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

E-mail: diane.haase@usda.gov

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Fall 2020

Dear TPN Reader

Welcome to another issue of Tree Planters' Notes!

In addition to 4 technical articles and the annual report on seedling production in the United States, this issue includes 10 proceedings papers from the 2019 annual nursery meetings:

- Joint Annual Meeting of the Northeast Forest and Conservation Nursery Association and the Southern Forest Nursery Association (Atlantic City, NJ, July 23–25, 2019)
- Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Associations (Sidney, BC, September 30-October 2, 2019)

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

Best Regards ~

Diane

Diane L. Haase

I grow plants for many reasons:

To please my eye or to please my soul,

to challenge the elements or to challenge my patience,

for novelty or nostalgia,

but mostly for the joy in seeing them grow.

~ **David Hobson**

Contents

SEE PAGE 18

SEE PAGE 54

Papers Presented at the Joint Annual Meeting of the Northeast Forest and Conservation Association and the Southern Forest Nursery Association (Atlantic City, NJ, July 23–25, 2019).

SEE PAGE 62

SEE PAGE 66

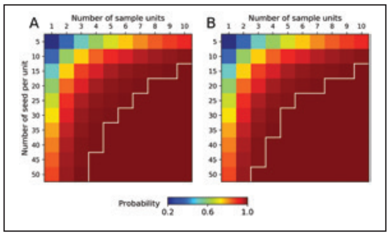
Contents

Papers Presented at the Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forestry and Conservation Nursery Association (Sidney, BC, September 30–October 2, 2019).

SEE PAGE 95

SEE PAGE 118

SEE PAGE 128



SEE PAGE 137

Fertilization Practices for Bareroot Hardwood Seedlings

David B. South and Robert E. Cross

Emeritus Professor, School of Forestry and Wildlife Sciences, Auburn University, AL;
Chief Productivity Manager, Cross Consultants, Shellman, GA.

Abstract

Large bareroot seedlings tend to be a preferred stocktype for hardwoods because they typically have larger root systems and are less expensive than seedlings grown in small containers. Fertilization can double or triple the dry mass of hardwood roots. A review of the use of fertilizers to produce bareroot hardwood seedlings revealed the total amount of nitrogen applied to seedlings depends on management objectives. The total annual rates can vary from 50 kg/ha to more than 500 kg/ha. Fertilizer regimes used to produce seedlings include a constant-rate method (i.e., each application contains similar amounts of nitrogen), a stepwise method (where initial rates are low and rates increase over the season), and formula method (where a formula is used to determine fertilizer rate). Due to a higher cost, most managers of bareroot nurseries do not use slow-release fertilizers. Some managers apply endomycorrhizal spores as insurance to prevent a phosphorus deficiency (caused by effective soil fumigation). Because micronutrient deficiencies are more likely to occur in neutral and alkaline soils, many hardwoods are grown at pH 4.5 to 5.5. Most trials in bareroot seedbeds indicate no growth benefit from K fertilization. Documented cases of Mg deficiencies in hardwood nurseries are rare and sulfur deficiencies might be overlooked in some nurseries. At nurseries with less than 1 percent organic matter, a proper fertilization regime will produce a good crop of hardwood seedlings.

Introduction

Methods used to produce hardwood seedlings have evolved over time. At one time, “shifting nurseries” were located close to reforestation sites. Once soil nutrients were depleted and weed populations increased, the temporary nurseries were abandoned. Permanent bareroot nurseries required “large needs” for fertilizer (Schenck 1907, p. 74). For example, fertilizers needed to produce 1 million seedlings might amount to 59, 7, and 36 kg of kainit (salts of potassium), superphosphate, and whale guano, respectively. Due to the cost of these fertilizers, Schenck (1907) would fertilize seedbeds using wood ashes, legumes, and compost made from street sweepings, kitchen refuse, loam, and burnt lime.

Fertilization practices today are quite different from those used 120 years ago and the species grown are different as well. In the Southern States, the demand for alder, cottonwood, and black locust (see table 1 for species’ scientific names) have declined, while demand for oaks has increased (table 1). Approximately 55 million hardwood seedlings were produced in the United States in 2016 (Hernández et al. 2018), with about 80 percent produced in bareroot nurseries in the South and Northeast (Enebak 2018, Pike et al. 2018). Demand for bareroot hardwoods in the South has doubled since 1966, but production has been declining since about 2004. In 2017, only about 39 million hardwood seedlings were produced in the United States (Haase et al. 2019), mostly in bareroot nurseries. For hardwood seedlings, the ratio of container seedlings to bareroot seedlings is about 1:8 in the Northeast (Pike et al. 2018) and about 1:142 in the South (figure 1). In the South, about 87 percent of bareroot hardwood seedlings are produced in five States (Alabama, Arkansas, Florida, Georgia, and Tennessee).

There are two primary reasons why bareroot hardwoods are the preferred stock type. First, bareroot hardwoods typically have larger roots than container-grown stock. Oak seedlings with larger root systems tend to survive better than those with smaller root systems (Alkire 2011, Kormanik et al. 1998, Schempf 2018). Oak seedlings grown in 0.75 L containers may have half the roots as 1-0 bareroot seedlings (Clark and Schlarbaum 2018, Dixon et al. 1981, dos Santos 2006, Salifu et al. 2009, Wilson et al. 2007). Although root mass of hardwoods grown in 11.3 L containers is larger than bareroot seedlings (Shaw et al. 2003, Walter et al.

Table 1. Annual production of hardwood seedlings in southern bareroot nurseries has varied by species and amount over time (based on data from Boyer and South 1984, Enebak 2018, Rowan 1972).

Species	Common Name	1966	1980	2017
		Million seedlings		
Acer spp.	Maple	–	–	–
Alnus glutinosa (L.) Gaertn.	Black alder	0.065	0.410	–
Alnus rubra Bong.	Red alder	–	–	–
Betula spp.	Birch	–	–	–
Caryea illinoensis (Wangenh.) K. Koch	Pecan	–	–	3.75
Cedrela odorata L	Cerdo	–	–	–
Cornus florida L.	Dogwood	0.395	0.492	0.401
Fraxinus pennsylvanica Marsh.	Green ash	0.313	0.647	0.542
Juglans nigra L.	Black walnut	0.240	0.147	0.114
Liquidambar styraciflua L.	Sweetgum	0.721	1.722	0.992
Liriodendron tuliperfera L.	Yellow poplar	2.078	0.601	0.642
Platanus occidentalis L.	Sycamore	0.635	1.243	0.858
Populus deltoides Bartr. Ex Marsh.	Cottonwood	3.120	0.610	–
Quercus spp.	Oaks	0.193	0.814	13.880
Quercus nigra L.	Water oak	–	–	–
Quercus rubra L.	Red oak	–	–	–
Quercus texana Buckley	Nuttall oak	–	–	–
Robinia pseudoacacia L.	Black locust	3.171	3.059	–
	Others	0.934	3.568	4.663
TOTAL		11.865	13.313	22.467

2013), the production cost is higher. This cost differential is the other reason why bareroot is preferred for hardwood seedlings. The retail price of oak seedlings may range from $0.40 (bareroot), to $1 (0.15 L container) to $11 (11.3 L container). Thus, due to the higher cost of container stock and the acceptable survival of properly planted bareroot stock, most oak plantations in the Eastern United States are planted with bareroot stock (Dey et al. 2008, Gentry 2020).

One million oak seedlings harvested in November may contain 552 kg N and 96 kg P (dos Santos 2006), but these estimates depend on several factors. For example, when oaks are grown without fertilization, one hectare of seedbeds might contain less than 120 kg N and weigh half as much as fertilized seedlings (table 2). Therefore, to maintain soil productivity and to produce good-quality seedlings, nursery managers use a variety of fertilization methods. To document the variability of bareroot hardwood fertilization practices, we conducted a literature review and also provide some observations from over 40 years of personal experience working in bareroot nurseries. It is ironic that most hardwood fertilizer research in the 21st century involves growing in containers, whereas most fertilizers used to produce hardwood seedlings are applied in bareroot nurseries.

[Abbreviations: Al = aluminum. AN = ammonium nitrate. B = boron. Ca = calcium. Cl = chloride. Cu = copper. Fe = iron. GA = green ash, K = potassium, Mg = magnesium. Mn = manganese. Mo = molybdenum. N = nitrogen. Na = sodium. P = phosphorus. S = sulfur. SG = sweetgum. YP = yellow poplar. Zn = zinc. ppm = parts per million. Cation exchange capacity = CEC. OM = organic matter. UAN = urea ammonium nitrate. US = unspecified state. Soil pH was measured in water except in one study where a calcium chloride buffer (CCB) solution was used.]

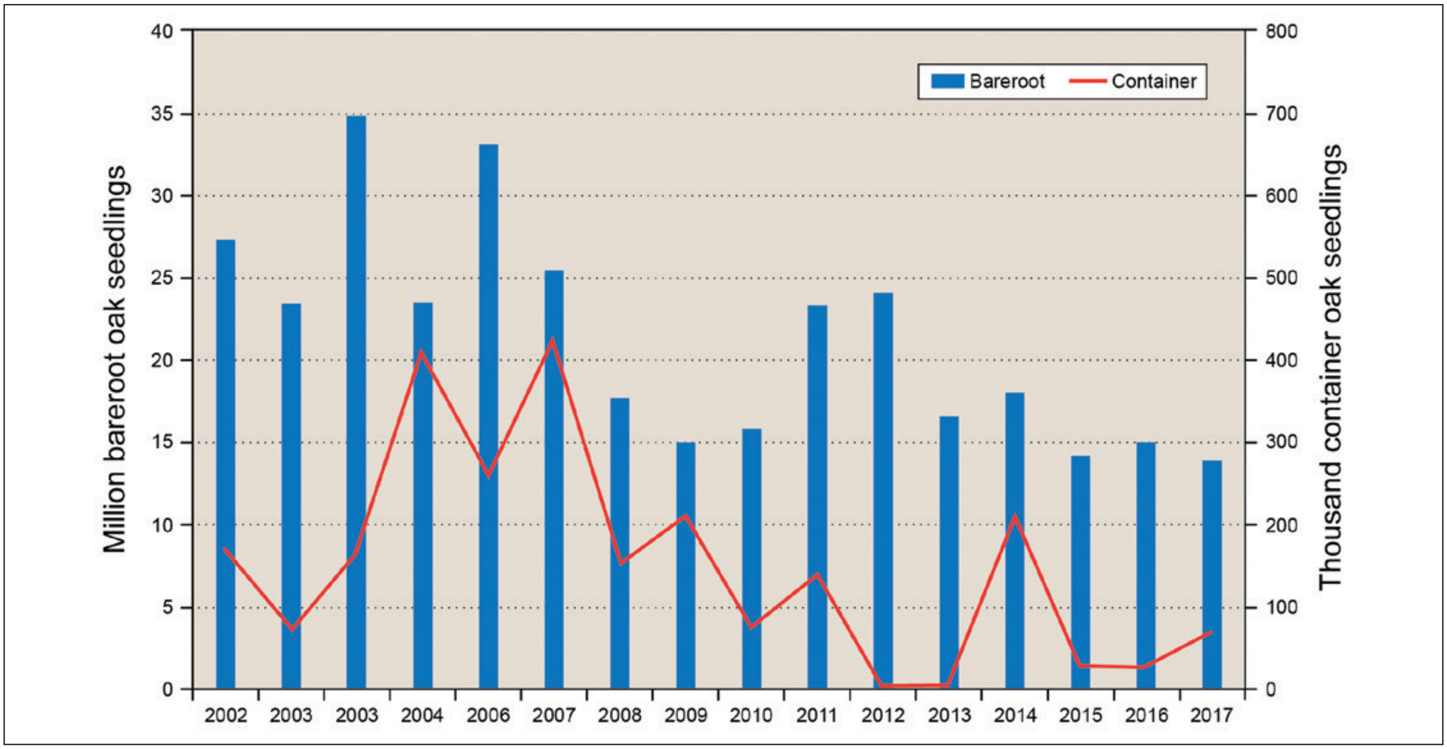


Figure 1. The trend in oak seedling production in the Southern United States (Enebak 2018). The average annual production from 2002 to 2017 was 21.3 million and 0.15 million for bareroot and container seedlings, respectively.

Fertilizer Types

Granular and Soluble Fertilizers

Common granular fertilizers applied before sowing include elemental S, KCl, gypsum (Ca-sulfate), dolomite (agricultural lime), and langbeinite (sul-po-mag). Granular AN was once commonly applied in hardwood nurseries; in fact, 1 nursery applied 14 applications over the growing season (Timmer 1985). However, due to safety reasons (Moore and Blaser 1960), AN is now applied as part of a liquid fertilizer mix (i.e., UAN).

Over time, most managers have shifted away from granular fertilizers and now apply soluble fertilizers after seedlings have formed true leaves. Some managers observe foliar burning when applying UAN (e.g., 37 kg N/ha) to young hardwood seedlings, and therefore, they switched to liquid urea (23-0-0) or urea traizone (28-0-0) to reduce phytotoxicity. Some managers apply liquid fertilizers using shielded sprayers (figure 2). Advantages to applying liquid top-dressings include: (1) greater application uniformity, (2) easier to apply with less labor (Triebwasser 2004), and (3) no need for leaves to be dry at the time of application. After application of soluble fertilizers, irrigation is used to remove fertilizer residue from foliage.

Figure 2. At some nurseries, liquid ammonium polyphosphate is applied to hardwood seedbeds in May, June, July, and August using directed sprayers. The shield reduces the amount of fertilizer applied to the tire-paths and increases the amount of phosphorus applied to the soil. (Photo by Robert Cross 2014).

Table 2. Estimates of harvested dry mass of bareroot hardwood seedlings from various nurseries (Mg/ha and #/ha are based on seedbed areas only; no unused land). Seedling dry mass at lifting depends on species, seedbed density, amount of nitrogen (N) fertilization, and seedling age at harvest. The amount of N harvested depends on the fertilization rate, fertilization method, length of time in the seedbeds and top-pruning prior to lifting. Fertilization methods include a constant rate of N per application (CON), an exponential rate of fertilization (EXP), and slow-release fertilizer application (SRF). Nitrogen use efficiency in this table was determined by dividing nitrogen harvested by nitrogen applied.

Species	Dry mass (Mg/ha)	Density (#/ha)	Dry mass (g)	Nitrogen harvested (kg/ha)	Nitrogen applied (kg/ha)	Nitrogen use efficiency (%)	Fertilization method	Lifting month	Reference
Oak	6	1,000,000	5.7	50	0	>100	NONE	September	Schmal et al. 2010
Oak	5	650,000	8.0	65	0	>100	NONE	December	Birge et al. 2006a
Oak	8	830,000	10.2	68	0	>100	NONE	December	Tilki et al. 2009
Oak	12	860,000	14.0	103	0	>100	NONE	October	Fujinuma 2009
Oak	15	860,000	18.0	120	0	>100	NONE	October	Fujinuma 2009
Oak	14	1,000,000	13.8	110*	0	>100	NONE	October	Dixon et al. 1981
Oak	7	1,000,000	7.1	85	180	47	CON	September	Schmal et al. 2010
Oak	16	860,000	18.7	96	269	40	CON	January	Williams and Stroupe 2002
Oak	12	650,000	19.0	195	273	71	EXP	December	Birge et al. 2006
Oak	23	840,000	27.5	180	287	63	CON	November	dos Santos 2006
Oak	14	650,000	21.0	286	546	52	CON	December	Birge et al. 2006
Oak	18	650,000	28.0	129	819	38	EXP	December	Birge et al. 2006
Oak	11	1,000,000	10.8	110	55	>100	CON	September	Dixon and Johnson 1992
Oak	29	860,000	33.7	158	259	61	CON	October	Fujinuma et al. 2011
Oak	25	860,000	29.0	195	157	>100	SRF	October	Fujinuma et al. 2011
Cerdo	4	4,060,000	0.9	130	660	20	CON	October	Mexal et al. 2002
Green ash	16	1,180,000	13.7	129	112	>100	CON	January	Lamar and Davey 1988
Green ash	12	920,000	13.1	86	239	36	CON	November	dos Santos 2006
Sweetgum	9	920,000	9.7	71*	140	51	CON	August	South et al. 1980
Yellow poplar	31	770,000	40.4	233	251	93	CON	November	dos Santos 2006
Walnut	20	960,000	20.9	160*	81	>100	CON	December	Brookshire et al. 2003
Walnut	18	400,000	44.7	143*	616	23	CON	November	Kormanik 1985

* Nitrogen harvested was estimated based on 0.8 percent N for seedling dry mass.

Slow-Release Fertilizers

Container nurseries use slow-release fertilizers (SRF) which can reduce waste that occurs with applying liquid fertilizers. Bareroot nurseries, however, rarely use SRF because the cost of N is 6 to 12 times more than that contained in liquid fertilizers (Timilsena et al. 2015, table 3). Applying SRF at 180 kg N/ha might cost $2,200 per ha and 484 kg N/ha (Garbaye et al. 1992) might cost $5,900.

SRF are sometimes referred to as “controlled release” but this can be misleading. Nursery managers can “control” the timing and rate of liquid fertilizer applications, but once SRF is incorporated into bareroot seedbeds, any “control” over nutrient release is gone. Greenhouse managers can control irrigation and temperature, but bareroot nursery managers do not have any control over rainfall or seedbed temperatures, which affect nutrient release rates. When SRF continue to release N in the late summer, shutting down seedling growth can be difficult (Steinfeld and Feigner 2004). Also, when soil stabilizers are not applied after sowing, some SRF pellets can work their way to the soil surface and wash away during downpours.

Table 3. Examples of nitrogen (N) fertilizers and the relative price per kg of N. Prices calculated assuming no value for Ca, K, P, and S.

Type	Fertilizer	% N	% P	% K	% S	% P_2O_5	% K_2O	Price per kg of N
Granular	Urea	46	0	0	0	0	0	$1.03
Granular	Urea + slow release coat	44	0	0	0	0	0	$1.20
Granular	Ammonium sulfate	21	0	0	24	0	0	$2.00
Granular	Diammonium phosphate	18	20	0	0	46	0	$3.00
Granular	Calcium nitrate	15.5	0	0	0	0	0	$3.65
Granular	Potassium nitrate	13	0	37	0	0	44	$10.70
Granular	Slow-release fertilizer	5	0	1.6	0	0	2	$13.20
Granular	Slow-release fertilizer	18	3	10	0	6	12	$12.30
Granular	Slow-release fertilizer	16	2	9	6	5	11	$6.80
Liquid	UAN	32	0	0	0	0	0	$1.00
Liquid	Urea	23	0	0	2	0	0	$1.20
Liquid	Ammonium thiosulfate	12	0	0	26	0	0	$2.70
Liquid	Liquid poly-phosphate	10	15	0	0	34	0	$6.00

When using SRF in bareroot seedbeds, the production of plantable seedlings is not as reliable as soluble fertilizer applications (Berenyl and Harrison 1992, van den Driessche 1988, Villarrubia 1980, Zarger 1964). In one study with bareroot pines, SRF produced 16 percent culls while liquid fertilization produced 3 percent culls (McNabb and Heser 1997). In another study, stunting occurred when seed were sown just above a band of SRF (Steinfeld and Feigner 2004). A valid economic comparison must include effects on seedbed density and cull percentages. Without a proper economic analysis, some growers may assume profits would increase after switching to SRF technology (Dobrahner et al. 2007, Timilsena et al. 2015).

Nitrogen (N)

The necessary amount of N applied to grow bareroot hardwood seedlings varies by species, year, rainfall, soil type, soil texture, and manager objectives (table 4). Slower growing species, such as water oak and pecan, may need more N than faster growing species, such as green ash and Nuttall oak. When fertilized at 434 kg N/ha, water oak may be half as tall as Nuttall oak (Kormanik et al. 1994). Typically, less N is needed when the target oak seedling height is 0.3 m, and more N will be needed when the target height is 1.2 m. Some species (e.g., alder and black locust) require little or no N in seedbeds, since they form symbionts that can utilize N from the air (Crannell et al. 1994, Hilger et al. 1991). The length of growing season affects seedling growth more than the N rate (figure 3).

Nitrogen Use Efficiency

For the purpose of this paper, nitrogen recovery efficiency and nitrogen use efficiency (NUE) are synonymous. NUE is determined by dividing N uptake (i.e., N in seedlings at harvest) by N applied as fertilizer (i.e., total N applied/seedling). When applying more than 400 kg N/ha per year, reducing the rate of N (per application) and increasing the frequency might increase NUE (Quiñones et al. 2003, South 1994).

A simple method to increase NUE is proper irrigation to reduce leaching of N. For sandy seedbeds, less N is leached at 2.5 cm water per week than at 5 cm per week (figure 4). NUE can also be increased by allowing soil acidity to gradually fall below pH 5.6. Uptake of selected nutrients in pecan was 23 to 88 percent greater when grown in pH 5.5 soil, compared with pH 6.5 soil (figure 5). Likewise, foliar N concentrations and seedling biomass were greater when oaks were

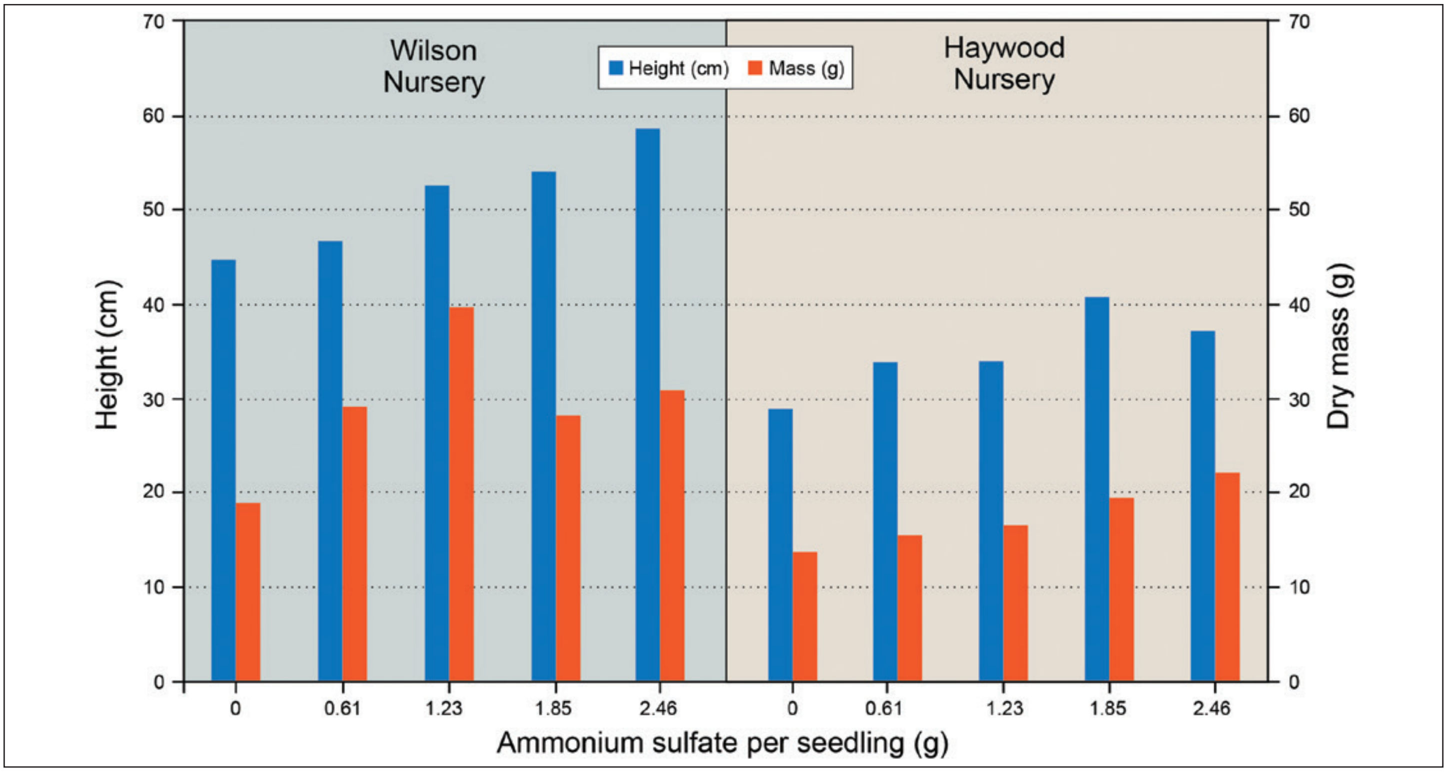


Figure 3. Effect of ammonium sulfate fertilizer and nursery location on seedling height and biomass of 2-0 northern red oak at nurseries in Wisconsin (Fujinuma 2009). The 2.46 g per seedling rate is equivalent to 445 kg N/ha and 508 kg S/ha. The growing season at the southern nursery (Wilson) is about a month longer than at the northern nursery (Haywood). The soil pH was initially 5.9 to 6.1; hardwoods tend to grow better in soils where ammonium sulfate has lowered the soil pH (Villarrubia 1980).

grown in $pH_{(CCB)}$ 4.3 soil vs. $pH_{(CCB)}$ 7.7 soil (Berger and Glatzel 2001).

Reducing soil nitrification rates can also increase NUE, especially in sandy nursery soils where rainfall leaches nitrates (Bengtson 1979, Radwan 1965). For example, fertilizing with ammonium thiosulfate can inhibit soil nitrification (Goos 2019) and will also lower soil pH. When applying nitrifying reducing products, it is important to remember that not all products work as expected (Franzen et al. 2011).

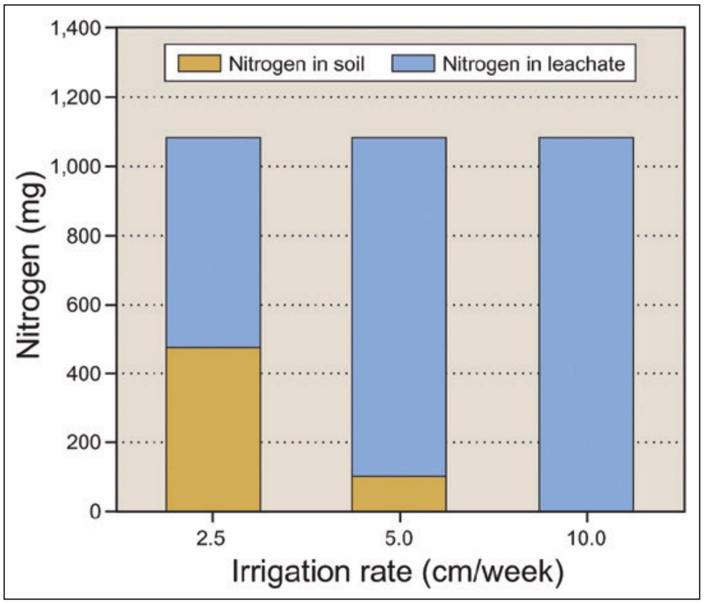


Figure 4. The effect of irrigation on the amount of nitrogen (N) leached from containers filled with sand (Bengtson and Voigt 1962). N (1,088 mg) was applied to the sand as ammonium nitrate. Although the results were presented after 17 weeks, most of the N was gone from the soil of the high irrigation rate after 4 weeks.

Seed efficiency (number of plantable seedlings produced per 100 pure live seed) can be reduced when managers lower fertilization rate in order to increase NUE. This is the main reason why NUE is not maximized at bareroot nurseries. For example, at one nursery, seed efficiency was reduced when the fertilization rate was decreased by 75 percent (O'Reilly et al. 2008).

When little or no fertilizer is applied, NUE will be above 100 percent, and when more than 200 kg N/ha is applied, NUE may average 50 percent (table 2). Therefore, when one-fourth the normal rate of N is applied to seedlings, researchers and practitioners can mistakenly attribute all the NUE increase to the use of SRF. Despite expectations that SRF applications will increase NUE, this is not true in all cases (Fujinuma et al. 2011, Fuller 1988, McNabb and Heser 1977, Villarrubia 1980, Zarger 1964).

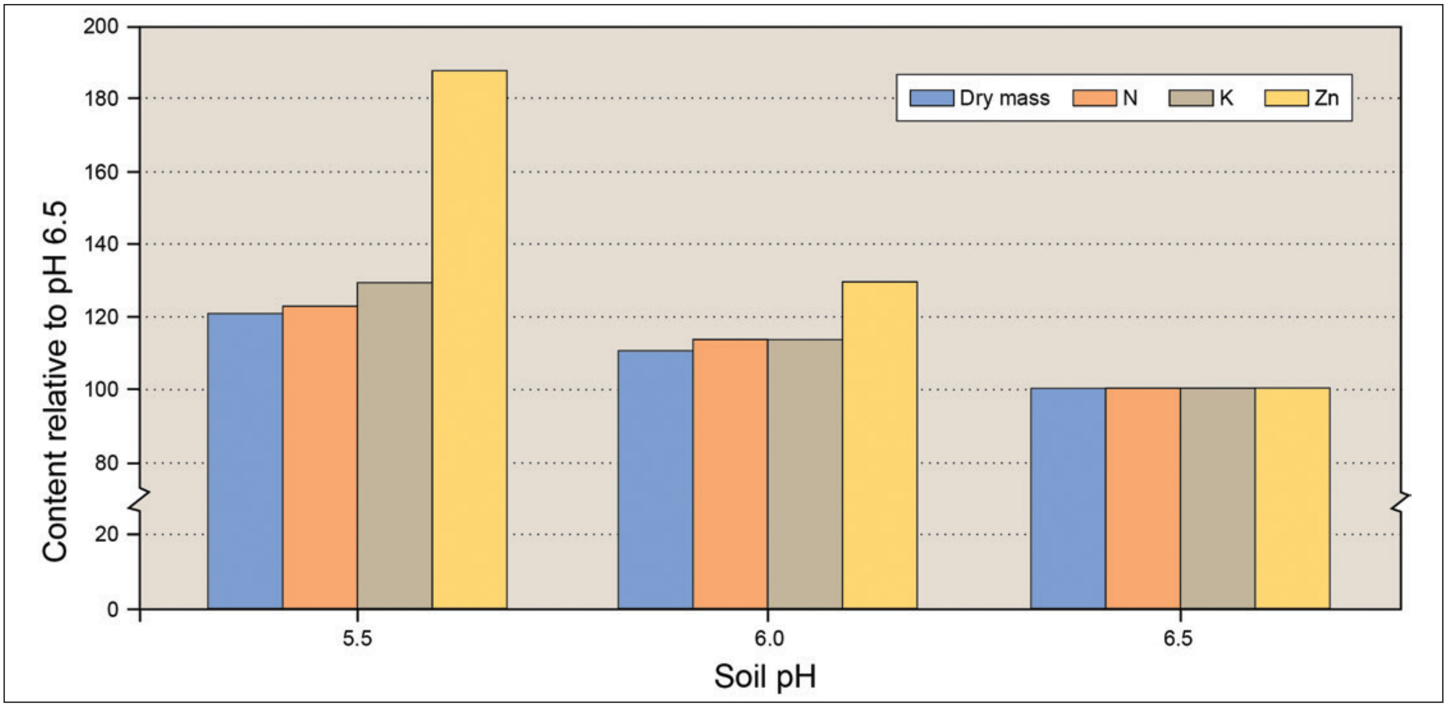


Figure 5. Nutrient use efficiency is sometimes greater when pecan seedlings are grown at soil pH 5.5 compared with soil pH 6.5. (Adapted from Sharpe and Marx 1986). Nutrient uptake of N (mg per seedling) was 23 percent greater at pH 5.5 than at pH 6.5. For pH 6.5 soil, average seedling values were 12.8 g dry mass, 145 mg N, 118 mg K, and 0.4 mg Zn.

Timing of N Application

When applied after sowing, an excessive delay in N fertilization can reduce seedling growth and NUE (Beckjord et al. 1980, Booze-Daniels et al. 1984). In some trials, a 21-week delay in N fertilization after sowing reduced seedling mass by 32 percent (Deines 1973). On the other hand, applying N too far ahead of sowing can waste resources and reduce NUE. For example, applying urea and ammonium nitrate before sowing oaks in the fall or before sowing small-seeded species in the spring is wasteful because rain can rapidly leach N, especially in sandy soils (Bengtson and Voigt 1962, Gaines and Gaines 1994). At some nurseries, AN only lasts in the soil about 6 weeks (Berenyl and Harrison 1991).

Benzian (1959, p. 639) wrote, “Nitrogen applied before sowing occasionally increased losses through ‘damping off’, and it has been better to apply soluble nitrogen fertilisers as top-dressings between June and September.” Because soil fumigation is often used prior to sowing hardwoods, problems with damping-off and root rot are reduced. Even so, some recommend keeping N fertilization low during the first 6 weeks after emergence (Enebak 2019, Filer and Cordell 1983). For example, applying 224 kg N/ha before sowing increased sycamore seedling mortality (Berenyl et al. 1970). For this reason, many authors recommend applying N only as top-dressings to bareroot hardwood seedlings (Aldhous and Mason 1994, Landis and Davey 2009, South 2019b).

When acorns and walnuts are sown in the fall, the first application of N occurs in the spring soon after true leaves emerge. At nurseries with relatively long growing seasons, the first N application is made in April or May. At nurseries with short growing seasons, fertilization begins in June (table 4). The final N application in bareroot nurseries is typically made before mid-September.

Total Rate of N

To grow bareroot hardwoods, the total amount of N applied in a year can vary from 50 kg/ha (Hauke-Kowalska and Kasprzyk 2017) to 112 kg/ha (Grieve and Barton 1960, Hoss 2004, Lamar and Davey 1988, Thor 1965) to 295 kg/ha, (Stone 1986) to 560 kg/ha (Garbaye et al.1992, Kormanik et al. 1998, Reazin et al. 2019), and some researchers have tested rates up to 900 kg/ha (Brown et al. 1981). As a comparison, recommended rates for horticultural greenhouses can exceed 1,400 kg N/ha (Chen et al. 2001).

Table 4. Examples of nitrogen (N) fertilizer rates (kg/ha) for spring-sown (March through May) and fall-sown (October through December) bareroot hardwoods. Except for one pre-sow application (in bold), applications were top-dressings. Application dates are approximate. Asterisk (*) indicates first year of 2-0 stock. Fertilization methods are: constant rate (CON), exponential rate (EXP), and stepwise rate (STEP). Species codes are: green ash (GA), sweetgum (SG), and yellow poplar (YP).

	Location	NC	VA	VA	GA	US	TN	US	IN	WI	IN	GA	IN	MO	WI	IR	AL
	Species Code	GA	SG	SG	SG	SG	YP	YP	Oak	Oak	Oak	Oak	Oak	Oak	Oak	Oak	Oak
	Sow date	5/15 1972	5/3 1978	Spring 1986	3/15 2004	4/17 2018	4/17 2006	4/17 2018	Fall 1984	Fall 1990	Fall 2000	Fall 2003	Fall 2003	Fall 2004	Fall 2005	Fall 2007	Fall 2009
	Method	Step	Step	Step	Step	Con	Con	Step	Con	Con	Con	Step	For	Con	Con	Con	Con
1	April 24																50
2	May 1				12												
3	May 8	**112**			12		39				78	6	42				
4	May 15				20												50
5	May 22										78	6	35				
6	May 29			42	28			20	85			18		28			
7	June 5						39			37	78		37	28		36	50
8	June 12		11	41	28			20				55		28	24		
9	June 19		11			28				37	78		48	28	23	36	
10	June 26		11	41	28	2	22	35	85			55		28	24		50
11	July 3					28				37	78	55	67			36	
12	July 10		28		28	30	34	40						28	23		
13	July 17	18		65		24				37	78	55	112	28	24	36	
14	July 24	28	28	53	28	24		35							23		50
15	July 31	28	37			20	39			37	78	55	112	28		36	
16	Aug 7				28	24	61								24		
17	Aug 14	28	37	53		24		40		37		55					
18	Aug 21				20	24									23		
19	Aug 28	22	37			24				37		37					
20	Sept 11	28	7									37					
Total kg N/ha		264	207	295	232	252	234	190	170	259	546	434	453	224	188*	180*	250

In 1930, grade-1 oak seedlings might be 21 cm tall (Guillebaud 1930) and 1 million seedlings might contain a total of 18 kg N. Now a million bareroot hardwood seedlings may average 50 to 70 cm tall and contain more than 400 kg N (table 2). A driving force for increased N fertilization in nurseries over the last several decades is because growth of hardwoods after planting is affected by seedling size at planting, which increases with N application (Jacobs et al. 2005, McNabb and Vanderschaaf 2005).

N Fertilizer Regimes

Fertilizer programs can be categorized into several regimes (Park et al. 2012). The constant regime (CON) employs the same N rate for each N application, while a stepwise (STEP) regime starts with a low N application (to increase NUE and avoid phytotoxicity) and then increases the N rate in two or three "steps." A slow-release regime (SRF) may involve one or two fertilizer applications per year

(Fujinuma et al. 2011, Garbaye et al. 1992, Iyer et al. 2002, Vande Hey 2007). A formula (FOR) regime employs a mathematical equation so that each N application has a unique N rate per hectare. Thus, application rates can vary greatly among nurseries. For example, one FOR regime applied urea (46-0-0) at 112 kg N/ha on July 17 (table 4), which is about five times as great as applying 23 kg N/ha using a CON regime.

Growers who follow the CON method typically apply 28 to 85 kg N/ha at each top dressing regardless of seedling age (Hoss 2004, table 4). Managers using the STEP method who are concerned about leaching apply top dressing at low initial rates; for example, the first application may be 6 to 12 kg N/ha followed by an application of 18 to 20 kg N/ha, and then 28 to 37 kg N/ha. When growing 2-0 seedlings, one STEP manager applies 50 kg N/ha during the first year and 60 kg N/ha during the second year (Hauke-Kowalska and Kasprzyk 2017).

Some researchers use an “exponential” formula (EXP) where the first application is the lowest N rate, the rate for each subsequent application is increased by a calculated amount, and the highest N rate is applied at the end of the season. For example, in one trial, each application contained 66 percent more N than the previous application (Chen et al. 2017). With some EXP regimes, more than 70 percent of the N is applied during the last two fertilizer applications (Chen et al. 2017, Schmal et al. 2011). A danger of the EXP regime is that seedling survival can be reduced if excessive salts are applied at the last application (Salifu et al. 2008). Therefore, others use a modified exponential formula (MEX) where the initial application has more N than subsequent applications made 4 to 6 weeks later (Birge et al. 2006, Hu et al. 2015, Imo and Timmer 1992, Reazin et al. 2019). With this regime, half of the total N may be applied in the last two applications. At some locations, hardwood seedlings that receive 50 percent of the fertilizer in the last few applications are referred to as “nutrient loaded” (Salifu et al. 2008).

In one trial, the fertilization regime had little impact on yellow poplar seedling growth although the CON method resulted in quicker early growth (Park et al. 2012). Weed control is easier after canopy closure so nursery managers favor regimes that result in rapid early growth of hardwoods.

Phosphorus (P)

Growers’ views vary regarding the minimum desired level for soil P in hardwood nurseries. These views also vary by the seedling species. Some growers set 15 to 22 ppm as the standard soil P level for hardwood seedbeds (Williams and Hanks 1994), while others set a target of 100 ppm (weak-Bray) (Kormanik et al. 2003) which is equivalent to 143 ppm Mehlich 3. Some growers conclude that seedbeds with more than 44 ppm P (Mehlich 3) do not need to be fertilized with P (Davey and McNabb 2019). A 300-ppm target (weak-Bray) is too high for sandy nurseries and can result in Cu and Zn deficiencies (Teng and Timmer 1990).

Endomycorrhizal hardwoods such as sweetgum, yellow poplar, green ash, and maple may become P deficient following soil fumigation. Therefore, some growers apply spores to increase the chance of endomycorrhizal formation on these hardwoods. In contrast, ectomycorrhizal species such as oak and pecan are less likely to be P deficient since windblown spores typically inoculate fumigated soil. With little or no P fertilization, mycorrhizal walnut, oak, and pecan seedlings can grow well in soil that contains as little as 8 ppm P (Marx 1979a, 1979b; Ponder 1979). Likewise, in fumigated soil with 16 ppm P, mycorrhizal plants can uptake enough P to grow well (figure 6). In contrast, non-mycorrhizal seedlings may need to be fertilized with P even when the soil has 100 ppm P. In a greenhouse trial (Yawney et al. 1982), mycorrhizal sweetgum seedlings grew taller in unlimed, fumigated soil (pH 4.5) when fertilized with enough dicalcium phosphate to raise the soil to 100 ppm P (figure 7).

For sweetgum, there is a subtle but practical difference between an unfertilized soil at 100 ppm P, and a recently fertilized soil at 100 ppm P. Growth of non-mycorrhizal sweetgum seedlings will benefit from P fertilization (figure 6), but there likely will be no growth benefit from 100 ppm in the soil if there has been no recent P fertilization because P becomes tied up and immobile in the soil. As a result, some growers apply 30 kg/ha of P (late May to early June) even when soil tests indicate 115 ppm (Mehlich 3) (South 2018). Soil P is typically high in operational hardwood seedbeds and, therefore, the difference among target values (15 ppm vs 100 ppm) may have little practical meaning.

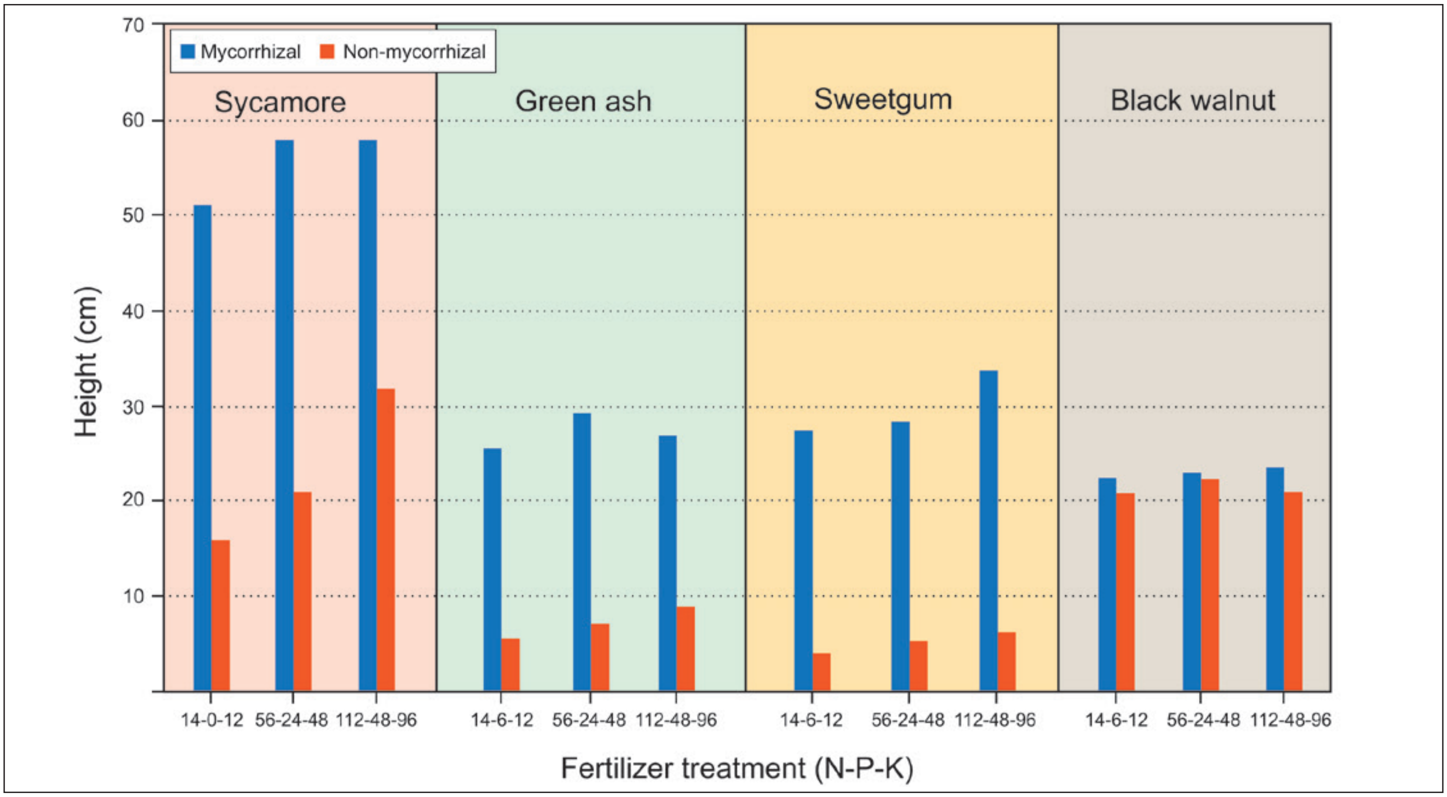


Figure 6. Effect of inoculation (about 850 Glomus spp. spores/m^2) and fertilization before sowing on height growth of bareroot hardwood seedlings (Schultz et al. 1981). Ten equal applications of $NH4NO_3$, totaling 1,680 kg/ha, (560 kg N/ha) were applied as top dressings. Pre-fertilization soil level was 12 ppm P. Fertilizer treatments did not affect heights of mycorrhizal seedlings but increased height of non-mycorrhizal sycamore, green ash, and sweetgum ($\alpha = 0.05$).

