clearly and strongly stated?
4. Is your information architecture constructed from the visitor's point of view?
5. Does your navigation anticipate and clearly support all reasonable path choices?
6. Does the layout reflect knowledge of eye-scanning patterns and "sweet spots?"
7. Does the choice of page elements reflect knowledge of how visitors use text versus graphics online as opposed to in-print?
8. Are the graphics and the text appropriate, well-chosen, and well-written?
9. Does the page reflect the principles of good usability?
10. Does the page utilize expert sales principles that encourage a buying decision?
11. Does the page utilize knowledge about consumer psychology and the different personality types?
12. Does the page make use of knowledge about online buying behavior?
13. Does the page inspire trust and build rapport? Currently security is more of an emotional issue than a technological one.
14. Is your contact information frequently available and easy to find?
15. Is help available? Is it user-centered versus technology-centered?
16. How many help channels, for example, E-mail, FAX, phone, live-on-web, do you provide?
17. Are the smallest details, such as fonts and colors, chosen with an understanding and knowledge of what maximizes sales?
18. Does the page delight visitors and inspire them to go deeper into the website? Does it actually guide them in doing so (Eisenberg and Eisenberg 2005)?

What is the most frequent, dangerous misunderstanding about the potential of websites? If it's there, the customers I'm seeking will find it. A website is a secondary marketing tool. Nearly essential today, yes. But, you need to take action to drive users to your site. If you are seeking a

broad audience, include the address in everything you print. Consumers expect to be able to pick up any piece of paper from your company and find the web address.

Packaging

Product shipping offers a rich opportunity to promote your company, reinforce selection of your product, and sell. The packaging itself should carry your logo and message. You can also be creative with your packing material and include information in the container.

A diesel engine rebuild company enjoyed high sales and very low damage at the receiving end. To streamline their procedures and achieve more sales, they hired a consultant. Many good ideas resulted from that exercise. However, their damage on the receiving end suddenly went from below industry standards to above those standards.

Why? Previously, the shipping department had been including individually wrapped candies among the packing material. Seeing that as an unnecessary expense, the consultant stopped the practice. The bottom line soon became clear. Searching for their candy treats, the customers' receiving department personnel were very careful in unpacking this unwieldy product. The candy was restored; damage statistics dropped again. This was indeed a beneficial case of thinking out of the box.

Direct Mail

The Direct Marketing Association (DMA) currently estimates that, in order to generate US$ 700 billion in sales, marketers will spend US$ 56 billion on direct mail and catalogs this year. That translates to every US$ 5.60 in marketing costs bringing in US$ 70 in sales—a glorious return on investment. The biggest expense is postage. Of that US$ 5.60, approximately US$ 1.50 is the cost of paper. If you're concerned about trees, consider that the US has 20% more trees than it had on the first Earth Day celebration more than 25 years ago (Hatch 2007).

Direct mail is accountable. It can be measured. It is an effective method of economically talking to your customers that is guaranteed to reach all of them. The biggest mistake made by small businesses is not building a database of their customers and contacts. You begin by answering that all-important question: Who is my customer? If you can't answer that one, how can you write or design for them?

Having built your database of customers and contacts and kept it up-to-date enables you to decide to use direct mail without a scramble to collect names and addresses. A direct mail professional will help you design your mail pieces that qualify for the lowest possible postal rates. You may or may not need your own Bulk Rate Permit, depending on your mailing frequency and charges by mailers in your area.

How often should you communicate with your customers? You should mail to them a minimum of four times per year. The life of your product influences this number, as do seasonal considerations.

E-mail

Almost 2 million E-mails are sent every second, roughly 171 billion per day, of which 90% are likely to be spam. To make a profit, a spammer needs a return of only 15 responses per million (Hatch 2007). With that kind of success rate, don't look for spam to let up anytime soon. Therefore, you need to be smart about how you use E-mail as a marketing tool.

Pay attention to the subject line. You have about 5 words to get the recipient's attention. A reader will decide within 2 or 3 lines if he or she is going to give you valuable time and thought. Get directly to the point, showing the reader how he or she will benefit from giving you more precious time.

It is essential to assure recipients you will not sell their address and to give them a clear, easy opt-out route. This is not the place to be clever about reader retention.

Trade Shows

If you present at trade shows, do so wholeheartedly. Set goals for contacts. Set goals for the outcome. How will you measure success? Educate those working your booth about your goals and how you want the company message presented. This ensures everyone visiting your booth will receive the same message.

Gather contact lists. One nearly indispensable technique is a sign-up of some kind. Some ask the visitor to leave a business card, while others require a form be filled out. Which would you rather do? Less writing is more powerful. Be sure to provide business card size blank paper for those who forgot theirs, or don't want to dispense so many.