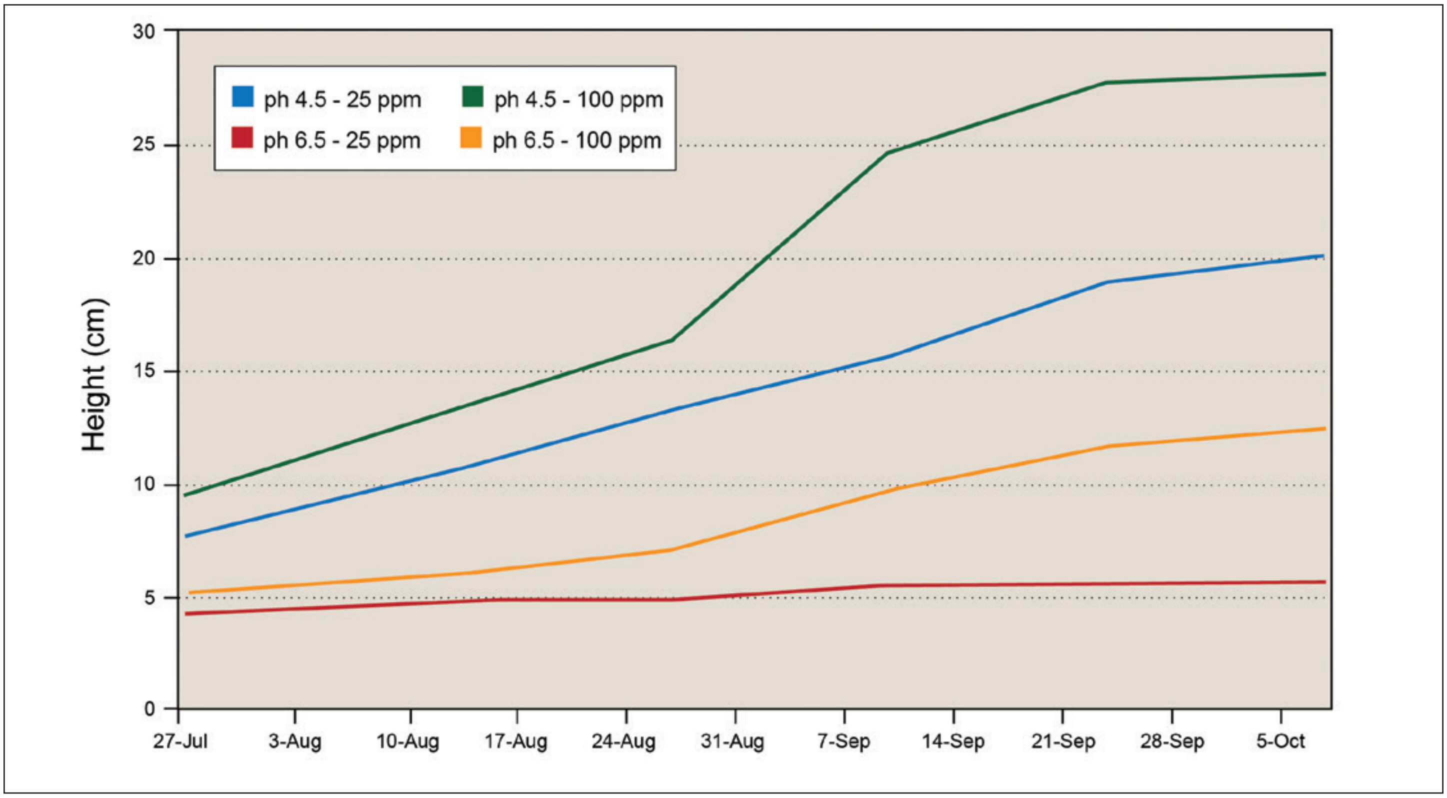


Figure 7. The beneficial effect of phosphorus fertilizer was greater for endomycorrhizal sweetgum seedlings grown without lime ($Ca(OH)_2$) compared with those with lime applied 4 weeks before sowing (soil pH 4.5 and 6.5, respectively). Dicalcium phosphate fertilization was applied at two rates (25 ppm and 100 ppm P). Seedlings were grown in a soil-perlite (4:1 v/v) medium in a greenhouse (Yawney et al. 1982).

Although research shows that a high level of soil P can reduce endomycorrhiza infection (Marx et al. 1989, Schultz et al. 1981, Yawney et al. 1982), nurseries with more than 370 ppm P have no problems growing endomycorrhizal seedlings (Han et al. 2016, Lambert 1982, Timmer 1985). In addition, fertilization with NPK can sometimes increase the percent infection with endomycorrhiza (Schultz et al. 1981). In fact, when two seedlings have an equal amount of endomycorrhizal biomass, the one with more roots will have fewer mycorrhizal biomass per m of root. Thus, larger seedlings with more roots (figure 8) can sometimes have the lowest percent infection.

Potassium (K)

There is little evidence to show that K fertilization will benefit growth of bareroot hardwood seedlings (figure 9). Soil with 21 to 40 ppm K produced 50 cm tall seedlings without any K fertilizer (Lamar and Davey 1988, South 1975). At one nursery, applying KCl (448 kg/ha) increased average sweetgum height by 6 cm (South 1975), but the same fertilizer treatment did not affect growth of sycamore and sweetgum at six other nurseries (Deines 1973, South 1975). In another trial, oak seedling growth was negatively related to foliar K (Phares 1971). Although some researchers recommend half of the K fertilizer be incorporated into the soil before sowing (Landis and Davey 2009), there is no evidence to show that hardwoods benefit from this practice. With sufficient rainfall, K can leach from irrigated sandy soil before roots can uptake nutrients. Freeze tolerance is not increased by KCl fertilization in August (Williams et al. 1974) and high levels of K can sometimes reduce freeze tolerance (Jozefek 1989, Koo 1985).

Calcium (Ca)

Most nursery soils contain more than 100 ppm Ca. Calcium deficiencies in hardwoods (Erdmann et al. 1979) are rare in bareroot nurseries (Davey 2005). There are only a few nursery studies that involve Ca treatments to hardwoods. At one nursery, a CaCl treatment temporarily lowered soil pH to 4.5, which increased yield of red alder seedlings (Crannell et al. 1994). In another trial, applying 1,121 kg/ha of Ca-carbonate after sowing (to a soil with more than 150 ppm Ca) had no effect on growth of green ash

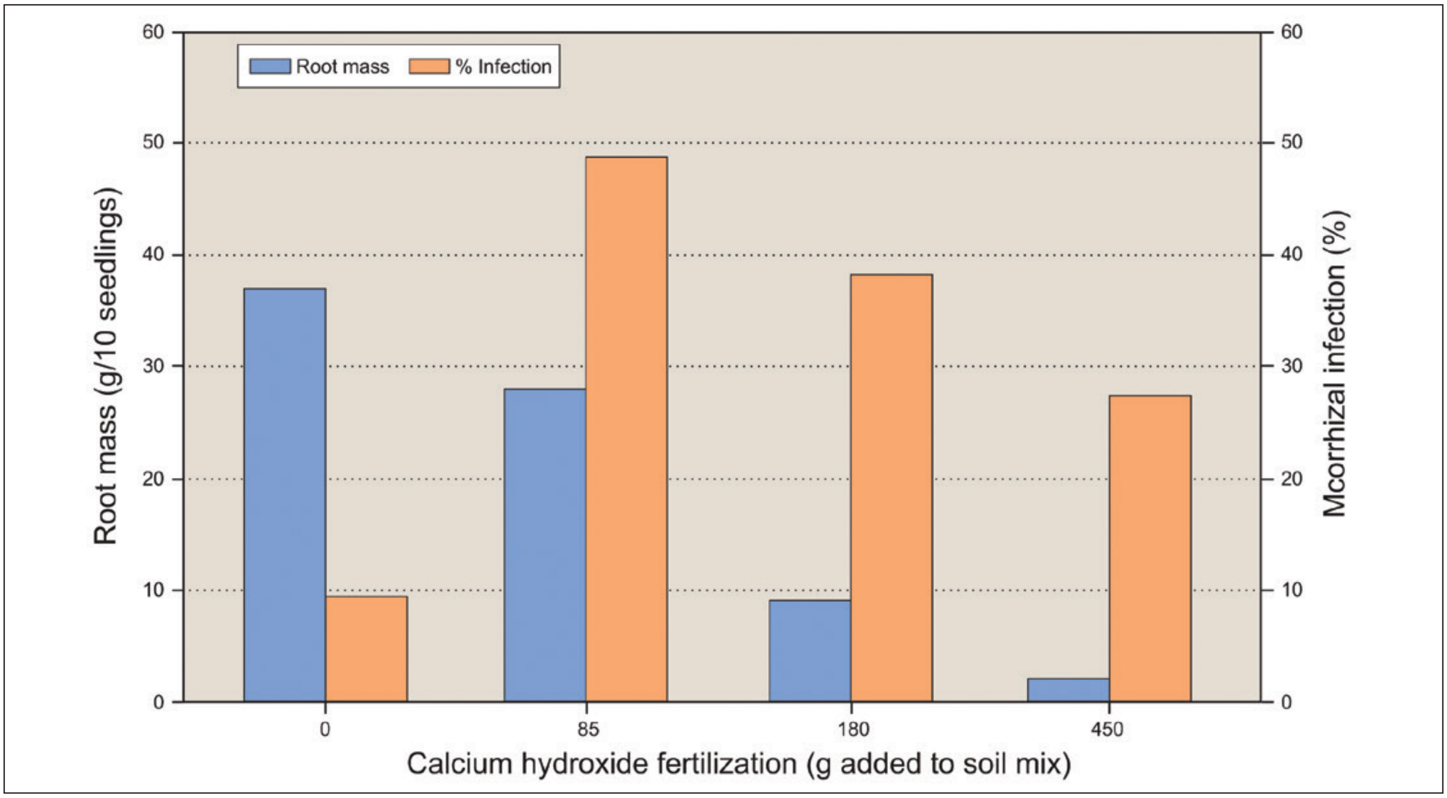


Figure 8. Fertilization with calcium hydroxide ($Ca(OH)_2$) reduced root growth of sweetgum seedlings but increased mycorrhizal infection of roots ($\alpha = 0.05$) (Yawney et al. 1982). By October, $Ca(OH)_2$ treatments resulted in pH levels of 4.6, 5.6, 6.5, and 7.8, respectively and soil calcium levels of 243, 622, 1,250, and 3,157 ppm, respectively. The largest seedlings, with the lowest percent infection of endomycorrhiza, were growing in pH 4.6 soil.

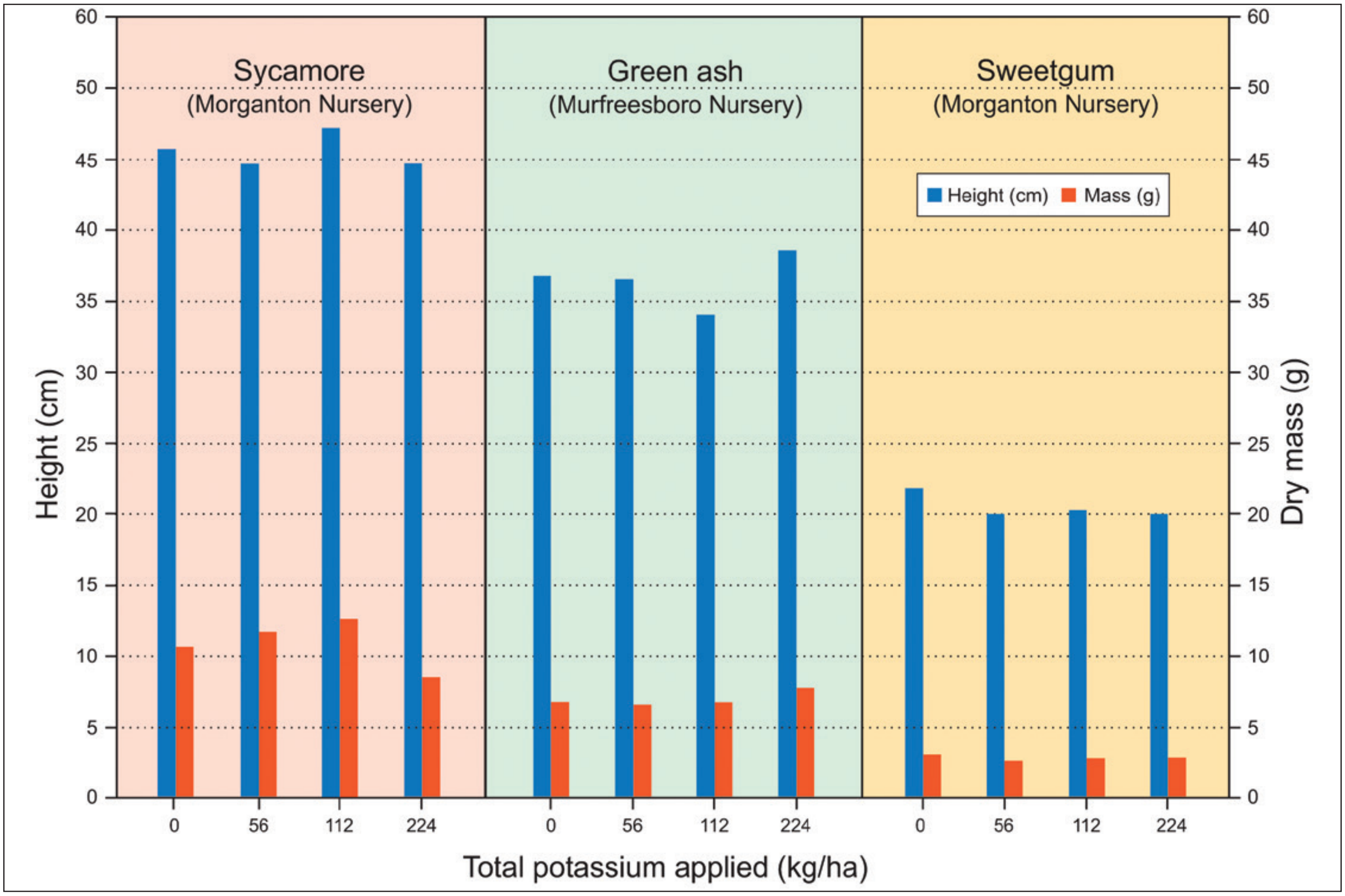


Figure 9. Four KCl fertilizer rates (divided over three equal applications) were applied to hardwood seedlings at two nurseries. At the Morganton Nursery, three applications were applied May 18 (before sowing), July 10, and August 22, 1972 on sycamore and sweetgum seedlings (Deines 1973). Similarly, three applications were made at the Murfreesboro Nursery on green ash after germination on July 17, August 14, and September 11, 1972. The KCl fertilizer had no effect ($\alpha = 0.05$) on height growth at either nursery. The high rate reduced seedling dry mass (standard error = 0.8 g) of sycamore but had no effect on biomass of green ash or sweetgum. The soil K level at the Morganton Nursery was 63 ppm in October 1972 and at the Murfreesboro Nursery, soil contained 32 ppm K in April 1974 (South 1975).

seedlings (Deines 1973). At a sweetgum nursery, seedbeds contained more than 250 ppm Ca and applying 1,121 kg/ha of Ca-carbonate (before sowing) decreased shoot dry mass (Deines 1973).

For sands and loamy sands, recommended Ca levels range from 200 ppm (South and Davey 1983) to 300 ppm (Davey and McNabb 2019) to 500 ppm (Kormanik et al. 2003). For high CEC soils in Western States, 1,000 ppm Ca has been recommended for seedbeds (Engstrom and Stoeckeler 1941, Landis 1988). However, when soil Ca levels increased to 622 ppm or higher with Ca-hydroxide fertilization, growth of sweetgum seedlings was reduced (figure 8). Similarly, adding too much lime can reduce hardwood seedling growth (Phares 1964, South 2019a, Timmer 1985) and root rots are most severe in seedbeds with a pH above 5.5 (Cordell et al. 1989).

Magnesium (Mg)

Documented cases of Mg deficiencies in hardwood nurseries are rare (Davey 2005, Hüttl and Schaaf 2012). This lack of deficiency may be due to applications of dolomitic lime or sul-po-mag. Some researchers adjust soil Mg to 50 ppm before sowing hardwoods (Kormanik et al. 1998) and some agronomists recommend Mg when soils contain 60 ppm Mg (South 2019b). In contrast, others see no need to apply Mg when soil levels are above 30 ppm (Davey and McNabb 2019). Although increasing K in solution will lower the amount of Mg in foliage (Cutter and Murphey 2007), excess K fertilization in hardwood seedbeds is not likely since K fertilization is not a practice used to "harden-off" deciduous hardwood seedlings.

Sulfur (S)

Stone (1980, p. 125) raised the question, "how much sulfur is needed for adequate hardwood growth?" The answer is still unclear and therefore some researchers set no target level for soil S. Davey and McNabb (2019) suggest S fertilization when soil levels fall below 10 ppm. Most nursery soils contain less than 20 ppm sulfate-S, which is the form available to plants. S deficiencies are rarely reported in hardwood seedlings (Aldhous and Mason 1994, Knight 1981, Leaf 1968). However, this is based on: (1) no obvious color symptoms in operational seedbeds (Stone 1980); (2) operational use of fertilizers that contain S; (3) no published photos of S deficiency from hardwood seedbeds; and (4) assuming type II statistical errors do not exist in nursery trials. Variability in hardwood seedbed density can be so high that a 25-percent increase in seedling production cannot be declared statistically significant.

Sulfur deficiencies may be overlooked, especially when the amount of S in the soil and foliage is not known. Applying 385 kg S/ha at one nursery increased height growth of sweetgum and green ash seedlings by 16 to 19 percent and raised the soil level from 0 to 7 ppm S to more than 19 ppm S (Stone 1980). Adding 1,344 kg S/ha to a loamy soil reduced soil pH and increased the number of plantable sycamore seedlings (table 5), which may have occurred due to reduced damping-off or increased seed germination (Siegel and Brock 1990).

When leaves are sampled during the summer, the S sufficiency range might be 1,200 to 1,600 ppm for oak (Kramer 2008, Van Sambeek et al. 2017) and 1,500 ppm for pecan (Hu et al. 1991). In greenhouses, growth of oak seedlings can be increased by adding K-sulfate (Browder et al. 2005), Al-sulfate (Davis 2003), or S plus micronutrients (Wright et al. 1999). Growth of other species can also be increased when sulfuric acid and nitric acid are added to irrigation water (South 2019a).

Sulfur fertilization rates less than 30 kg/ha are used to correct a potential S deficiency; but when the goal is to lower soil pH, rates can exceed 400 kg/ha. Rates for sandy soils vary from 400 to 900 kg S/ha (Davey and McNabb 2019, Mullen 1969), while rates for fine-textured soils may exceed 1,000 kg/ha (table 5). Managers who apply high rates of elemental S should

Table 5. Effect of elemental sulfur on soil pH (December 1983) and hardwood seedling morphology at a nursery in Mississippi (CEC = 11; loam soil with 29 percent sand). Sulfur was mixed into the soil on March 10 (sweetgum; sow date March 12) and April 11 (sycamore; sow date May 18). There were four replications for each test (n=12) and seedlings were lifted February 10–13, 1984.

Sulfur treatment (kg/ha)	Statistics	Density (#/m²)	Height (cm)	Plantable seedlings (#/m²)	Dry mass (g)	Soil pH
Sycamore						
0		50	100	36	16	5.4
672		58	108	44	18	5.2
1344		62	103	51	15	5.2
	LSD α = 0.10	13.3	8.4	12.1	3.2	0.16
	LSD α = 0.05	16.8	10.5	15.2	4.0	0.19
	Linear P>F	0.1462	0.1405	0.0547	0.5757	0.0481
Sweetgum						
0		91	69	68	11	5.9
672		104	73	78	12	6.0
1344		82	66	61	11	5.9
	LSD α = 0.10	23.4	7.3	19.1	2.8	0.61
	LSD α = 0.05	29.4	9.3	24.1	3.5	0.77
	Linear P>F	0.4789	0.4300	0.1747	0.7033	0.5987

LSD = least significant difference.

do so at least 2 to 3 months before sowing (Armson and Sadreika 1979); applying elemental S just 2 weeks before planting can injure some hardwoods (Timmer 1985). The risk of injury decreases when sufficient rainfall occurs after application but before germination (Carey et al. 2002).

Micronutrients

There are three approaches to micronutrient fertilization: (1) wait until visual symptoms appear (Altland 2006, Benzian 1959), test foliage, and then fertilize; (2) apply low rates of chelated micronutrients to foliage as a preventive measure; and (3) apply micronutrients when soil tests indicate low levels. During the 1950s, laboratories did not routinely test soils for micronutrient levels and, as a result, many managers did not apply micronutrient fertilizers (Iyer and Love 1974). Typically, micronutrient deficiencies did not occur on hardwoods when soils were more acid than pH 6.5 but Fe, Cu, and Zn deficiencies did occur on alkaline soils (Hoch 2018, Stoeckeler and Jones 1957, Timmer and Leyden 1980). The second approach is rarely used in bareroot hardwood nurseries.

Most managers follow the third approach and apply micronutrients when soil tests indicate low levels. Minimum soil values for hardwood seedbeds are: Mn-5 ppm, Zn-1 ppm, Cu-0.8 ppm, and B-0.4 ppm (Davey and McNabb 2019). Although a 20-ppm soil level is deemed adequate for Fe, a deficiency in hardwood seedlings is rare when soil pH is below 6.5. When soil pH is near neutral, applying high rates of Cu chelates may improve growth of some hardwoods (Timmer and Leyden 1980) but low rates of Cu may have no effect on growth (figure 10). In fact, it may be difficult to induce Cu deficiencies using sandy soils (Van den Burg 1983).

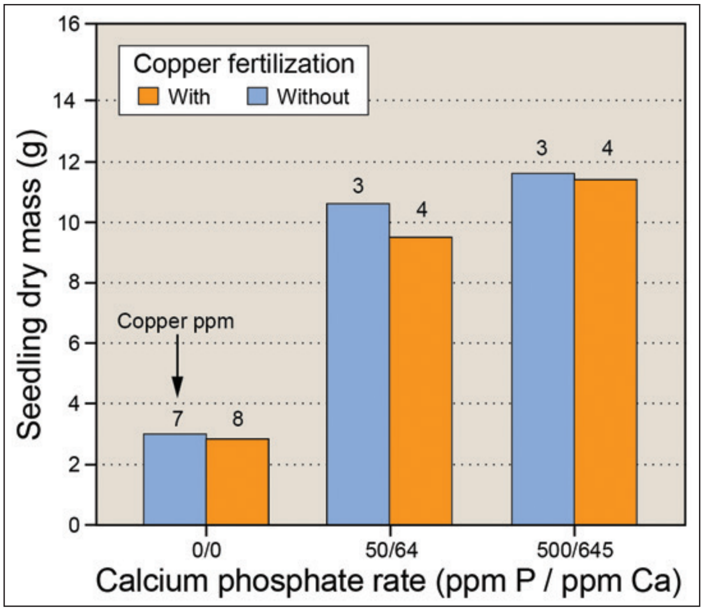


Figure 10. Copper and calcium phosphate treatments were applied to a peat:sand:sandy loam medium (pH 7.4 to 7.9; 6 ppm P). Fertilization with calcium phosphate increased growth of sweetgum seedlings (inoculated with *Glomus fasciculatus*) but fertilization with copper did not increase growth (Lambert 1982). Seedlings growing in the copper-treated treatments (soil = 5 ppm Cu) had slightly higher foliar copper (values above bars indicate foliar copper in ppm) but the 1 ppm increase was not significant (α =0.05).

Although foliar tests are used to help diagnose problems such as stunting for unknown reasons, foliar samples are not routinely used as a tool for deciding when to apply chelated micronutrients. Instead of spending money for foliar tests, money is allocated toward the purchase of micronutrients. In some cases, applying 250 g Cu/ha may cost $12 per ha for one application, while one foliar test might cost $26. In addition, interpretation of lab results is problematic for several micronutrients. A foliar test result of 3 ppm Cu (figure 10) does not necessarily mean that seedlings are not growing well. Likewise, results from oak foliage are not useful in determining if chlorosis is a result of Fe deficiency (Hauer and Dawson 1996, Hoch 2018). In some cases, use of chelates may even reduce micronutrient levels in foliar tests (Kramer 2008, Wallace et al. 1983).

Organic Matter

Nurseries in Washington and Oregon may have more than 4 percent OM (Crannell et al. 1994), while nurseries in warmer environments can have less than 1 percent (South 1975, 1992). Although experienced managers typically have no nutrient-related problems growing hardwoods in low OM soils, some managers still strive to increase OM levels. In Georgia, maintaining OM at 1 percent can be accomplished by making small, frequent additions of organic materials. Small applications can reduce problems associated with too much OM (e.g., N chlorosis and stunting). A 5-cm depth of sawdust can be added prior to growing 2 years of cover-crops (Cross 1984) but, if applied just prior to sowing, sawdust might cause problems (Davey 1953, Rose et al. 1995). At $22/m^3, a 2.5 cm depth of sawdust would cost $5,500/ha and might not increase OM by the time seed are sown a year later (Koll 2009, Munson 1982, Tran 2005). Applying more expensive compost or peat might increase soil OM by perhaps 1 percent (Brener 1971, Munson 1982) but will likely have no effect on height and diameter of red oak seedlings (Buchschacher et al. 1991).

The use of fertilizers can produce a good crop of hardwood seedlings at nurseries with less than 1 percent OM. Although much has been written about the benefits of OM to growing bareroot hardwood seedlings (Davey 1994, 1996; Davis et al. 2006), reports that show a positive economic benefit from incorporating OM before sowing hardwoods either do not exist or have not been published. Some managers have been disappointed when free or inexpensive sources of OM resulted in weed problems and high soil pH.

Additional Considerations

Statistics

Most nursery trials have only three or four replications and therefore the statistical power of the test is low (South and VanderSchaaf 2017, VanderSchaaf et al. 2003). As a result, even a 30-percent (table 5) to 100-percent increase in plantable seedlings may not be statistically significant. Also, researchers sometimes thin seedbeds to a common spacing in order to minimize the effects of variations in seedling density. Although this practice is good for research, it eliminates the ability to detect treatment effects on density. With a few exceptions (Mexal et al. 2002, O'Reilly et al. 2008, South 1977, Villarrubia 1980), most fertilizer trials in hardwood seedbeds do not report treatment effects on seedbed density. Furthermore, nursery conditions and management approaches differ from nursery to nursery. Therefore, research results from another region may have limited applicability. Managers most often adopt fertilizer regimes based on nursery records, observations from check plots, publications, assumptions, and economics.

Leave Check Plots

"In general, it is advisable to leave some nursery beds without fertilizer application to serve as controls" (Iyer and Love 1974, p. 14). Periodically, fertilizer treatments should be reevaluated with check plots, as treatments, conditions, objectives, and managers change. For example, managers who left check plots (figure 11) learned that routine applications of K before sowing had no effect on crop production (Kahn et al. 2014, South 1975). From our experience, check plots can be installed by temporarily covering seedlings with a tarp (just prior to applying fertilizers). When applying less than 7 kg/ha of micronutrients, check plots might reveal no detectable effect on seedling color or levels of foliar nutrients.

Figure 11. Check plots without fertilization (foreground) in a study to evaluate nitrogen source on sweetgum growth (beds on right) and green ash seedlings (beds on left) at the Capron Nursery in Virginia (Villarrubia 1980). (Photo by Chuck Davey 1978).

Final comment

For more than a century, nursery managers have grown bareroot hardwood seedlings using a variety of fertilizer sources and methods of application. Since 1900, fertilizer rates have increased for hardwoods and advances in equipment have reduced labor costs. With effective cultural practices, bareroot hardwood seedlings can be grown at a cost of less than $0.20 each. From our findings, it seems fertilizer practices will continue to evolve over the next few decades. Nursery managers will have to evaluate new technologies, new techniques, and new fertilizer regimes to determine the combination that produces the best seedling quality and economic results for their facility.

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Address correspondence to—

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

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Forest Nursery Seedling Production in the United States—Fiscal Year 2019

Diane L. Haase, Carolyn Pike, Scott Enebak, Lori Mackey, Zhao Ma, and Chelsea Silva

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

Abstract

Forest nursery production for the 2019 planting season was more than 1.3 billion tree seedlings (including about 18 million container seedlings imported from Canada). Approximately 75 percent of seedlings were produced as bareroot stock. Only a small portion (3 percent) of seedlings were hardwood species. Based on this total number of seedlings and estimated planting densities in each State, approximately 2.5 million ac (1.0 million ha) of trees were planted. More than 80 percent of production and planting occurred in the Southern States.

Background

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

Methodology

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

Table 1. Hardwood and conifer tree seedling production and acres planted for each State and each region during the 2019 planting year.

State	Hardwood seedlings produced	Hardwood acres planted[1]	Conifer seedlings produced	Canadian conifer imports	Conifer acres planted[1]	Total seedlings produced	Total acres planted[1]	FIA data acres planted[10]
Southeast								
Florida[2]	1,457,000	2,649	45,046,000	—	81,902	46,503,000	84,551	150,006
Georgia[2]	4,573,000	8,315	326,070,000	—	592,855	330,643,000	601,169	212,353
North Carolina[2]	223,000	405	63,258,000	—	115,015	63,481,000	115,420	108,401
South Carolina[2]	450,000	818	161,005,000	—	292,736	161,455,000	293,555	88,362
Virginia[2]	893,000	1,624	28,245,000	—	51,355	29,138,000	52,978	57,031
Regional Totals	**7,596,000**	**13,811**	**623,624,000**	**0**	**1,133,862**	**631,220,000**	**1,147,673**	**616,153**
South Central								
Alabama[2]	2,913,000	5,296	107,473,000	—	195,405	110,386,000	200,702	218,748
Arkansas[2]	8,449,000	15,362	103,468,000	—	188,124	111,917,000	203,485	89,136
Kentucky[3]	450,320	1,035	114270	—	263	564,590	1,298	1,142
Louisiana[2]	—	—	50,559,000	—	91,925	50,559,000	91,925	160,561
Mississippi[2]	1,154,000	2,098	84,691,000	—	153,984	85,845,000	156,082	140,495
Oklahoma[2]	413,000	751	2,341,000	—	4,256	2,754,000	5,007	31,659
Tennessee[2]	2,519,000	4,580	3,535,000	—	6,427	6,054,000	11,007	24,386
Texas[2]	—	—	89,328,000	—	162,415	89,328,000	162,415	126,044
Regional Totals	**15,898,320**	**29,122**	**441,509,270**	**0**	**802,799**	**457,407,590**	**831,922**	**792,171**
Northeast								
Connecticut[3]	200	—	100	—	—	300	1	0
Delaware[2]	—	—	—	—	—	—	—	515
Maine[5]	—	—	—	4,000,000	6,667	4,000,000	6,667	4,069
Maryland[2]	989, 717	1,799	664,300	—	1,208	1,654,017	3,007	0
Massachusetts[3]	10,000	23	5,000	—	11	15,000	34	0
New Hampshire[3]	24,500	56	297,600	—	684	322,100	740	402
New Jersey[3]	93,035	214	145,050	—	333	238,085	547	0
New York[5]	99,300	166	584,500	—	—	683,800	166	2,0 77
Pennsylvania[3]	5,283,020	12,145	3,888,215	—	8,938	9,171,235	21,083	1,847
Rhode Island	—	—	—	—	—	—	—	0
Vermont[3]	2,000	5	100	—	—	2,100	5	0
West Virginia[3]	149,242	343	74,555	—	171	223,797	514	0
Regional Totals	**6,651,014**	**14,751**	**5,659,420**	**4,000,000**	**18,014**	**16,310,434**	**32,765**	**8,910**
North Central								
Illinois[3]	486,440	1,118	183,050	—	421	669,490	1,539	1,667
Indiana[4]	1,627,595	2,504	637,241	—	980	2,264,836	3,484	2,413
Iowa[5]	458,338	764	110,350	—	184	568,688	948	0
Michigan[2,9]	2,230,217	4,055	11,135,281	2,894,960	25,510	16,260,458	29,564	6,330
Minnesota[2,9]	317,033	576	2,480,170	3,376,626	10,649	6,173,829	11,225	8,403
Missouri[3]	976,845	2,246	500,845	—	1,151	1,477,690	3,397	223
Ohio[3]	10,100	23	50	—	—	10,150	23	2,173
Wisconsin[6,9]	784,950	981	2,295,993	874,580	3,963	3,955,523	4,944	8,256
Regional Totals	**6,891,518**	**12,268**	**17,342,980**	**7,146,166**	**42,858**	**31,380,664**	**55,126**	**29,465**

State	Hardwood seedlings produced	Hardwood acres planted[1]	Conifer seedlings produced	Canadian conifer imports	Conifer acres planted[1]	Total seedlings produced	Total acres planted[1]	FIA data acres planted[10]
Great Plains								
Kansas[2]	22,000	40	46,000	—	84	68,000	124	1,012
Nebraska[2]	701,500	1275	1,250,000	—	2,273	1,951,500	3,548	0
North Dakota[2]	48,803	89	754,441	—	1,372	803,244	1,460	0
South Dakota[2]	557,436	1,014	263,099	—	478	820,535	1,492	164
Regional Totals	**1,329,739**	**2,418**	**2,313,540**	**0**	**4,206**	**3,643,279**	**6,624**	**1,176**
Intermountain								
Arizona[2]	3,360	6	680	—	1	4,040	7	0
Colorado[2]	146,000	265	168,700	32,400	366	314,700	631	669
Idaho[2]	267,396	486	8,804,450	3,749,040	22,825	12,820,866	23,311	10,016
Montana[2]	30,780	56	454,755	52,000	921	537,535	977	4,506
Nevada[2]	2,435	4	355	—	1	2,990	5	0
New Mexico[2]	4,000	7	48,000	—	87	52,000	95	0
Utah[2]	300,000	545	125,000	—	227	425,000	773	0
Wyoming	—	—	—	—	—	—	—	846
Regional Totals	**753,971**	**1,371**	**9,601,940**	**3,833,440**	**24,428**	**14,156,951**	**25,799**	**16,037**
Alaska								
Alaska[2]	12,000	22	9,000	324,544	606	21,000	628	0
Pacific Northwest								
Oregon[7,9]	3,627,300	10,364	66,872,937	390,000	192,180	70,890,237	202,544	118,350
Washington[7,9]	388,113	1,109	61,702,571	2,298,952	182,861	64,389,636	183,970	96,376
Regional Totals	**4,015,413**	**11,473**	**128,575,508**	**2,668,952**	**375,041**	**135,279,873**	**386,514**	**214,726**
Pacific Southwest								
California[8]	81,428	181	12,855,259	—	28,567	12,936,687	28,748	36,986
Hawaii[8]	5,900	13	—	—	—	5,900	13	568
Regional Totals	**87,328**	**194**	**12,855,259**	**0**	**28,567**	**12,942,587**	**28,761**	**37,554**
Totals	**43,235,303**	**85,429**	**1,241,490,917**	**17,993,102**	**2,430,381**	**1,302,362,378**	**2,515,811**	**1,716,192**

[1] Acres planted were estimated assuming:

[2] 550 stems/acre.

[3] 435 stems/acre.

[4] 650 stems/acre.

[5] 600 stems/acre.

[6] 800 stems/acre.

[7] 350 stems/acre.

[8] 450 stems/acre.

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

[10] FFIA = Forest Inventory and Analysis; average annual acreage planted estimated for all States (2020) on 5-year cycles, except for Alabama, Louisiana, Mississippi, and North Carolina, which are on 7-year cycles, and for Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, and Washington, which are on 10-year cycles. Data generated by Andy Hartsell, USDA Forest Service.

Table 2. Bareroot and container tree seedling production for each State and each region during the 2019 planting year.

State	Bareroot	Container[1]	Total Seedlings Produced
Southeast			
Florida	42,752,000	3,751,000	46,503,000
Georgia	202,373,000	128,270,000	330,643,000
North Carolina	50,885,000	12,596,000	63,481,000
South Carolina	161,441,000	14,000	161,455,000
Virginia	28,648,000	490,000	29,138,000
Regional Totals	**486,099,000**	**145,121,000**	**631,220,000**
South Central			
Alabama	88,165,000	22,221,000	110,386,000
Arkansas	111,792,000	125,000	111,917,000
Kentucky	564,590	—	564,590
Louisiana	—	50,559,000	50,559,000
Mississippi	75,691,000	10,154,000	85,845,000
Oklahoma	2,651,000	103,000	2,754,000
Tennessee	6,054,000	—	6,054,000
Texas	89,328,000	—	89,328,000
Regional Totals	**374,245,590**	**83,162,000**	**457,407,590**
Northeast			
Connecticut	—	300	300
Delaware	—	—	0
Maine	—	4,000,000	4,000,000
Maryland	1,555,617	98,400	1,654,017
Massachusetts	—	15,000	15,000
New Hampshire	322,100	—	322,100
New Jersey	198,223	39,862	238,085
New York	683,800	—	683,800
Pennsylvania	9,159,880	11,355	9,171,235
Rhode Island	—	—	0
Vermont	1,000	1,100	2,100
West Virginia	223,797	—	223,797
Regional Totals	**12,144,417**	**4,166,017**	**16,310,434**
North Central			
Illinois	647,100	22,390	669,490
Indiana	2,102,583	162,253	2,264,836
Iowa	558,988	9,700	568,688
Michigan	13,001,055	3,259,403	16,260,458
Minnesota	2,776,580	3,397,249	6,173,829
Missouri	1,477,690	—	1,477,690
Ohio	—	10,150	10,150
Wisconsin	3,028,643	926,880	3,955,523
Regional Totals	**23,592,639**	**7,788,025**	**31,380,664**
Great Plains			
Kansas	—	68,000	68,000
Nebraska	1,125,000	826,500	1,951,500
North Dakota	704,389	98,855	803,244
South Dakota	813,421	7,114	820,535
Regional Totals	**2,642,810**	**1,000,469**	**3,643,279**
Intermountain			
Arizona	—	4,040	4,040
Colorado	134,700	180,000	314,700
Idaho	1,906,035	10,914,851	12,820,886
Montana	19,558	517,977	537,535
New Mexico	—	2,790	2,790
Nevada	—	52,000	52,000
Utah	—	425,000	425,000
Wyoming	—	—	0
Regional Totals	**2,060,293**	**12,096,658**	**14,156,951**
Alaska			
Alaska	0	21,000	21,000
Pacific Northwest			
Oregon	39,776,011	31,114,226	70,890,237
Washington	35,996,236	28,393,400	64,389,636
Regional Totals	**75,772,247**	**59,507,626**	**135,279,873**
Pacific Southwest			
California	—	12,936,687	12,936,687
Hawaii	—	5,900	5,900
Regional Totals	**0**	**12,942,587**	**12,942,587**
Totals	**976,556,996**	**325,805,382**	**1,302,362,378**

[1] Ten States (Alaska, Colorado, Idaho, Maine, Michigan, Minnesota, Montana, Oregon, Washington, and Wisconsin) received container seedlings produced in Canada.

Assumptions

The following assumptions were used in compiling this report.

1. *The number of seedlings reported by the participating forest and conservation nurseries was the number of shippable seedlings produced for distribution in the 2019 planting season (i.e., seedlings that were planted from fall of 2018 through spring of 2019).*

Some species of forest seedlings require two or more growing seasons to reach accepted forest and conservation seedling size standards, so not all seedlings in production at a nursery at any given time are considered shippable (i.e., available for distribution). Therefore, only shippable seedlings were counted.

2. *All seedling production reported in this survey met the grading standards for the respective nurseries (i.e., cull seedlings were not included in the estimates).*

Table 3. Annual forest nursery seedling production in each region for FY 2012 to FY 2019.

Year	Total seedling production	West (17 States)	East (20 States)	South (13 States)
FY 2019	1,302,362,378	166,043,690	47,691,098	1,088,627,590
FY 2018	1,187,282,896	76,253,776	46,667,266	1,064,361,854
FY 2017	1,284,824,689	151,321,764	67,595,266	1,065,907,659
FY 2016	1,260,216,076	152,785,327	72,314,630	1,035,094,369
FY 2015	1,302,237,795	175,464,446	95,417,986	1,031,355,363
FY 2014	1,217,607,888	115,620,820	85,684,417	1,015,564,370
FY 2013	1,181,554,535	96,344,063	102,066,671	983,143,801
FY 2012	1,190,552,819	170,975,830	81,672,547	936,918,542

FY = fiscal year.
Sources: This report, Haase et al. (2019), Harper et al. (2013, 2014), Hernández et al. (2015, 2016, 2017, 2018)

Production estimates are often based on seedbed inventories of seedlings meeting grading standards. For cases in which nurseries ship seedlings by weight, as opposed to examining and counting each seedling, landowners and tree planters often plant every seedling that is shipped to them.

3. *Seedling production data were collected from all the major nurseries that produced forest and conservation tree seedlings for the planting season.*

Considerable effort was made to contact all major producers of forest and conservation seedlings. The universities collecting the survey data reported, with few exceptions, that the major producers were included in the results.

4. *All seedlings reported in this survey were produced for reforestation and conservation projects.*

Some of the nurseries that participated in this survey also produce seedlings for ornamental use, Christmas tree production, or other horticultural purposes. Private nurseries were asked to report only seedling production destined for conservation and reforestation planting.

5. *Forest tree seedlings remain in the general area where they are produced.*

Forest and conservation seedlings are routinely shipped across State borders and at times across international borders. It is assumed that, on average, the number of seedlings imported into a State is equal to the number of seedlings exported from that State. In some States, a significant number of container seedlings are produced in Canada and imported for planting in those States. Estimates of the number of seedlings shipped from Canada were obtained from Canadian nurseries that routinely export seedlings to the United States.

6. *Dividing the number of seedlings shipped from forest and conservation nurseries by the average number of stems planted per acre in a specific State is an appropriate proxy of the number of acres of trees planted during the planting season.*

These estimations do not include direct seeding or natural forest regeneration activities. Average tree planting densities for each State were provided by FIA.

7. *Respondents to the production survey reported only hardwood and conifer trees produced.*

Nurseries were asked not to include shrubs in their production estimates. Many conservation and restoration plantings include shrubs and herbaceous plants to address wildlife, biodiversity, or other management objectives. Using only tree production to estimate acres planted results in an underestimate of planted acreage where a mixed planting of shrubs and trees occurred.

Data Trends

More than 1.3 billion forest tree seedlings were planted in the United States in fiscal year (FY) 2019. This production level is an increase from previous years. Variation is attributed to inconsistent participation from nurseries during data collection each year (particularly in the Western States), as well as increased planting in recent years following wildfires, pests, and harvests. Based on the total number of seedlings shipped and the average number of seedlings planted per acre in each State, approximately 2.5 million ac (1.0 million ha) of trees were planted during the fall 2018 through spring 2019 planting season.

Address correspondence to—

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

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Enhancing Direct Seeding Efforts With Unmanned Aerial Vehicle (UAV) “Swarms” and Seed Technology

Matthew Aghai and Tiffani Manteuffel-Ross

Director of Biological Research & Development, DroneSeed Co, Seattle, WA;
Research Scientist, Biological Research & Development, DroneSeed Co, Seattle, WA

Abstract

Technological advances of unmanned aerial vehicles (UAVs) are creating new possibilities for establishing trees and native plants across large areas and have the potential to serve as a rapid response tool in post-disturbance environments. The advanced machinery and automation are also extending the possibility for “enhanced” seeding methods as an intermediate between conventional direct seeding and planting of nursery stock. This approach may allow managers to overcome limitations of cost, labor, safety, and viability. Here we present components of our novel software, hardware, and seeding systems designed to address payload delivery efficiently, precisely, safely, at scale, and within the regulatory framework of the United States Federal Aviation Administration.

Introduction

Artificial regeneration approaches for landscape management must meet myriad ownership objectives and account for economic, regulatory, and ecological considerations. Seeds are often the basis for artificial regeneration, whether as the first step in a nursery’s investment in a seedling or applied directly on the landscape with little other intervention. Direct seeding for reforestation and native plant restoration is currently used in cases where rapid response is necessary (e.g., soil stabilization with grasses, Kruse et al. 2004, Peppin et al. 2010), where the ecology of a species regenerated from seed contributes to sound silvicultural practice (e.g., a reduction in lag time for establishing appropriate species and stocking goals), or where restoration objectives (e.g., vegetation/canopy cover or habitat) can be met.

In forest operations, direct seeding is relatively fast to implement, but several disadvantages lead to a nearly 80-percent failure for individual seeds, and greater than 50-percent failure by project to meet stocking targets (Grossnickle and Ivetić 2017). Direct seeding, however, can be more cost effective at scale (Baumhauer et al. 2005) with some recent research highlighting up to a 64-percent reduction in reforestation costs (Pérez et al. 2019). Historically, direct seeding in forestry has been used successfully to meet landscape objectives where high volumes of seed are deployed with the expectation that poor survival and self-thinning will lend to appropriate stocking (Ceccon et al. 2016, Duryea 1987, Palma and Laurance 2015, Scott 1970). Alternatively, in restoration efforts, particularly for large-scale projects, direct seeding is the primary revegetation approach because it is typically 10 to 30 times cheaper than planting nursery stock (Masarei et al. 2019) and is less labor intensive. Difficulties for direct seeding also exist in restoration, including high incidence of desiccation, predation, and wind erosion that contribute to low plant establishment rates—ranging from 10-percent emergence to outright failure (Commander et al. 2013, Masarei et al. 2019, Merritt and Dixon 2011).

Although direct seeding can be practical, low cost, and responsive to immediate need, conventional approaches of this method have been impeded by crude dispersal mechanisms, coarse spatial distribution techniques, and unrefined seed handling (Grossnickle and Ivetić 2017). For large treatment areas, from rangelands to large post-disturbance forestry units, seed deployment is often non-uniform when applied using aerial systems with broadcasting machines, sling-pod buckets, or boom dispersing systems (Hallman and Larson 1980). For example, the distribution of most aerially broadcast seed is highly irregular when using airplanes and helicopters due to aircraft speed, bridging or jamming in hoppers, and scattering as influenced by propeller or rotor wash or the aerodynamic properties of the seed (Becker 2001). Additionally, after seed lands on the ground, a number of abiotic and biotic factors can limit germination, survival, viability, and persistence. Without controlled selection of microsites, a large amount of aerially broadcast seed lands in unsuitable or inhospitable places that will not support plant establishment (e.g., surface rock or large woody detritus and erosion-prone or crusted surfaces). Surface deposition of seed is at a risk of predation, undesirable seed transport from wind or precipitation, and potential damage or mortality from desiccation (Gornish et al. 2019, Madsen et al. 2016). Where ground-based machine access is possible, seed can be deployed using tractors with drill-seeding attachments or other agricultural-style equipment, with the intent of achieving some control over subsurface seed placement resulting in potentially higher establishment rates (Masarei et al. 2019).

A consequence of seeding using conventional systems is the loss of substantial quantities of seed. Seed is an increasingly valuable commodity to various industries, including governments, resource companies, and nonprofit organizations, as they position themselves for addressing large climate change mitigation efforts and landscape-scale restoration efforts through increased planting (Broadhurst et al. 2016, Jalonen et al. 2016, Nevill et al. 2016). Given the increased size and frequency of disturbances on the landscape due to climate change-driven phenomena like wildfire, beetle-kill, drought (Seidl and Rammer 2017, Stephens et al. 2014), and the reduced likelihood of natural regeneration from seed rain and recruitment (Kemp et al. 2016, 2019), seed-use efficiency is tantamount to sustainable land-management practices and risk mitigation. Updating the technology and methods of direct seeding provides an opportunity for reduced seed usage, improved spatial distribution and targeting, and greater survival outcomes for direct seeding in forest, restoration, and rangeland settings (Grossnickle and Ivetić 2017, Maserai et al. 2019).

Technological advances of unmanned aerial vehicles (UAVs) are creating new possibilities for natural resources management. This technology gives the ability to survey a landscape, use high-quality aerial imagery to classify sites, then deploy materials (such as seed) over large areas quickly and efficiently with battery-powered, propeller-based aircraft that use slow flight speeds and are highly maneuverable (figure 1). Until recently, use of UAVs for reforestation and restoration work has been limited to imaging for reconnaissance and monitoring (for regulatory and technological reasons, see Baena et al. 2018, Belmonte

Figure 1. A DroneSeed custom-engineered hexacopter (patent pending) capable of carrying up to 57 lb (25 kg) of payload with an "all-up" weight up to 115 lb (52 kg). This aircraft is typically flown autonomously as part of multiple, coordinated, high-capacity, autonomous aircraft, also known as "swarms," and operated by a limited number of ground personnel to service battery and payload replacements between missions. (Photo courtesy of DroneSeed 2019)

et al. 2019, Sankey et al. 2017); however, unmanned commercially available aircraft are increasingly becoming capable of achieving precise direct seeding on complex and remote landscapes.

Technological and Regulatory Limitations

According to the Federal Aviation Administration (FAA) regulations (USDOT 2020), a typical commercial UAV is restricted to an all up weight of 57 lbs (25 kg), and pilots can only fly a single aircraft in which they must maintain "line-of-sight" of the UAV unless they have a waiver or exemption (identified as Federal statutes as "part #" waivers). Typical commercial UAVs are also usually limited by technological capacity (hardware limitations) to flight times of 15 minutes (internal DroneSeed communication). These regulatory restrictions limit acreage, operational ability, and payload (herbicide, seed, etc.) size in a given flight, creating a mismatch between the application capacity and treatment need, because many management units cover vast areas. The seemingly simple exercise of increasing the number of drones and corresponding operators will not directly result in incremental improvements to throughput.

A pathway to working on the landscape scale of significant acres with UAV systems requires technology to achieve "swarm" operations. Swarms are multiple, coordinated, high-capacity, autonomous aircraft, operated by a limited number of ground personnel. Thus, for resource management beyond remote sensing, revegetation operations with UAV swarms need to meet several primary requirements: (1) ability to carry substantial weight (payloads) with support systems (such as battery charging systems) to prioritize flight over time aircraft are on the ground; (2) regulatory consent to scale operations to multiple coordinated UAVs over long distances and beyond visual line of sight; (3) UAV programmability through targeted software development; and (4) improved handling, deposition, and efficiency of seed dispersal.

Seed Distribution and Enablement Technology

Handling, delivery, and efficacy of materials (e.g., seed) deployed from UAVs also needs improvement. Aerial broadcast systems, to date, have largely relied on attachments that can be described as hopper-fed buckets with a motorized sling that emit seed in a coarse manner (Stevens 1999). These systems further rely on the aircraft's altitude, speed, and GPS accuracy to achieve their target seeding rates, often on difficult or remote terrain. In direct seeding efforts, multi-species mixes can be composed of forbs, grasses, and shrub seeds with a wide range of sizes and morphologies. During aerial broadcasting, seed mixtures are subject to intense vibrations that can cause segregation by size and species, and mechanical processes that are unable to precisely control flow rate, often resulting in uneven seed distributions (Becker 2001). To stabilize seed and normalize distribution patterns, seed can be coated or pelleted as individual seed or agglomerates (Madsen et al. 2016, Masarei et al. 2019, Pedrini et al. 2020). While many seed coating and processing technologies have been applied to native plant species for easing the aerial seeding process, these technologies have rarely been applied to forest tree seed (particularly conifers) (Grossnickle and Ivetić 2017).

A holistic approach to seed technology should increase the probability of seed germination, root egress, and plant establishment without hindering the evolutionary potential of that particular species. To mitigate predation of the seed, seed-coating amendments can include olfactory and/or gustatory deterrents (Pearson et al. 2018), camouflaging agents (Porter 2013, Van Damme 1988), and/or masking agents and physical barriers (Taylor et al. 2020). Efficacy of seed treatments as a predation deterrent should be mindful of regulatory standards, and trophic consequences of toxic/noxious properties. Beneficial seed-coating amendments should enable the survival and development process, including a rooting substrate, nutrients, phytohormones, mycorrhizal and bacterial symbionts, all of which can mitigate desiccation and other limiting edaphic conditions.

Developing successful seed treatments will require a thorough understanding of species-specific biological traits, such as seed morphology, dormancy requirements, and viability, in addition to site-specific biotic and abiotic conditions that will impact seed after deposition. To date, direct seeding efforts—particularly with native plants—have employed a wide range of treatments. Controlled stratification (Barnett 2014) and/or dormancy alleviation treatments (Kildisheva 2019, Kildisheva et al. 2020) can enable better germination and establishment. A number of experiments and field operations have evaluated poisons, chemical deterrents, and supplementary feeding to alleviate predation from granivores (Campbell 1981, Sullivan 1979). A shift in environmental laws and best practices has more recently led to an exploration of plant-derived deterrents like capsaicin (i.e., hot pepper), activated carbon, or essential oils (Taylor et al. 2020).

DroneSeed Case Studies

Much of the equipment, infrastructure, and software required for the premise of swarm operations did not exist when DroneSeed began operations in 2016. Our interdisciplinary team (composed of software and hardware engineering, aviation, forestry, geographic information systems, and ecology professionals), based in Seattle, WA, is advancing the UAV-based aerial-seeding technology and techniques. Our customers' typical "pain-points" include the need for large-scale, post-disturbance (specifically wildfire) revegetation/stabilization tools, difficulty accessing remote and rough terrain, limited labor pools or the increasing costs of planting, stressful site conditions (e.g., drought), and the high cost of planting stock. Our team has developed a number of novel solutions for UAV-based revegetation, including software guidance systems, hardware such as aircrafts and support vehicles with power systems, and standard operating procedures to safely sustain operations. Our multi-component process (figure 2) can provide landowners and managers with a comprehensive survey, payload delivery, and

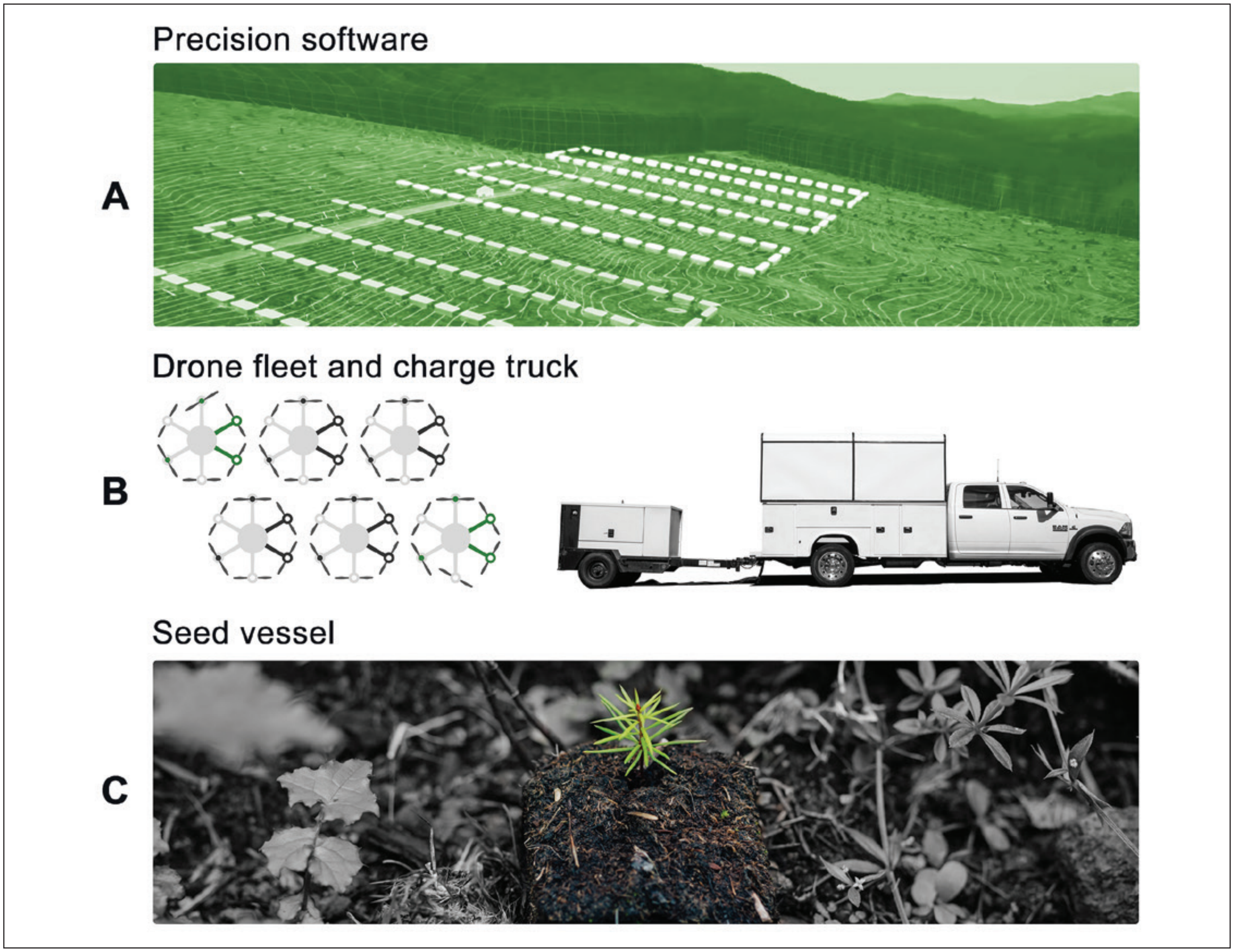

Figure 2. DroneSeed's three-part solution (patent pending) for revegetation consists of (a) proprietary software to survey, create swarm flight plans, and identify areas for seed deployment; (b) mobile charging truck that can keep five drones that each carry a 57 lb (25 kg) payload continuously in the air; and (c) seed vessels— "pucks"—that can boost seedling survival rate by reducing predation and desiccation. (Images courtesy of DroneSeed 2020)

monitoring solution for myriad site conditions and terrain complexities.

The following case studies, presented in chronological order, capture the onset of our program development for aerial seeding from mid-2018 through late 2019 when we began to service larger land areas with the technology. The case studies intend to provide the reader with an overview of the early development process and application of our technology as we use rapid scaling and adaptive management to continue to develop tools for forest managers and restoration practitioners.

Case Study 1: Payload Size and Line of Sight Waivers

Since 2017, DroneSeed has achieved a number of precedent-setting regulatory approvals to pioneer the swarm-based revegetation platform. DroneSeed's first waiver was a 15-aircraft "swarm" waiver, under FAA part 107. Aircraft in this waiver must be under 55 lb (25 kg) and are allowed to be flown by one pilot. Achieving the part 137 (to dispense fertilizer, herbicides, and water for up to five aircraft under 55 lb [25 kg]) required a "Knowledge and Skills" test in which the chief pilot commands an aircraft in front of FAA inspectors from one of the regional Flight Standards District Offices (FSDO).

Late in 2018, we set another precedent in the heavy-lift UAV industry by achieving the over-55 lb (25 kg) per aircraft swarm (FAA part 137 approval). This approval granted us the ability to fly up to five aircraft, each with a 57-lb (25.9 kg) payload and total weight of 115 lb (52 kg) with one pilot. The waivers were granted to deploy herbicides and other registered products from the aircraft, specifically seed and seed vessels conducive to revegetation operations. In 2019, the latest regulatory permission allowed DroneSeed to conduct field operations that require beyond visual line of site (BVLOS) capability. A summary of regulatory achievements can be found in table 1.

Case Study 2: Biotechnology ("Pucks") for Seeding

We developed biotechnology for seeding methods intermediate to direct seeding and planting nursery stock (figure 3) that can be deployed by UAVs to address key establishment issues. We created manufacturing processes for customized seed treatment and embedding into vessels ("pucks") to optimize seedling germination and establishment after dispersal from the aircraft. The pucks consist of a fiber-based substrate and provide risk-mitigating amendments to the seed (e.g., to reduce predation). The puck substrate simulates optimum seeding depth and acts as a germination bed on site, providing optimal pH, some water retention, and addition of beneficial abiotic and biotic amendments for germination and seedling establishment.

The puck, named for its appearance and compressed configuration when dry, is not a "one-size-fits-all" technology, as different ecosystems and species require different base materials, amendments, and configurations. Current sizes range from the smaller

Table 1. Summary of DroneSeed regulatory achievements with corresponding dates and descriptions.

Agency	Permission/waiver*	Date obtained	Description
FAA	Part 107	11-16-2016	Allows 1 pilot to fly 15 drones under 55 lbs simultaneously
FAA	Part 137	3-17-2017	Allows dispensing pesticides with drones under 55 lbs
FAA	Part 137	4-25-2017	Allows dispensing pesticides with drones over 55 lbs
FAA	333 Exemption	8-13-2018	Allows 1 pilot to fly 5 drones over 55 lbs simultaneously
FAA	333 Exemption	9-11-2018	Allows 1 pilot to fly 5 drones over 55 lbs simultaneously and added a DroneSeed aircraft type to permissions
FAA	333 Exemption	7-26-2019	Allows Beyond Visual Line of Site operations

*Further detail on regulatory information can be found at https://www.faa.gov/uas/

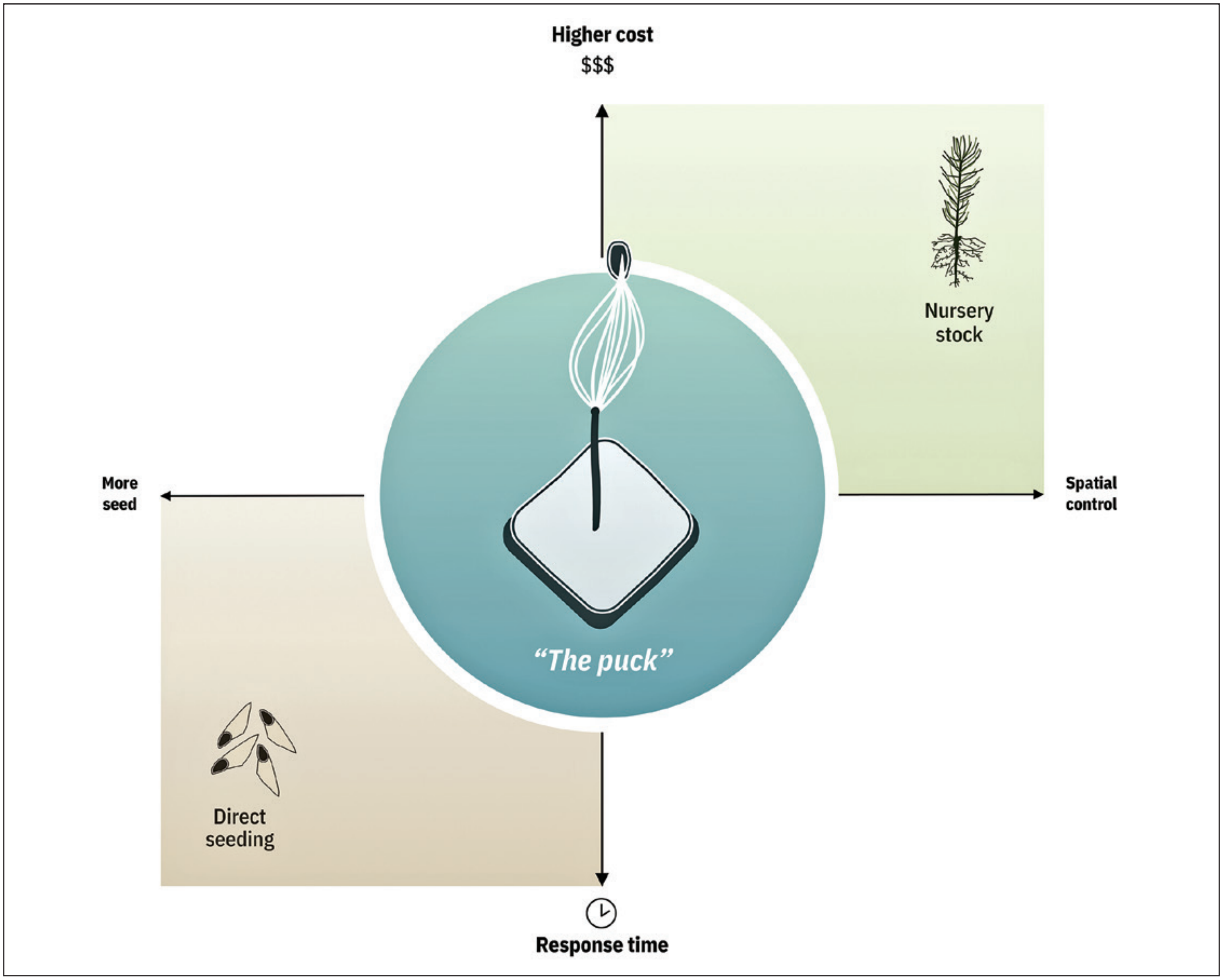

Figure 3. DroneSeed enablement strategy is a fiber-based vessel ("puck") with amendments suited to species and site conditions designed to increase seed germination and seedlings establishment (patent pending). The puck is designed to be an intermediate product between conventional direct seeding and nursery stock options for artificial regeneration. (Image courtesy of DroneSeed 2020)

2 by 2 by 0.4 in (5 by 5 by 1 cm) up to the largest 27 by 27 by 2 in (70 by 70 by 5 cm). Additional puck dimensions are developed to meet new species and ecosystem needs as the customer base expands—typically a 3-month process is required to meet scalable manufacturability for a new configuration. In addition to the puck, species-specific treatments are applied directly to seeds to alleviate dormancy (as needed), or to add coatings to decrease risk of predation, pathogens, and desiccation.

As a payload, the homogenous puck has advantages including a consistent quantity of seed, easier transport and deployment, and reliable behavior after deployment. Additionally, the puck lends itself to rapid and efficient manufacturing, packaging, and reloading of the aircraft between missions. During manufacturing, we track seed lot information (e.g., provenance, elevation, age, germination rate, etc.), seed treatment, and amendment information all the way to the deployment site.

Since the technology is novel, limited field data are available. Using greenhouse and bench trials prior to operations and accounting for significant mortality rates common in true field conditions, we set initial seeding rates for a species and calibrate in subsequent operations with similar species and edaphic conditions. Much of the development work has centered on puck functionality for conifer systems, with ponderosa pine

(*Pinus ponderosa* Lawson & C. Lawson) serving as the model species. Typically, three or more conifer seeds are amended into the puck, with up to six seeds per puck. A prescriptive range of 500 to 2,000 pucks may be applied per acre (1,250 to 5,000 pucks per hectare), with the intention of achieving up to 20-percent survival and establishment without overstocking a unit. With native plant seed, variation is higher, as grass, forb, and shrub species have variable seed characteristics. Higher seed quantities can be used by changing the configuration of the puck during production.

Since DroneSeed first developed and field-tested the pucks in 2018, a variety of commercial project sites have been seeded with more than 400,000 pucks. To expedite availability of data on puck performance, DroneSeed manufactured early versions and deployed them in small trials in the northern and southern hemispheres to generate two growing seasons of data regarding puck performance, as described in the following sections.

Trial Site: Southwestern Washington State USA

DroneSeed was granted access to a 5-ac (1.6-ha) recently harvested site on the University of Washington Pack Experimental Forest to test microsite variables in relation to seedling emergence from DroneSeed's proprietary puck. The Pack Forest is located in the foothills of Mt. Rainier, approximately 50 miles (80 km) southeast of Seattle, WA, and is dominated by second growth Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco).

A total of 40 unequal-size plots were installed across 1 ac (0.4 ha) on September 24 and 25, 2018. Quadrats followed an east-west and elevational gradient and varied in size to increase relative proportion of exposed mineral soil (figure 4). A total of 1,000 early-version (V1, table 2) peat-based puck prototypes were used in this test, 25 per plot in groups of 5 to 10. Microsites were identified as locations with "nurse materials" along stumps and next to downed logs or coarse, woody detritus, but also as exposed patches of mineral soil. In plots where microsites were not available (or less present), pucks were placed randomly on surface conditions which included duff, slash, or fine woody detritus. The "clusters" were located with a Tersus GPS for tracking purposes.

Three Douglas-fir seeds of local provenance were embedded into each puck. No deterrents, fertilizers, or fungicides were included in this "beta" test.

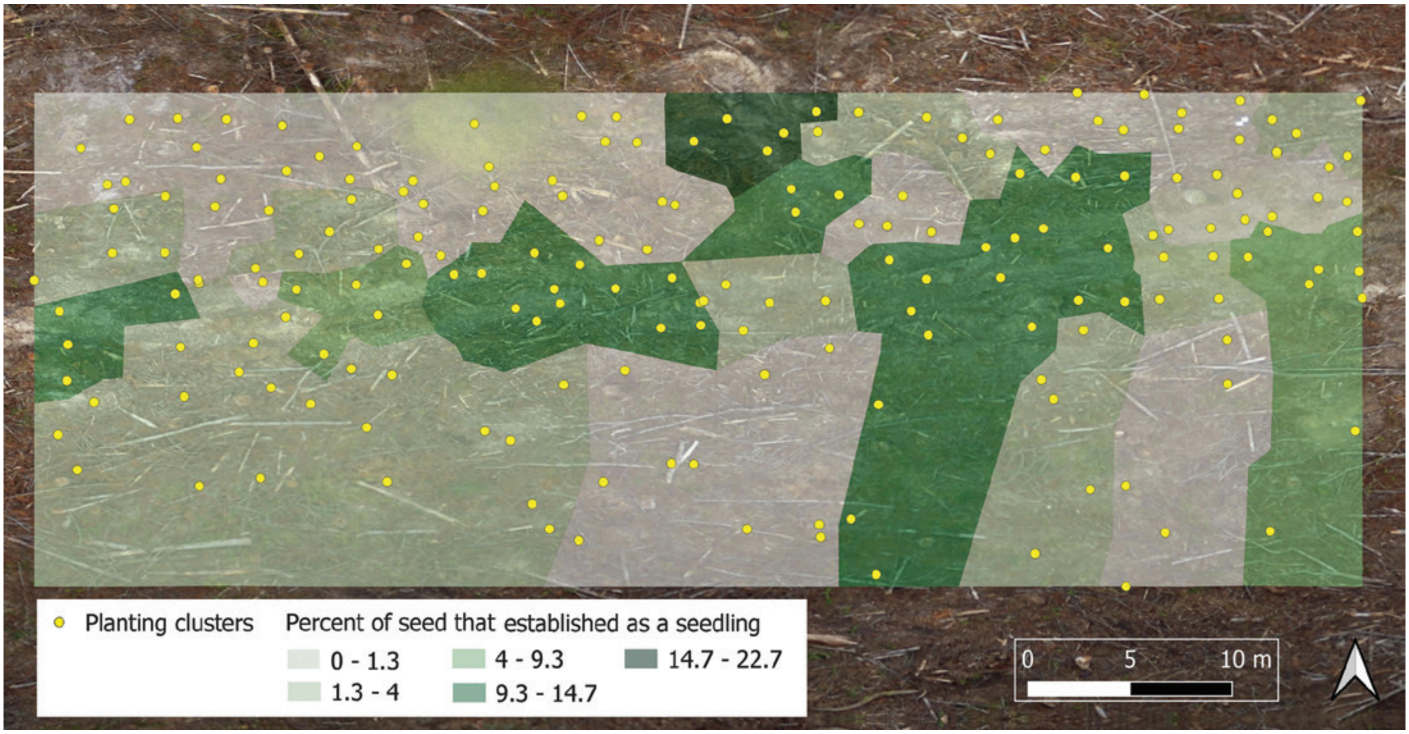

Figure 4. A trial site segmented by seeding quadrants where the puck with Douglas-fir (Pseudotsuga menziesii Mirb. Franco) seed was deployed at 25 per quadrant. The figure is color coded to represent percentage establishment 12 months after seeding. Note the faintly visible edaphic conditions, including extensive debris and post-harvest conditions. It appears that mineral soil exposed by logging skid tracks is correlated to increased percentage of established seedlings (see figure 5). (Photo courtesy of DroneSeed 2018)

Table 2. Versions and corresponding features and amendments of the DroneSeed "puck," a seed-planting vessel used to improve likelihood of seed germination and establishment.

Seed vessel version (year deployed)	Design features and amendments
"Beta" - Version 1 (2018)	Fiber-based pellet Single-sided seed configuration pH stabilized
"V2" - Version 2 (2018)	Fiber-based pellet Double-sided configuration pH stabilized
"V3" - Version 3 (2019)	Fiber-based pellet Double-sided configuration pH stabilized Olfactory and gustatory predatory deterrents (plant-based)
"V4" - Version 4 (2019)	Fiber-based pellet Double-sided configuration pH stabilized Olfactory and gustatory predatory deterrents (plant-based) Pathogen risk mitigation
"V5" - Version 5 (2020)	Advanced materials for fiber-based pellet (2 varieties) Double-sided configuration pH stabilized Olfactory and gustatory predatory deterrents (plant-based) Enhanced manufacturing process for amendments/seed Nutrients and beneficial organisms (optional) Biochar and other carbon or mineral material supplements (optional)

Prior to manufacturing, a subset of seed was stratified (surfaced sterilized with bleach, then soaked for 48 hours followed by storage at 3 ˚C for 30 to 90 days, at high relative humidity, see Dumroese et al. 1988). Each puck had one unstratified (dormant from storage) and two stratified seeds, as a means of bet-hedging. Pucks were transported to the project site and deployed by hand within 48 hours of manufacturing.

Throughout the 2019 growing season, we monitored seedlings emerging from pucks and distinguished them from seed rain from nearby mature canopy. We determined seedlings had germinated from our pucks based on known puck locations, puck residue surrounding seedlings, and seedling age.

At the final measurement (September 2019), 14 percent of the pucks produced seedlings within a 12-month period. Given that there were 3 seeds per puck for this trial, this translates to a 4.7-percent seedling to seed ratio. Grossnickle and Ivetic (2017) found the average seedling establishment rate of 16 percent (range of 0 to 52 percent calculated as survival rate following >1 growing season per/total number of seeds planted) with temperate conifers, influenced by biotic pressure (predation and competition), seedbed receptivity (microsites), and seed viability. In our trial, plots with majority mineral soil had the highest survival and those with a majority of slash had the lowest survival (figure 5). It is likely that this new mineral soil in skid tracks from cable logging and other harvesting operations improved soil contact and water or nutrient availability (Barker et al. 2014).

This particular site represented relatively difficult regeneration conditions due to recalcitrant native vegetation (e.g., sword fern [*Polystichum munitum* (Kaulf.) C. Presl] and Oregon grape [*Mahonia aquifolium* (Pursh) Nutt.]), heterogeneity in surface conditions, and the lack of site preparation. Additionally, the trial site was surrounded by undisturbed second growth forest, which likely increased granivore predation (anecdotal evidence of rodent activity was captured on game cameras placed on the site).

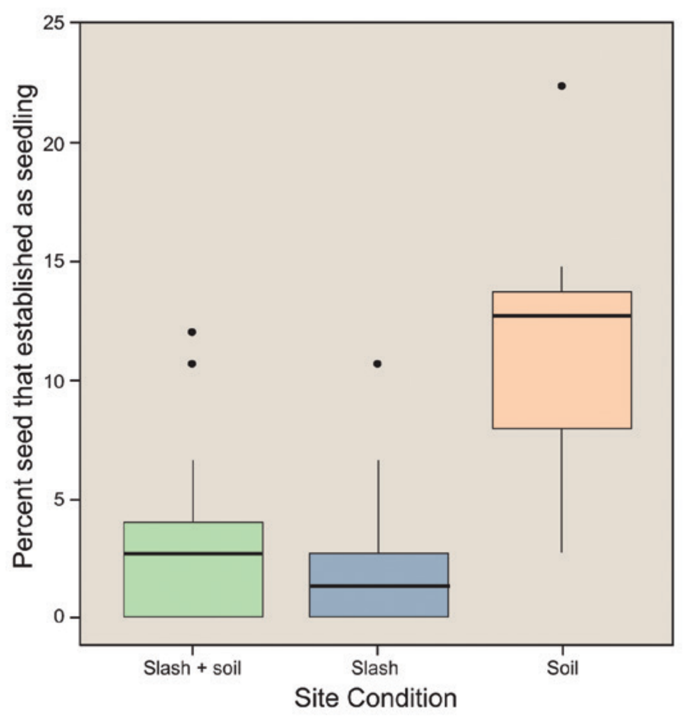

Figure 5. In a comparison of edaphic conditions, mineral soil conditions appear most favorable for rooting and establishment of seedlings from pucks.

Trial Site: New Zealand

In 2019, DroneSeed established several test plots using approximately 10,000 V3 pucks (table 2) across the North and South Islands of New Zealand. Three species were included: radiata pine (*Pinus radiata* D. Don), Douglas-fir, and mānuka (*Leptospermum scoparium* J.R. Forst. & G. Forst.), all with significant economic and ecological relevance to the region and reciprocal regions where DroneSeed operates. Radiata pine and Douglas-fir are the primary timber species commercially grown across New Zealand, and mānuka is a fast-growing plant native to New Zealand that has been subject to many eradication efforts over the last century but is now the focus of many commercial and restoration planting efforts because of its applications as a soil stabilizer, an important ecosystem component, and a major contributor to the oil and honey (pollinator) marketplace (Stephens et al. 2005).

A total of 16 plots were established across seven ownerships, on both the North and South islands. The sites ranged from cutover forestland (recent harvests), to earthquake-damaged hillsides, to pastureland that was slated for afforestation. Each test plot was approximately 2.5 acre (~1 ha) and was selected on the basis of recent disturbance (harvest or erosion) or vegetation-clearing by grazing stock (pasture). We stratified our experiments latitudinally across both islands, thus providing a variety of climatic, edaphic, and biophysical conditions. No chemical site preparation was implemented prior to deploying the pucks, but grazing animals were allowed access on some plots ahead of the trial.

Pucks and materials were shipped to New Zealand, where a local group finished the manufacturing process. All pucks included amendments intended to deter granivore predation (table 2). There were two treatment groups for radiata pine (either stratified or dormant seed treatments), two treatment groups on two site types for Douglas-fir (also either stratified and dormant seed), and one untreated group for mānuka. The radiata pine and Douglas-fir had four seeds per puck. The mānuka seed averaged ten seeds per puck.

Over a 10-day period in August 2019, pucks were hand distributed over the 16 plots. The distribution of blocks and transects varied to match the landform, vegetation status, and edaphic conditions provided by landowners for testing. In pasture rehabilitation areas or on erosion points, for example, a randomized block distribution was used to capture variability over a concentrated area of interest. In operational forestry settings, multiple pucks were distributed per point over long transects between rows of planted seedlings and/or between rows of slash.

In November 2019, we collected data to estimate puck residual material, survived seedlings, microsite presence/absence, and edaphic conditions, along with any relevant supplementary observations. No pucks of conifers had more than a single seedling. In cases where multiple pucks were deployed per point, multiple seedlings were present and counted individually. In the case of mānuka, we counted each puck as a single seedling, although there were often more than five emerged plants per puck (figure 6).

In 11 of the 16 plots, the outcomes met our operational hypothesis that survival (pucks with an established seedling) would be less than 5 percent by quantity of pucks deployed for each plot. In the other five plots, survival (established seedlings at the time of monitoring) exceeded 5 percent of all pucks deployed, and in some cases up to 37 percent of pucks deployed resulted in seedlings (table 3). The

Table 3. Range of results from 16 plots installed in New Zealand to trial an early version of the DroneSeed "puck." Pucks were distributed to plots in early August and measurements were collected in late November 2019.

Species	Seed treatments	Sample size[1]	Number of plots	Site types	Seed to seedling ratio (percent established)	Percent of pucks with seedling establishment	Trees per acre[2]
Radiata pine	Stratified or dormant	500 to 1075	8	Cutover	0.1 to 3.7	0.4 to 14.8	3 to 159
Douglas-fir	Stratified or dormant	400	4	Pasture rehabilitation and cutover	0.1 to 1.1	0.5 to 4.3	2 to 17
Mānuka	N/A	550 to 565	4	Earthquake restoration	0.1 to 3.8	0.5 to 37.5	3 to 212

[1] Range of puck quantities per plot; mānuka was amended with approximately 10 seeds/puck; Douglas-fir and radiata pine were amended with 4 seeds/puck.

[2] Estimated established, per plot.

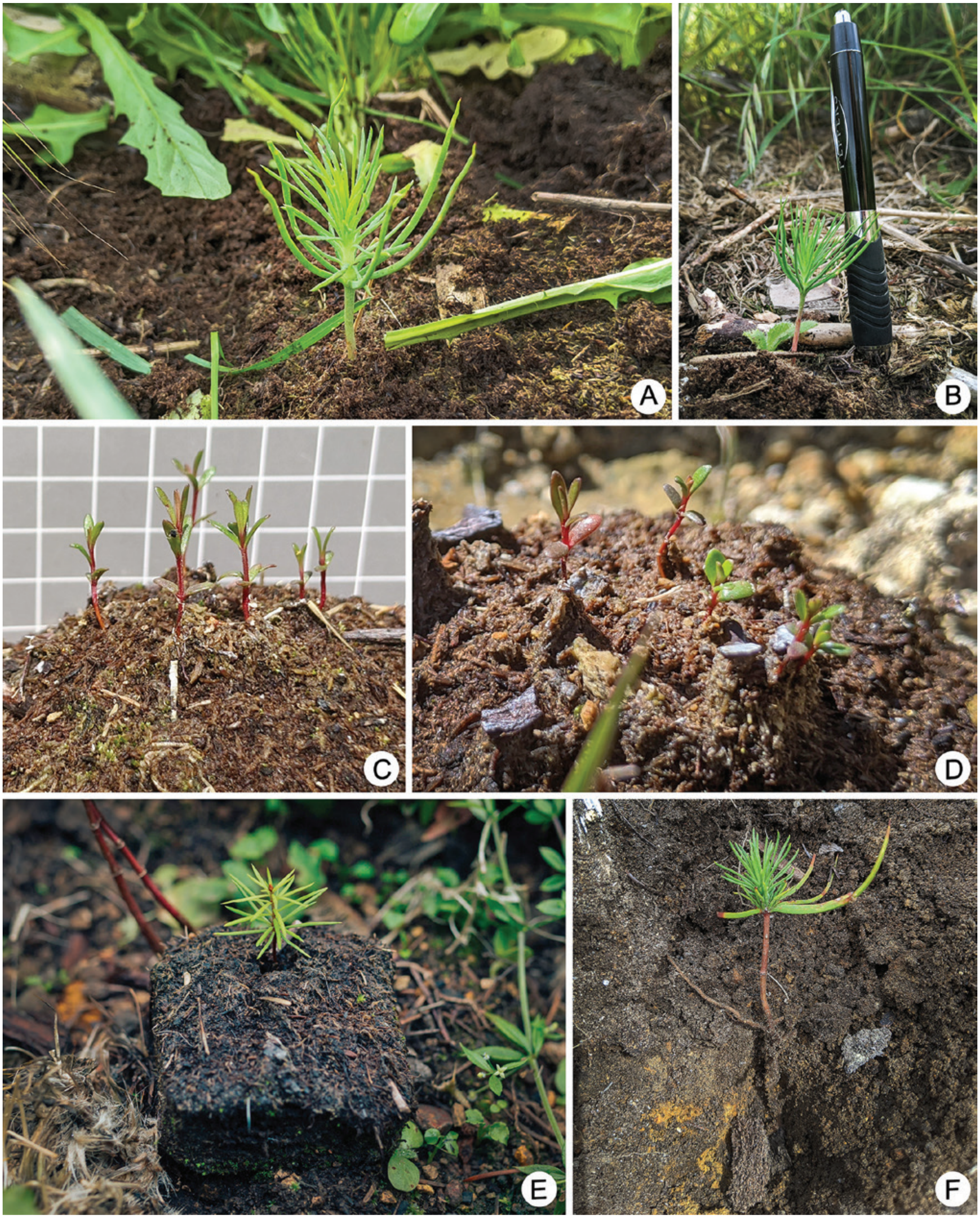

Figure 6. DroneSeed seed vessels (pucks) 6 months after deployment to field sites in New Zealand. (a) Radiata pine seedlings on degraded puck material. (b) Pen for scale next to a germinated radiata pine seedling. (c) Mānuka seedlings emerging from pucks in multiples with cm scale background grid. (d) Mānuka seedlings emerging from a degraded puck. (e) A single Douglas-fir emerged from a puck. (f) An excavated radiata pine seedling showing taproot egress and lateral root formation. (Photos courtesy of DroneSeed 2019)

Douglas-fir pucks averaged 1.6 percent seedling establishment (pucks with a seedling), radiata pine averaged 5.4 percent seedling establishment, and mānuka averaged 16.3 percent seedling establishment (table 3). Stratification was not implemented for mānuka, a typically photosensitive seed that had highly variable germination in our plots. Stratification improved Douglas-fir establishment, but not radiata pine (figure 7). Survival appeared to be primarily driven by moisture availability and soil type. On the South Island, where overgrazed or degraded clay soils were common, we saw a significantly limited germination rate. Clay soils limit surface water retention; so, while hydration of the pucks is possible during rain events, degradation or desiccation of the pucks due to surface flows or drying soils can occur between rain events. Other causes of low survival are likely predation and pathogens. While we did mitigate some predation with capsaicin deterrent, we did not account for potential damping off, or post-germination mortality from bird or insect predation (both of the latter were anecdotally observed).

A distinct observation from the test sites, and something we hope to demonstrate in future trials, is the correlation of microsites to survival and early development of seedlings. Depressions in the ground and shade from objects (e.g., woody detritus, adjacent vegetation, etc.) appeared to provide a favorable microclimate or shelter from predation.

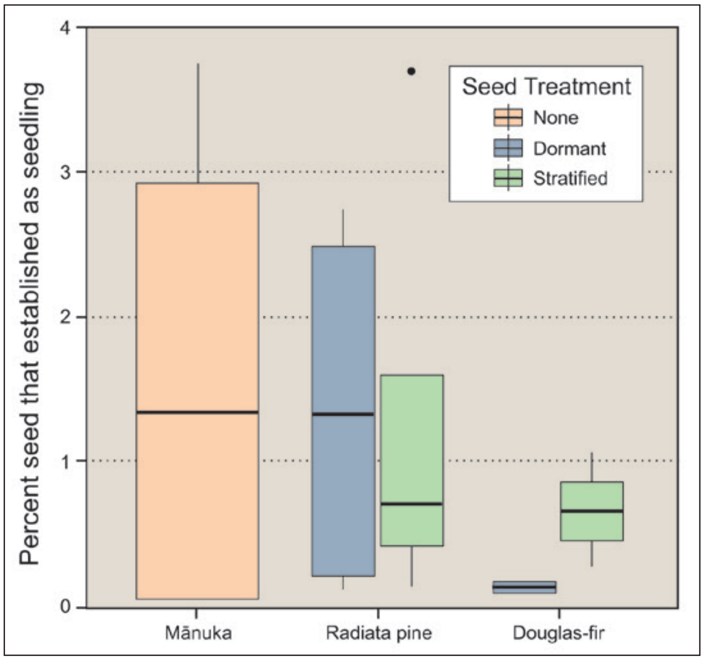

Figure 7. Seed germination from pucks varied by species across several plots in New Zealand. Stratification was critical for Douglas-fir seedlings but unnecessary for radiata pine.

Case Study 3: Custom UAV Systems and Operations for Dispersion of Seed

To carry a sufficient payload for successful vegetation management operations, we developed custom-engineered UAVs, using heavily modified, off-the-shelf components. Each UAV consisted of a central body housing a flight control computer, long-range telemetry radio, co-computer, redundant power supplies, redundant GPS modules, and batteries, with six radial arms supporting electric motors and propellers. Flight-control computers and long-range telemetry radios allow UAVs to receive pre-programmed flight plans and operate on autopilot, but with an observing pilot to take control if necessary. In 2019, when the next case study was completed, aircraft had a capable range of up to 7 mi (11.3 km), operating time of 8 to 18 minutes, and capacity to carry 57 lb (25.9 kg) per aircraft. The pucks deployed are tracked in a semi-controlled manner along a 3-m (10.8-ft) wide swath for each operational transect (figure 8), allowing for tracking genetic material from collection through revegetation.

Using a fusion of LiDAR (light detection and ranging), RGB (red, green, blue) imagery, and NIR (near infrared imagery), DroneSeed creates 3D models of a survey site, which can be used for planning heavy-lift swarm missions, but are also useful for many other survey objectives relevant to landowner objectives such as locations of site preparation, microsite and mineral soil identification, and general suitability of surfaces for seeding operations.