Try your best not to let anyone leave without a "take-away." The more it invokes play, the longer it will be kept and remembered. There are lots of tri-fold brochures. Don't blend into the bottom of the bag or the file at the back of the drawer.

Enthusiastic contact persons are essential. Nothing is more deadly at a trade show than a table across the front of your booth with 1, 2, or 3 tired persons slouching on folding chairs. Stand up. Smile. Two effective booth elements are a front display at an angle, which will drive traffic into your booth and mirrors. We are all attracted to a mirror. It grabs your well-positioned lighting and flashes it into the attention of those in the aisle. You can spend thousands of dollars on booth design and production, but you don't have to do so to be effective. Much depends on expectations you've established for your company. For example, a simple booth from a company such as Sony Electronics would be shocking and disappointing, but not from XYZ Vintage Vegetable Seed Company.

Your booth design must reflect your other marketing tools. This is not the time to wander off the road of consistency. Your logo, colors, style, and so on should be hard at work in your booth design.

The hardest work is after the show. Follow-up on your contacts. Refer to your conversation, about which you did make notes, of course. Include a comment about their business, which you noted on your contact sheet.

Advertising

Advertising refers to paid messages. Especially effective right now is radio. Write dynamic, streamlined copy that puts the listener in the situation. If you're a smaller company, radio is most useful for retail business, as national buys are costly. Notice, "costly" and "expensive" are not interchangeable. By "expensive" we often mean a lot of money was spent. But actually, it means the return on investment (ROI) was small or negative.

For most companies in attendance at this conference, you can most effectively present your message in print. Geography is a primary consideration. Your target market is grouped by interest, not geography. Print publications overcome the challenge of reaching potential customers who are not co-located.

Repetition is important. It buoys up your image-building. The time to advertise is when you are successful. If you wait until you *need* more business, it is too late to build an image and reputation. Funds may be too short for you to make the huge impact necessary for quick response.

Branding

Branding is listed last because it is the culmination of your effectively employing the appropriate elements previously discussed. People say the word "branding" as though it's a mysterious and complex proposition. But when you peel off all the layers of hype, it comes down to this: if advertising is "getting your name out," then branding is simply "attaching something to your name." A brand is the sum total of all the mental associations, good and bad, that are triggered by a name. What does your name stand for in the mind of the public? What are the mental associations triggered by your company name (Williams 2001)?

Even though it may seem counterintuitive, the simple truth is that the advertiser's message, itself,

is far more important than the vehicle of its delivery. Successful branding depends on your ability to speak to the customer in the language of the customer about what matters to the customer (Williams 2001).

Conclusion

When you can answer the questions above, you know your company. You understand where you are going, and how. Being content with who you are frees you to reach for the stars. Will you succeed by accident? Most "fortunate accidents" are not really accidents at all. They happen as the direct result of hope, faith, and passion. What do you want to make happen? The first step is to see it vividly in your mind. The second step is to cause all those around you to see it just as vividly in theirs. Dreams are highly contagious. What's yours (Williams 2001)?

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Recent Workforce Trends and their Effects on the Silviculture Program in British Columbia

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British Columbia's entrepreneurial silviculture sector provides a reliable just-in-time service delivery of forestry activities from planting trees to fighting wildfires. Transient, and seeming to rely often on improvisation, contractors actually run logistically sophisticated businesses that are able to match varying field conditions to the biological and administrative imperatives of their forest company and government clients. The sector has adapted well to keeping up with the next surprise.

The industry is labour intensive and relies on an elite workforce willing to consistently work hard under often remote and challenging circumstances. Just what attracts manual workers to the sector and keeps them there has been studied in 2 recent reports undertaken by the Western Silvicultural Contractors' Association's (WSCA) BC SAFE Silviculture Project, which is under the aegis of the BC Forest Safety Council. The results show a significant demographic shift to younger and less experienced workers. This has implications for the productivity of the sector, its safety performance, and the cultural underpinnings that have kept it successfully planting almost 6 billion trees to date.

Other work by the WSCA has tracked economic trends that appear to have contributed to

the draining of experience from the sector. Principally, planters earnings over the last decade have not matched trends in the consumer price index. The SAFE silviculture sector studies show workers are attracted to the sector and will remain with the contractors based on earnings and the safety and organizational competence of their employers. The shortness of the tree planting season, and the unreliability of other forestry activity opportunities, have led prospective workers to look elsewhere for employment. In 2006, almost a third of those workers interviewed said they would not return in 2007. Anecdotal reports from this year suggest the ranks of tree planters continue to fill with younger, less experienced workers.

In the short term, the applications from inexperienced workers are low, but seem to sufficient to fill the ranks of the exiting workers. As for the long term, the sector appears faced with the conundrum of having to do more forestry work, including stand tending and ecosystem restoration, with a smaller workforce.

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July 16 to 19, 2007

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