UAV-Assisted Artificial Regeneration

We were contracted in 2019 to survey and seed a unit that was part of the 2015 North Star Complex fire in northeastern Washington State. The property ownership experienced catastrophic, stand-replacing fire throughout the project area and well beyond those boundaries (Engel et al. 2019). The high-intensity fire resulted in almost complete destruction of the understory and canopy biomass, therefore limiting

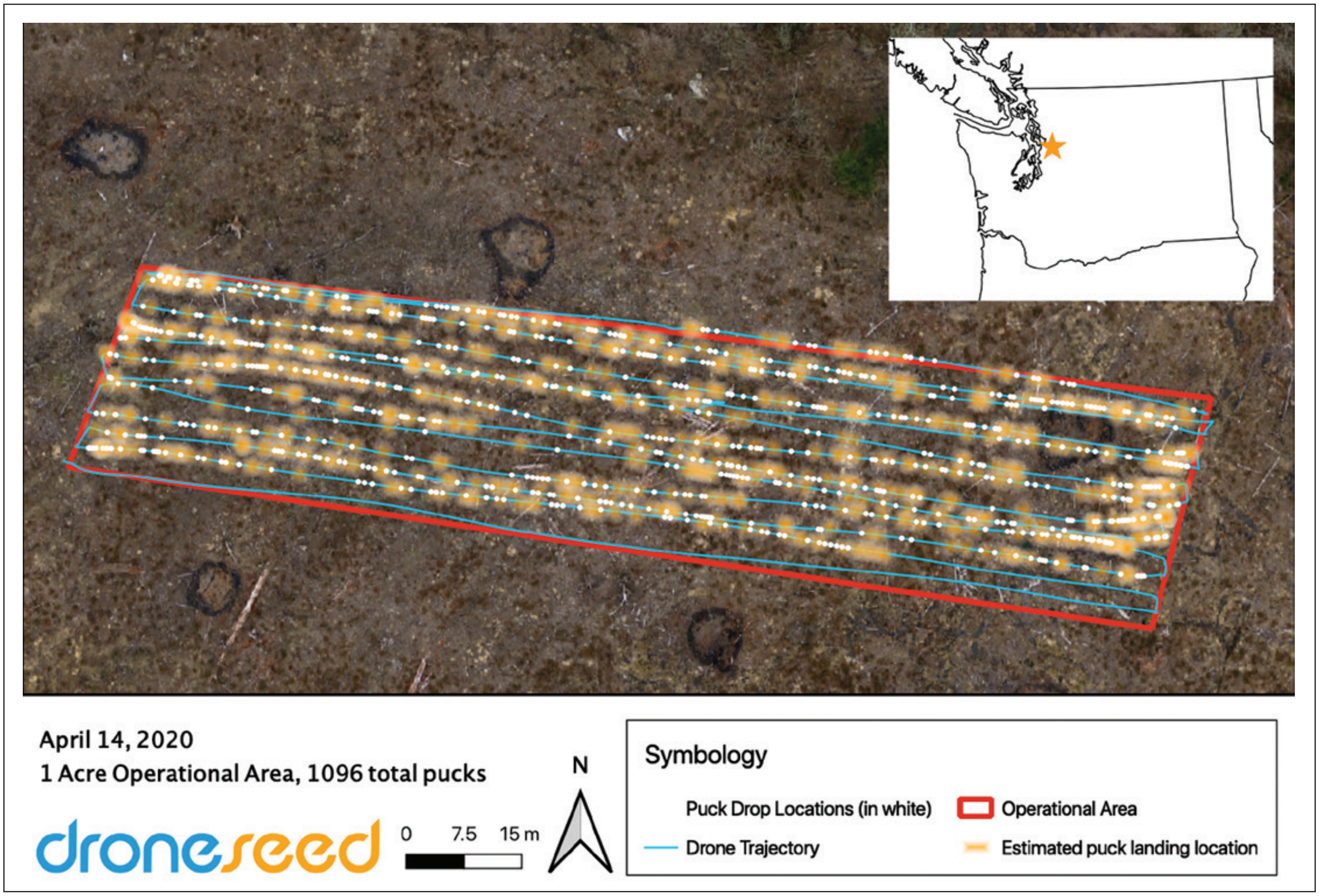

Figure 8. DroneSeed puck dispersion tracking is mapped and landing position is estimated within a 3-m swath using an onboard sensor system. This enables tracking of payloads with a high degree of accuracy from seed procurement and vessel manufacturing through to the field site. (Image courtesy of DroneSeed 2020)

opportunities for timely natural regeneration from seed rain. In subsequent years, recalcitrant native vegetation had grown to dominate the project area which had not yet reforested with conventional planting efforts.

The landowner objective was to establish economically and ecologically relevant stands of native trees across the unit. The edaphic conditions were deemed difficult and insufficient for conventional regeneration using nursery stock. The non-timber species dominating these conditions could not be controlled using chemical site preparation given the current regulatory situation on this ownership which prevents herbicide use based on environmental concerns. As an alternative to herbicide application, mechanical site preparation can create optimal edaphic conditions through scarification using excavators for turning over vegetation and surface materials, downing snags, collecting slash into concentrated points, and exposing mineral soil. Scarification was completed in fall 2019 (figure 9a) immediately prior to DroneSeed survey and seeding operations. We identified “No-Plant Zones” (NPZ) that were to be excluded from seeding due to substrate (e.g., large rocky outcroppings, moraine fields, etc.) and persistent vegetation cover (e.g., areas with dense, live canopy). We also excluded areas within the unit boundaries that were designated by the land manager to not be seeded, such as buffers around roads (figure 9b).

The land management provided a shapefile denoting the scarified area to be aerially surveyed for this project. Aerial drone survey with multispectral (RGB and NIR) and LiDAR imaging provided immediate insight into vegetation and soil status as well as landscape features (figures 10a and 10b). For the landowner, the aerial survey provided a series of high-resolution imagery data sets that can inform future land management practices. The LiDAR survey data informed UAV programming

Figure 9. (a) An aerial view of a DroneSeed customer site in northeastern Washington where mechanical scarification treatments removed vegetation that established over 4+ years following a large fire. (b) DroneSeed used multispectral survey imagery to designate no-plant zones and buffer roads (in red) to efficiently target optimal site conditions for seeding. (Photos courtesy of DroneSeed 2019)

for obstacle avoidance and terrain (figure 10c). The aerial survey data assisted with the development of a prescription for deploying enhanced seed over ground conditions that were most conducive to germination and establishment (such as site-prepped areas).

Seed for the project was provided by the land management 6 weeks prior to onsite operations so that manufacturing and assembly times for the pucks could be accommodated. Three species were included in this project: ponderosa pine, Douglas-fir, and western larch (*Larix occidentalis* Nutt.). Each puck contained 3 to 6 seeds, depending on species and management preference, and a total of 1,000 pucks were deployed across the project area.

Using heavy-lift UAV swarms, DroneSeed operators treated 51.3 acres (20.8 hectares) using up to three autonomously flown coordinated UAVs for each mission to achieve puck deployment. Operations were conducted immediately prior to, or during, snowfall events, leading pucks with dormant conifer seed to be buried under snow for the duration of winter. DroneSeed, along with the landowners, installed fixed radius plots and transects across the treated area to monitor dispersion pattern and germination/establishment rates. As of June 2020 (upon submission of this article), there was initial germination and rooting at some sites. The DroneSeed team will be reporting outcomes in future publications.

Conclusions

Aerial seeding and the supporting technology largely rely on dated technology (Becker 2001). DroneSeed has been working with stakeholders in the forestry and native plant restoration industries to develop products that address post-disturbance needs. Specifically, we have focused on the post-fire environment, where seedling production and response times are

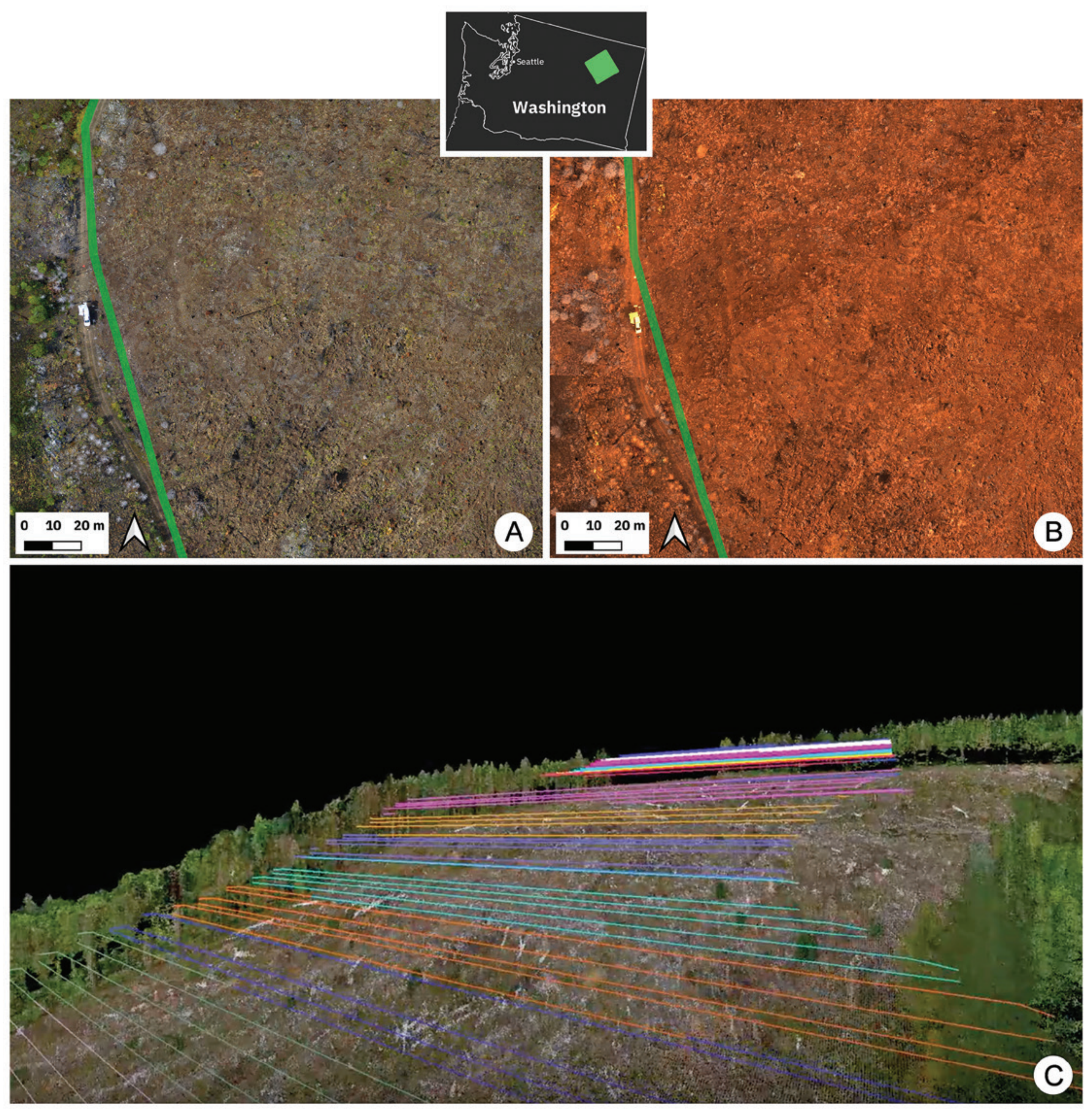

Figure 10. (a) RGB and (b) NIR imagery of a DroneSeed field site following drone survey operations. (c) Our survey process also collects LiDAR imagery, which is used to plan heavy-lift UAV operations designated by these overlaid multi-colored "mission lines." (Photos courtesy of DroneSeed 2019)

constrained by swift response needs and limitations within conventional reforestation supply chain and labor pools.

We offer improvement from broadcast payload applications— currently focused on seed. At the time of these projects, we were able to service up to 25 ac (10 ha) per day with a single team of four people and a three-aircraft drone fleet. The technical capacity for five aircraft in simultaneous flights exists; however, we are reviewing landing area protocols to safely achieve this by 2021 which should improve our daily acreage rate by 20 to 40 percent. These protocols are anticipated to lead to a daily service capacity of 200 ac (80 ha) per fleet by mid-2022. In the meantime, we are developing standard operating procedures for all UAV field operations, as they are a critical, and often overlooked, component of safe and scalable performance.

Seed "enablement" or "enhancement" strategies will continue to be a critical component of all machine-deployed seed, whether for aerial or ground-based applications. We anticipate monitoring academia and industry for improved materials and techniques, but also continuing fast throughput research, engineering, and manufacturing. Our primary goal is to improve seed-use efficiency and survival rates with each iteration of our technology and seed treatment processes. We currently focus on using non-improved, abundant seed sources, as improved genetic stock is often better suited for nursery investment. Our working species list is growing to include many economically important conifer species, a variety of rangeland grasses, and native plant species from across North America, Hawaii, and Oceania.

We do not see this technology as a replacement to conventional and time-tested regeneration strategies involving nursery stock production and manual planting operations. We anticipate developing this tool to assist with the growing backlog of reforestation and revegetation on private and public lands as a consequence of disturbance and initiatives to address climate change. In situations where native plant restoration is critical, landscapes prove challenging, and lag times in the conventional reforestation supply chain exist, seed distributed by UAVs may be opportune. Our puck can be stored in large quantities, much like raw seed, thus eliminating the economic risk of growing vast amounts of stock for unknown future use, and puck deployment can provide cost and safety advantages compared with hand-planting because each UAV can rapidly cover more terrain than manual planting.

Address correspondence to—

Matthew Aghai, DroneSeed Co, 1123 NW 51st Seattle, WA; phone: 425-659-3931 x 1007 email: matthew@droneseed.co

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Efficacy of Spinetoram, Methoxyfenozide and Lambda-Cyhalothrin for Control of European Pine Shoot Moth (*Rhyacionia bouliana*)

Mario Lanthier and Stefanie Harder

CropHealth Advising & Research, Kelowna, BC

Abstract

Four trials were conducted in 2017 and 2018 in British Columbia, Canada, to test newer insecticides for control of European pine shoot moth in a conifer seed orchard. In each of the four trials, the insecticides spinetoram and lambda-cyhalothrin gave over 80-percent reduction in insect damage when compared to untreated. The insecticide methoxyfenozide was tested in one trial and also provided over 80-percent control. In two trials, excellent control of the pest was obtained with one application made in late April, targeting the larvae moving from overwintering sites to new developing shoots. In the other two trials, excellent control was obtained with two consecutive applications made in mid-June and early July, targeting adults and newly hatched larvae on new plant shoots. This work helped generate data for label extension of the products.

Background

Forest seed orchards are managed similarly to tree fruit orchards except cones are harvested, from which seeds are extracted for later sowing in nurseries (figure 1). In British Columbia, more than 250 million trees are produced annually specifically for reforestation efforts after logging of forests (BC Ministry of Forests 2017). Seed orchards grow mostly conifer trees, including a large component of lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. Ex. S. Watson) (BC Ministry of Forests 2020).

European pine shoot moth (*Rhyacionia bouliana*, Lepidoptera: Tortricidae) is a pest of pine plantations in many areas of Canada. This pest was first reported in North America in 1914 and has since migrated across the continent (Pointing 1967). A night flying moth (figure 2), European pine shoot moth is also found on mugo pines (*Pinus mugo* Turra) in ornamental landscapes and production nurseries.

All of the damage from European pine shoot moth is done by the larva (figure 3), which attacks new shoots and reduces conelet production. The European pine shoot moth overwinters as a third instar larva in

Figure 1. Forest seed orchards are managed similarly to fruit tree orchards except cones are harvested, from which seeds are extracted for later sowing in nurseries. (Photo by Stefanie Harder 2019)

Figure 2. The European pine shoot moth adult has orange or bright ochre forewings with irregular, diagonal silvery lines and a wingspread of 15 to 20 mm. This insect flies mostly at dusk. (Photo by Cora Watts 2018)

Figure 3. The European pine shoot moth larva has a smooth, dark brown abdomen with a shiny black head and thoracic shield. This caterpillar may reach 16 mm in length. (Photo by Mario Lanthier 2017)

Figure 5. In early spring, presence of European pine shoot moth is noted by a dying terminal bud or a crust of dried pitch on the host tree. Most of the feeding is done in April and May when the elongating shoots are tunneled by the larvae. (Photo by Mario Lanthier 2017)

hibernacula (pitchy web) beside the terminal bud, or on smaller buds next to the terminal bud. In spring, the larvae becomes active and moves into the terminal bud (figure 4). During May and June, the larva feeds within the terminal bud, also damaging the stem (figure 5). Attacked shoots are visible as wilting terminals with pitch accumulation at the base of buds (figure 6). Terminal shoots may be killed (figure 7). The larvae pupate inside the shoot before exiting in late spring to early summer (figure 8). Adults live for about 1 month, with females laying eggs on twigs or on sheaths of new needles. Eggs hatch shortly after and young larvae bore into new needles (Martineau 1984). Over time, a high population of this insect may cause substantial reductions in shoot growth and losses to cone production sites (figure 9). Additionally, field observations indicate that feeding damage to the shoot may cause young conelets to abort.

Figure 6. During May and June, the European pine shoot moth larvae feed within the terminal bud and terminal stem. Attacked shoots are visible as wilting terminals with pitch accumulation at the base of buds. (Photo by Mario Lanthier 2018)

Figure 4. The European pine shoot moth overwinters as a third instar larva resting on, or inside, the terminal bud. (Photo by Mario Lanthier 2017)

Figure 7. Terminal shoots are killed following feeding by European pine shoot moth. (Photo by Mario Lanthier 2018)

Figure 8. The larvae of European pine shoot moth will pupate inside the shoot before exiting in late spring to early summer. (Photo by Mario Lanthier 2018)

In conifer seed orchards, European pine shoot moth was previously considered a minor pest but the population has increased in recent years. Control treatments are now applied at many facilities. At one location in south-central British Columbia, infestation by larvae on lodgepole pine increased from 25 percent of trees affected 1 year to 80 percent of trees affected the following year (Heeley 2003).

The insect population can be managed by manual removal of infected shoots before the larvae pupate into adults. This method is useful in landscapes but is slow and labor-intensive on tall trees typical of conifer seed orchards. Recently grafted young trees may require a pesticide treatment to protect newly elongating shoots.

Figure 9. In conifer seed orchards, damage by European pine shoot moth negatively impacts subsequent cone production. The photo shows an unaffected shoot (left) and an affected shoot (right). (Photo by Mario Lanthier 2018)

In Canada, no pesticide product is registered for European pine shoot moth in conifer seed orchards. Formulations of dimethoate (trade names Cygon® 480EC and Lagon® 480E) are registered for this pest on pine trees grown as ornamentals or Christmas trees (PMRA 2019). The label rate is 2 L in 1000 L of water, or 0.2 percent concentration. Some conifer growers report better efficacy for this pest at 0.5 percent concentration. The label rate of dimethoate for other seed cone pests is 1 to 2 percent.

Dimethoate is an organophosphate compound of moderate to high toxicity to mammals, based on laboratory studies on rats and rabbits (Health Canada 2011). Since 2016, the active ingredient is subject to long restricted re-entry after application: 18 days for thinning of pine trees in Christmas tree plantations and 49 days for seed cone harvest of spruces (*Picea* spp.) in seed orchards (Health Canada 2015). The objective of our study was to evaluate newer insecticides of lower acute toxicity for their efficacy against European pine shoot moth in pine seed orchards.

Methodology

Various insecticides were tested over four distinct trials (table 1). The products were applied on lodgepole pine trees at Vernon Seed Orchard Co. Ltd., British Columbia (50˚13' north, 119˚19' west, elevation 500 metres). The trees were field-grown, grafted, and planted in 1995 at a spacing of 3.5 m within the tree row and 6.0 m across the tractor alley. Each trial was set up in a randomized, complete block design.

Trials 1 and 2 were conducted in spring 2017 and 2018, respectively, and targeted larvae moving from overwintering sites to new developing shoots. Six treatments were applied over eight replicates in trial 1 and nine replicates in trial 2 (tables 2 and 3). Each replicate was an individual tree surrounded by untreated buffer trees. One application was made in each trial, on April 21, 2017 (trial 1) and April 23, 2018 (trial 2). The spray solution was prepared with municipal water. Each treatment was applied at a rate of 2 L per tree using hand-held backpack sprayers (Solo 475, Solo Inc., Newport News, VA, hollow cone nozzles 1.8 mm orifice) (figure 10).

Trials 3 and 4 were both conducted during summer 2017, and targeted adults and newly hatched larvae on new plant shoots. Three treatments were applied over five replicates in trial 3 and four replicates in

Table 1. Products selected for trials to control European pine shoot moth.

Active ingredient (a.i.)	Trade name	Concentration of a.i.	Label rate
Dimethoate	Lagon® 480E	480 g/L	0.2 L / 100 L (0.2%)
Lambda-cyhalothrin	Matador® 120EC	120 g/L	104 ml / 1000 L / ha
Methoxyfenozide	Intrepid™ 240F	240 g/L	1.0 L / 1000 L / ha
Spinetoram	Delegate™ WG	25%	420 g / 1000 L / ha
Thiamethoxam	Flagship® WG	25%	35 g / 100 L

Table 2. Trial 1 (spring application, 2017) treatments and results. Treatments were applied April 21 except dimethoate 0.5% on April 28 and thiamethoxam was repeated May 2.

Treatment	Trial rate	Mean flagging shoots per tree (sd)
Untreated	n/a	36.6 (7.9)
Dimethoate 480 g/L	2 ml / L	14.8 (10.6) *
Dimethoate 480 g/L	5 ml / L	5.4 (3.8) *
Lambda-cyhalothrin 120 g/L	0.10 ml / L	2.9 (3.9) *
Spinetoram 25%	0.42 g / L	2.3 (2.6) *
Thiamethoxam 25%	0.32 g / L	27.0 (17.6)
Treatment probability (F 5,42)		0.0001

Means followed by * are statistically different from the untreated treatment at p=0.05 Tukey's HSD.
sd = standard deviation.

Table 3. Trial 2 (spring application, 2018) treatments and results. Treatments were applied April 23 except dimethoate 0.5% was applied on April 27.

Treatment	Trial rate	Phytotoxicity Mean (sd)	Mean # flagging shoots per tree (sd)
Untreated	n/a	2.0 (1.12)	27.9 (13.1)
Dimethoate 480 g/L	2 ml / L	1.9 (0.93)	12.4 (5.8) *
Dimethoate 480 g/L	5 ml / L	1.7 (0.71)	9.1 (8.1) *
Lambda-cyhalothrin 120 g/L	0.10 ml / L	1.8 (0.97)	3.0 (2.3) *
Methoxyfenozide 240 g/L	1 ml / L	1.4 (0.73)	0.2 (0.7) *
Spinetoram 25%	0.42 g / L	2.0 (0.50)	1.1 (1.8) *
Treatment probability (F 5,48)		0.7154	0.0001

Means followed by * are statistically different from the untreated treatment at p=0.05 Tukey's HSD.
sd = standard deviation.

Figure 10. For trials 1 and 2, insecticide treatments were applied with a backpack sprayer at a rate of approximately 2 L of spray solution per tree. (Photo by Stefanie Harder 2018)

trial 4 (tables 4 and 5). Each replicate consisted of two rows totalling 100 trees (trial 3) or 120 trees (trial 4), separated from the next replicate by three unsprayed rows. Treatments were applied on June 19 and again on July 5 with an air-blast sprayer (Slimline Manufacturing, Penticton BC) (figure 11). This is the standard spray equipment for commercial applications at these facilities. The sprayer was calibrated on June 15 to determine delivery rate per hectare. Treatments were applied with the sprayer in low range third gear, middle 8 nozzles, giving a delivery of 840 L/ha. Calibration was done using the standard formula:

$$\text{Delivery rate (L / ha)} = \frac{\text{Output (L/min)} \times 600 \text{ (conversion factor)}}{\text{Speed (km/h)} \times \text{Row spacing (metres)}}$$

Application timing mimicked standard grower practices for the target pest. Dimethoate was applied as a grower control. Trial plants were managed following normal practices. No other pesticide applications were made in the trial areas and weather was seasonal for the duration of the project.

Table 4. Trial 3 (summer application, 2017) treatments and results. Treatments were applied June 19 and July 5, 2017. Damage (flagging shoots) was assessed on June 27, 2018.

Treatment	Trial rate	Phytotoxicity 8 days after second treatment (sd)	Mean # flagging shoots per tree (sd) 1 year after treatment
Untreated	n/a	1.30 (0.64)	14.5 (7.19)
Lambda-cyhalothrin 120 g/L	104 ml / ha	1.45 (0.74)	1.22 (1.66) *
Spinetoram 25%	420 g / ha	1.48 (0.75)	0.72 (1.05) *
Treatment probability F (2,146)		not significant	< 0.0001

Means followed by * are statistically different from the untreated treatment at p=0.05 Tukey's HSD.
sd = standard deviation.

Table 5. Trial 4 (summer application, 2017) treatments and results. Treatments were applied June 19 and July 5, 2017. Damage (flagging shoots) was assessed on June 15, 2018.

Treatment	Trial rate	Phytotoxicity 22 days after second treatment (sd)	Mean # flagging shoots per tree (sd) 1 year after treatment
Untreated	n/a	1.93 (0.97)	7.5 (5.16)
Lambda-cyhalothrin 120 g/L	104 ml / ha	2.13 (1.22)	0.78 (1.49) *
Spinetoram 25%	420 g / ha	1.98 (1.07)	0.73 (1.85) *
Treatment probability F (2,117)		not significant	< 0.0167

Number followed by * is statistically different from untreated at p=0.05 Tukey's HSD.
sd = standard deviation.

Figure 11. For trials 3 and 4, insecticide treatments were applied with a commercial air blast sprayer. This is the standard application equipment in commercial forest seed orchards. (Photo by Mario Lanthier 2017)

Figure 12. Assessment of European pine shoot moth damage was made by visually counting flagging shoots in mid-June. (Photo by Mario Lanthier 2018)

Measurements

Phytotoxicity was evaluated prior to and after pesticide applications. Plants were visually examined for symptoms typical of pesticide injury (leaf spots, speckles, tips brown, margins brown, needles brown, tips chlorotic, needles chlorotic) (Costello 2003). Plant injury was rated from 0 (no damage) to 10 (100 percent of the plant is affected), by increments of 10 percent. All plants were examined in trials 1 and 2, whereas 20 or 10 randomly selected plants were examined in trials 3 and 4, respectively.

Insect damage was evaluated in mid-June for all trials (51 to 55 days after the spring application in trials 1 and 2 and approximately 1 year after application in trials 3 and 4). The number of flagged shoots per tree was recorded as an indirect measure of insect activity (figure 12). Each trial tree was examined by two persons simultaneously doing a visual count. The count was repeated and the results compared to ensure consistency. All trees were examined in trials 1 and 2 and 10 trees per replicate were randomly selected for examination in trials 3 and 4. Pest identity was confirmed by visual examination of larvae by a specialist on European pine shoot moth.

Data analyses

All data were subjected to analysis of variance (ANOVA) with F-test set at p=0.05. Where results indicated statistical significance, pairwise comparison was done with Tukey's HSD to determine significant differences between sample means. The analysis was done with ARM software (https://www.gdmdata.com/Products/ARM).

Results

This project relied on natural infestation of the target pest, as the site had extensive damage in 2016. Untreated trees showed extensive damage (figure 13). In all four trials, most treatments significantly reduced the number of flagged shoots per tree when compared to the untreated trees (tables 2, 3, 4 and 5). Some treatments differed significantly from the grower control.

Figure 13. The site had severe damage by European pine shoot moth in 2016. Trees left untreated for the trials showed extensive damage in 2017 and 2018. (Photo by Mario Lanthier, 2018)

Figure 14. Treated trees showed little damage by European pine shoot moth. In all four trials, the test products lambda-cyhalothrin and spinetoram provided over 90-percent control compared with untreated treatments. (Photo by Mario Lanthier 2018)

In all trials, the test products lambda-cyhalothrin and spinetoram provided 90-percent control or better when compared with untreated treatments (figure 14). Methoxyfenozide was applied in trial 2 and also provided more than 90-percent control. Thiamethoxam was applied in trial 1 and provided poor control of the target pest. No treatment-related phytotoxicity was associated with any of the products in any of the trials.

Discussion

Based on the conditions of these trials, the insecticides lambda-cyhalothrin and spinetoram provided effective control of European pine shoot moth, defined as more than 80-percent reduction in insect damage compared with untreated trees. Results were consistent when products were applied either once in the spring at the start of larvae moving from overwintering sites to newly developing shoots, or twice in early summer when newly hatched larvae are present on new plant shoots. The insecticide methoxyfenozide also provided effective control in one trial and is a candidate for further studies.

The main objective of this project was to confirm efficacy of newer insecticides for the target pest, for the purpose of label registration. Registration of pesticides in Canada is subject to a number of conditions (PMRA 2003). For insecticides, an adequate number of trials, usually three studies over 2 years, must demonstrate consistent performance with the proposed rates at the expected pest pressures. A statement of "control" indicates the product consistently reduces the pest damage to a commercially acceptable level and the performance provided should match or exceed that of a commercially acceptable standard treatment.

Dimethoate is a broad-spectrum organophosphate insecticide belonging to Resistance Management Mode of Action Group 1B, which inhibits the enzyme acetylcholinesterase, interrupting the transmission of nerve impulses in insects (IRAC 2019). It works by systemic and contact action. It is considered of high oral acute toxicity to mammals, with identified occupational risks when applied in seed cone orchards (Health Canada 2011).

Spinetoram has contact and translaminar activity: the compound crosses the leaf cuticle to provide control of insects feeding inside the tissue, such as leafminers (Bacci 2016). This mode of action is also called "locally systemic" and likely explains the excellent results in trials 1 and 2 when applied in early spring while the larvae are feeding inside terminal shoots (figure 15). The active ingredient is currently registered in Canada for fir coneworm (*Dioryctria abietivorella*), another important pest in conifer seed orchards (PMRA 2019). Spinetoram is a semi-synthetic spinosyn, a derivative of biological active substances produced by the soil actinomycete *Saccharopolyspora spinosa* (Sato 2012). It belongs to the Group 5 insecticides, acetylcholine receptor modulators that cause persistent activation of nicotinic acetylcholine receptors, thus disrupting normal synaptic signal transmission

Figure 15. Spinetoram has translaminar activity, providing control of insects feeding inside plant tissue. In this project, some terminal shoots were opened and revealed a dead caterpillar of European pine shoot moth. (Photo by Mario Lanthier 2018)

in the insect central nervous system. This particular mode of action is unique to spinetoram and spinosad, the only two active ingredients in Group 5 (Health Canada 2008).

Methoxyfenozide is a molting accelerating compound, also called insect growth regulator. It has low acute toxicity to mammals and is not a concern for chronic exposure (Health Canada 2004). It is not significantly leaf-systemic (Carlson 2001). Feeding on a treated plant surface induces a precocious moult in lepidopteran larvae, leading to cessation of feeding and premature head capsule slippage and death (Nauen 2002). This mode of action likely explains the excellent results in trial 4.

Lambda-cyhalothrin is currently registered in Canada for western conifer-seed bug (*Leptoglossus occidentalis*), a pest in conifer seed orchards (PMRA 2019). It is a non-systemic, contact or stomach poison with some repellent properties, with rapid knockdown and long residual activity (Health Canada 2003). It is the long residual activity that provided the excellent results noted in this project. Lambda-cyhalothrin is a synthetic pyrethroid insecticide of Group 3A (IRAC 2019). It acts as an axonic poison on both the peripheral and central nervous systems of the insect. A recent review determined there are potential risks of concern from dietary exposures. Cancellation was proposed for all applications on food crops but uses would remain for ornamentals and trees (Health Canada 2017).

Effective control of European pine shoot moth in seed cone orchards looks promising with newer insecticides such as spinetoram and methoxyfenozide. The compounds are fairly safe to humans and the environment and gave excellent control of the target pest in a series of trials conducted in a commercial facility in 2017 and 2018. Another effective product is lambda-cyhalothrin, especially because of its long residual on plant surfaces. Future registration for use in seed orchards, however, is uncertain.

Address Correspondence To—

Mario Lanthier, CropHealth Advising & Research, P.O. Box 28098, Kelowna BC, V1W 4A6, Canada; email: office@crophealth.com; phone: 250-717-1898.

Acknowledgments

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Double Trouble Historic Village: A Window Into Pinelands Industries

Andrew J. Anderson

Resource Interpretive Specialist, Double Trouble Village State Historic Site, New Jersey State Park Service, New Jersey Department of Environmental Protection, Bayville, NJ

Abstract

The New Jersey Pinelands Village of Double Trouble was an industrial center for over 2 centuries. The natural environment of cedar forest and the rapidly flowing Cedar Creek provided both raw materials and water power for an extensive lumber industry from the 1700s to the 1900s. As timber was cut, the cleared swampland created bog habitat ideal for growing cranberries. Cranberry culture began at Double Trouble Village in the 1860s. By the 20th century, the Double Trouble Company was one of the largest cranberry operations in the State. Today the aptly named Double Trouble Village State Historic Site provides a window into these past Pine Barrens industries, with a complete company town, sawmill, and cranberry sorting and packing house. The Double Trouble Historic District (National Register Reference # 78001787) occupies more than 200 ac (approximately 80 ha) and includes the village and surrounding bogs. This paper was presented at the 2019 Joint Annual Meeting of the Northeast and Southern Forest Conservation Nursery Associations (Atlantic City, NJ, July 23–25, 2019).

Historical Overview

Located on the northeastern edge of the New Jersey's Pinelands National Reserve (figure 1), the historic Double Trouble Village provides a window into past Pine Barrens industries with a complete company town, sawmill, and cranberry sorting and packing house (figure 2).

The Pinelands National Reserve was created by Congress through the passage of the National Parks and Recreation Act of 1978. The reserve occupies 22 percent of New Jersey's land area and is the largest body of open space on the Mid-Atlantic seaboard between Richmond and Boston. The reserve encompasses approximately 1.1 million ac (approximately 445,000 ha) and spans portions of 7 counties and all or part of 56 municipalities. The reserve is home to vast oak-pine forests, extensive wetlands, dozens of rare plant and animal species, and the Kirkwood-Cohansey aquifer system which contains an estimated 17 trillion gal (approximately 64 trillion L) of water.

The natural environment at Double Trouble Village consists of Atlantic white cedar (*Chamaecyparis thyoides* [L.] Britton, Sterns & Poggenb.) forest and the rapidly flowing Cedar Creek. These resources provided both raw materials and water power for an extensive lumber industry from the 1700s to the 1900s. As timber was cut, the cleared swampland created bog habitat ideal for growing cranberries (*Vaccinium macrocarpon* Aiton). Cranberry culture began at Double Trouble in the 1860s. By the 20th century, the Double Trouble Company was one of the largest cranberry operations in the State.

The area's name harkens back to the colonial era when an earthen dam on Cedar Creek provided a constant flow to turn the sawmill's waterwheel. After muskrat gnawed through the dam causing a breach of gushing water, the owner declared they had trouble. When these same muskrats gnawed through the repaired dam later that week, the exasperated owner threw up his hands in defeat stating they now had "Double Trouble."

Lumber Era

Irish merchant Anthony Sharp became the first recorded landowner of what would eventually become Double Trouble when he acquired the property in 1698. The tract included a portion of Cedar Creek and an abundant supply of Atlantic white cedar. By 1765, his son, Joseph Sharp, operated a sawmill on the site. Sea Captain William Giberson purchased the Double Trouble

Figure 1. New Jersey's Pinelands National Reserve was established by Congress in 1978 as one of the Nation's first national reserves. (Courtesy New Jersey Pinelands Commission, 2008)

Figure 2. The former company town of Double Trouble has several preserved original buildings including workers' cottages, the general store, the cranberry packing house, and a sawmill. (Courtesy New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, 2005)

property by 1806. His son, Sea Captain George Giberson, inherited the tract in the early 1850s. During the heyday of the lumber industry in the Giberson era, Double Trouble had two sawmills and reportedly employed more than 2,400 people (figure 3). From the seaport in nearby Toms River, lumber was shipped to ports up and down the East Coast.

Atlantic white cedar is native to the Atlantic and Gulf Coasts of North America and is found from Southern Maine to Mississippi. Locally known as Jersey cedar, the trees grow in forested wetlands where they dominate the canopy. It takes about 70 years for a cedar to grow to a harvestable size. The hardy wood is resistant to decay and warping. It was often milled as roof shingles and clapboard siding (figure 4). Local shipbuilders used this prized wood for constructing the Barnegat Bay Sneakbox, a melon-seed shaped boat with a shallow draft that was often used for duck hunting (figure 5).

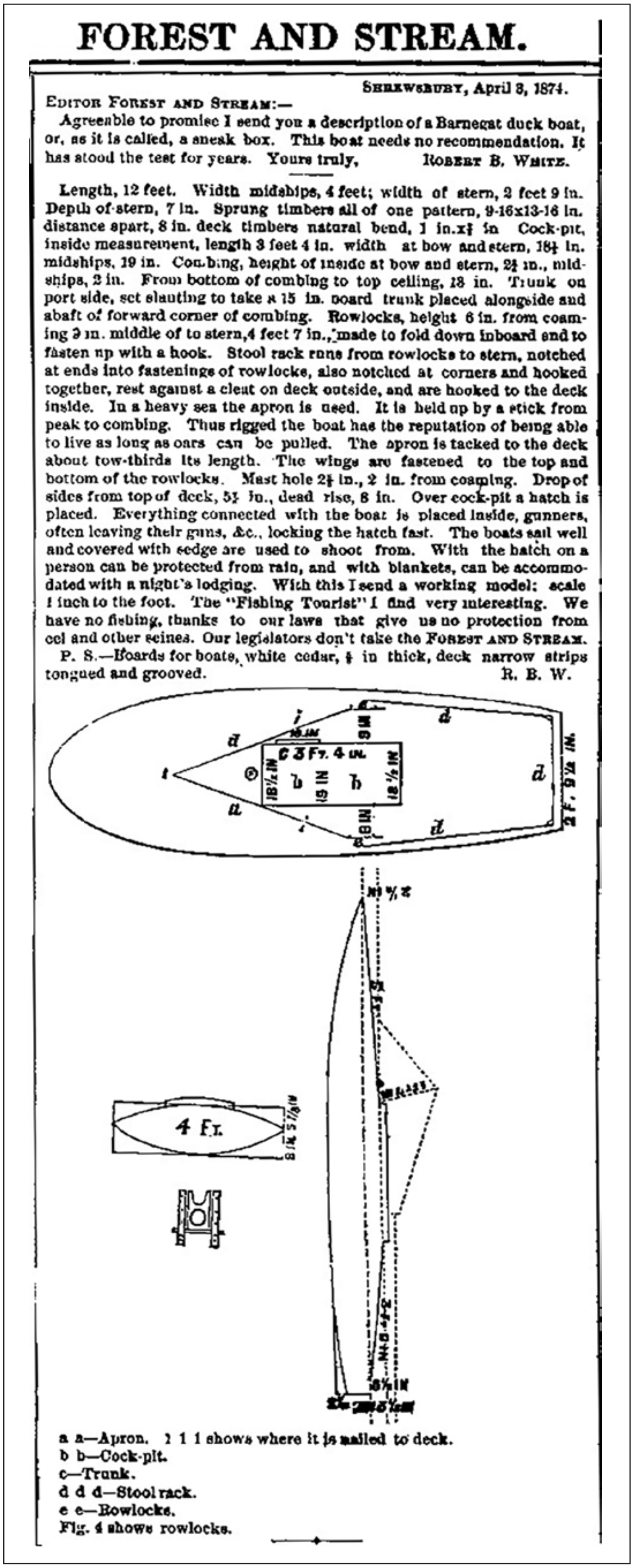
Figure 3. The Double Trouble Lumber Company employed 2,400 people to harvest, mill, transport, and sell lumber in the mid-1800s. (Courtesy New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, circa 1910)

Figure 4. The Double Trouble Lumber Company sold shingles, clapboard siding, posts, rails, channel markers, and bean poles. (Courtesy New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, circa 1910)

Cranberry Era

As increasingly large areas of Atlantic white cedar swamp were cleared for the timber operation, George Giberson looked for methods to reclaim the land for additional income. Cranberry farming afforded such an opportunity (figure 6).

Cranberries are a group of evergreen dwarf shrubs with trailing vines and slender, wiry uprights with small leaves that grow wild in acidic bogs in North America. Because the blossom—the expanding flower, stem, calyx, and petals—resembles the neck and head of a crane, an English missionary coined the plant a "craneberry" in 1647. Soon after, the "e" was dropped and the name shortened to "cranberry." The fruit is initially light green, turning red when ripe in the fall. Revolutionary War veteran Captain Henry Hall first cultivated cranberries in Cape Cod,



FOREST AND STREAM.

SHREWSBURY, April 3, 1874.

EDITOR FOREST AND STREAM:—

Agreeable to promise I send you a description of a Barnegat duck boat, or, as it is called, a sneak box. This boat needs no recommendation. It has stood the test for years. Yours truly, ROBERT B. WHITE.

Length, 12 feet. Width midships, 4 feet; width of stern, 2 feet 9 in. Depth of stern, 7 in. Sprung timbers all of one pattern, 9-16x13-16 in. distance apart, 8 in. deck timbers natural bend, 1 in.x¾ in. Cock-pit, inside measurement, length 3 feet 4 in. width at bow and stern, 18½ in. midships, 19 in. Combing, height of inside at bow and stern, 2½ in., midships, 2 in. From bottom of combing to top ceiling, 18 in. Trunk on port side, set slanting to take a 15 in. board trunk placed alongside and abaft of forward corner of combing. Rowlocks, height 6 in. from coaming 3 in. middle of to stern, 4 feet 7 in., made to fold down inboard and to fasten up with a hook. Stool rack runs from rowlocks to stern, notched at ends into fastenings of rowlocks, also notched at corners and hooked together, rest against a cleat on deck outside, and are hooked to the deck inside. In a heavy sea the apron is used. It is held up by a stick from peak to combing. Thus rigged the boat has the reputation of being able to live as long as oars can be pulled. The apron is tacked to the deck about tow-thirds its length. The wings are fastened to the top and bottom of the rowlocks. Mast hole 2½ in., 2 in. from coaming. Drop of sides from top of deck, 5½ in., dead rise, 8 in. Over cock-pit a hatch is placed. Everything connected with the boat is placed inside, gunners, often leaving their guns, &c., locking the hatch fast. The boats sail well and covered with sedge are used to shoot from. With the hatch on a person can be protected from rain, and with blankets, can be accommodated with a night's lodging. With this I send a working model: scale 1 inch to the foot. The "Fishing Tourist" I find very interesting. We have no fishing, thanks to our laws that give us no protection from eel and other seines. Our legislators don't take the FOREST AND STREAM.

P. S.—Boards for boats, white cedar, ¾ in thick, deck narrow strips tongued and grooved. R. B. W.

a a—Apron. 1 1 1 shows where it is nailed to deck.
b b—Cock-pit.
c—Trunk.
d d d—Stool rack.
e e—Rowlocks.
Fig. 4 shows rowlocks.

Figure 5. The first printed description of a Barnegat Bay Sneakbox appeared in Forest and Stream on April 3, 1874, in a short letter from Robert B. White, including a rough dimensional drawing. (Courtesy New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, 1874)

Figure 6. Workers and their families outside the Double Trouble General Store. (Courtesy New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, circa 1910)

MA, about 1816. Farmers soon saw cranberries as a viable commercial crop and started to convert swampland into manmade bog environments.

Civil War Captain Ralph Gowdy is credited with planting the initial cranberry bog at Double Trouble in 1863. Soon after, George Giberson's son-in-law, sawmill operator Thomas Hooper, planted two bogs now known as the Upper and Lower Hooper Bogs. These cranberry bogs were gravity fed and irrigated with water that traveled through sluiceways from Cedar Creek. Following the deaths of Thomas Hooper in 1871 and George Giberson in 1893, the Double Trouble tract started to fall into disrepair.

Giberson's daughter sold the property to Edward Crabbe in 1903. Six years later, Crabbe formed the Double Trouble Company and expanded the cranberry industry. The sawmill was rebuilt to run on steam and later a Witte engine (figure 7). Under the Crabbe family's management, 260 ac (approximately 105 ha) of cranberry bogs were cultivated. The 56-ac (approximately 23 ha) Mill Pond Bog, formerly the mill pond for the sawmill, was the largest in New Jersey. A new reservoir was constructed upstream to provide water for irrigation and maintenance flooding.

Edward Crabbe built a modern cranberry sorting and packing house. Cottages were constructed for migrant workers to stay during the harvest season. With Crabbe's leadership, the Double Trouble Company became one of the largest growers in the business. They sold fresh cranberries as a member of the American Cranberry Exchange.

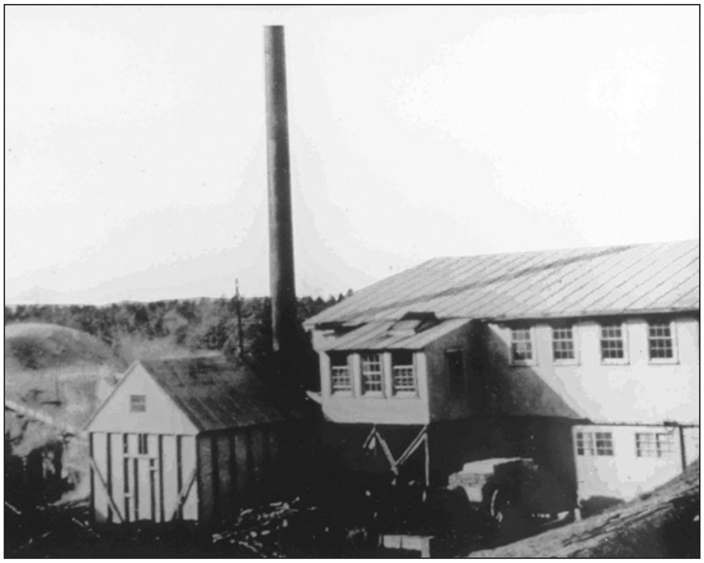


Figure 7. The Double Trouble sawmill was powered by steam in the early 1900s. (Courtesy New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, circa 1910)

For almost a century, cranberries were "dry" harvested at Double Trouble. Berries were originally picked by hand one at a time. As the industry expanded, migrant workers raked berries off the vine with a cranberry scoop—a wooden box with metal tines (figure 8). The fresh cranberries were then sorted and packaged on site for shipment to market (figures 9 and 10). Starting in the mid-1960s the Double Trouble cranberry bogs were "wet" harvested. Bogs were flooded with water from the reservoir. A machine was then used to knock the buoyant berries off the submerged vines. These floating cranberries were corralled to one side of the bog and removed for shipment to a central receiving plant in Chatsworth, NJ (figure 11). As the cranberry industry

Figure 8. Migrant workers hand harvesting cranberries with a scoop at Double Trouble Village in Ocean County, New Jersey. (Courtesy of New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, 1959)

Figure 9. Hayden and Bailey Separators isolate the good berries from bad berries at the Double Trouble sorting and packing house. (Courtesy of New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, 1959)

Figure 10. Local women hand sorting cranberries at the Double Trouble sorting and packing house. (Courtesy of New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, 1959)

Figure 11. One of the last modern "wet" cranberry harvests at Double Trouble Village. (Courtesy of New Jersey Department of Environmental Protection, Double Trouble Village State Historic Site archives, 2004)

shifted from fresh, dry-harvested berries to faster processed, wet-harvested berries, the large number of migrant workers was no longer needed and many of the cottages were abandoned.

Double Trouble Village Today

Following the construction of the Garden State Parkway in the early 1950s, more than half of the county's cranberry bogs gave way to housing developments, shopping centers, highways, and parklands. In 1940 there were more than 100 cranberry growers in Ocean County. Two decades later, only 10 growers remained. The Double Trouble Company was one of the last. After Edward Crabbe passed away and a fluctuation in the market brought down the price of cranberries, the Double Trouble Company offered its land for sale. Negotiations with several developers fell through, and the village and surrounding land were purchased by the State of New Jersey in 1964, in part to protect the Cedar Creek watershed. The Double Trouble Historic District (National Register Reference # 78001787), within the 8,000-ac (approximately 3,250 ha) Double Trouble State Park, includes the village, reservoir, and cranberry bogs, and was placed on the State and national registries of historic places in 1977 and 1978, respectively.

Some of the original cranberry bogs are still visible at Double Trouble Village. They were maintained and harvested through an agricultural lease until a decade ago, when the last Ocean County-based commercial cranberry farmers retired. Other bogs, including the Mill Pond Bog, were long abandoned and have successional growth of red maple (*Acer rubrum* L.) and Atlantic white cedar competing for sunlight. While New Jersey ranks third in cranberry production in the United States, the industry is now almost exclusive to the heart of the Pinelands National Reserve in Burlington County. Cedar Creek is now a protected waterway, popular with canoers and kayakers, and surrounded by miles of hiking trails and the historic village.

Address correspondence to—

Andrew J. Anderson, Double Trouble State Park, 581 Pinewald Keswick Road, Bayville, NJ 08721; email: andrew.anderson@dep.nj.gov; phone: 732–341–4098; https://njparksandforests.com/parks/double.html

Acknowledgments

All information and photographs are courtesy of the Double Trouble Village State Historic Site archives and manuscript collection.

A Comparison Among Four Commonly Used Soil Fumigation Techniques in a Wisconsin Bareroot Seedling Nursery

Roger A. Bohringer

Assistant Manager, Wilson State Nursery, Wisconsin Department of Natural Resources, Division of Forestry, Boscobel, WI

Abstract

In 2016, the Wisconsin Department of Natural Resources initiated a study comparing several soil fumigant options in a side-by-side trial at the Wilson State Nursery. A 77:33 ratio of methyl bromide/chloropicrin (MBC33) was the operational treatment, as this was the soil fumigant historically used at Wilson Nursery with consistent success. The alternatives tested were metam sodium, 100-percent chloropicrin, and a no fumigation control. Three replicates of each treatment were sown with jack pine (*Pinus banksiana* Lamb.), red pine (*P. resinosa* Aiton), white pine (*P. strobus* L.), and white oak (*Quercus alba* L.). Germination was evaluated weekly in each treatment plot. At lifting, seedlings were measured for height, stem diameter, shoot dry mass, and root dry mass. In addition, weed mass was measured in each plot. Germination was relatively poor in all plots due to erratic weather conditions that season. Weed biomass was least in methyl bromide plots. Seedlings were largest in chloropicrin and methyl bromide plots. This paper was presented at the 2019 Joint Annual Meeting of the Northeast and Southern Forest Conservation Nursery Associations (Atlantic City, NJ, July 23–25, 2019).

Background

Wisconsin Department of Natural Resources' bareroot seedling nurseries have long depended on soil sterilization via fumigation as a necessary first step in preparing ground for planting. Over the years, many products have been tried with varying success, but the standard treatment for Wisconsin became a shank-injected and tarped application of methyl bromide and chloropicrin (MBC33). However, due to environmental concerns regarding methyl bromide and ozone depletion, the nurseries came under increasing political pressure to find an alternative.

Metam sodium is commonly used in Central Wisconsin to sterilize potato and vegetable fields. This fumigant is shank-injected and water sealed, rather than tarped, and has overall fewer environmental concerns. Because of its proven history in vegetable production in Wisconsin, metam sodium seemed like an effective and low-cost alternative to methyl bromide for the State nurseries, and the switch was made in 2013. After a couple of years of metam sodium use, however, Wilson State Nursery (Boscobel, WI) observed conifer stunting and increasingly frequent problems with root rots in various species. Additionally, delayed germination and poor bed densities were noted since switching fumigants. While no clear cause and effect could be drawn, the problems were troubling enough to justify a return to MBC33, and to establish a trial to compare the efficacy and phytotoxicity among fumigation alternatives.

Methodology

Four fumigation treatments were randomly assigned locations in each of three replications in the bareroot field at Wilson State Nursery by dividing each block into four plots, writing treatments on cards, shuffling, and drawing a treatment card for each plot. The same card-draw method was used within each treatment to randomly assign species locations (figure 1). The four treatments were: 100-percent chloropicrin (CP), metam sodium (MS), 77:33 methyl bromide + chloropicrin (MB), and a nonfumigated control (NONE). Metam sodium was applied at 75 gal/ac (700 L/ha) on August 15, 2016 (figure 2).

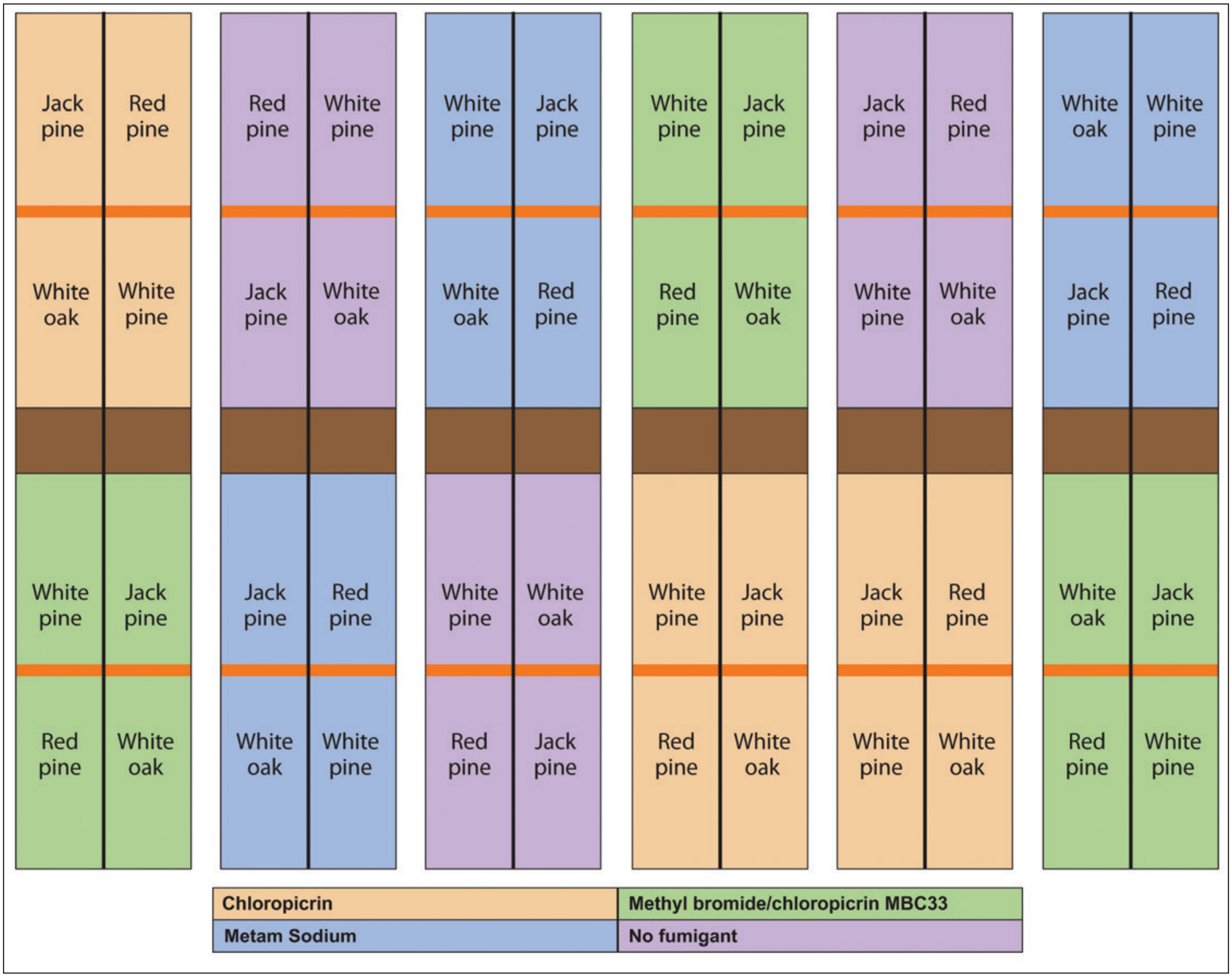


Figure 1. Randomly assigned spatial distribution of trial plots in nursery beds at the Wilson State Nursery to evaluate four fumigation treatments on four species.

Chloropicrin 100 and methyl bromide-chloropicrin (MBC33) were applied on September 17, 2016, both at 240 lbs/ac (270 kg/ha). A two-bed buffer (12 ft [3.7 m]) was established between all treatments to reduce edge effects.

The four species included in the study were: jack pine (*Pinus banksiana* Lamb.), red pine (*P. resinosa* Aiton), white pine (*P. strobus* L.), and white oak (*Quercus alba* L.). Seed for all species was sown October 20–24, 2016. Each species/treatment plot was approximately 120 ft by 4 ft (36.6 by 1.2 m). All plots were treated regularly, at approximately 5-week intervals, with pre-emergent herbicides (oxyflourfen and pendimethelin), at the same time as the rest of the nursery's production pine beds. The fungicide mefenoxam was applied to all conifer beds at the beginning of germination as a precaution against damping off, and prophylactic applications of thiophanate methyl, mancozeb, and chlorothalonil were applied according to the nursery's regular fungicide spraying schedule to prevent various shoot and foliar diseases. All stock was irrigated as needed, as determined by nursery staff.

Three seedling-sampling grids, each 6 by 48 in (15.2 by 121.9 cm), were established in each species/treatment plot, roughly 25 ft (7.6 m) apart from each other. These grids were inventoried weekly to monitor germination, survival, and growth. In addition, 2 by 4 ft (0.6 by 1.2 m) weed-sampling grids were established in each plot to measure weed development. To assess weed-control efficacy, weed mass in each grid was evaluated on July 20 of the first growing season

Figure 2. Metam sodium application rig. (Photo by Kyoko Scanlon 2016, Wisconsin Department of Natural Resources)

by removing all weeds at the ground line, then drying and weighing them.

Jack pine and white oak seedlings were harvested in April 2018 as 1-0 seedlings using standard nursery lifting techniques. Twenty jack pine seedlings from each of three replications (seedbeds) were measured. No data were collected from the white oak, as the population was too low to get valid data. Red pine and white pine (60 seedlings per replication) were harvested the following spring (April 2019) as 2-0 seedlings. At lifting, pine seedlings of each species were measured for height from root collar to terminal bud, and for stem diameter just above the root collar. Seedlings were then thoroughly washed, roots were severed at the root collar, and both shoot and root dry weights were measured using standard lab procedures.

Results

Bed Density

One of the concerns with metam sodium, based on anecdotal evidence, is the possibility of delayed germination and low bed densities. Unfortunately, we were unable to evaluate this adequately due to low bed densities across all trial plots in spring 2017. Our target seedling density for conifer beds is 31 trees/ft2 (335 trees/m^2). Actual densities across the various treatments in 2017 were less than 10 trees/ft^2 (less than 110 trees/m^2). In fact, Wilson Nursery had very poor germination in nearly all fall-planted seed beds in 2017, with total failures in several species, presumably due to the erratic winter weather.

Although germination was poor for all treatments, red pine germination was statistically significantly lower in the chloropicrin plots (figure 3). There was a similar, but not statistically significant, decrease in white pine germination in chloropicrin plots (data not shown). Interestingly, however, the nursery's saleable inventory sampling conducted in August showed the chloropicrin plots produced fewer cull seedlings, so the final saleable yield was comparable with the other treatments, despite lower germination (table 1). This lower germination on chloropicrin treated ground is concerning, but it is a problem that should be easy to correct by increasing seeding rates.

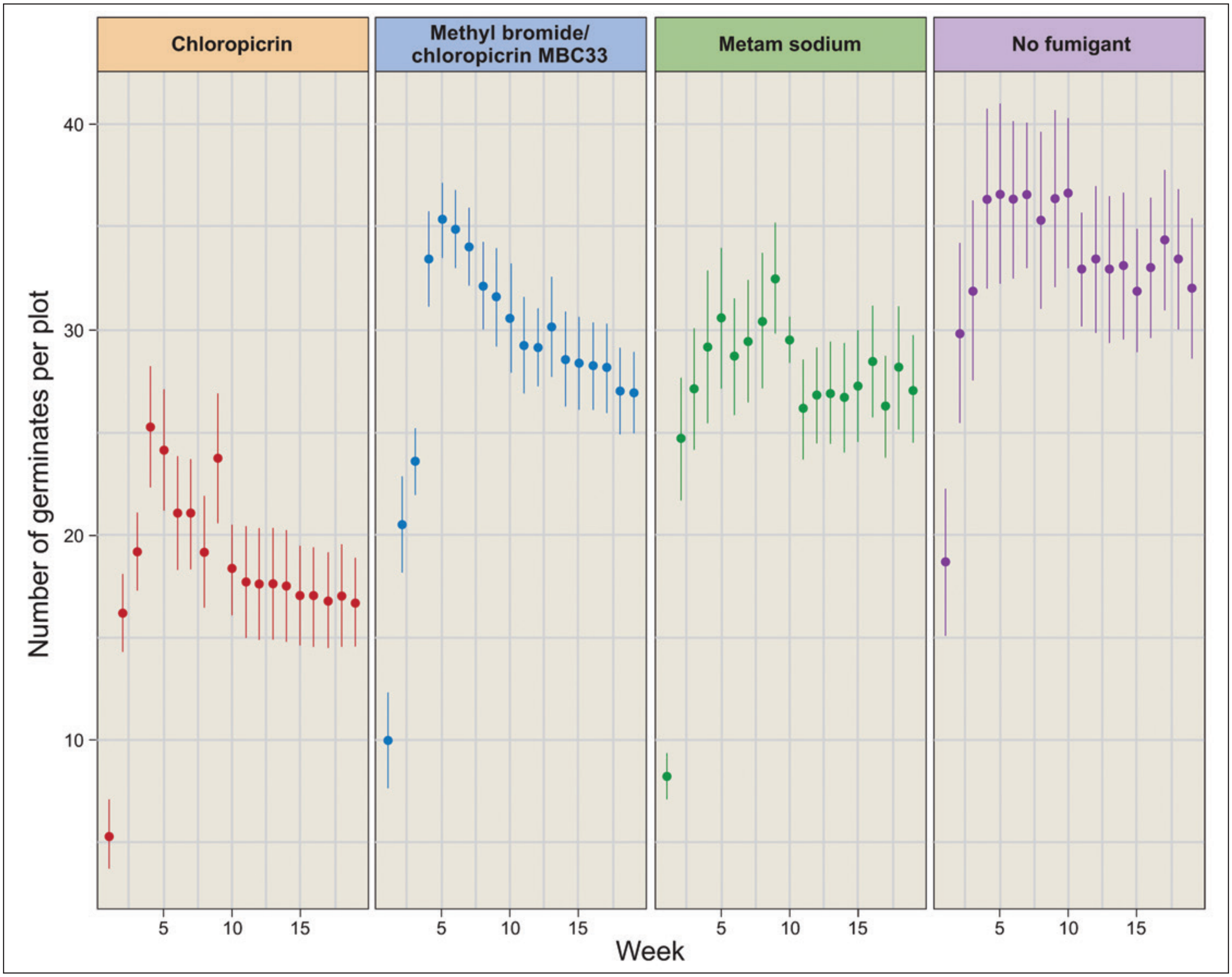


Figure 3. Red pine bed density was lowest in chloropicrin plots.

Weed Biomass

White oak seedbeds require a layer of chopped straw mulch, which introduced a considerable amount of weed seed, primarily hawksbeard (*Crepis* spp.) and groundsel (*Senecio vulgaris* L.) into the fumigated study area. Thus, the mulch likely increased the overall weed biomass across all plots. However, all blocks should have been affected to the same degree.

As expected, methyl bromide provided the best weed control, although all three fumigant treatments had significantly less weed biomass than the unfumigated treatment (figure 4). This differential between fumigated and unfumigated plots would likely have been even greater without the addition of the chopped straw in the white oak plots.

Seedling Morphology

Seedlings grown in the chloropicrin plots were largest for all three pine species (figure 5). Those grown in the methyl bromide plots were also consistently larger than those in the nonfumigated plots. Jack pine seedlings performed quite well on metam sodium (figure 5), which was unexpected based on previous anecdotal observations at Wilson State Nursery. On the other hand, white pine seedlings grown in metam sodium plots tended to be smaller than all other treatments, including the nonfumigated control. White pine stunting was an issue the nursery struggled with previously while using metam sodium operationally and was one of the main reasons for discontinuing its use. Height and stem diameter results among treatments for each species were similar to the biomass results (data not shown).

Table 1. Estimated cull seedling percentages during August sale inventory for each species/treatment.

Treatment	Average trees/ft²	Field cull (%)	Saleable trees/ft²
	Jack pine		
Chloropicrin	11.3	7.1	10.5
Methyl bromide + chloropicrin	10.2	17.1	8.5
Metam sodium	11.3	14.0	9.7
Control (untreated)	10.6	41.6	6.2
	Red pine		
Chloropicrin	11.7	23.5	8.9
Methyl bromide + chloropicrin	13.1	30.3	9.1
Metam sodium	13.2	30.6	9.2
Control (untreated)	13.4	54.1	6.2
	White pine		
Chloropicrin	12.0	39.3	7.3
Methyl bromide + chloropicrin	14.1	42.1	8.1
Metam sodium	13.4	66.2	4.5
Control (untreated)	12.8	50.4	6.3

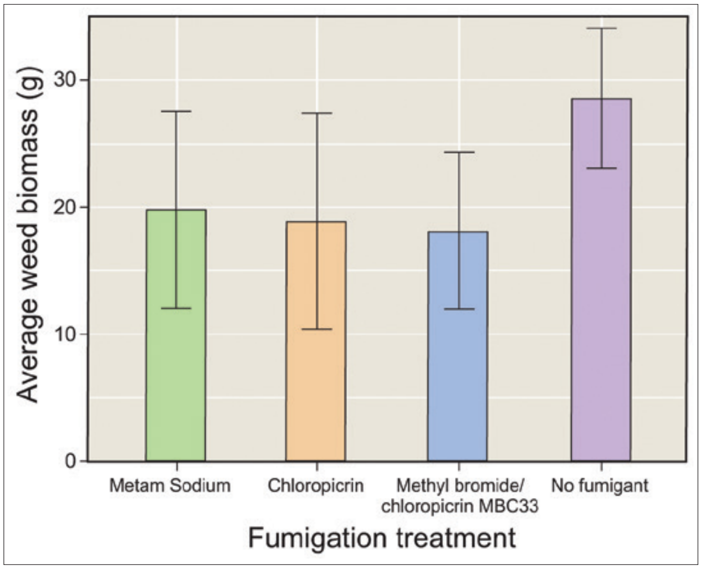


Figure 4. Weed biomass (July 20, 2017) was highest in untreated plots.

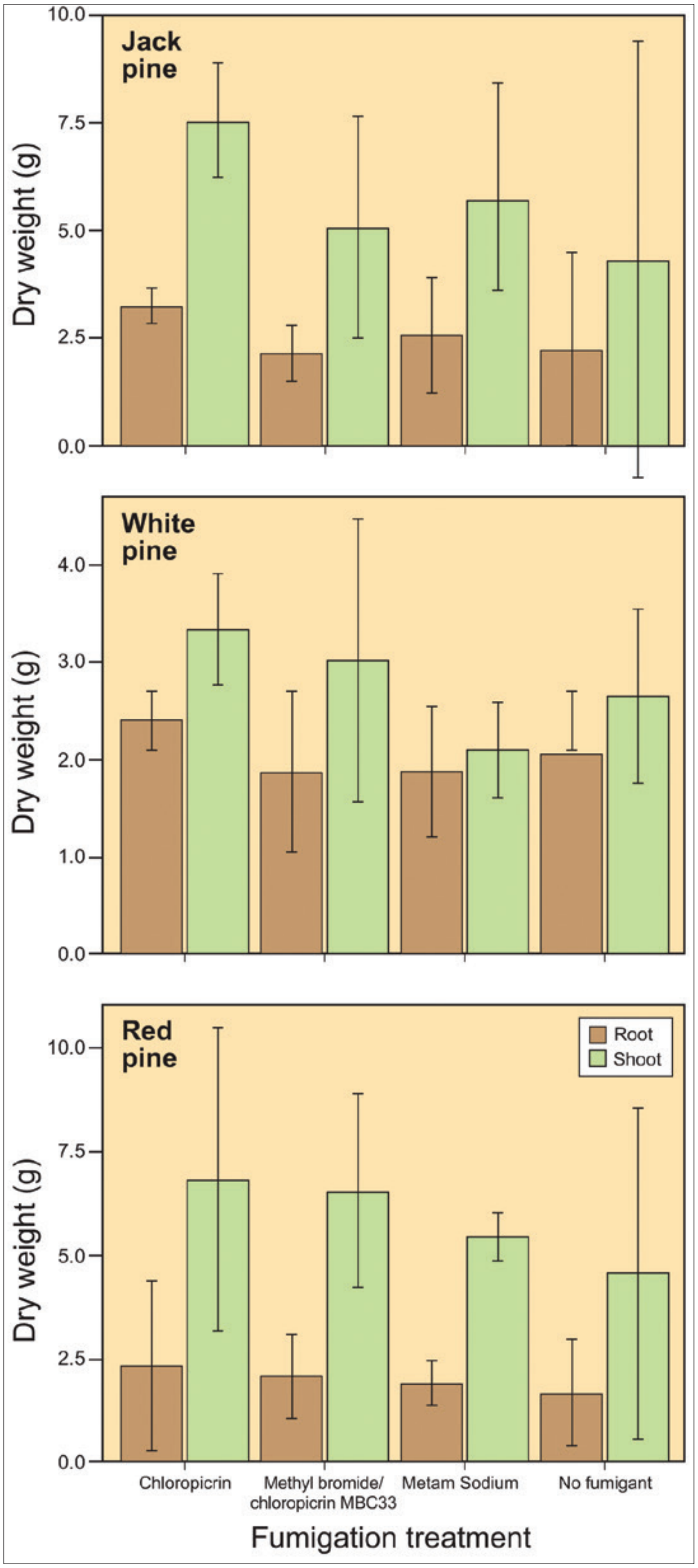


Figure 5. Shoot and root biomass varied among fumigation treatments for three pine species.

Management Implications

This trial did not give clear answers to all of the nursery's fumigation questions. It did, however, yield some information that will prove useful in making future management decisions. While MBC33 did not consistently outperform the other fumigants, it produced solid results on all species, and provided good weed control. This, along with the long history of successful MBC33 use in Wisconsin nurseries, makes it the preferred fumigation option. Chloropicrin's solid performance on all species show that it is a viable alternative should methyl bromide be unavailable, but the poor germination shown would need to be compensated for with higher seeding rates. The comparatively poor growth of all species grown under the no fumigation treatment confirms that this approach is not a viable option for our operation, which strives to consistently produce 5- to 8-inch (13- to 20-cm) jack pine seedlings in one growing season, and similar sized red pine and white pine in two growing seasons.

Address correspondence to—

Roger Bohringer, Wilson State Nursery, Wisconsin Department of Natural Resources, 5350 Highway 133 East, Boscobel, WI 53805; email: Roger.Bohringer@wisconsin.gov; phone: 608-485-1425.

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The author thanks all Wisconsin Department of Natural Resources Division of Forestry personnel who helped in planning and implementing this trial, especially Reforestation and Forest Health staff. Special thanks to Dustin Bronson for his statistical expertise in designing the study, analyzing, and graphing the data, and to Michael Ard, Kyoko Scanlon, and the Wilson State Nursery staff for the many hours spent collecting samples and processing data.

The USDA Natural Resources Conservation Service Plant Materials Program and the New Jersey Plant Materials Center

Scott Snell

Natural Resource Specialist, U.S. Department of Agriculture, Natural Resources Conservation Service, Cape May Plant Materials Center, Cape May Court House, NJ

Abstract

The New Jersey Plant Materials Center (NJPMC) has been providing plant solutions for natural resource conservation concerns since 1965. As one of 25 Plant Materials Centers (PMC) nationwide that constitute the U.S. Department of Agriculture, Natural Resources Conservation Service Plant Materials Program (PMP), the NJPMC is uniquely situated to focus on coastal ecosystem conservation concerns. The NJPMC and other PMCs achieve their shared task of developing and delivering vegetative solutions and conservation technology primarily via three products: conservation plant releases, published documents, and presentations/training sessions. The PMP benefits from internal partnerships between PMCs and external partnerships with other Federal/State agencies, nonprofit groups, and academia to achieve shared goals as efficiently as possible. This paper was presented at the 2019 Joint Annual Meeting of the Northeast and Southern Forest Conservation Nursery Associations (Atlantic City, NJ, July 23–25, 2019).

Introduction

The New Jersey Plant Materials Center (NJPMC) is one of 25 Plant Materials Centers (PMC) within the U.S. Department of Agriculture (USDA), Natural Resources Conservation Service (NRCS) Plant Materials Program (PMP), that form a nationwide network of strategically located PMCs based on soil and climatic conditions (figure 1) with a common mission and vision. The overall mission is to find plant solutions to solve conservation problems. This mission is achieved under the PMP's overall vision to function as the plant experts for the NRCS, fully integrated and coordinated with technical and field office staff, developing and delivering vegetative solutions and conservation technology for NRCS customers. The PMP conducts its plant evaluation activities under the guiding philosophy of Dr. Franklin J. Crider, first head of the PMP, who held the belief that nature has evolved a plant for almost every growing condition (Sharp 2013).

Plant Materials Program History

In the early 1930s, Congress responded to the "Dust Bowl" by creating the Soil Conservation Service (SCS) Division of Nurseries under the USDA in 1935. Over time, the agency's responsibilities increased, as did the types of resource concerns addressed. The program was later renamed the SCS Plant Materials Program, and in 1994, when the SCS was renamed NRCS to more accurately describe the increased scope of resource concerns addressed by the agency, it became the NRCS Plant Materials Program. The reorganization and renaming also acted as a sign of the continued commitment of the Federal Government to address a wide array of conservation challenges using science-based tools and standards.

Since its inception, the PMP had performed the function of a nursery program, producing hundreds of millions of plants annually while conducting observational trials for the purpose of plant selection and development. The 1954 USDA appropriations act designated PMCs as "observational nurseries," distinguishing them from "production nurseries," thereby relieving PMCs of the responsibility for mass production of plants and allowing them to concentrate their efforts on the development of plant technology in the form of varietal plant releases and other products (Helms 2008).

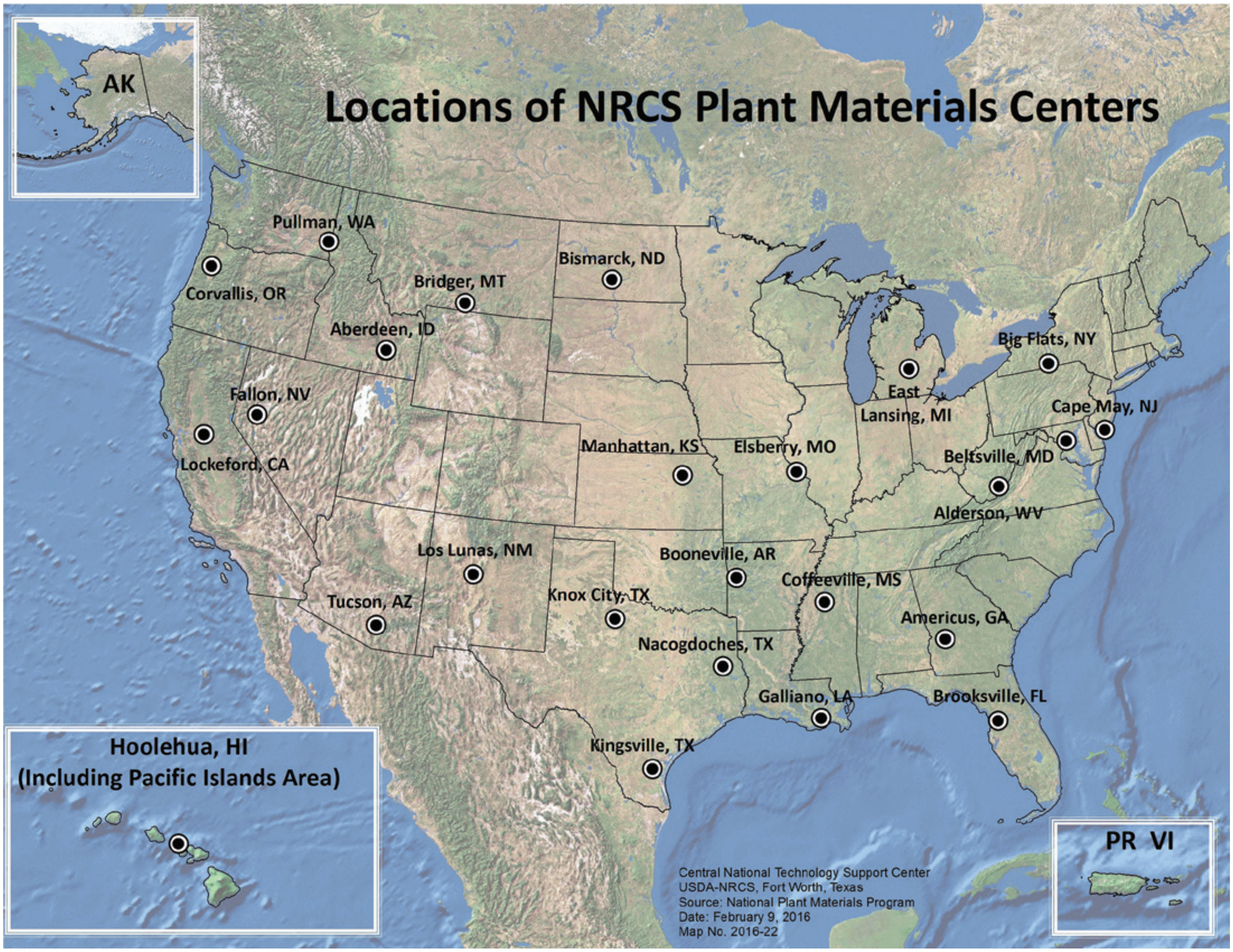

Figure 1. NRCS Plant Materials Centers are located throughout the United States. (Source: National Plant Materials Program, USDA-NRCS 2016)

Plant Materials Program Products

The major products offered by the PMP are conservation plant releases, published documents, and presentations/training sessions. The PMP has four levels of plant releases. Those levels in ascending order of the amount of testing required to meet the minimum criteria for each release level are source identified, selected, tested, and cultivar. On average, from the start of planning to the time of release to commercial growers, each level of release respectively takes 3, 5, 8, and 10 years.

Regardless of the release level, the release process is best described as a six-step process. The first step is to define the conservation need. The intention of the PMP is to have these needs percolate from private landowners and partners to NRCS staff and then be reported by the State's plant materials committee via the State plant materials needs assessment survey. The second step is germplasm collections. Depending on the scope and details of the defined need, these collections could be extensive over a large geographic area or concentrated on more site-specific conditions. The third step is to select from the germplasms collected based on desired traits (e.g., stem count, plant height, drought resistance, flower abundance, seed production) that are most applicable for addressing the conservation need. This step is usually conducted at a PMC. The fourth step is in situ testing of the selected germplasms to determine adaptation to the intended site conditions and evaluate the degree of success in addressing the resource concern. The fifth step is to increase the selected germplasm(s). This increase can be done vegetatively or by seed, depending on the release level and plant species. The final step is to officially release the selection as a named release to commercial nurseries and seed producers for large-scale production and public availability. Public notice of a new conservation release is announced by a Notice of Release publication. Ultimately, the goal is for the plant release to be used for restoration projects and conservation practices on both private and public lands.

The PMP began to phase out the exploration of nonnative plants for conservation purposes during the 1970s and refocused efforts on native plant releases. Of more than 700 releases nationally from the PMP, 570 are still active and more than two-thirds are native to their intended areas of use. Stressing the importance of native plant releases was a forward-thinking and proactive move on the part of the PMP to combat the spread of invasive species, given that a miniscule amount of research existed on nonnative, invasive species until the mid-1990s (Lowry et al. 2013).

Published documents constitute another major PMP product. These documents range from technical, peer-reviewed articles in refereed journals to nontechnical newsletters and informational brochures or flyers intended for the general public. Other common PMP publications include plant guides (featured on the USDA Plants Database), release brochures, posters, final study reports, technical notes, and annual progress reports of activities. All publications can be found on the authoring PMC's website.

Presentations and trainings make up the PMP's third product area. They take a wide variety of forms, depending on the circumstances and intended audience. The PMP delivers formal speaking presentations and poster presentations at professional conferences, tours of PMC facilities for nursery and agriculture industry personnel, and field trainings and equipment demonstrations on specific conservation topics for NRCS, Conservation District, partner agency, and nonprofit groups.

New Jersey Plant Materials Center

The New Jersey PMC (NJPMC) was established in 1965, making it one of the more recent additions to the PMP—the majority of PMCs opened before 1960 and only six PMCs opened after the New Jersey Center. The NJPMC was mandated by Congress to test and develop plants for shoreline restoration and make them available to the public through the commercial nursery industry. A catalyst that motivated Congress to appropriate funding for the creation of the NJPMC was the devastation caused by a 1962 nor'easter storm, the Ash Wednesday Storm (Sharp 2013). Considered by the U.S. Geological Survey to be one of the most destructive storms ever to impact the Mid-Atlantic States, the Ash Wednesday Storm lasted 3 days (5 tide cycles) and caused hundreds of millions of dollars of property damage in 6 States, over 1,000 injuries, and 40 deaths on the Northeast Coast (Cooperman and Rosendal 1962, Morton 2003, Savadove and Buschholz 1993). According to Morton et al. (2003), the majority of property damage occurred where healthy dune systems were not established to protect structures from storm surges. To this day, minimizing the impacts of coastal storms via plant solutions on the dune systems is a task that falls within the realm of the NJPMC's responsibility to focus primarily on highly erodible critical areas of the Mid-Atlantic coastal plains.

The NJPMC is located in Swainton, NJ, (figure 2) on approximately 80 ac (approximately 32 ha) of land leased from the State of New Jersey for production and field studies; all infrastructure is situated on 4 ac (1.6 ha) of adjacent federally owned land. The NJPMC is ideally situated to focus on coastal ecosystem conservation concerns given its location near tidal marshes, coastal dune communities, and extensive wetlands. The NJPMC provides plant solutions for natural resource conservation concerns pertaining to coastal shorelines, sand dunes, mined lands, and coastal grassland habitat

Figure 2. An aerial view of the NJPMC facility located in Swainton, NJ. (Photo courtesy of USDA-NRCS Earth Team 2017)

serving a nine-State area, including all or portions of Connecticut, Delaware, Maryland, Massachusetts, New York, New Jersey, North Carolina, Rhode Island, and Virginia. Like its parent agency, the responsibilities and scope of resource concerns addressed by the NJPMC has altered and increased over time to become more applicable to, and in line with, NRCS programs. The NJPMC addresses many of the same concerns that affect agricultural lands in its service area. This primarily includes addressing erosion of the sandy soils of the coastal plains in cultivated fields, impacts due to saltwater intrusion, water quality degradation from nutrient runoff, and strategies for improvement and maintenance of soil health. To remedy conservation concerns, the NJPMC has developed and released 19 conservation plant releases, 15 of which are currently in active production (table 1; figure 3). Additionally, NJPMC staff have written or contributed to more than 85 publications addressing resource concerns. Recent publications from the NJPMC can be found in table 2.

The NJPMC recently hosted an all-day field training. Michael Yacovelli (biological science technician, NRCS-NJPMC) and Scott Snell (natural resource specialist, NRCS-NJPMC) covered native grass seeding considerations: site preparation, seed application options, drill calibration, use of nurse crops, and maintenance (figure 4a). Paul Salon (soil health specialist, NRCS [retired]) and Kaitlin Farbotnik (conservation agronomist, NRCS) led a session on cover crop mix species selection and soil health (figure 4b). Betsy McShane (New Jersey State biologist, NRCS) and Brittany Dobrzynski (stewardship specialist, New Jersey Audubon) led a session on creating pollinator habitat with an emphasis on pollinator hedgerows and species selection (figure 4c). Kaitlin Farbotnik and Michael Yacovelli demonstrated the operation of a spader and reviewed a variety of tillage equipment options, the recommended use for each piece of equipment, the level of soil disturbance and remaining residue cover expected with each, and the resulting soil health implications. In addition, Becky Watson (biological science technician, Cape Atlantic Conservation District partner employee) gave an overview and tour of the PMC seed cleaning facility, and the Cape May County Beach Plum Association and Jenny Carleo (county agriculture agent, Rutgers) staffed a table offering information and tastings of beach plums and beach plum value-added products. Lastly, David Steinmann (major land resource areas soil scientist, NRCS) presented information on the process of extracting subaqueous soil cores and displayed examples.

Table 1. Conservation plant releases by the New Jersey Plant Materials Center.

Release Name	Common Name	Scientific Name	Applications[1]	Origin
Cape	American beachgrass	*Ammophila breviligulata* Fernald	E, S, W	MA
Suther Germplasm	big bluestem	*Andropogon gerardii* Vitman	B, E, F, W	NC
Wildwood	northern bayberry	*Morella pensylvanica* (Mirb.) Kartesz	E, H, S, W	NJ, NC
Atlantic	coastal panicgrass	*Panicum amarum* Ell. var. amarulum (Hitchc. & Chase) P.G. Palmer	E, F, H, S, W	VA
Carthage	switchgrass	*Panicum virgatum* L.	E, F, W	NC
High Tide Germplasm	switchgrass	*Panicum virgatum* L.	B, E, F, H, ST, W, WL	MD
Timber Germplasm	switchgrass	*Panicum virgatum* L.	B, H	NC
Ocean View	beach plum	*Prunus maritima* Marshall	E, S, W	DE, MA, NJ
Dune Crest Germplasm	shore little bluestem	*Schizachyrium littorale* (Nash) E.P. Bicknell	E, W	DE, NJ
Suther Germplasm	little bluestem	*Schizachyrium scoparium* (Michx.) Nash	E, F, W	NC
Monarch Germplasm	seaside goldenrod	*Solidago sempervirens* L.	E, S, W	DE, NJ, VA
Coastal Germplasm	Indiangrass	*Sorghastrum nutans* (L.) Nash	E, F, W	CT, RI, MA
Suther Germplasm	Indiangrass	*Sorghastrum nutans* (L.) Nash	B, E, F, W	NC
Avalon	saltmeadow cordgrass	*Spartina patens* (Aiton) Muhl.	E, S, W, WL	NJ
Southampton Germplasm	prairie cordgrass	*Spartina pectinata* Bosc ex Link	B, E, S, ST, W, WL	NY

[1] Application codes: B = biomass; S = salt tolerant; E = erosion control; ST = streambank; F = forage; W = wildlife benefits; H = hedgerow; WL = wetland applications.

Strength in Partnerships

A major strength of the PMP is that it allows PMCs to form internal partnerships between PMCs as well as external partnerships with other Federal/State agencies, nonprofits, and academia working towards common goals. With 25 PMCs strategically located throughout the United States, the PMP has the unique ability to conduct replicated, consistent studies over widespread, diverse regions and conditions. The diversity of geographic locations of PMCs provides the means for each center to test the range of adaptability of their conservation plant releases by forming internal partnerships between PMCs. In recent years, PMCs implemented a national study to determine which areas of the country could effectively use ‘Tropic Sun’ sunn hemp (*Crotalaria juncea* L.) as a cover crop and green manure (Clark 2016). Currently, the PMP is conducting a national cool-season study to examine 59 varieties of nine common cover-crop species: cereal rye (*Secale cereale* L.); common oat (*Avena sativa* L.); black oats (*Avena strigosa* Schreb.);

Table 2. Publications produced by the New Jersey Plant Materials Center. All publications are available online at: https://www.nrcs.usda.gov/wps/portal/nrcs/publications/plantmaterials/pmc/northeast/njpmc/pub/

Publication type	Title	Year
Annual progress report of activities	Cape May Plant Materials Center 2019 Annual Progress Report of Activities	2020
Information brochure	New Jersey Plant Materials Center Conservation Plant Releases and Suppliers	2019
Annual progress report of activities	Cape May Plant Materials Center 2018 Annual Progress Report of Activities	2019
Newsletter	Conserving the Sweetgrass Tradition I Mashpee Wampanoag Tribe	2019
Plant guide	Northern Bayberry (*Morella pensylvanica*) Plant Guide	2019
Annual progress report of activities	Cape May Plant Materials Center 2017 Annual Progress Report of Activities	2018
Plant guide	Beach plum (*Prunus maritima*) Plant Guide	2018
Newsletter	Coastal Bluff Erosion in the Atlantic Coastal Plain-How are Shoreline Property Owners Coping with Bluff Erosion	2018
Newsletter	Developing Climate Change Resilience in Conservation Plants	2018
Poster	Developing Coastal Grassland Technologies	2018
Poster	Revegetation Success of Native Species Following Chemical and Mechanical Treatment of *Phragmites australis*	2018
Final study report	Monarch Germplasm seaside goldenrod (*Solidago sempervirens*) direct seeding trials	2018
Plant guide	Virginia saltmarsh mallow (*Kosteletzkya virginica*) Plant Guide	2018
Major publication	New Jersey Sea Grant Consortium - Dune Manual	2017
Poster	The Cape May PMC-Developing Plant Technologies for Coastal Ecosystem Restoration	2017

Figure 3. Conservation plants released by the NJPMC include (a) ‘Ocean View’ beach plum, (b) Virginia saltmarsh mallow (in development), and (c) ‘Wildwood’ northern bayberry. (Photos by Scott Snell 2017–19)

Figure 4. A 2018 NJPMC field day training included (a) native grass seeding considerations, (b) cover crop mix species selection and soil health, and (c) creating pollinator habitat. (Photos courtesy of Cape May Plant Materials Center 2018)

hairy vetch (*Vicia villosa* Roth); Austrian winter pea (*Pisum sativum* L.); daikon radish (*Raphanus sativus* L.); crimson clover (*Trifolium incarnatum* L.); red clover (*Trifolium pratense* L.); and Balansa clover (*Trifolium michelianum* Savi). Participating PMCs have completed the field trials, collected the necessary data, and transferred the data to the USDA Agricultural Research Service (ARS) for statistical analysis. Individual PMCs are in the process of writing final study reports. Several are already publicly available (Bullard 2019, Pickett et al. 2019, Young-Mathews 2019) and the remainder should be published later this year.

The NJPMC has worked with a diverse range of external partners as well. Most recently, an agreement was established with the Bureau of Land Management (BLM) and their Seeds of Success (SOS) program. The mission of SOS is to collect native seed for long-term storage and for the development of commercially available, ecologically appropriate germplasms (Haidet and Olwell 2015). The NJPMC's responsibility thus far with this project has been to process more than 2,100 unique seed collections of 359 plant species. NJPMC staff clean each collection to obtain high seed purity and then pull a sample from each collection to send to the ARS Western Regional Plant Introduction Station in Pullman, WA. ARS staff catalog and preserve each sample for long-term storage and provide samples upon request for basic and applied plant research. The remainder of each collection is either sent direct to a restoration project for immediate use or stored for future development/increase. The most noteworthy restoration project to receive SOS seed collections was a revegetation site at Prime Hook National Wildlife Refuge (NWR) managed by the U.S. Fish and Wildlife Service (USFWS). Superstorm Sandy severely altered the ecology of Prime Hook NWR by inundating managed freshwater wetlands with saline storm surges and damaging protective dune barrier systems. In 2017, the USFWS aerially applied a locally collected seed mix provided by the SOS program to restore about 4,000 ac (approximately 1,620 ha) of tidal marsh and barrier beach ecosystems (figure 5). Since the seeding, USFWS staff have reported excellent native vegetation recruitment.


Figure 5. Smooth cordgrass (*Spartina alterniflora* Loisel.) seed (lower left corner) being loaded for aerial seeding at Prime Hook National Wildlife Refuge. (Photo courtesy of U.S. Fish and Wildlife Service 2016)

The NJPMC has also partnered with Rutgers University in a series of studies focused on pollinator and plant interactions and plant species longevity. Rutgers led a pollinator specificity study at the NJPMC to examine pollinator preference and mutualistic relationships between pollinators and commonly recommended plant species for pollinator habitat (MacLeod 2016). In 2018, during his third year as a seasonal employee at the NJPMC, Luis Almeyda (biological science technician, Cape Atlantic Conservation District) completed his Master of Science in Environmental Science at Stockton University with his capstone project using the plots from the Rutgers study at NJPMC which had remained in place unmaintained for 5 years. In his study, Almeyda (2018) examined the longevity and long-term vigor of the pollinator plant species by assessing survival rates, spread, and stem counts of the remaining pollinator plant species. His findings showed distinct variations in the survival and vigor of the 20 plant species examined, with several species showing substantially greater stem counts, survival, and spread (figure 6). The information gained from this study could be of use to planners recommending flowering plant species for pollinator wildlife habitat in the Mid-Atlantic region.

Address correspondence to—

Scott Snell, Natural Resource Specialist, USDA – NRCS Cape May Plant Materials Center, 1536 Route 9 North, Cape May Court House, NJ 08210; email: scott.snell@usda.gov; phone: 609-536-6354.

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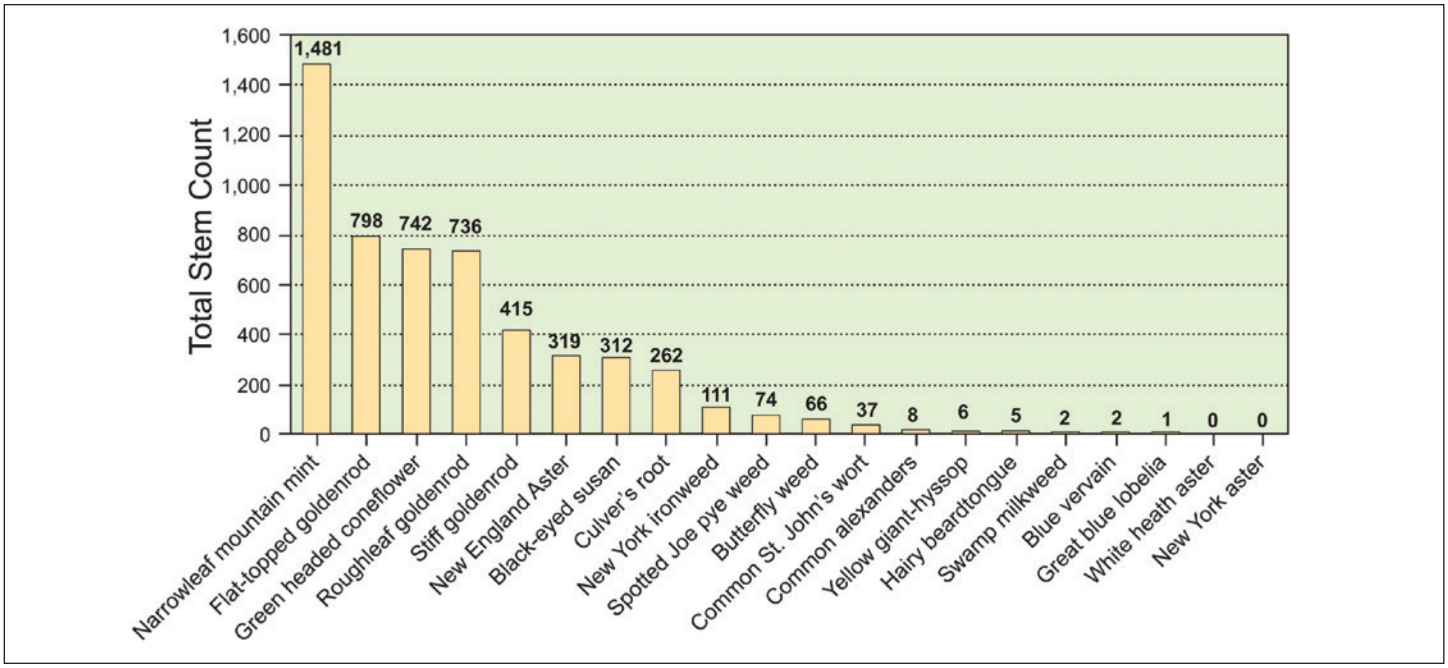

Figure 6. Total stem count data for 20 plant species examined in Almeyda's (2018) Capstone Project at the NJPMC.

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New Seed Collection Zones Are a Mid-Level Descriptor of Seed Origin for the Eastern United States

Carolyn Pike

Regeneration Specialist, USDA Forest Service, Eastern Region State and Private Forestry, West Lafayette, IN

Abstract

This paper provides a brief overview about the Eastern Seed Zone Forum and the new seed-collection zones that were developed for the Eastern United States. A detailed description of the zones and their development has been published in the *Journal of Forestry* (Pike et al. 2020). This paper was presented at the 2019 Joint Annual Meeting of the Northeast and Southern Forest Conservation Nursery Associations (Atlantic City, NJ, July 23–25, 2019) and at the 2019 Annual Meeting of the Intertribal Nursery Council (Tulsa, OK, June 12, 2019).

Overview

In 2015, the U.S. Department of Agriculture, Forest Service assembled a team called the ESZF (Eastern Seed Zone Forum) to develop seed-collection zones for the Eastern United States. As part of that effort, a webinar series was held in 2018, and a Seed Zone Summit was held in May 2018 in Lexington, KY. The team then developed a new map of seed-collection zones for the 37 Eastern States by combining two layers: plant hardiness zones (USDA ARS 2012) and eco-regions (Cleland et al. 2017). In total, 245 unique seed collection zones were created (figure 1). The seed zones are denoted by continuous colors on the map, and are sequentially numbered. The latest version of the map, the archived webinar series, and other resources are available at: www.easternseedzones.com. A full description of the methodology and map development has been published in the *Journal of Forestry* (Pike et al. 2020). A list of the seed collection zones by county can be downloaded directly from the Arc GIS map page, accessible at the website, to facilitate sorting and utility by nurseries, land managers, researchers, and other users.

The seed-collection zones were intended to be used for trees and plants to define seed origin. The zones are relatively large, and therefore may serve as a mid-level descriptor of seed origin to help seed collectors, dealers, and nurseries that commonly move plant material among States. This system may be too coarse for gene conservation purposes, where collectors may rely on GPS coordinates to pinpoint the origin of plant material or seed.

For most purposes, the State and numeric seed-collection zone can be used to describe a seedlot's origin. For example, seed collected in Carlton County, MN, (zone 7) would be labeled as MN-7, and could be lumped with other Minnesota counties in zone 7 (e.g., Itasca, Aitkin, and Cass Counties). Douglas County, WI, is also in zone 7, but this seed would be designated as WI-7. Some seed collectors may decide to maintain separate seedlots by county or include additional provenance information to meet their needs. Nurseries may decide to lump seed from several different zones into one nursery bed, or they may decide to split by State, depending on their nursery practices and logistical needs.

Common garden studies remain the gold standard for determining how far to move seed from, or within, any particular seed-collection zone. For species that have not been field-tested, limiting seed movement within a seed collection zone or between adjacent seed collection zones is a reasonable, general guideline. Seed-transfer decisions for improved seed are based on progeny tests at multiple locations; for recommendations, consult with the improvement program staff that established and analyzed the progeny tests.

The next phase of this project will include a summary of best practices for seed transfer of the most commonly planted species (workhorse species) in the Eastern United States. A team of geneticists will

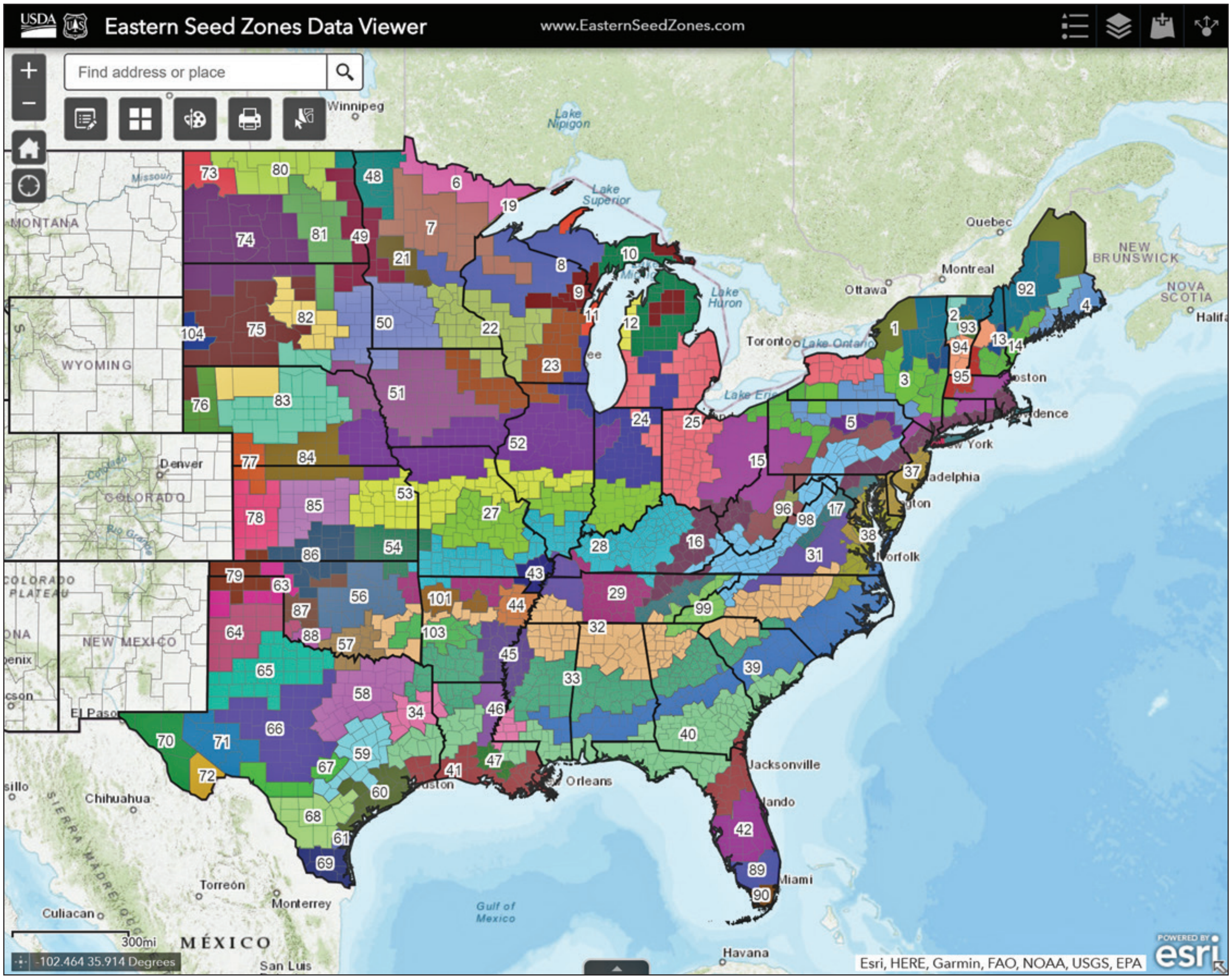

Figure 1. Latest version of the Eastern Seed Zone Forum map, version 2.2, available at www.easternseedzones.com.

review the literature of common gardens (with a particular focus on provenance trials), and make general seed-transfer recommendations for these workhorse species. This information will be compiled into a document for nursery workers and seed collectors.

Address correspondence to—

Carrie Pike, USDA Forest Service, 715 W. State St., West Lafayette, IN 47907; email: carolyn.c.pike@usda.gov; phone: 765-496-6417.

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Lifting Dates, Chilling Hours, and Storage Duration on Slash Pine Seedling Root Growth Potential, Growth, and Survival

Ryan L. Nadel, Nina D. Payne, Thomas A. Stokes, and Scott A. Enebak

Assistant Research Professor, Southern Forest Nursery Management Cooperative (SFNMC), School of Forestry and Wildlife Sciences (SFWS), Alabama Agricultural Experiment Station (AAES), Auburn University, Auburn, AL; Research Assistant, SFNMC, SFWS, Auburn University, Auburn, AL; Research Associate, SFNMC, SFWS, Auburn University, Auburn, AL; Professor and Director, SFNMC, SFWS, AAES, Auburn University, Auburn, AL.

Abstract

Annually, the Southern United States produces over 1 billion forest tree seedlings, the majority of which are conifers (pine) produced as bareroot seedlings. Typically grown for less than a year, seedlings are then lifted from the soil, packed in boxes, bags or bundles, and placed in cold storage before being transported to sites for reforestation. Lifting operations typically occur from late November to early March each year. On occasion, however, circumstances require seedlings to be lifted later or stored longer than recommended. Over three lifting seasons, we investigated pine seedling storability based on a series of delayed lift dates (January through March) and varying storage durations (0 to 14 weeks). Data patterns varied among the three seasons. In general, later lift dates and longer storage durations resulted in reduced seedling growth and survival. This paper was presented at the 2019 Joint Annual Meeting of the Northeast and Southern Forest Conservation Nursery Associations (Atlantic City, NJ, July 23–25, 2019).

Introduction

The United States annually produces more than 1.2 billion seedlings for reforestation, of which 80 to 90 percent is produced in the 13 Southern States (Haase et al. 2019). The majority of seedlings produced in the South are conifers, produced as bareroot seedlings and grown in a similar manner to that of regular agricultural crops (Haase et al. 2019, Starkey et al. 2015). Seedlings are typically grown in native soils within open fields for approximately 9 months before they are removed from the soil during harvesting, or what is called lifting (Starkey et al. 2015).

Lifting usually occurs between late November and late February, the optimum time when seedlings are dormant. These seedlings are packed in boxes, bags, or bundles and placed in cold storage for a 2- to 3-week period before being shipped to the field, where they are planted in areas that have recently been harvested and prepared for reforestation (Carlson 1991, Johnson and Cline 1991, Starkey et al. 2015), or into fields for converting land back to forests. Seedling storage avoids issues of mold and decay, which can decrease seedling survival after outplanting (Grossnickle and South 2014, Landis and Haase 2008). Weather conditions, however, are not always favorable and may delay outplanting, thus requiring longer storage durations than recommended. With fluctuating freezing and above-normal temperatures occurring more often across the Southern United States during the winter months of December, January, and February, there are concerns regarding optimum lifting time, seedling storability, and seedling growth and survival after outplanting (Harrington and Gould 2015).

Environmental conditions during seedling growth in the nursery impact seedling quality and physiological readiness for storage and outplanting (Carlson 1991). Seedling quality can be defined as a seedling that can survive periods of environmental stress and produce vigorous growth following outplanting (Grossnickle and MacDonald 2018, Grossnickle and South 2017). Seedlings must be physiologically ready to grow. Photoperiod and environmental cues during the growing season within the nursery are fundamental to this physiological readiness (Haase et al. 2016). Seedling dormancy, cold hardiness, and the accumulation of carbohydrate reserves (Deligöz 2013) are the main physiological processes that ensure seedlings are physiologically ready to withstand the stresses of lifting, handling, and planting such that optimal root and shoot growth can occur after outplanting (Burdett and Simpson 1984).

Bud dormancy commences in mid-fall (October–November) and the development of cold hardiness in all seedling tissues follows in early winter (December–January) (Burr 1990, Haase 2011). Note that the physiological processes of dormancy and cold hardiness are complex and not well understood for southern pine species (Johnson and Cline 1991).

Dormancy

Physiologically, bud dormancy can arise whenever stressful environmental conditions occur, even during the phenological phase of active growth. For example, drought, temperature extremes, or nutrition limitations can cause bud dormancy (Ritchie and Dunlap 1980). Bud dormancy during the active growth stage is, however, reversible with the removal of the environmental stress (Ritchie and Landis 2004). Typically, active growth of conifer seedlings slows in summer and bud formation occurs with the initiation of quiescence (ectodormancy) (Burr 1990). For northern provenances, photoperiod also plays an important role (Cooke et al. 2012, Haase 2011). If favorable conditions occur for seedling growth, ectodormancy is reversible. Beginning late fall (October/ November), true internal dormancy (endodormancy) starts and continues into December. During endodormancy, seedlings will not resume growth even when favorable conditions for growth are present. Growth resumes after seedlings experience a certain period of low temperatures (chilling requirement). Once the chilling requirement is met, the seedling re-enters ectodormancy and will resume growth if favorable conditions (primarily warmer temperatures) are present (Ritchie and Landis 2004).

For southern pine species, little is known about the dormancy cycle and its relation to photoperiod and temperature. Both nutrition and water availability can influence bud development timing (Cooke et al. 2012, Harrington and Gould 2015, Larsen et al. 1986, Ritchie and Dunlap 1980). Decreased photoperiod was found to partially substitute for chilling temperatures (Cooke et al. 2012). While southern pine species have chilling requirements, the quantity is still unclear when compared with that of northern pine species (Cooke et al. 2012, Hallgren and Tauer 1989, Johnson and Cline 1991, Kolb et al. 1985, Larsen et al. 1986). Exposure to low (but above-freezing) temperatures has been observed to enhance bud break and root growth potential (RGP) (Cooke et al. 2012, Ritchie and Dunlap 1980).

Cold Hardiness

Cold hardiness develops with physiological changes throughout all seedling tissues following the suspension of rapid cell expansion. For southern pine species, cold temperature acclimation in response to decreasing air temperatures is referred to as hardening initiating. Thus, cold hardiness initiates after bud dormancy initiation (Johnson and Cline 1991). Although the two are often referred to synonymously, they are completely separate processes (Haase 2011). For southern pines species, tissues within the seedling acclimate differently (Hallgren and Tauer 1989, Kolb et al. 1985). Once a sufficient amount of chilling hours are met (maximum usually achieved in winter), we assume there is a corresponding increase in seedling freeze tolerance (Burr 1990, Grossnickle and South 2017, Haase 2011). The level of cold hardiness for a species is genotype-specific (Grossnickle and South 2014). In contrast to bud endodormancy, cold hardiness can decrease or “be lost” if seedlings are exposed to increasing temperatures. Seedlings have been shown to de-acclimate with as little as 3 to 7 warm nights (South et al. 2008, 2009).

Chilling Hours and Seedling Storage

Seedling chilling hours are quantified based on the cumulative number of hours of exposure to a specified range of cold temperatures. The accepted temperature range to define a chilling hour is often species- and nursery-dependent (Burdett and Simpson 1984, Carlson 1991, Johnson and Cline 1991). In the Southeastern United States, chilling hours are usually quantified in the range of 32 to 46 °F (0 to 8 °C), and temperatures below 32 °F do not count (Grossnickle and South 2014, South 2012). Using this preferred method of chilling hour calculation, nurseries target 200 to 400 chilling hours for loblolly pine (*Pinus taeda* L.) to overcome rest (internal dormancy) depen ding on geographic origin (Johnson and Cline 1991, Ritchie and Dunlap 1980). For the Southeastern States, this chilling hour target is usually met in early to mid-December, after which point seedlings can be lifted for long- or short-term storage.

Successful long-term storage (1 to 3 months) of seedlings requires that they be able to tolerate extended periods in cool, dark conditions while maintaining physiological quality (Grossnickle and South 2014). Short-term storage (1 to 3 weeks) is often used for keeping a supply of seedlings available in the cooler but does not necessarily require chilling hours (Grossnickle and South 2014). Studies have shown that container loblolly pine seedlings could be stored successfully for a month without exposure to any chilling hours prior to storage (Grossnickle and South 2014, Larsen et al. 1986, Boyer and South 1985). In two studies, bareroot seedlings exposed to 113 or 223 chilling hours tolerated 4 weeks or 11 weeks of storage, respectively (South 2013, South and Donald 2002). Inadequate number of chilling hours has occasionally been used to explain low outplanting survival following a hard freeze (Larsen et al. 1986). Cold storage can partially satisfy chilling requirements for several northern species, but this is unlikely to occur for southern pine species due to their likely short chilling requirements (Harrington and Gould 2015, Ritchie and Dunlap 1980).

Although chilling hours is known to be beneficial to pine seedlings, the impact of chilling hours on seedling storability and their subsequent growth is poorly understood, as evidenced by several popularly held myths (South 2012). Thus, this area of seedling quality needs further research. The objective of our 3-year study was to better understand the relationships between chilling hours and seedling storability, as well as seedling growth and survival after outplanting.

Materials and Methods

Seedlings, Chilling Hours, and Outplanting

For this study, a single seedlot (genotype) of slash pine (*Pinus elliottii* Engelm.) was used over three seedling production and lifting seasons (2016–2017; 2017–2018; and 2018–2019). Seedlings were grown and lifted from a commercial bareroot forest tree nursery in Georgia using standard operational procedures (figure 1a). Seedlings were lifted and stored (figure 1b and c) at varying intervals (see description in subsequent sections). Chilling hours were calculated using the Utah chill-hour model from 1 November until each lifting date (table 1). The Utah chill hour model is a weighted function assigning different chilling efficiencies to different temperature ranges, including negative contributions for higher temperatures (Anderson and Seeley 1992).

Figure 1. Slash seedlings were (a) grown under operational conditions in a nursery bed. After lifting on a range of dates, seedlings were stored for varying durations in (b) boxes within (c) a cooler. (Photos by Ryan Nadel, 2017)

Table 1. Chilling hours calculated for each seedling-lifting period over three lifting seasons.

	Number of chilling hours at the time of lifting					
Year	**Time_0**	**Time_1**	**Time_2**	**Time_3**	**Time_4**	**Time_5**
2017	-302	-426	-428	-637	-820	-1016
2018	97	142	121	-103	-91	-152
2019	118	135	-6	-67	-142	-262

Following storage, seedlings were planted at the Auburn University trophotron (deep plastic-lined beds filled with sand) (figure 2), where they grew without supplemental water, weed control, or fertilization for 12 months before being harvested and measured.

Lift Dates and Storage Durations

For each lifting season, 1,000 seedlings (equivalent to a full box of seedlings) were hand lifted from the bareroot nursery bed at the start of the study period (January) (figure 1). Every 2 weeks thereafter, for a total of 6 lift dates ending in late April/early May each year (Time_0, Time_1, Time_2, Time_3, Time_4, Time_5).

At each lift date, 15 seedlings were randomly selected for measurement and outplanting (storage duration = 0); the rest were placed into cooler storage (33 to 37 °F [0.6 to 2.8 °C]) in standard shipping boxes. Over a 14-week period, 15 seedlings from each lift date were randomly removed from the cooler, measured, and outplanted for a maximum of eight storage durations (table 2). A total

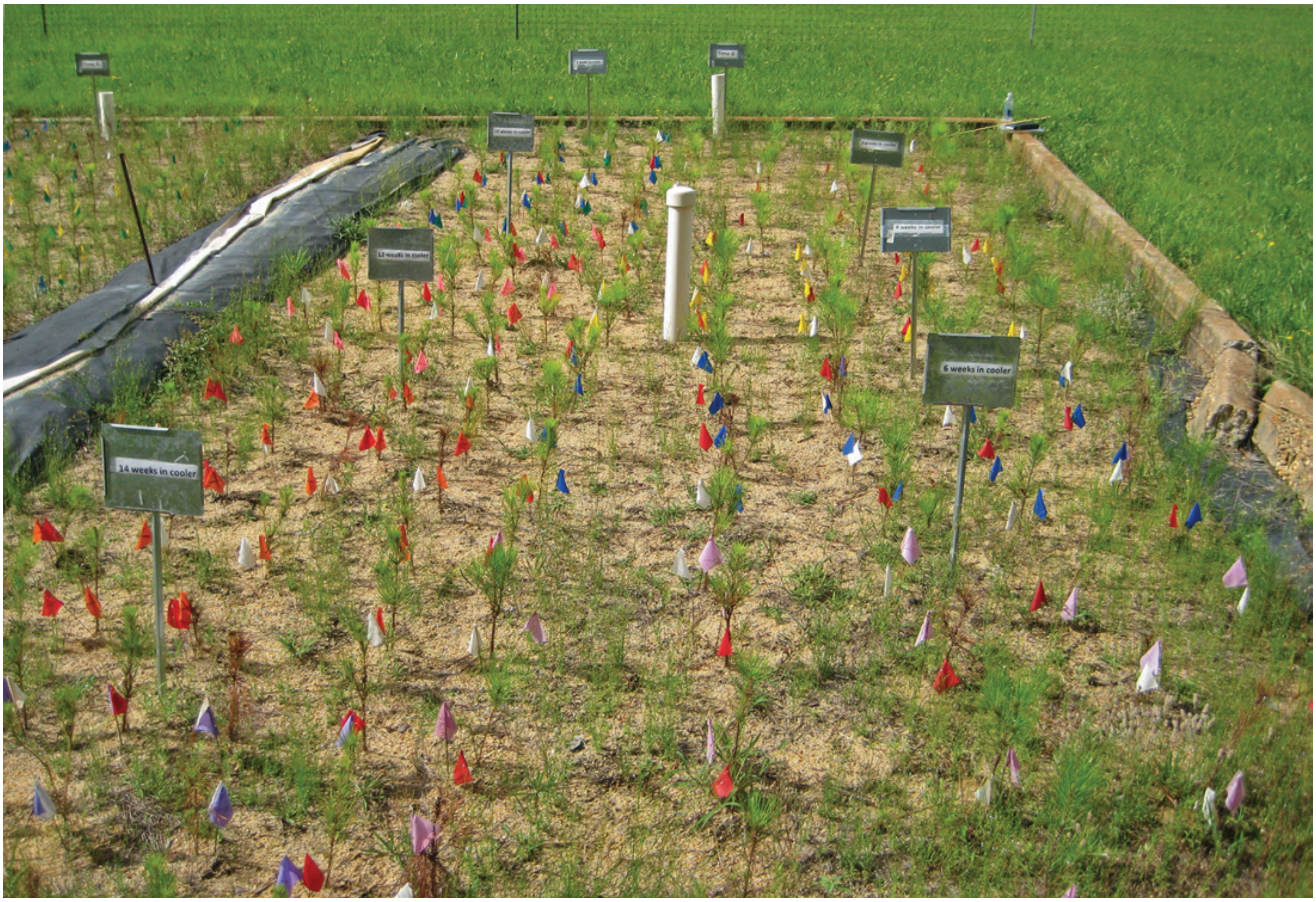


Figure 2. Seedlings from varying lift dates and storage durations were outplanted in the trophotron at Auburn University to evaluate first season shoot and root development. (Photo by Ryan Nadel, 2017)

Table 2. Sample groups of seedlings (indicated by X) each year for the different combinations of lift date and storage duration.

Storage duration (weeks)	2017						2018						2019			
	Lift date						Lift date						Lift date			
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3
0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
6	X	X	X	X	X		X	X	X	X			X	X	X	X
8	X	X	X	X			X	X	X				X	X	X	
10	X	X	X				X	X					X	X		
12	X	X					X						X			
14	X															

of 1,230 seedlings (820 outplanted) over the study's three seasons represented a total of 82 lift date/storage duration sample groups (table 2).

Measurements

Root growth potential (RGP) was determined on five seedlings for all Time_0/storage duration combinations and for all lift date/storage duration = 0 combinations. RGP is a measure of a seedling's ability to rapidly produce new root growth (Ritchie and Dunlap 1980). To measure RGP, seedlings were placed in aquarium tanks filled with continuously aerated water. After 30 days, the number of new white root tips greater than 0.2 inches (0.5 cm) for each seedling was recorded.

Root collar diameter (RCD) and height (Ht) were measured on all 15 seedlings for each sample group. Additionally, the 10 outplanted seedlings for each sample group were re-measured for RCD and Ht as well as percent survival in June and December. At the conclusion of the study each year (December 2017, 2018, and 2019), seedlings were harvested. Shoots and roots of harvested seedlings were separated and pooled by sample group, then placed in a drying oven until constant mass was achieved. Mean shoot and root mass were calculated from the total mass of pooled seedlings divided by the number of living seedlings. The ratio of shoot-to-root was calculated by simple division. In addition, root weight ratios (RWR) were calculated as follows:

$$RWR\,(\%) = \frac{dry\ root\ weight}{(dry\ root\ weight\ +\ dry\ shoot\ weight)} \times 100$$

Experimental Design and Data Analyses

The study design was an incomplete factorial (lift date by storage duration) with limited replication. It was not possible to apply valid statistical analyses without a replicated, full factorial design. We elected not to include all combinations because later lift dates with longer storage durations are too far beyond operational procedures and the seedling phenological cycle. Nonetheless, the data generated from this study represent a logistical feat for demonstrating seedling responses to a wide range of lift dates and storage durations. Despite the inability to apply statistics to the data, we calculated the means for each sample groups and noted several trends, discussed below.

Results

Root Growth Potential and Morphology at the Time of Outplanting

The relationship between seedling RGP and storage duration (lift date = Time_0) varied by season with a decreasing, neutral, and increasing relationship with increasing storage duration for the 2017, 2018, and 2019 seasons, respectively (figure 3a). Similarly, RGP response to lift date (storage = 0 weeks) varied by season with decreasing RGP at later lift dates in 2017 but the opposite in 2018 and 2019 (figure 3b).

RCD tended to increase with later lift dates but was not affected by storage duration (table 3). Seedling height did not follow any consistent pattern with regard to lift date or storage duration (table 3).

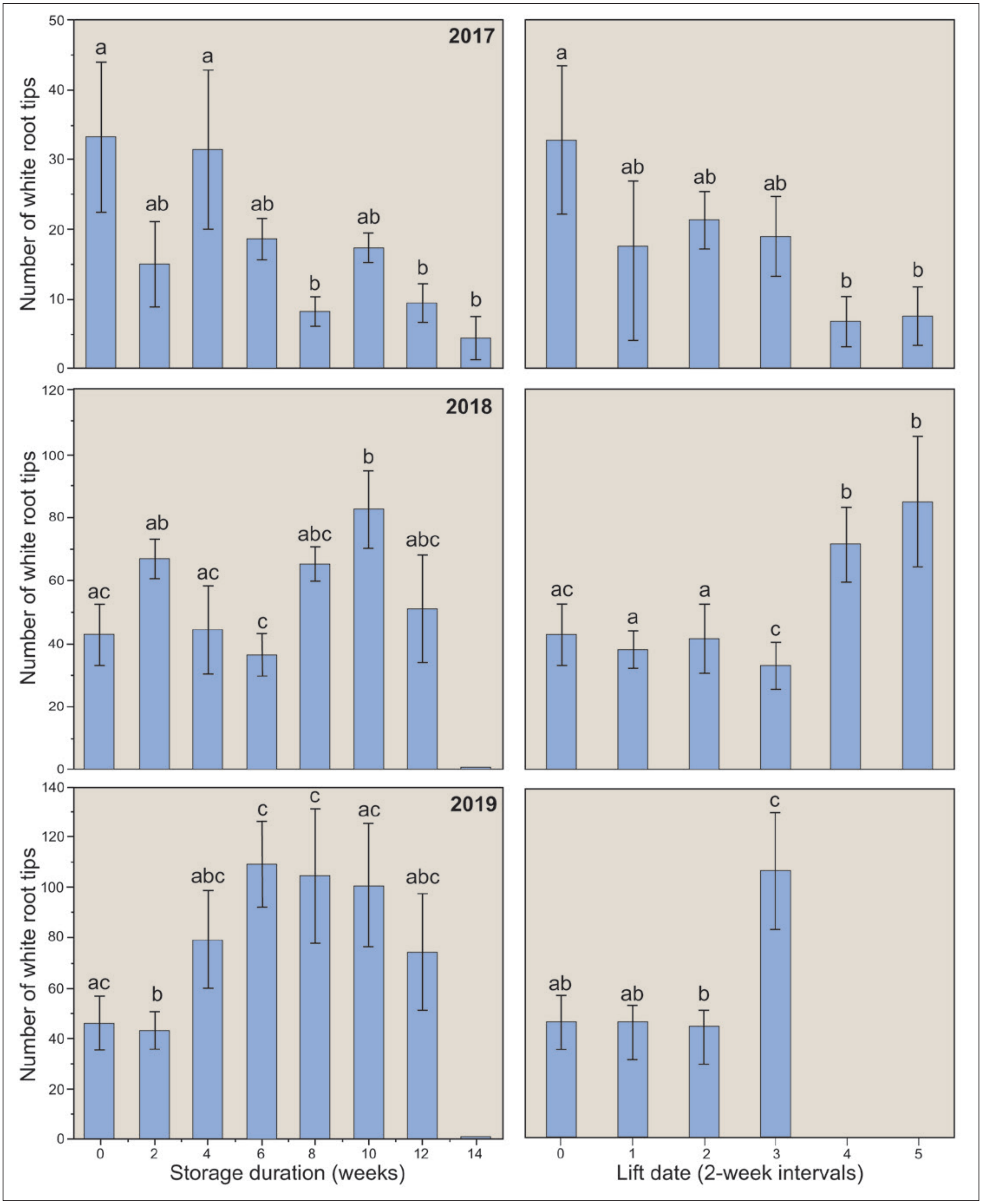


Figure 3. Mean root growth potential (number of white root tips) of tree seedlings based on (a) storage duration or (b) lift date over three seasons. Within each graph, bars with different letters are significantly different ($\alpha < 0.05$).

Table 3. Morphology at the time of outplanting for seedlings with varying combinations of lift date and storage duration.

Storage duration (weeks)	Initial RCD (mm)						Initial Height (cm)					
	Year 2017											
	Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5
0	4.42	4.36	5.20	5.22	5.59	5.63	33.5	32.3	33.4	30.8	35.3	34.2
2	4.70	5.54	5.66	6.46	4.82	5.51	32.6	32.0	31.4	34.6	32.9	33.6
4	5.69	4.92	5.31	5.19	5.76	4.69	32.5	31.0	30.5	32.2	34.9	32.6
6	5.49	5.73	6.43	6.43	7.04	.	32.3	30.8	33.1	31.0	34.3	.
8	4.79	6.21	6.13	5.47	.	.	30.6	32.3	31.8	31.6	.	.
10	6.10	5.58	4.81	.	.	.	33.9	33.1	30.7	.	.	.
12	5.29	5.15	.	.	.	.	31.5	32.9	.	.	.	.
14	5.01	.	.	.	.	.	31.5	.	.	.	.	.

Storage duration (weeks)	Year 2018											
	Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5
0	5.13	6.41	5.75	6.05	5.82	5.78	26.3	28.6	26.6	28.9	28.9	28.6
2	5.88	5.43	5.78	5.61	6.08	6.01	27.2	27.8	29.3	27.6	27.7	30.3
4	5.16	5.37	6.10	6.14	6.13	.	25.9	30.3	26.4	29.3	26.7	.
6	5.22	6.02	6.00	6.18	.	.	27.9	26.1	26.9	27.3	.	.
8	5.56	5.35	5.74	.	.	.	25.5	26.9	25.0	.	.	.
10	5.58	5.77	.	.	.	.	27.2	27.9	.	.	.	.
12	5.59		.	.	.	.	25.7	.	.	.	.	.

Storage duration (weeks)	Year 2019											
	Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5
0	5.52	5.43	6.13	6.00	.	.	30.2	30.3	33.9	34.2	.	.
2	5.68	5.91	6.06	6.41	.	.	27.2	31.7	32.5	34.1	.	.
4	5.98	6.43	6.08	6.23	.	.	29.9	30.7	31.4	35.9	.	.
6	5.31	5.74	5.78	6.05	.	.	28.5	29.9	32.9	34.7	.	.
8	5.00	6.12	5.98	.	.	.	28.0	29.9	33.2	.	.	.
10	5.17	5.95	.	.	.	.	28.0	29.6	.	.	.	.
12	6.16	.	.	.	.	.	28.5	.	.	.	.	.

RCD = Root collar diameter.

Morphology and Survival After One Growing Season

Shoot and root mass tended to decrease with increasing storage duration for each lifting date, especially in 2017 and 2019 (table 4). Shoot:root was highest (greater than 3) during the 2017 season and lowest (less than 2) during the 2018 season, but did not vary notably due to lift date or storage duration (table 4). RWR at the end of one growing season tended to increase with increasing storage duration (table 4).

Both RCD and height growth at the end of each growing season tended to decrease with later lift dates and with longer storage durations (table 5). Seedling survival varied by season but tended to decrease with increasing storage duration, especially for later lift dates (table 5).

Discussion

Root Growth Potential and Morphology at the Time of Outplanting

The relationship between RGP and increasing storage duration differed among years (figure 3a). Studies on other southern pine species have also shown annual variations in RGP in response to storage and attributed these variations to lifting date, storage temperature, and storage duration (Deligöz 2013, Grossnickle and South 2014, Haase et al. 2016, Hallgren and Tauer 1989, Ritchie and Dunlap 1980). Root growth potential is linked to the bud dormancy cycle and peaks in mid- to late winter, just before bud break when seedling shoots are ectodormant (Carlson 1991, Deligöz 2013, Ritchie and Dunlap 1980). With increasing temperatures, seedling RGP

Table 4. Morphology after one growing season for outplanted seedlings following different lift dates and storage durations.

Storage duration (weeks)	Shoot Mass (g)						Root Mass (g)						shoot: root						Root weight ratio					
	Year 2017																							
	Lift date						Lift date						Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
0	36.32	26.31	.	36.92	10.77	9.66	8.83	8.15	.	10.27	2.38	3.07	4.1	3.2	.	3.6	4.5	3.1	19.6	23.7	.	21.8	18.1	24.1
2	26.42	22.65	22.72	28.35	10.59	4.65	8.34	7.63	7.45	9.37	2.32	0.36	3.2	3.0	3.0	3.0	4.6	12.9	24.0	25.2	24.7	24.8	18.0	7.2
4	15.48	20.12	17.66	19.05	10.65	5.07	4.64	7.34	5.60	6.49	2.42	1.01	3.3	2.7	3.2	2.9	4.4	5.0	23.1	26.7	24.1	25.4	18.5	16.6
6	13.85	19.82	30.01	21.02	12.52	.	5.31	6.70	12.64	6.40	3.36	.	2.6	3.0	2.4	3.3	3.7	.	27.7	25.3	29.6	23.3	21.2	.
8	12.45	32.33	13.14	13.85	.	.	3.24	12.17	5.31	3.23	.	.	3.8	2.7	2.5	4.3	.	.	20.7	27.4	28.8	18.9	.	.
10	16.98	19.34	7.37	.	.	.	4.01	6.91	2.62	.	.	.	4.2	2.8	2.8	.	.	.	19.1	26.3	26.2	.	.	.
12	8.70	12.95	.	.	.	.	1.69	6.10	.	.	.	.	5.1	2.1	.	.	.	.	16.3	32.0	.	.	.	.
14	7.93	.	.	.	.	.	2.26	.	.	.	.	.	3.5	.	.	.	.	.	22.2	.	.	.	.	.

Storage duration (weeks)	Year 2018																							
	Lift date						Lift date						Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
0	6.35	10.80	9.54	3.92	5.85	6.86	3.63	4.72	5.06	1.92	2.89	2.85	1.7	2.3	1.9	2.0	2.0	2.4	36.37	30.41	34.66	32.85	33.07	29.35
2	6.47	13.98	9.22	6.25	5.20	7.96	4.12	7.68	5.67	3.46	2.32	3.22	1.6	1.8	1.6	1.8	2.2	2.5	38.90	35.46	38.08	35.63	30.85	28.81
4	8.50	9.43	10.49	7.13	4.83	.	4.88	5.18	6.45	4.27	1.87	.	1.7	1.8	1.6	1.7	2.6	.	36.47	35.46	38.08	37.46	27.91	.
6	4.61	9.53	7.50	5.52	.	.	3.09	5.51	5.31	2.95	.	.	1.5	1.7	1.4	1.9	.	.	40.13	36.64	41.45	34.83	.	.
8	7.01	5.08	8.00	.	.	.	3.82	4.71	4.19	.	.	.	1.8	1.1	1.9	.	.	.	35.27	48.11	34.37	.	.	.
10	5.36	7.66	.	.	.	.	3.25	3.86	.	.	.	.	1.6	2.0	.	.	.	.	37.75	33.51	.	.	.	.
12	5.79	.	.	.	.	.	4.96	.	.	.	.	.	1.2	.	.	.	.	.	46.14	.	.	.	.	.

Storage duration (weeks)	Year 2019																							
	Lift date						Lift date						Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
0	7.51	7.83	6.99	5.94	.	.	2.35	2.36	1.87	1.86	.	.	3.2	3.3	3.7	3.2	.	.	23.8	23.2	21.1	23.8	.	.
2	3.65	6.67	5.01	5.33	.	.	1.31	1.52	1.65	1.57	.	.	2.8	4.4	3.0	3.4	.	.	26.4	18.6	24.8	22.8	.	.
4	4.84	7.41	3.79	3.22	.	.	1.56	1.92	0.64	1.02	.	.	3.1	3.9	5.9	3.2	.	.	24.4	20.6	14.4	24.1	.	.
6	4.35	3.69	2.31	.	.	.	1.27	1.11	0.9	.	.	.	3.4	3.3	2.6	.	.	.	22.6	23.1	28.0	.	.	.
8	2.08	2.93	1.82	.	.	.	0.93	0.85	0.91	.	.	.	2.2	3.4	2.0	.	.	.	30.9	22.5	33.3	.	.	.
10	3.08	7.34	.	.	.	.	1.02	1.98	.	.	.	.	3.0	3.7	.	.	.	.	24.9	21.2	.	.	.	.
12	2.7	.	.	.	.	.	1.22	.	.	.	.	.	2.2	.	.	.	.	.	31.1	.	.	.	.	.

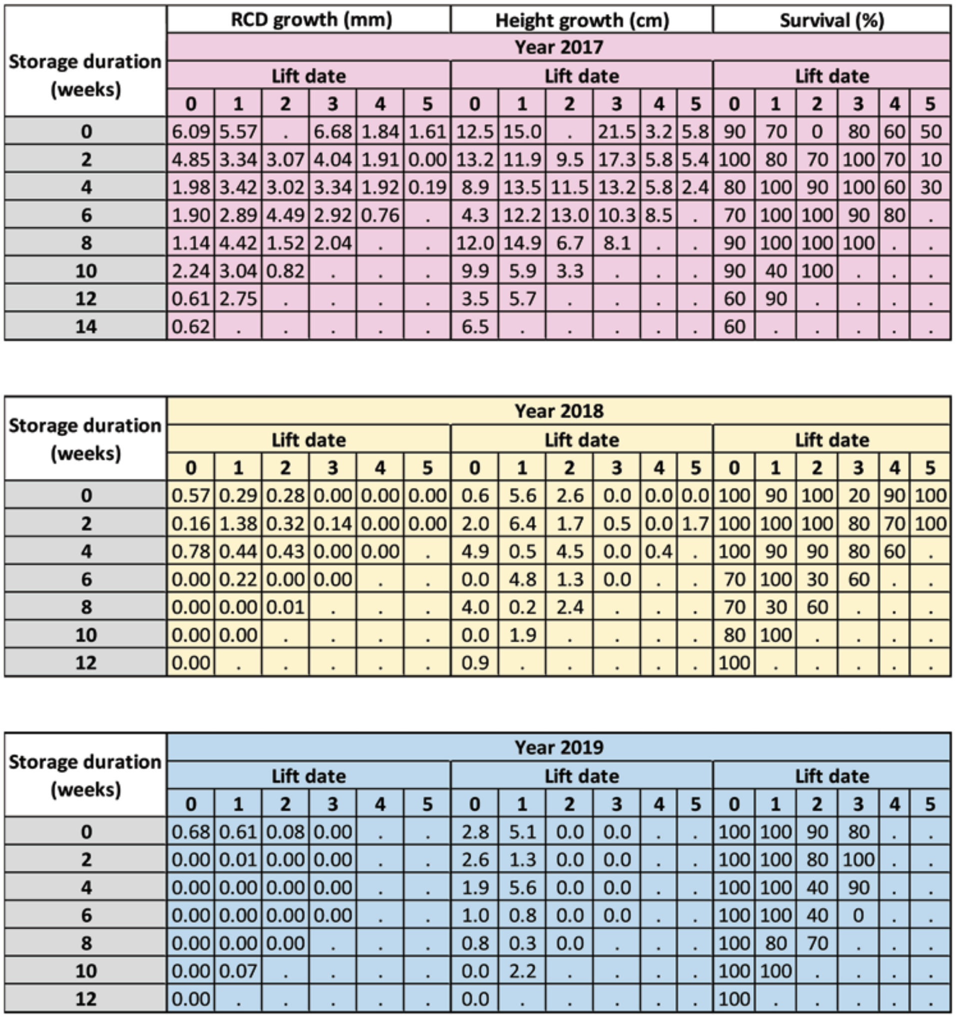
usually decreases as competition for resources (water and nutrients) transitions from root growth to bud elongation (Deligöz 2013, Ritchie and Dunlap 1980). Studies with loblolly pine found RGP increased with increasing chilling hours, and seedlings with a high proportion of ectodormant buds at planting had higher RGP (Carlson 1991, Larsen et al. 1986, Ritchie and Dunlap 1980). This relationship was also observed in our study with nearly twice the RGP values occurring in 2019 and 2018 as compared with those for 2017, when chilling hours were very low (table 1; figure 3). Our 2018 and 2019 data also show that RGP increased with later lift dates and longer storage times, likely due to a further increase in chilling hours (figure 3).

As would be expected, initial RCD tended to increase with later lift dates because root growth and RCD growth continue after budset as long as soils are warm enough. Height, on the other hand, did not show a pattern with regard to lift date (table 3). Any height variations can be attributed to normal sampling error, since seedlings had already set bud by the onset of the study each season.

Morphology and Survival After One Growing Season

After outplanting, the most notable differences were among seasons. Those planted in 2017 received no chilling at the time of lifting (table 1) and shoots were already starting to grow inside the storage boxes. This early growth is evident in the higher initial values for height (table 3) and likely resulted in a growth advantage after planting as evidenced by the higher values for shoot and root biomass (table 4) and for RCD and

Table 5. Growth (RCD and Ht) and survival after one growing season for outplanted seedlings following different storage and lifting periods.



Storage duration (weeks)	RCD growth (mm)						Height growth (cm)						Survival (%)					
	Year 2017																	
	Lift date						Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
0	6.09	5.57	.	6.68	1.84	1.61	12.5	15.0	.	21.5	3.2	5.8	90	70	0	80	60	50
2	4.85	3.34	3.07	4.04	1.91	0.00	13.2	11.9	9.5	17.3	5.8	5.4	100	80	70	100	70	10
4	1.98	3.42	3.02	3.34	1.92	0.19	8.9	13.5	11.5	13.2	5.8	2.4	80	100	90	100	60	30
6	1.90	2.89	4.49	2.92	0.76	.	4.3	12.2	13.0	10.3	8.5	.	70	100	100	90	80	.
8	1.14	4.42	1.52	2.04	.	.	12.0	14.9	6.7	8.1	.	.	90	100	100	100	.	.
10	2.24	3.04	0.82	.	.	.	9.9	5.9	3.3	.	.	.	90	40	100	.	.	.
12	0.61	2.75	.	.	.	.	3.5	5.7	.	.	.	.	60	90	.	.	.	.
14	0.62	.	.	.	.	.	6.5	.	.	.	.	.	60	.	.	.	.	.

Storage duration (weeks)	Year 2018																	
	Lift date						Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
0	0.57	0.29	0.28	0.00	0.00	0.00	0.6	5.6	2.6	0.0	0.0	0.0	100	90	100	20	90	100
2	0.16	1.38	0.32	0.14	0.00	0.00	2.0	6.4	1.7	0.5	0.0	1.7	100	100	100	80	70	100
4	0.78	0.44	0.43	0.00	0.00	.	4.9	0.5	4.5	0.0	0.4	.	100	90	90	80	60	.
6	0.00	0.22	0.00	0.00	.	.	0.0	4.8	1.3	0.0	.	.	70	100	30	60	.	.
8	0.00	0.00	0.01	.	.	.	4.0	0.2	2.4	.	.	.	70	30	60	.	.	.
10	0.00	0.00	.	.	.	.	0.0	1.9	.	.	.	.	80	100	.	.	.	.
12	0.00	.	.	.	.	.	0.9	.	.	.	.	.	100	.	.	.	.	.

Storage duration (weeks)	Year 2019																	
	Lift date						Lift date						Lift date					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
0	0.68	0.61	0.08	0.00	.	.	2.8	5.1	0.0	0.0	.	.	100	100	90	80	.	.
2	0.00	0.01	0.00	0.00	.	.	2.6	1.3	0.0	0.0	.	.	100	100	80	100	.	.
4	0.00	0.00	0.00	0.00	.	.	1.9	5.6	0.0	0.0	.	.	100	100	40	90	.	.
6	0.00	0.00	0.00	0.00	.	.	1.0	0.8	0.0	0.0	.	.	100	100	40	0	.	.
8	0.00	0.00	0.00	.	.	.	0.8	0.3	0.0	.	.	.	100	80	70	.	.	.
10	0.00	0.07	.	.	.	.	0.0	2.2	.	.	.	.	100	100	.	.	.	.
12	0.00	.	.	.	.	.	0.0	.	.	.	.	.	100	.	.	.	.	.

RCD = Root Collar Diameter

height growth (table 5) at the end of the 2017 growing season compared with the other two seasons, in spite of relatively dry and warm growing conditions in 2017. On the other hand, seedlings planted in 2018 and 2019 were exposed to chilling hours and were likely still ectodormant at the time of outplanting. In those seasons, seedlings lifted in Time$_0$, Time$_1$, and Time$_2$ (2018 only) had approximately 100 or more chilling hours at the time of lift, while those lifted at later dates had negative chilling hours as temperatures increased (table 1). Interestingly, 2018 and 2019 seedlings lifted during those later dates with negative chilling had little or no growth after outplanting. They broke bud but did not elongate. Because we used the Utah chill hour model to calculate chilling (Anderson and Seeley 1992), we were able to show this interactive effect of warming before lifting and subsequent field performance. Similarly, Haase et al. (2016) demonstrated differences in chilling hour accumulation using two calculation methods, though that study did not have notable negative values. For regions prone to warm temperatures after bud set, a weighted chilling model can provide vital information.

Within each growing season, seedling morphology and survival tended to decrease with increasing storage duration and later lift dates (tables 4 and 5). Studies have shown a significant effect of lift date on subsequent root and shoot growth (Deligöz 2013, Haase et al. 2016). Furthermore, as cold storage duration increases, a corresponding reduction in growth after outplanting can occur (Deligöz 2013, South and Donald 2002). For other conifer species, reduction in growth was related to total seedling carbohydrate content at planting affecting early root growth (Deligöz 2013). Roots and shoots compete within the plant for carbohydrates. Lifting into late winter or early spring usually means seedlings have been exposed to higher temperatures, which stimulate bud elongation and reduce root growth (Ritchie and Dunlap 1980). The ability of seedlings to grow roots shortly after planting is positively correlated to improved seeding survival after outplanting (Grossnickle 2005, Mena-Petite et al. 2001). Our results reflect these phenological cycles, with those from earlier lift dates and shorter storage durations likely having the advantage to grow roots and establish on the outplanting site before bud break.

Shoot:root is commonly used as an indicator of drought avoidance potential (Grossnickle 2012). The ratio indicates the balance between transpiring shoot tissues and moisture-absorbing root tissues. Loblolly pine seedling survival after outplanting is negatively correlated with increasing shoot:root (Larsen et al. 1986). Sufficient root growth is important for seedling survival and successful establishment (Carlson 1986, Grossnickle and South 2017, Larsen et al. 1986, Mena-Petite et al. 2001). Greater root mass indicates there is a greater absorptive surface for water and nutrient uptake (Grossnickle 2012, Mena-Petite et al. 2001). Thus, seedlings with shoot:root greater than 3 have lower survival potential compared with seedlings that have shoot:root between 1 and 3 (Grossnickle 2012). Similarly, lower RWR (which follows shoot:root patterns) will have lower survival potential. In our study, shoot:root in 2017 was greater than 3 for nearly every lift date/storage duration group, which is likely linked to the low initial RGP coupled with immediate height growth following planting during that season. In turn, the high shoot:root likely contributed to the lower survival of those lifted later in the 2017 growing season (table 4).

Our study shows potential effects of lift date and storage duration on subsequent seedling quality and performance. These effects, however, were strongly influenced by seasonal variations in chilling hour accumulation (and de-accumulation). This variation demonstrates the importance of multi-year assessments. Conclusions based on just 1 year would not have captured the range of seasonal variability. The interactive effect of seasonal weather patterns and lift date makes it challenging to offer management recommendations, though it is clear that late lift dates and long storage durations can reduce field growth and survival after outplanting.

Address correspondence to—

Ryan L. Nadel, Southern Forest Nursery Management Cooperative, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849; email: ryan.nadel@auburn.edu; phone: 334-844-8069.

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Southern Pine Beetle: Damage and Consequences in Forests of the Mid-Atlantic Region, USA

Kenneth L. Clark, Michael Gallagher, Carissa Aoki, and Matthew Ayres

Research Forester, U.S. Department of Agriculture (USDA) Forest Service, Northern Research Station (NRS), Silas Little Experimental Forest, New Lisbon, NJ; Research Ecologist, USDA Forest Service, NRS, Silas Little Experimental Forest, New Lisbon, NJ; Lecturer in Environmental Studies, Bates College, Lewiston, ME; Professor, Department of Biological Sciences, Dartmouth College, Hanover, NH.

Abstract

Coastal pitch pine (*Pinus rigida* Mill.) forests on the Mid-Atlantic Coastal Plain are threatened by the northerly migration of southern pine beetle (*Dendroctonus frontalis* Zimmerman). We quantified effects of southern pine beetle infestations and suppression treatments on composition and structure of pine-dominated forests in the Pinelands National Reserve of New Jersey. We then used a synthesis of forest census measurements, carbon (C) flux measurements, and simulations to evaluate potential effects on C sequestration. Pine tree mortality was extensive in infested areas, resulting in 94-percent reduction in basal area and 96-percent reduction in aboveground biomass, though pine seedlings and saplings were mostly unaffected in untreated infested areas. Beetle suppression treatments (cut and leave or cut and chip) further reduced pine sapling basal area whereas hardwoods were largely unaffected. Estimated leaf area recovered to 50 percent of pre-infestation levels 3 to 5 years following infestations, and estimated annual gross ecosystem production averaged 67 percent of values in uninfested areas. Estimated net ecosystem productivity, a measure of C sequestration, was lowest for cut and leave treatments and highest for cut and chip treatments where the majority of chips were hauled offsite for commercial use. Managing for pine-oak mixedwood stands can increase resistance to future outbreaks of bark beetles and other defoliators. This paper was presented at the 2019 Joint Annual Meeting of the Northeast and Southern Forest Conservation Nursery Associations (Atlantic City, NJ, July 23–25, 2019).

Introduction

Throughout the Northeast and Mid-Atlantic regions of the United States, intermediate-age forests with median tree ages of approximately 70 to 110 years have regenerated following farm abandonment, the cessation of intensive forestry practices such as clearcutting and charcoal production, and severe wildfires (Duveneck et al. 2017, Pan et al. 2011, Stambaugh et al. 2018).

On the Atlantic Coastal Plain, continued, but less extensive, wildfire activity through the 20th century, followed by active fire management with frequent prescribed burning, has limited the regeneration of oaks (*Quercus* spp.) and other hardwoods, and favored the persistence of forests dominated by pitch pine (*Pinus rigida* Mill.) and shortleaf pine (*P. echinata* Mill.) (Forman and Boerner 1981, La Puma et al. 2013, Little 1979). These globally rare pine ecosystems encompass high plant species diversity, but are threatened by land-use change and development. In addition, increasing fire suppression limits pine regeneration and recruitment and allows encroachment of oaks and other hardwoods (Gallagher 2017, La Puma et al. 2013). Preserved areas include the Pinelands National Reserve of New Jersey, the Central Pine Barrens of Long Island, NY, and small areas in coastal New England.

In addition to their unique characteristics and high biodiversity, pine-dominated forests in the Mid-Atlantic region play important roles in providing ecosystem services. These forests are as productive as other major forest types in the Mid-Atlantic region and sequester equivalent amounts of atmospheric carbon dioxide (CO_2) on an annual basis (table 1). Net primary productivity estimated for pine-dominated, oak-hickory, and mixed oak-pine stands from the U.S. Department of Agriculture, Forest Service, Forest Inventory and Analysis (FIA) data are consistent with simulations using PnET CN, a

Table 1. Productivity of pine-dominated, oak-dominated, and mixed pine-oak forests in the Mid-Atlantic region. Data are net primary production and net ecosystem production from USDA Forest Service Forest Inventory and Analysis data, simulation results using PnET CN, and carbon (C) flux measurements in the Pinelands National Reserve.

Site	Pine-dominated T C ha^{-1} yr^{-1}	Oak-dominated T C ha^{-1} yr^{-1}	Mixed pine-oak T C ha^{-1} yr^{-1}
Net primary productivity (NPP)			
FIA data[1]	4.2 ± 0.5	4.6 ± 0.5	3.8 ± 0.6
PnET CN[2]	4.3 ± 0.3	5.0 ± 0.5	3.6 ± 0.4
Net ecosystem productivity (NEP)			
FIA data[1]	1.0 to 1.6	1.7 to 2.1	1.2 to 1.7
Flux data[3]	1.8 ± 0.3	1.8 ± 0.3	1.4

T C ha^{-1} yr^{-1} = tons of carbon per hectare per year.

[1] Forest Inventory and Analysis, [2] Pan et al. 2009, 2011, [3] Clark et al. 2010, 2018.

process-based forest productivity model (Pan et al. 2006, 2009, 2011). Estimated net ecosystem production (NEP), a measure of carbon (C) sequestration, by pine-dominated, oak-dominated, and mixed pine-oak forests across the region derived from FIA data are consistent with annual NEP values calculated from eddy covariance measurements of net exchange of CO_2 (NEE) during undisturbed years in intermediate age forests of the Pinelands National Reserve of New Jersey (table 1; Clark et al. 2010, 2018).

Disturbance regimes in intermediate-age forests throughout the Northeast and Mid-Atlantic regions are now dominated by infestations of native and non-native insects, which account for a large proportion of tree damage and mortality, while windstorms, harvest activities, and managed wildland fire have become secondary (Fei et al. 2019, Kautz et al. 2017, Kosiba et al. 2018, Lovett et al. 2016, Pasquarella et al. 2018). On the Mid-Atlantic Coastal Plain, outbreaks of gypsy moth (*Lymantria dispar* L.) have resulted in oak and other hardwood mortality in oak-dominated stands, and southern pine beetle (*Dendroctonus frontalis* Zimmerman) infestations have resulted in pine (*Pinus* spp.) mortality in pine-dominated stands over the last decade. These outbreaks have been the dominant insect-driven disturbances and have far exceeded the area impacted by wildfires or windstorms (Gallagher 2017, Heuss et al. 2019, Weed et al. 2013). Pine tree mortality caused by southern pine beetle infestations can be extensive in infested stands throughout the Southeastern United States without aggressive suppression activities (Dodds et al. 2018, Guldin 2011). Without suppression activities, infestations have resulted in increased populations of oaks and other hardwoods, thereby accelerating successional changes. Although FIA data has captured the long-term impacts of other invasive insects on host species (e.g., gypsy moth and red oak decline; Morin and Leibhold 2015, Fei et al. 2019), until recently, little information existed for changes in composition and structure in pitch and shortleaf pine stands infested by southern pine beetle (Aoki et al. 2018, Clark et al. 2017, Heuss et al. 2019; reviewed in Dodds et al. 2018).

Short-term impacts of insect infestations on ecosystem functioning in Mid-Atlantic forests are well-characterized (e.g., Clark et al. 2010, 2014, 2018; Deel et al. 2012; Renninger et al. 2014), and a number of simulation models have captured the overall dynamics of C and hydrologic cycling associated with these disturbances (Kretchun et al. 2014, Medvigy et al. 2012, Xu et al. 2017). In summary, infestations of bark beetles and defoliators initially reduce leaf area of impacted stands, causing an immediate reduction in photosynthetic capacity and autotrophic respiration, which decreases NEP and reduces evapotranspiration (Amiro et al. 2010, Clark et al. 2010, 2012, 2018). Compensatory photosynthesis by the remaining foliage, which is typically exposed to higher light levels, and the rapid cycling of nutrients from nutrient-rich frass and litter facilitates resprouting of new foliage (Curtis and Gough 2018, Hornslein et al. 2019). As a result, gross ecosystem productivity (GEP), evapotranspiration (Et), and ecosystem water use efficiency (WUE_e), defined as the amount of CO_2 assimilated per unit of water transpired, often recover rapidly following insect damage (Clark et al. 2014, Guerrieri et al. 2019). Long-term consequences of insect infestations on C fluxes have been documented less frequently. These efforts have indicated that increases in standing dead and coarse woody debris following repeated defoliation or bark beetle infestations result in increased heterotrophic respiration and a long-term depression of NEP (Clark et al. 2018, Renninger et al. 2014, Xu et al. 2017).

In this research, we quantified how infestations of southern pine beetle and two frequently employed suppression treatments affected forest composition and structure of intermediate-age, pine-dominated forests, focusing on changes to leaf area and canopy nitrogen (N) content. We then evaluated how changes in forest composition and structure potentially affect ecosystem functioning, especially C sequestration, by employing a synthesis of forest census measurements, C flux measurements, and simulation models. Finally, we addressed how changes in composition of forests impacted by southern pine beetle could affect the capacity of forests to respond to future disturbances. We suggest that management for mixedwood stands, consisting of mixtures of pine and oaks, would increase associational resistance to insect infestations, reducing impacts to continuity in ecosystem services.

Methods and Materials

Site Description

Research sites were located in upland and lowland forests in Atlantic, Burlington, Cumberland, and Ocean Counties in the Pinelands National Reserve (PNR) of southern New Jersey. The PNR is 4,452 km^2 in size and is the largest continuous forested landscape on the Mid-Atlantic Coastal Plain. Approximately 4,380 km^2 of the PNR were designated as a UNESCO Biosphere Reserve in 1988 (https://nj.gov/pinelands/reserve/). Pine-dominated, mixed-composition, and oak-dominated stands comprise the upland forests, and lowland forests are dominated by pitch pine, mixed hardwoods, and Atlantic white cedar (*Chamaecyparis thyoides* (L.) B.S.P) (McCormick and Jones 1973). Most stands have regenerated naturally following the cessation of timber harvesting and charcoal production toward the end of the 19th century, and severe wildfires throughout the 20th century (Forman and Boerner 1981, La Puma et al. 2013, Little 1979). The climate is cool temperate, with mean monthly temperatures of 0.7 and 24.6 °C in January and July, respectively (1988 to 2018; State Climatologist of New Jersey). Mean annual precipitation is 1,183 ± 168 mm. Soils are derived from the Cohansey and Kirkwood formations, are sandy, coarse-grained, and have low nutrient status, cation exchange capacity, and base saturation (Tedrow 1986). The landscape is characterized by a relatively high frequency of wildfires and prescribed burns compared with other forest ecosystems in the Northeastern United States; from 2004 to 2016, over 15,000 wildfires burned 36,654 ha and prescribed fires were conducted on 84,096 ha (Gallagher 2017, La Puma et al. 2013, NIFC 2019). On average, the annual area burned in prescribed fires now exceeds that burned in wildfires by a factor of two.

Southern pine beetle infests primarily hard pines (Dodds et al. 2018, Nowak et al. 2015). In the Mid-Atlantic region, pitch pine, shortleaf pine, Virginia pine (*Pinus virginiana* Mill.), and loblolly pine (*P. taeda* L.) are vulnerable to infestations. The recent southern pine beetle outbreak in New Jersey started in approximately 2000, and by 2016, over 19,500 ha had been infested, followed by 13,520 ha of damage in Long Island, NY, by 2019 (Dodds et al. 2018, Heuss et al. 2018, NY Department of Environmental Conservation 2019). Pitch pine-dominated lowlands have been impacted to a greater extent than upland forests (Aoki et al. 2018).

Southern Pine Beetle Infestations and Forest Structure

Comparative forest census plots based on FIA protocols were installed in uninfested and infested areas in 51 stands throughout the research sites in the southern portion of the PNR in 2014 and 2015, 2 to 5 years following infestation by southern pine beetle (Clark et al. 2017). Aerial and ground-based surveys conducted by New Jersey Department of Environmental Protection (NJDEP) and Dartmouth College researchers were used to locate beetle-damaged areas on public lands (primarily State forests and wildlife-management areas). Infested areas ranged from 0.5 to 35 ha in size. All stands were dominated by pitch pine, with shortleaf and Virginia pine also present in some stands.

Sampled pine trees averaged 77 ± 24 years old (Aoki et al. 2018). Upland stands also contained mixed oaks, sassafras (*Sassafrass albidum* (Nutt.) Nees), and an occasional beech (*Fagus grandifolia* Ehrh.). Lowland stands also contained red maple (*Acer rubrum* L.), black gum (*Nyssa sylvatica* Marshall), American holly (*Ilex opaca* Aiton), and sweetgum (*Liquidambar styraciflua* L).

Both infested and uninfested areas within each of the 51sampled stands were subjected to one of three treatment strategies employed by NJDEP staff and contractors: (1) untreated, where no management occurred ($n = 12$); (2) cut and leave, where infested and buffer pine trees were felled and left in place ($n = 27$); and (3) cut and chip, where infested and buffer pine trees were felled, and either bunched and chipped and all chips scattered onsite, or chips were hauled offsite for commercial use ($n = 12$). Following FIA sampling protocols, we took measurements in four subplots (168 m^2) within each treatment strategy area although, because of size limitations, fewer subplots were sampled in some infested areas. In each subplot, species, diameter-at-breast height (dbh, 1.37 m), height, and crown condition were recorded for all live and dead trees (stems greater than 12.5 cm dbh), and all live and dead saplings (stems between 2.5 cm and 12.5 cm dbh). Additionally, each subplot was evaluated for canopy cover (visual estimate), understory height, understory species composition, cover by species (including tree seedlings, defined as stems less than 2.5 cm dbh), and the number of pine seedlings. Basal area was calculated from dbh measurements and expressed as m^2 stems ha^{-1}.

(1) $$\text{Basal area} = \pi\,(\text{dbh}/2)^2$$

Allometric equations based on destructive harvests were used to estimate total aboveground biomass, foliar biomass, and available fuel mass of pine trees and saplings in each subplot (Clark et al. 2013, 2017). Published values were used to estimate aboveground biomass and foliar biomass of oaks and other hardwoods (Chojnacky et al. 2014, Fatemi et al. 2011, Whittaker and Woodwell 1968).

Specific leaf area (SLA; m^2 g dry weight^{-1}) of the dominant canopy and understory species was measured with a leaf area meter (LI-3000a, LI-COR Inc., Lincoln, NE) and a conveyer belt (LI-3050c, LI-COR Inc.) using fresh needle fascicles and leaves sampled at six reference sites in Burlington and Ocean counties, which were then dried at 70 ºC and weighed. Canopy leaf area index (LAI; m^2 m^{-2} ground area) was estimated by multiplying leaf and needle mass calculated from allometric equations for each species by the appropriate SLA value and then summing results for all species. Projected leaf area of pine needle fascicles was multiplied by $\pi/2$ to calculate one-sided LAI. Canopy and understory foliage were sampled for N content at the time of peak leaf area during the growing season at representative stands in the PNR. Oven-dry foliar samples were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and analyzed for N concentration using a modified Kjeldahl method (Allen 1989). An Astoria 2 Analyzer (Astoria-Pacific International, Clackamas, OR) was used to measure the ammonium concentration of each sample, and results were converted to N concentration in foliage samples. Additional values for foliar N content were obtained from sampling conducted by Renninger et al. (2013, 2015) and Guerrieri et al. (2016, 2019). Nitrogen mass (g N m^{-2} ground area) in canopy and understory foliage was calculated for dominant species by multiplying species-specific N concentrations by corresponding estimates of foliar biomass. Literature values were used for foliar N content of the hardwood species that we did not sample.

Forest Productivity Simulations

To understand how infestations of southern pine beetle and associated suppression treatments affected C fluxes, we estimated gross ecosystem production (GEP), ecosystem respiration (R_{eco}), and NEP for uninfested, infested but untreated, and treated areas. Estimates of GEP for all areas were based on the relationship between maximum LAI during the growing season and GEP calculated from eddy covariance measurements made over 25 combined years at pine- and oak-dominated stands, documented in Clark et al. (2018).

(2) $$\text{GEP} = 232.8\,(\text{LAI}) + 388.4$$

For this relationship, $r^2 = 0.667$, $F = 49.0$, and $P < 0.01$. We assumed a baseline ecosystem respiration rate of 15.3 ± 1.2 T C m^{-2} yr^{-1} for uninfested stands, reflecting average R_{eco} for pine-dominated forests measured during undisturbed years in Clark et al. (2018). We then used relationships derived from Renninger et al. (2014) and Clark et al. (2018) to estimate C release from “excess” standing dead trees and coarse woody debris in infested but untreated and treated areas. In infested but untreated stands, we assumed that snags accounted for approximately half of the dead stem mass, and the remaining half consisted of coarse woody debris, consistent with field observations (figure 1). For the cut-and-leave treatment areas, we assumed that all of the dead pine tree and sapling stem mass was coarse woody debris. We simulated two scenarios for the cut-and-chip treatments: (1) pine trees and saplings were bunched and chipped, with all chips then broadcast scattered across the site; or (2) 70 percent of chips were hauled offsite for commercial use. We averaged decomposition rates for the 3- to 5-year period following treatments, consistent with the timing of our field census measurements. We then calculated annual R_{eco} and NEP for each treatment 3 to 5 years following infestations and suppression treatments.

Statistical Analyses

Values for basal area and aboveground biomass of trees and saplings, LAI, foliar N content, and pine seedling counts were compared using ANOVA analyses (SYSTAT 12, SYSTAT Software, Inc., San Jose, CA). Comparisons among treatments were made with Tukey´s Honestly Significant Difference (HSD) tests that adjusted significance levels for multiple comparisons. Paired sample T-tests were used to compare values for uninfested and infested areas within stands.

Results

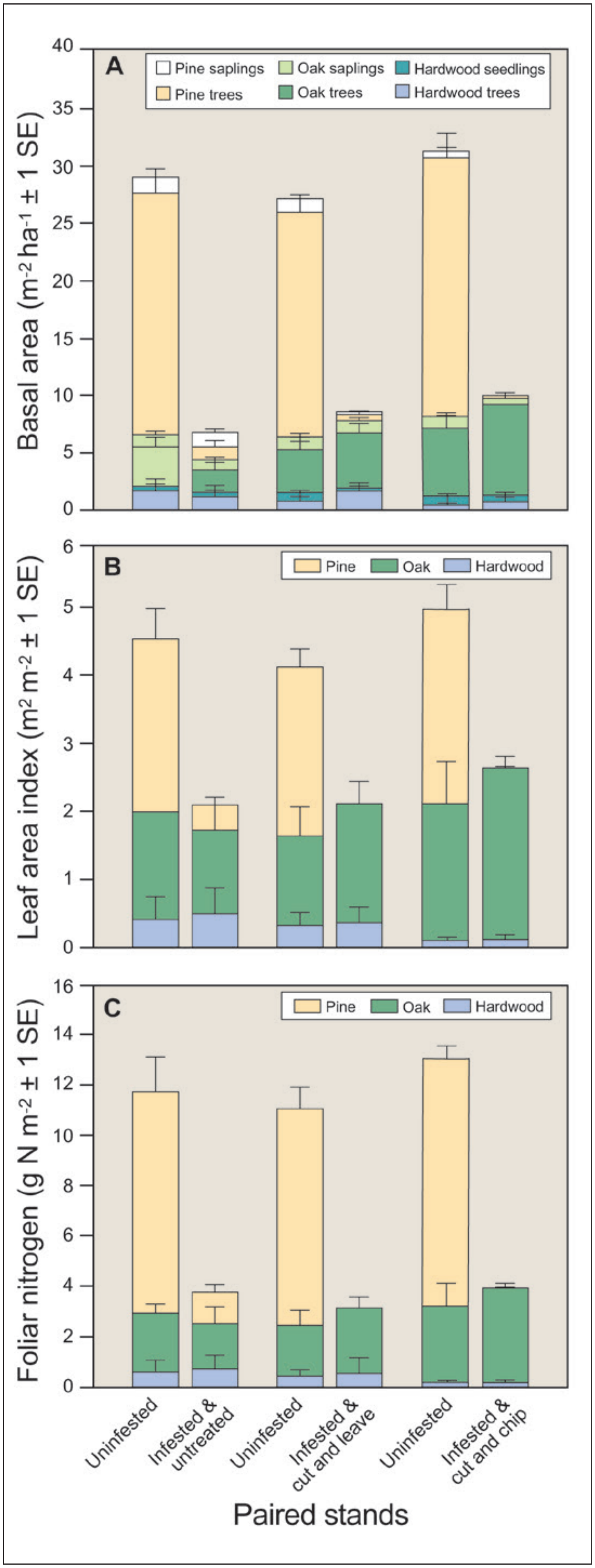
Southern Pine Beetle Infestations and Forest Structure

Pine tree basal area and aboveground biomass averaged 21.4 ± 1.0 m^2 ha^{-1} and 74 ± 4 T ha^{-1}, respectively, in uninfested areas in the 51 stands sampled across southern New Jersey (figure 2a, table 2). Total basal area, leaf area, and foliar N in uninfested areas did not differ among treatments, with approximately equivalent distributions occurring among pines and the sum of oaks and other hardwoods. Pine trees and saplings in uninfested areas accounted for 76 percent of total basal area, 58 percent of tree and sapling leaf area, and 76 percent of tree and sapling foliar N (figure 2).

Figure 1. Extensive pine tree mortality following an infestation of southern pine beetle in Tuckahoe Wildlife Management Area, Pinelands National Reserve of Southern New Jersey. Standing pine trees are dead, and coarse woody debris has accumulated on the forest floor, while red maple (*Acer rubrum* L.) and black gum (*Nyssa sylvatica* Marshall) trees and saplings are unaffected. (Photo by Kenneth Clark 2015)

Infestations of southern pine beetle resulted in extensive mortality of pitch, shortleaf, and Virginia pine trees (figure 2). Pine tree basal area and aboveground biomass in untreated, infested areas were reduced by 94 and 96 percent compared with uninfested areas, respectively, while pine sapling basal area and aboveground biomass, and basal area of oaks and other hardwoods were nearly unaffected (figure 2a and table 2). Pine tree and sapling LAI and foliar N in untreated, infested areas averaged 14 and 15 percent of values in adjacent uninfested areas, respectively (figures 2b and 2c). Suppression treatments in infested areas reduced pine tree and sapling basal area and aboveground biomass by more than 95 percent and more than 99 percent compared to adjacent uninfested areas (figure 2a, table 2). Similarly, pine tree and sapling LAI and foliar N mass in treated areas

Figure 2. Effects of southern pine beetle and suppression treatments on forest composition and structure in the Pinelands National Reserve of southern New Jersey. Data are shown for (a) basal area of trees and saplings, (b) leaf area index of trees and saplings, and (c) nitrogen content in foliage of trees and saplings in uninfested areas, infested areas that were untreated, infested areas where cut-and-leave suppression treatments were conducted, and infested areas where cut-and-chip suppression treatments were conducted. Other hardwoods include red maple (*Acer rubrum* L.), black gum (*Nyssa sylvatica* Marshall), sassafras (*Sassafras albidum* [Nutt.] Nees), American holly (*Ilex opaca* Aiton), and sweet gum (*Liquidambar styraciflua* L.).



averaged only 1 and 2 percent, respectively, of the values in adjacent uninfested areas (figures 2b and 2c). Pine seedlings were most abundant in cut-and-chip treatments, where extensive disturbance of the forest floor occurred during vehicle and equipment use, exposing the bare, sandy soil (figure 3, table 2). Size-class sampling of seedlings indicated a strong decline in seedling number with height, suggesting high rates of mortality in all areas (figure 4). In contrast to pines, infestation and suppression treatments had little effect on basal area, LAI, or N mass of oak trees and saplings in upland areas or of other hardwood trees and saplings such as red maple and black gum in lowland areas (figure 2).

The distribution of snags and coarse woody debris in untreated, infested areas was highly variable, with some areas composed of nearly all standing dead trees, and other areas with a majority of beetle-killed trees already on the forest floor (figure 1). Coarse woody debris averaged 77.8 T ha^{-1} in cut-and-leave treatments, with maximum amounts of 105 ± 12 T ha^{-1} in a pitch pine lowland stand. Coarse woody debris was minimal in many of the cut-and-chip treatments, since the chips had been either scattered or removed from the site.

Forest Productivity Simulations

Estimated GEP of uninfested areas averaged 17.1 T C ha^{-1} yr^{-1}, and NEP averaged 1.8 T C ha^{-1} yr^{-1} (table 3). Estimated GEP following infestation by southern pine beetle was largely driven by oaks, other hardwoods, and understory vegetation, and averaged 11.6 T C ha^{-1} yr^{-1}, approximately 67 percent of rates in uninfested areas. Estimated R_{eco} in infested stands ranged from 16.0 to 17.9 T C ha^{-1} yr^{-1} and was a function of both the position (snags vs. coarse wood on the forest floor) and size of wood fragments following treatments. In the 3- to 5-year period simulated following infestations and treatments, reduced leaf area had a larger effect on NEP values than variation in R_{eco}. However, enhanced coarse wood mass potentially increases R_{eco} for varying lengths of time among treatments. For example, in our simulations, coarse wood in the cut-and-leave treatments took 19 years to reach 50 percent of original C mass, and 57 years to reach 10 percent of original C mass, while debris in cut-and-chip treatments took 7 years to reach 50 percent of original C mass and 22 years to reach 10 percent of original C mass. Estimated NEP was negative for all suppression treatments, reflecting a net loss of C (table 3).

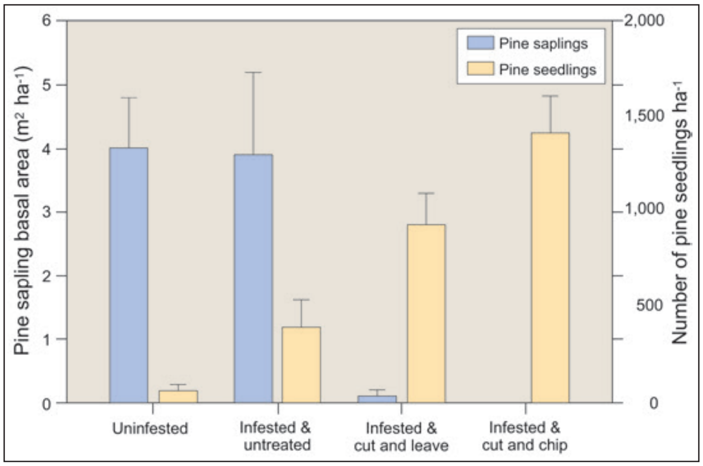


Figure 3. Basal area of pine saplings per hectare (left axis) and number of pine seedlings per hectare (right axis) in uninfested areas, infested areas that were untreated, infested areas where cut-and-leave suppression treatments were conducted, and infested areas where cut-and-chip suppression treatments were conducted.

Discussion

Stand Density and Composition

The extensive mortality of pine trees in infested stands reported here is consistent with the impacts reported for southern pine beetle in pine-dominated forests of the Southeastern United States (Guldin 2011, Nowak et al. 2015), and more recently, further north on the Atlantic Coastal Plain on Long Island, NY (Dodds et al. 2018, Heuss et al. 2019). Overall, stand density and the proportion of pine trees and saplings are critical factors in the probability of southern pine beetle aggregation and infestation leading to pine tree and sapling mortality, with basal areas greater than 28 m^2 ha^{-1} considered highly susceptible to infestations (Guldin 2011). Stand density (as reflected in basal area measurements reported here) is proportional to turbulence regimes within forest canopies, altering the dispersion of aggregation pheromone released by southern pine beetles (Thistle et al. 2004). The recent infestations in New Jersey and Long Island occurred in relatively dense pine-dominated stands with an average pine tree and sapling basal area of 21.4 ± 1.0 m^2 ha^{-1} and 23.8 ± 2.0 m^2 ha^{-1}, respectively, considerably greater than the target basal area of 18 m^2 ha^{-1} that has been effective in mitigating southern pine beetle damage in Southeastern U.S. forests (Guldin 2011, Nowak et al. 2015).

We found that oak trees and saplings in upland stands and other hardwood trees and saplings in lowland

Table 2. Structural characteristics of the canopy and understory in uninfested, infested but untreated, and infested and treated areas impacted by southern pine beetle. Values are means ± 1 SE. Significance levels were tested using ANOVAs and Tukey's HSD tests, and values indicated with different letters among treatment types are significantly different.

Variable	Uninfested (n=51)	Infested: Untreated (n=12)	Infested: Cut and leave (n=27)	Infested: Cut and chip (n=12)
Canopy				
Height (m)	15.2 ± 0.3a	10.1 ± 1.0b	10.4 ± 1.0b	12.0 ± 1.5b
Cover (%)	61.9 ± 2.4a	30.8 ± 6.0b	20.2 ± 4.8b	16.6 ± 6.1b
Aboveground pine biomass (T ha^{-1})				
Trees	74.2 ± 4.2a	2.6 ± 0.9b	0.3 ± 0.2b	0.7 ± 0.4b
Saplings	4.0 ± 0.8a	3.9 ± 1.3a	0.1 ± 0.1b	0.0 ± 0.0b
Understory				
Height (m)	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Cover (%)	71.6 ± 4.6	71.3 ± 8.3	72.0 ± 5.5	88.7 ± 1.0
Number of seedlings (ha^{-1})				
Count	67 ± 33a	400 ± 143b	930 ± 169c	1411 ± 201d

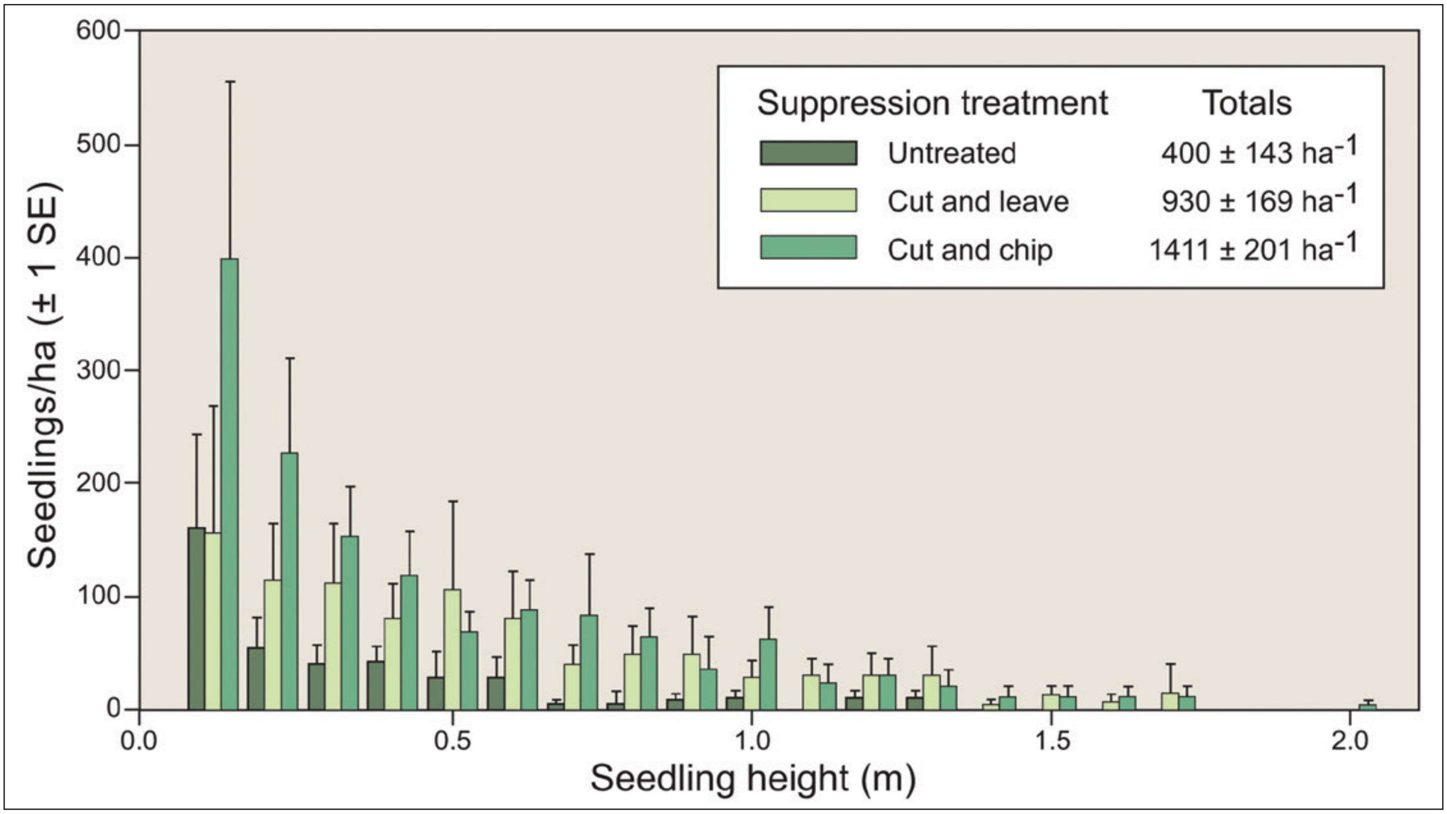


Figure 4. Number of pine seedlings by height class in 0.1-meter increments for infested but untreated areas, cut-and-leave treatments, and cut-and-chip treatments.

stands were essentially unaffected in untreated infested areas and retained to a large extent in treated areas in the Pinelands, similar to results reported by Heuss et al. (2019) for southern pine beetle infestations in Long Island, NY. In contrast to pine-dominated stands, southern pine beetle only rarely impacted pines in oak-dominated stands, and tree mortality was lower in mixed pine-oak stands in the PNR, a pattern also documented by Heuss et al. (2019) for Long Island. In their study, mortality averaged 60 percent of total basal area in pitch pine-dominated stands, and 50 percent and 35 percent in unmanaged and managed pine-oak stands, respectively.

Southern pine beetle damage accelerates succession in infested forests on the Atlantic Coastal Plain, which may ultimately result in the formation and persistence of uneven age, mixed composition stands (La Puma et al. 2013, Clark et al., in preparation). Stands consisting of mixtures of conifers and hardwoods, termed mixedwood stands, have

Table 3. Structural characteristics of the canopy and understory in uninfested, infested but untreated, and infested and treated areas impacted by southern pine beetle. Values are means ± 1 SE. Significance levels were tested using ANOVAs and Tukey's HSD tests, and values indicated with different letters among treatment types are significantly different.

Treatment	Growth Ecosystem Production (GEP) T C ha^{-1} yr^{-1}	Ecosystem Respiration (R_{eco}) T C ha^{-1} yr^{-1}	Net Ecosystem Production (NEP) T C ha^{-1} yr^{-1}
Uninfested	17.1 ± 1.1	15.3 ± 1.2	1.8 (0.7 to 2.9)
Infested, untreated	11.1 ± 2.4	16.3 ± 1.5	-5.2 (-2.8 to -7.6)
Infested, cut and leave	11.4 ± 1.5	16.6 ± 1.6	-5.2 (-3.7 to -6.7)
Infested, cut and chip[1]	12.4 ± 0.6	17.9 ± 1.9	-5.5 (-4.9 to -6.1)
Infested, cut and chip[2]	12.4 ± 0.6	16.0 ± 1.4	-3.6 (-3.0 to -4.2)

T C ha^{-1} yr^{-1} = tons of carbon per hectare per year.

[1] Assuming all chips were broadcast scattered across the area.

[2] Assuming 70 percent of chips were hauled off site for commercial use.

greater associational resistance to insect infestations and other disturbances, and tree mortality is typically reduced compared to forests dominated by a single genus or species, especially for infestations of monophagous insects (Jactel et al. 2017, Kabrick et al. 2017). In infested but untreated pine stands in the current study, the relative basal area of pine, oak, and other hardwood trees and saplings has shifted and converged on the relative basal area of trees and saplings characterizing uninfested mixed composition forests (figure 5, center bar). A similar convergence in species composition and structure towards mixedwood stands has occurred in oak-dominated stands following repeated infestations of gypsy moth and oak tree mortality in the PNR (figure 5; Clark et al. 2018). Both insect infestations are leading to the formation of pine-oak mixedwood stands that will likely persist because they may incur less damage than pine-dominated or oak-dominated stands during future insect infestations (Clark et al., in preparation). In untreated stands impacted by southern pine beetle, basal area of pine trees and saplings are well below the critical density (approximately 18 m^2 ha^{-1}) that would support future aggregations of beetles (Aoki et al. 2018, Dodds et al. 2018, Nowak et al. 2015). Similarly, oak tree and sapling density are relatively low in mixedwood stands and they experience lower mortality than oak-dominated forests, which are especially vulnerable to recurring gypsy moth infestations throughout the Mid-Atlantic region (Clark et al. 2018, Fei et al. 2019, Morin and Liebhold 2015).

Stand Productivity

Net C assimilation and stand productivity are driven by the recovery of leaf area and foliar N levels following southern pine beetle infestations and suppression treatments (Amiro et al. 2010, Clark et al. 2018, Medvigy et al. 2012). When canopy openings occur, either because of needle abscission from standing dead

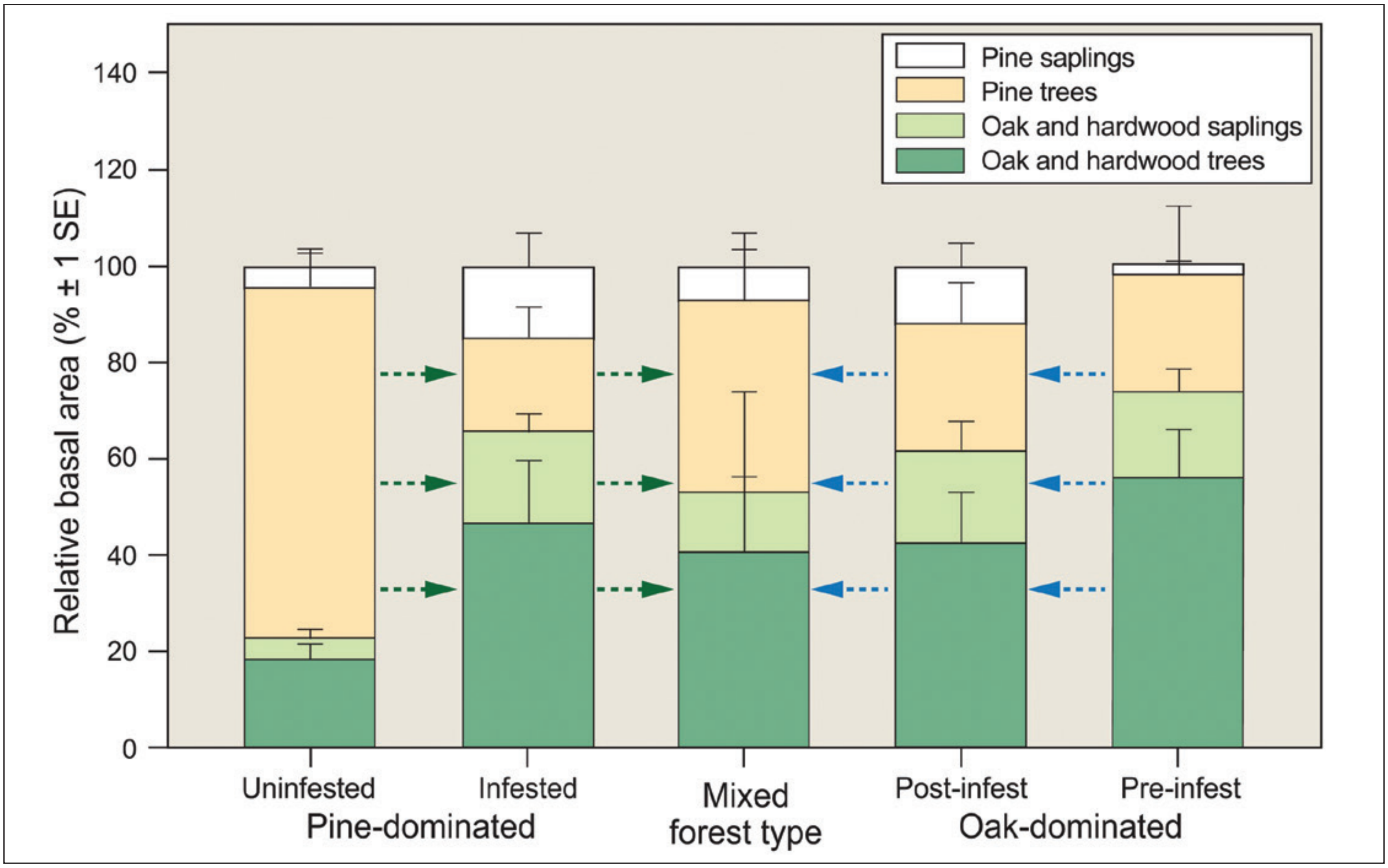


Figure 5. Relative basal area of pines and hardwood trees and saplings pre- and post-insect infestations. Data are from pine-dominated uninfested areas, untreated areas following infestation by southern pine beetle, an uninfested mixed composition stand at Fort Dix in the Pinelands National Reserve, an oak stand before gypsy moth infestation in 2005 (Pre-infest) and following tree and sapling mortality in 2018 due to gypsy moth infestations in 2007 and 2008 (Post-infest) at the Silas Little Experimental Forest in the Pinelands National Reserve (see Clark et al. 2018 for details of the field sampling). Oaks and other hardwoods have been combined as "hardwoods." Infestation results in a convergence in species composition and structure towards mixedwood stands (indicated by arrows).

trees and saplings, treefalls following pine mortality, or damage during suppression treatments, leaf area of remaining trees, saplings, and the understory can respond rapidly (Curtis and Gough 2018). Numerous forest tree species in the Mid-Atlantic region are characterized by regeneration strategies that enhance survival following disturbance (e.g., epicormic budding in pitch and shortleaf pines, prolific resprouting in most oaks and red maple). Clark et al. (2014, 2018) showed an approximate doubling of understory and sub-canopy leaf area in the next growing season following defoliation and tree mortality during gypsy moth infestations in PNR forests, indicating a rapid recovery response to insect damage. Although gypsy moth defoliation was severe and oak tree mortality was approximately 40 percent of stand basal area, leaf area recovered rapidly and GEP and ecosystem WUE_e reached pre-defoliation levels 3 to 4 years after peak defoliation (Clark et al. 2014, 2018; Guerrieri et al. 2019). In our simulations with southern pine beetle infestations, GEP recovered to approximately 67 percent of pre-infestation levels 3 to 5 years following infestation and suppression treatments, and will likely approach pre-infestation levels within a few years.

In contrast to the rapid recovery of GEP, recovery of NEP following southern pine beetle infestations and suppression treatments will be delayed because enhanced R_{eco} following insect infestations and tree mortality is a strong function of C release from decomposing snags and coarse woody debris. We observed similar results in oak-dominated forests following gypsy moth defoliation and tree mortality; while LAI and GEP recovered rapidly, enhanced R_{eco} depressed NEP for at least a decade (Clark et al. 2018). Renninger et al. (2014) projected that NEP in oak-dominated stands where significant tree mortality occurred would be reduced for at least 2 decades as coarse wood decomposes. Although our simulations indicated that reduced GEP was more important than enhanced R_{eco} in reducing NEP, this pattern will likely reverse within a few years as leaf area and foliar N mass recover to pre-defoliation levels. NEP will then be partially a function of the fate of standing dead and coarse woody debris, and the size of the residual wood. In our study, we assumed that chips had a higher decomposition rate than whole stems, and quantification of chip decomposition through time would improve our estimates of NEP.

Management Implications

Our study (and many others) suggests a number of management practices are appropriate for reducing the impact of future southern pine beetle infestations in the Mid-Atlantic region. Reducing stand basal area by thinning to a basal area at or below 18 $m^2\ ha^{-1}$, or at least reducing sub-canopy stem density using prescribed fire, will increase resistance to infestations (Dodds et al. 2018, Gallagher 2017, Guldin 2011, Nowak et al. 2015). Many of the unmanaged pine-dominated stands in the PNR and on Long Island are currently at or above this level, and thus will be vulnerable to future infestations (Dodds et al. 2018; Clark et al., in preparation; USDA FIA data).

Once stands have been infested, two strategies could be used to enhance ecosystem functioning and to reduce the probability of stand damage from future insect infestations. First, utilizing wood from suppression treatments following insect infestations will reduce ecosystem respiration. Our analyses indicate that when pine stems are harvested and removed from site, such as partial removal of chips in the cut-and-chip treatments, estimated R_{eco} is reduced, resulting in less negative NEP values for a shorter period of time. With that management regime, NEP, and thus C sequestration, recovers more rapidly compared to untreated or cut-and-leave treatments in infested areas. If management options for coarse, woody debris are limited, prescribed burning to reduce the risk of wildfires has two benefits: calcium, phosphorous, and other nutrients stored in coarse woody debris is released to vegetation, thereby increasing photosynthetic assimilation (Carlo et al. 2016, Renniger et al. 2013); and competition from understory vegetation is reduced to encourage pine regeneration and establishment. The second management strategy is to ensure that sufficient regeneration of pines occurs in treated areas following infestations so that future stands are composed of mixtures of pines and hardwoods. This strategy can result in uneven-age, mixedwood stands, which have greater resistance to insect infestations than either even-age and monospecific or monogeneric stands (Jactel et al. 2017). Our forest census data indicate that pine seedling and sapling densities are very low in areas where suppression treatments were conducted. Enrichment planting of pine seedlings should be considered in targeted areas where pine regeneration has failed following prescribed burn treatments to reduce competition from understory vegetation. Ensuring the regeneration of pine-oak mixedwood stands that are relatively resistant to future outbreaks of bark beetles and other defoliators will reduce economic costs associated with tree mortality and suppression treatments, as well as mitigate short-term impacts to ecosystem functioning resulting from insect damage, especially C sequestration.

Address correspondence to—

Kenneth Clark, Silas Little Experimental Forest,
501 Four Mile Road, New Lisbon, NJ 08064; email:
kenneth.clark@usda.gov

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Survey of Pest Problems and Pesticide Use in Canadian Forest Seedling Nurseries

Mario Lanthier and Cora Watts

CropHealth Advising & Research, Kelowna, BC, Canada

Abstract

A survey regarding pest problems and pesticide use was distributed to forest seedling nurseries across Canada in the spring of 2017. Growers were asked to volunteer information relevant to their site for the pests found and the pesticides applied over the previous 5 years. Botrytis gray mold was identified as the main disease of concern, requiring at least one pesticide application over the previous 5 years at 89 percent of nurseries. Fusarium root rot is also a disease of concern. The survey identified Lygus bug as the main insect problem and liverwort as the main weed problem. The results highlight the need for new pesticide registrations for forest seedling nurseries in Canada. A full summary of the survey methodology and findings are reported. This paper was presented at the 2019 Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Associations (Sydney, British Columbia, September 30–October 2).

Background

Forest nurseries produce tree seedlings to meet reforestation needs after logging. In Canada, tree seedlings are grown from seeds within a greenhouse environment (figure 1) with approximately 95 percent grown in Styrofoam™ containers, also called Styroblocks® (Peterson 1991). Pest management is an important part of the production and frequently requires the use of pesticides.

In British Columbia, annual nursery production is approximately 250 to 300 million trees (BC Ministry of Forests 2017). This accounts for about 50 percent of the total Canadian seedling production (Canadian Council of Forests Ministers 2020). The most commonly produced seedlings are conifers, including lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. Ex. S. Watson), interior spruce (referring to white spruce: *Picea glauca* (Moench) Voss; Engelmann spruce: *P. engelmannii* Parry ex Engelm.; and their hybrids), Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco), western redcedar (*Thuja plicata* Donn ex D. Don), and western larch (*Larix occidentalis* Nutt.).

The federal agency in charge of pesticide registrations is the Pest Management Regulatory Agency (PMRA), a branch of Health Canada. In 2016, the PMRA published preliminary decisions on chlorothalonil and iprodione, two fungicides commonly applied by forest seedling nurseries. Both products were proposed for cancellation on conifer crops (Health Canada 2016b, 2016c). The Forest Nursery Association of British Columbia (FNABC, http://www.fnabc.com/) recognized the major impact on the industry from the proposed changes. A survey was undertaken to assess pest problems and pesticide use by Canadian forest seedling nurseries to clarify the need for new pesticide registrations. The project was funded by FNABC and conducted by CropHealth Advising & Research (Kelowna, BC, http://www.crophealth.com). This article summarizes the results of that survey.

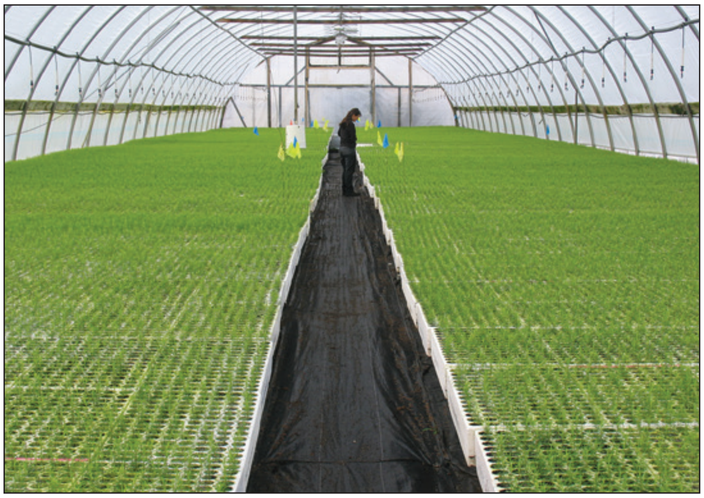

Figure 1. Forest nurseries produce tree seedlings to meet reforestation needs after logging. Most trees in Canada are grown from seeds in containers within a greenhouse environment. (Photo by Mario Lanthier 2007)

Methodology

Survey questions were developed based on pest problems commonly reported in the industry. Nurseries were asked 30 questions about insect pests, diseases, weeds, rodents, and disinfection. Pest problems were listed by their common English names without attempting to identify at the species level at each seedling nursery. Some questions required yes or no answers (e.g., respondents were asked to check a box to indicate whether or not a pest was found at their operation). In other questions, participants were asked to rate the importance of each pest based on expected damage if left unmanaged: 3 for the first pest in importance, 2 for the second in importance, and 1 for the third in importance. The values were summed, then divided by the total number of responses to each question to calculate the overall relative importance of each pest; a higher numerical value indicates a pest of higher importance.

The list of forest seedling nurseries was prepared from industry sources, government listings, a search of the internet, and suggestions by participants. For British Columbia, a list was compiled from the BC Ministry of Forests and Range and from the FNABC membership list. The final list of 27 entries was exhaustive for this province. For nurseries outside of British Columbia, a list was prepared using the Canadian Forests website (http://www.canadian-forests.com/silviculture-nurseries.html), a search of companies on the web, and other suggestions by participants. The final list of 28 nurseries was incomplete for Ontario and Québec, where there were no industry organizations to consult. The survey materials were translated into French for distribution to nurseries in Québec.

All operations were contacted via email or personal telephone calls. Growers were asked to volunteer information relevant to their site. Approximately 1 month was given for participants to respond via Survey Monkey (https://www.surveymonkey.com/), or by entering answers into a Word document, or by filling space within an email. No attempt was made to verify the information provided.

Of the 53 operations contacted across Canada, 38 sent replies (72 percent of the total). The response rate was 88 percent in British Columbia and 57 percent for the remainder of the country (table 1).

Table 1. Forest seedling nurseries contacted, and replies received, by province across Canada.

Province	Contacted	Responded
British Columbia	25	22
Alberta	5	5
Saskatchewan	2	1
Manitoba	1	0
Ontario	9	2
Québec	9	7
New-Brunswick	2	1
Newfoundland	0	0
Total across Canada	53	38

Survey Results for Diseases and Fungicides

The survey showed that Botrytis gray mold is, by far, the main disease of concern in forest seedling nurseries across Canada, requiring at least one pesticide application over the previous 5 years at 89 percent of nurseries (table 2). On a scale of 0 (lowest concern) to 3 (highest concern), this disease was rated at 2.1, whereas all other diseases were rated 1.0 or lower.

Fusarium root rot is another disease of concern, requiring at least one pesticide application over the past 5 years at 61 percent of nurseries across Canada (71 percent in British Columbia). Sirococcus tip blight was reported as a “top-3 disease of concern” by 30 percent of respondents (table 2).

The relative importance of various diseases is markedly different across Canada. Fusarium root rot was rated as a top-3 disease by 77 percent of facilities in British Columbia but only 20 percent of facilities elsewhere in Canada. By contrast, Scleroderris canker was not mentioned by British Columbia nurseries but was rated as a top-3 disease elsewhere in Canada.

Fungicides made with thiophanate-methyl, chlorothalonil, and iprodione are used extensively in the Canadian nursery industry (table 3). In British Columbia, the preferred formulations are iprodione (such as Rovral) and thiophanate-methyl (such as Senator), each being applied by 86 percent of respondents. Outside of British Columbia, however, the preferred formulations were chlorothalonil and thiophanate-methyl, applied by 93 and 80 percent of respondents, respectively.

Table 2. Diseases that required a pesticide application between 2012 and 2017 (based on 36 replies) and diseases of most concern, ranked from first to third (based on 37 replies).

Disease	Pathogen	Number requiring an application	Importance			Weighted rating
			1st	2nd	3rd	
Gray mold	*Botrytis cinerea*	32	21	6	3	2.1
Root rot	*Fusarium* spp.	22	5	6	9	1.0
Tip blight	*Sirococcus strobilinus*	18	2	8	1	0.6
Root rot	*Pythium* spp.	16	1	3	6	0.4
Damping off	Various pathogens	15	2	2	4	0.4
Root rot	Various pathogens	11	2	2	7	0.5
Root rot	*Cylindrocarpon* spp.	9	0	2	0	0.1
Needle dieback	*Phoma* spp.	4	0	1	2	0.1
Shoot blight	*Phomopsis* spp.	3	0	1	0	0.1
Scleroderris canker	*Gremmeniella abietina*	3	2	1	0	0.2
Snow mold	Not mentioned	3	0	2	0	0.1
Keithia needle blight	*Didymascella thujina*	3	0	0	0	0
Root rot	*Phytophthora* spp.	2	0	0	0	0
Needle blight	*Dothistroma septosporum*	1	0	0	0	0
Root rot	*Thielaviopsis basicola*	1	0	0	0	0
Others	*Diplodia*, *Melaspora*, *Meria*, poplar rust	4	0	0	1	0.0

Across Canada, 81 percent (based on 37 respondents) of nurseries rely on past experience with the disease to determine the main pathogens affecting their crop. Other methods used to diagnose were: recognizing visual symptoms (78 percent); commercial diagnostic laboratories (76 percent); comparing symptoms with publication photos (46 percent); and consulting with outside specialists (41 percent).

Table 3. Fungicides applied between 2012 and 2017 (based on 36 replies).

Active ingredient	Examples of trade names	Number of responses
thiophanate-methyl	Senator®	30
chlorothalonil	Daconil 2787®	29
iprodione	Rovral®	27
metalaxyl-m	Subdue Maxx®	14
captan	Maestro® / Captan®	14
Streptomyces strain K61	Mycostop® Biofungicide	7
Trichoderma h.	Rootshield® Biofungicide	6
fludioxonil	Medallion®	5
fenhexamid	Decree®	4
propiconazole	Banner Maxx®, Pivot ®, Topas®	3
Streptomyces lydicus	Actinovate®	3
fludioxonil + cyprodinil	Palladium®	1
Other	Banner Maxx®, Pivot®, Topas®	3

Survey Results for Insects and Insecticides

Survey results showed that *Lygus* bug is, by far, the main insect problem of forest seedling nurseries across Canada. This pest, also called tarnished plant bug, required at least one pesticide application over the previous 5 years at 83 percent of nurseries across Canada (table 4). On a scale of 0 (lowest concern) to 3 (highest concern), lygus bug was rated as 2.2 across Canada. The rating was 2.4 in British Columbia and 1.9 elsewhere in Canada. All other insect pests were rated below 1.0. Other insect pests of concern are aphids, cutworms, and fungus gnats. Root weevil is a large concern in British Columbia but less elsewhere in Canada.

The insecticides most commonly applied across Canada are permethrin and cypermethrin (table 5). In British Columbia, the preferred products are formulations of cypermethrin and permethrin, applied by 70 and 50 percent of respondents, respectively. Outside of British Columbia, the preferred products are formulations of permethrin and chlorpyrifos, applied by 60 and 40 percent of respondents, respectively.

Table 4. Insect pests and mites that required a pesticide application between 2012 and 2017 (based on 35 replies) and of most concern, ranked from first to third (based on 38 replies).

Insect or Mite	Latin name	Number requiring an application	Importance			Weighted rating
			1st	2nd	3rd	
Lygus bug	*Lygus* spp.	29	23	5	4	2.2
Aphid	Various species	18	5	4	8	0.8
Cutworm	Various species	15	3	4	5	0.6
Root weevil	Various species	13	4	6	0	0.6
Caterpillars[1]	Various species	10	0	4	4	0.3
Fungus gnat	*Bradysia* spp.	8	0	6	5	0.5
Spider mite	*Olygonychus ununguis*	6	2	1	1	0.2
Thrips	Various spp.	5	0	1	1	0.1
Shore fly	*Scatella stagnalis*	5	0	3	4	0.3
Cranberry girdler	*Chrysoteuchia topiarius*	3	0	0	0	0.0
Other (slugs, cranefly, beetles)		3	0	0	1	0.0

[1] Caterpillars included tussock moth (*Orgyia detrita*), spruce budworm (*Choristoneura fumiferana*), western black headed budworm (*Choristoneura freemani*), webworm (*Hyphantria cunea*), tent caterpillar (*Malacosoma* spp.).

Survey Results for Weeds and Herbicides

Liverwort is the main weed problem of forest seedling nurseries across Canada. This weed required at least one pesticide application over the previous 5 years at 73 percent of nurseries across Canada (table 6). On a scale of 0 (lowest priority) to 3 (highest priority), liverwort was rated as 1.6 across the country. All other weeds were rated below 1.0.

Glyphosate-based products are the most widely used herbicide in forest seedling nurseries, being applied by 89 percent of respondents across Canada (table 7). Other commonly applied herbicides are formulations of flumioxazin and simazine, applied by 57 and 51 percent of respondents, respectively.

Table 5. Insecticides and miticides applied between 2012 and 2017 (based on 35 answers).

Active ingredient	Examples of trade names	Number of responses
Permethrin	Ambush®, Perm-UP, Pounce®	19
Cypermethrin	Cymbush®, Ripcord ™	15
Carbaryl	Sevin®	9
Chlorpyrifos	Citadel®, Pyrate, Pyrinex™	6
Potassium salts of fatty acids	Safer® Insecticidal Soap	6
Malathion	Malathion	4
Diazinon	Diazinon	2
Others[1]		9

[1] Other products mentioned were abamectin (Avid®), acephate (Orthene®), acetamiprid (TriStar), Bacillus thuringiensis var. kurstaki (Dipel®), bifenazate (Floramite®), deltamethrin (Decis®), diflubenzuron (Dimilin®), dimethoate (Cygon®), endosulfan (Thiodan®), fenbutatin oxide (Vendex®), and spirotetramat (Movento®).

Survey Results for Rodents and Rodenticides

In British Columbia, the house mouse required a pesticide treatment at 86 percent of facilities, compared with 50 percent of facilities outside the province. Outside of British Columbia, the field mouse required a pesticide treatment at 79 percent of facilities, compared with 48 percent at BC facilities. Other rodents mentioned were rats, gophers, marmots, skunks, and squirrels.

The house mouse (*Mus musculus*), the meadow vole (*Microtus* sp.) and the roof rat (*Rattus rattus*) are common rodents in Canadian agriculture production. Where they are present, mice and voles can cause severe damage to crops.

Based on this survey, the most commonly used rodenticides are made of warfarin, diphacinone, and chlorophacinone (table 8).

Survey Results for Sanitation and Disinfectants

Based on 35 nursery respondents across Canada, 49 percent annually sanitize growing areas such as benches, floors, and walls, 26 percent seldom sani-

Table 6. Weeds that required a pesticide application between 2012 and 2017 (based on 33 replies) and weeds of most concern (based on 36 replies).

Weed	Latin name	Number requiring an application	Importance			Weighted rating
			1st	2nd	3rd	
Liverwort	*Marchantia polymorpha*	24	15	4	3	1.6
Dandelion	*Taraxacum officinale*	22	2	1	5	0.4
Grasses	Various species	21	4	7	1	0.8
Chickweed	*Stellaria media*	19	0	4	5	0.4
Moss	Various species	19	3	3	3	0.5
Pearlwort	*Sagina procumbens*	15	2	1	3	0.3
Fireweed	*Chamaenerion angustifolium*	15	1	3	3	0.3
Horsetail	*Equisetum arvense*	13	1	3	2	0.3
Bittercress	*Cardamine hirsute*	12	4	4	1	0.6
Groundsel	*Senecio vulgaris*	11	1	0	1	0.1
Bindweed	*Convolvulus arvensis*	8	0	0	0	0.0
Woodsorrel (Oxalis)	*Oxalis* spp.	8	0	0	0	0.0
Pineapple weed	*Matricaria discoidea*	7	0	0	1	0.0
Nostoc algae	*Nostoc commune*	6				
Other perennial broadleaf[1]	Various species	9	2	3	2	0.4
Other annual broadlea[f1]	Various species	8	0	2	1	0.1

[1] Other weeds mentioned were amaranth, annual bluegrass, aspen seedlings, birch seedlings, Canada thistle, cattail, elm seedlings, fleabane, knapweed, kochia, henbit, lamb's quarters, mustard, nettle, night shade, *Poa annua*, poplar seedlings, popweed, portulaca, purslane, quack grass, red root pigweed, Russian thistle, sensitive onoclea, shepherd's purse, *Spergularia rubra*.

tize, 14 percent sanitize between each crop, 9 percent sanitize only when practical, and 3 percent sanitize irregularly. Chlorine-based products are used by 63 percent of nurseries (table 9). Quaternary ammonia products are also used across Canada and sodium metabisulphite is reported only in British Columbia.

Table 7. Herbicides applied between 2012 and 2017 (based on 37 replies).

Active ingredient	Examples of trade names	Number of answers	% of responses
Glyphosate	Roundup® or other brands	33	89
Flumioxazin	SureGuard®, Broadstar®	21	57
Simazine	Simazine, Princep Nine-T®	19	51
Paraquat	Gramoxone®	5	14
2, 4-D	Par III®, Target®, Trillion®	5	14
MCPA	MCPA Amine 500	2	1
Indaziflam	Alion	1	0
Other[1]		5	14

[1] Other products mentioned were amitrole (Amitrol), baking soda, hydrogen peroxide, isoxaben (Gallery™), napropamide (Devrinol®), oxyfluorfen (Goal™), propyzamide (Kerb™), and horticultural vinegar.

Discussion

Survey Findings

This survey is based on common English pest names. It is likely that some participants reported for the same pest problem caused by different pest organisms.

Participants were not asked to report the non-pesticide methods of their management program. One participant mentioned their operation has been "pesticide free for the last 5 years." The industry makes extensive use of cultural pest control methods, especially to reduce conditions that favour specific diseases. Examples include management of relative humidity and air temperature to reduce incidence of gray mold (Peterson et al. 1988) and heat treatments for sanitation of Styrofoam™ containers (Peterson 1991). Many participants reported using hot water or steam in their operations.

For diseases, the survey identified gray mold as the main concern. Caused mostly by *Botrytis cinerea*, this disease is a concern in late summer to early fall when

Table 8. Rodenticides applied between 2012 and 2017 (based on 36 replies).

Active ingredient	Examples of trade names	Number of responses
Warfarin	Hillcrest, Warfarin Baitpaks®	9
Diphacinone	Ramik®, Ditrac®	8
Chlorophacinone	Ground Force™, Rozol®	7
Brodifacoum	Jaguar®, Ratak®	4
Cellulose from corn cobs	Wilsarin® Rat & Mouse Killer	2
Bromadiolone	Boot Hill®, Hawk®	1
Traps without pesticide		8
Other products, difethialone	FastDraw®, Hombre®	3
Other	fungicide repellent	1

Figure 3. *Lygus* bug is the main insect of concern in forest seedling nurseries in Canada. Feeding on rapidly growing tissue can affect growing tips, buds, and flowers, leading to a loss of apical dominance and the development of weak, multiple leaders as seen on the two seedlings on the right. (Photo by Mario Lanthier 2019)

plants reach their desired size, creating stagnant air within the canopy. Mold may be visible and affected needles may be killed (figure 2). The problem then moves with the plants during outplanting (Sutherland and van Eerden 1980). Fusarium root rot is a major concern on Douglas-fir; several species are involved, the most common being *F. oxysporum* and *F. acuminatum* (James et al. 1990).

For insects, the survey identified *Lygus* bug as the main insect problem. *Lygus* bug, also called tarnished plant bug, refers to various species but most commonly *L. lineolaris* and *L. hesperus*. The insect feeds on rapidly growing tissue such as growing tips, buds, and flowers, leading to a loss of apical dominance and the development of weak, multiple leaders (Sutherland et al. 1989) (figure 3). The pest is often a concern at forest nursery facilities adjacent to agriculture fields. The insects are displaced when the agriculture crop is mowed or harvested and winged adults fly into the nearby seedling nursery.

For weeds, liverwort was the main problem. The common liverwort (*Marchantia polymorpha* L.) is commonly found in greenhouses and has been reported as "probably the most severe weed problem in container nurseries," especially in 2-year-old crops (Scagel and Evans 1990). In general, this weed can reduce seedling growth by competition for light, water, and nutrients, may be a reservoir for insects and disease pests, and can give the impression of overall poor nursery management (Landis 1989) (figure 4).

Figure 2. Gray mold, caused mostly by *Botrytis cinerea*, is the main disease of concern in forest seedling nurseries in Canada. The pathogen can develop rapidly in late summer to early fall when plants reach their desired size, creating stagnant air within the canopy. The mold becomes visible and affected needles may be killed. (Photo by Mario Lanthier 2018)

Figure 4. Liverwort is the main weed problem reported by forest seedling nurseries in Canada. The common liverwort (*Marchantia polymorpha* L.) can reduce seedling growth by competition for light, water, and nutrients, and may be a reservoir for insects and disease pests. (Photo by Mario Lanthier 2013)

Table 9. Disinfectant products applied between 2012 and 2017 (based on 35 replies).

Active ingredient	Number of responses
Chlorine and Sodium hypochlorite (bleach)	22
Quaternary ammonia products	12
Sodium metabisulphite	7
Hydrogen peroxide + peroxyacetic acid (Sanidate®)	5
Hydrogen dioxide + peroxyacetic acid (Zerotol®)	2
Hydrogen peroxide	2
Others[1]	8

[1] Other answers provided were Chemprocide, dish soap, Horti-Klor™, Lysol®, sweeping, Velosan, and vinegar.

Pesticide Registrations

Results from this survey highlighted the need for new fungicide registrations for forest seedling nurseries in Canada. Fungicides made with chlorothalonil, iprodione, and thiophanate-methyl are used in more than 75 percent of nurseries. All three active ingredients have been recently reviewed by the Pest Management Regulatory Agency (PMRA), the federal agency in charge of pesticide registrations. For chlorothalonil, the final decision is to limit the number of applications per year for outdoor and greenhouse-grown conifers, with a restricted re-entry of 15 days for harvesting in seedling production (Health Canada 2018a). For iprodione, the final decision is to limit the number of applications per year, with a restricted re-entry of 1 day for greenhouse production (Health Canada 2018b). For thiophanate-methyl, the current proposal is for the product to remain available but label wording will include extensive personal protective equipment and a maximum of 2 applications per season (Health Canada 2019b).

Additionally, new insecticide registrations may also be required for forest seedling nurseries in Canada. Insecticides most commonly applied across the country are pyrethroids (active ingredients cypermethrin and permethrin). This is Group 3 for resistance classification (IRAC 2019). Many of the other insecticides reported are currently subject to review by Health Canada. Insecticides based on acephate, carbaryl, chlorpyrifos, diazinon, dimethoate, and endosulfan were applied in the past 5 years by 66 percent of respondents. Carbaryl is no longer registered for use in greenhouses (Health Canada 2016a); chlorpyrifos is proposed for cancellation for most uses, except greenhouse ornamentals (Health Canada 2019a); diazinon is no longer registered for ornamental plants (Health Canada 2009); dimethoate is now subject to long restricted re-entry in seed orchards (Health Canada 2015); and endosulfan has been discontinued since 2016 (Health Canada 2011).

Following the initial distribution of the survey results, efforts were undertaken to pursue new pesticide registrations, especially for fungicides. The British Columbia industry association has secured funding for efficacy trials with newer fungicides to generate data that will support label extension and include the forest seedling industry. This work will likely continue for multiple years until the registration of a range of products with varied modes of action. At the same time, clarifications were obtained from the federal agency on pesticide label wording. Based on the User Site Classification in place in Canada, forest seedling nurseries are considered “ornamentals” (Health Canada 2003). This clarification has opened access to more products previously thought to be unavailable and forest seedling nurseries in Canada can now legally use many pesticides registered for ornamental plants.

Address correspondence to—

Mario Lanthier, CropHealth Advising & Research, P.O. Box 28098, Kelowna BC, V1W 4A6, Canada; email office@crophealth.com; phone 250-717-1898.

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Seedling Ecophysiology: Five Questions To Explore in the Nursery for Optimizing Subsequent Field Success

Steven C. Grossnickle, Steven B. Kiiskila, and Diane L. Haase

*Consultant, Nursery*To*Forest Solutions, North Saanich, BC; Crop Manager, Arbutus Grove Nursery, Victoria, BC; Western Nursery Specialist, U.S. Department of Agriculture, Forest Service, Portland, OR*

Abstract

This paper is based on questions from an audience participation discussion with the author S. Grossnickle during the Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Association (Sidney, BC, September 30-October 2, 2019). The five question topics presented herein were, by consensus, the most discussed questions presented by the audience of nursery practitioners and foresters. Topics explored in this paper relate to nursery hardening practices, irrigation management to promote stress resistance, cultural strategies to promote vigorous root growth, storage practices for hot-lifted seedlings, and storage length for overwinter stored seedlings. The following answers to these specific topics are the authors' combined views on these nursery cultural practices.

Introduction

Nursery cultural practices have a direct impact on seedling quality and subsequent field performance (Dumroese et al. 2016, Grossnickle 2012, Grossnickle and MacDonald 2018a, Mattsson 1997, Sutton 1979). Culturing seedlings requires specialized knowledge and skill to produce adequate quantities of high-quality plants from appropriate genetic seed sources in a timely manner. This process starts with a partnership between the client and the nursery manager to determine plant specifications that are matched to the outplanting site (Dumroese et al. 2016). These plant specifications include species, seed source, and stocktype, as well as particular morphological and physiological characteristics that will maximize the seedling potential to survive and thrive after outplanting (Haase 2008).

For any given seedling crop, it is important to define and refine the path required to go from start to finish. Plants are biological organisms and must be treated as such; they are not widgets in a factory. Morphology is relatively easy to see and measure, and most target specifications are based on these measures. Nonetheless, physiological function must also be considered because seedling physiological responses to the environment determine their survival and morphological development (Grossnickle 2000).

Plants' physiological function is ever changing and responding to their external environment. Thus, constant monitoring of seedling development in the nursery is essential, especially for identifying and addressing any problems (Duryea 1985, Grossnickle and MacDonald 2018b). Throughout the process, growers must manage risks to maximize yield and performance. Without good quality upon leaving the nursery, seedlings with the best genetics cannot do well in the field.

This paper explores five questions about seedling ecophysiology and nursery culturing raised during the 2019 Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Association.

Question 1 – What Are the Best Cultural Hardening Practices To Maintain Physiological Quality?

Cultural hardening practices that improve seedling "physiological quality" have long been considered important for increasing survival and growth potential after field planting for both bareroot (Wakeley 1948, 1954) and container-grown (Landis et al. 2010, Lavender and Cleary 1974, Tinus 1974) seedlings. This is because hardened seedlings usually have quality attributes necessary to become established after planting on restoration sites (Grossnickle 2012, Grossnickle and MacDonald 2018a). As nursery-grown seedlings reach a desired morphological size, cultural practices to modify daylength, temperature, watering, and fertilization can be applied to harden seedlings (Landis et al. 1999, Landis 2013, Tinus and McDonald 1979).

Stress resistance is not considered to be related to plant age (e.g., freezing resistance [Sakai and Larcher 1987]; drought resistance [Teskey et al. 1984]), but rather to its morphological, physiological, and phenological state (Fuchigami et al. 1982, Lavender 1985). Changes in phenological and physiological parameters are known to occur in parallel (Fuchigami et al. 1982, Fuchigami and Nee 1987, Lang et al. 1985) with stress resistance varying seasonally with plant development (Bigras 1996, Burr 1990, Grossnickle 2000) in temperate zone tree species (figure 1). In addition, root growth is related to seasonal shoot dormancy patterns, decreasing as shoot endodormancy (regulated by internal factors) intensifies in the fall and increasing as seedlings move toward ecodormancy (regulated by environmental factors) (Ritchie and Dunlap 1980, Ritchie and Tanaka 1990). This knowledge of plant acclimation in relation to the phenological state can be used for scheduling hardening practices during the last stages of a nursery cultural program, thereby improving seedling quality and enhancing subsequent field performance (Landis et al. 2010, Lavender and Cleary 1974, Tinus 1974).

Acclimation of seedlings is based on the concept of "slowly increasing stresses to induce physiological adjustments in plants" (Kozlowski and Pallardy 2002); thus, cultural practices that enhance stress tolerance or avoidance can help seedlings develop morphological and physiological protection from potentially limiting field site conditions (Landis et al. 1999, Lavender and Cleary 1974, Tinus 1974, Wakeley 1954). The following sections describe cultural practices of modified daylength (photoperiod), temperature, and fertilization to promote seedling hardening while maintaining

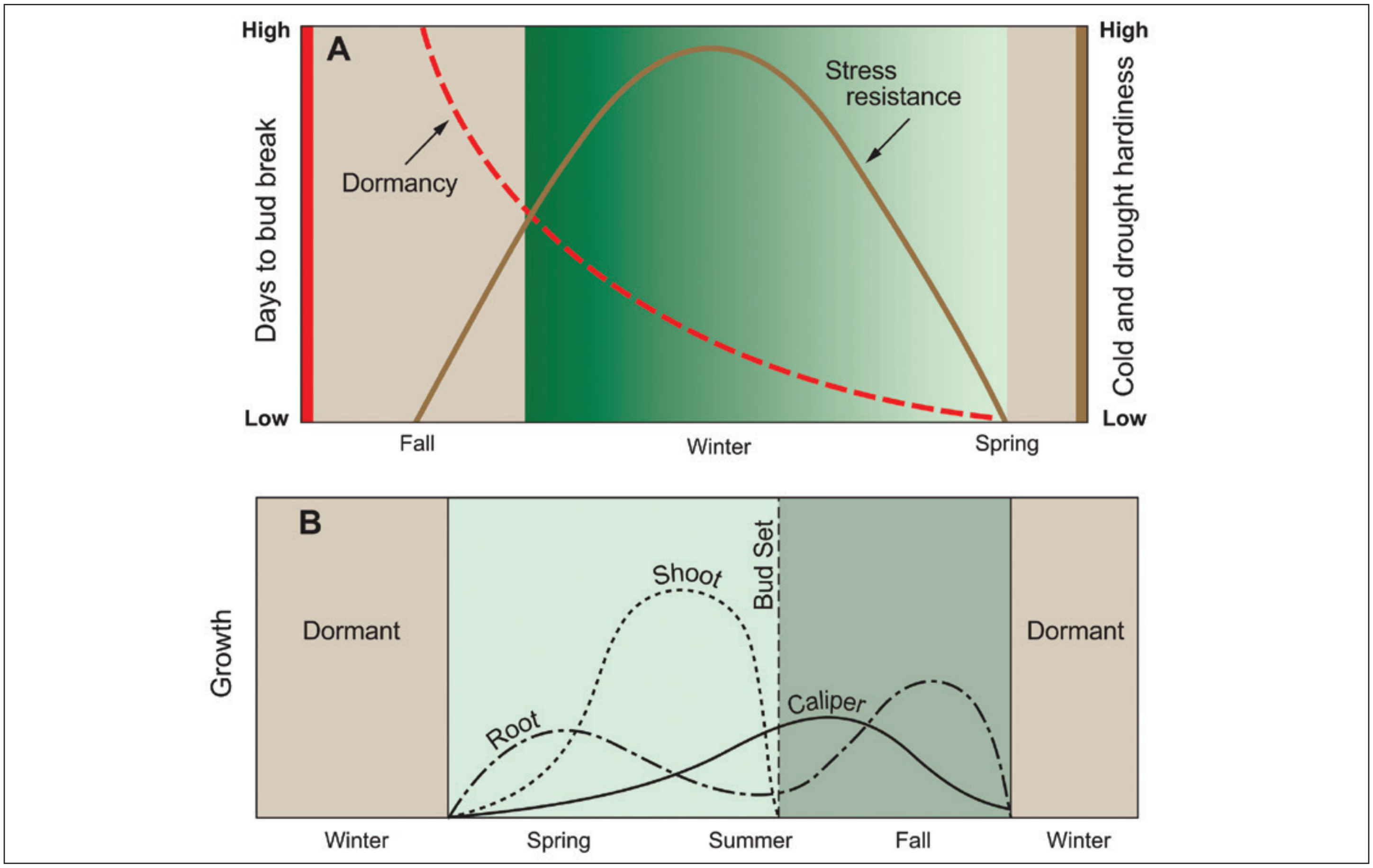

Figure 1. Seedlings have distinct phenological cycles which can vary somewhat based on species, geographic seed source, and weather patterns. (a) Bud dormancy (measured as days to budbreak) is high in the fall and declines through the winter and early spring, while stress resistance and cold hardiness peak in winter. (b) Root and shoot growth follow different patterns. (a - adapted from Landis et al. 2010; b – adapted from Landis et al. 1999)

physiological quality. A detailed discussion on watering as a seedling hardening cultural practice is described in the answer to Question 2.

Daylength

After the summer solstice, daylength shortens, promoting development of endodormancy. With northern-latitude tree species, the end of shoot elongation and development of terminal buds is considered to be the first stage of fall acclimation to low temperatures (Weiser 1970) and an overall increase in stress resistance (Levitt 1980). Seedlings normally enter the first stage of fall acclimation to low temperatures (Grossnickle 2000, Lang et al. 1985, Levitt 1980, Weiser 1970) and develop increased drought resistance (Abrams 1988, Teskey et al. 1984) in the latter half of summer, when shoot elongation has ended and terminal buds are developing (Burr 1990). As seedlings develop a "hard bud," they are considered endodormant and will not break bud even if they are exposed to optimal environmental conditions (see Temperature section). In this state, they continue to grow roots, though root growth is declining (Ritchie and Dunlap 1980, Ritchie and Tanaka 1990).

Because temperate tree species respond to seasonal decreases in daylength, short-day treatments have been developed in northern-latitude container nurseries to induce shoot growth cessation and bud formation (Landis et al. 1999, Tinus and McDonald 1979). The typical short-day treatment for spring-planted seedlings is initiated in August with an 8- to 10-h day and 14- to 16-h night treatment for 10 to 12 days, with variations depending on species and genetic sources (Grossnickle 2000, Landis et al. 1999). Seedlings are then placed under a cultural regime to maintain budset (i.e., moderate water stress, shortened photoperiod, and low N fertilization; Landis et al. 1999, Lavender and Cleary 1974). During hardening, the reduction of N fertilization is an optional practice that brings N levels below their optimum range, with N levels returned to their optimum range when limiting seasonal environmental conditions ensure seedlings remain endodormant and will not reflush. These practices are then maintained until they are lifted for storage in late fall or early winter. For summer-planted (Grossnickle and Folk 2003, Luoranen et al. 2006) and fall-planted (Luoranen and Rikala 2015; MacDonald and Owens 2006, 2010) seedlings, short-day treatment (as defined above) is initiated approximately 2 months before seedlings are lifted and shipped to the field to allow for a 5- to 6-week exposure to seasonal shortening photoperiods and ambient temperatures. This approach recognizes the annual seedling phenological and physiological cycles (figure 1), and utilizes them to promote budset development, dormancy, freezing tolerance, and drought resistance (Colombo et al. 2001, Grossnickle 2000, Landis et al. 2010), thereby producing hardened seedlings. The advantage of using photoperiod manipulation is that it allows for the application of a uniform cultural treatment over the entire crop (Landis et al. 1999).

Temperature

As seedlings are exposed to cold fall temperatures and accumulate chilling hours, they move through the endodormancy phase, with maximum days to budbreak in late summer and early fall decreasing through the fall and into winter (Burr 1990, Fuchigami et al. 1982) and increasing stress resistance (Grossnickle 2000) peaking in winter (figure 1). When seedlings complete the endodormancy phase and move into the ecodormancy phase, root growth potential increases (Burr 1990, Ritchie and Tanaka 1990) and seedlings only remain inactive as long as environmental conditions are unfavorable for growth (Burr 1990, Fuchigami et al. 1982, Lang et al. 1985).

Chilling hours, rather than calendar date, are used by nursery practitioners to track fall acclimation because temperate conifers require a period of chilling to move through endodormancy and become ready for overwinter storage. Chilling hours are quantified based on specific temperature ranges. For example, in the Pacific Northwest and Canada, chilling hours are often recorded from 0 to 4.4 °C (32 to 40 °F) (Timmis et al. 1994, van den Driessche 1977), or to 10 °C (50 °F) (Burdett and Simpson 1984, Ritchie et al. 1985), while in the southern United States, chilling hours are typically reported within the range of 0 to 8 °C (32 to 46.5 °F) (Carlson 1985, Garber 1983). In some instances, temperatures above or below a certain level are given partial or negative chilling hours (Haase et al. 2016, Harrington et al. 2010). Chill days (O'Reilly et al. 1999), degree-hardening-days (Landis et al. 2010), or hardening degree days (Carles et al. 2012) are sometimes reported when hourly data are not available. As chilling hours increase, the days to budbreak decrease and stress resistance increases for a wide range of temperate tree species (Grossnickle and South 2014) (figure 1).

Fertilization

Reduction, reformulation, or withdrawal of fertilizer toward the end of the growing season is an effective means to slow growth and induce bud formation (Landis et al. 1999, Tinus and McDonald 1979). This practice is sometimes done in concert with short-day treatments at container nurseries. Typically, N fertilization is reduced by 50 to 90 percent from rates used during the rapid growth phase of seedling development (Landis et al. 1989). Fall fertilization regimes, applied after the hardening fertilization treatment, have been developed to result in optimum nutrient levels available for growth after outplanting (Dumroese 2003, Hawkins 2011, Landis 1985), while fall nutrient loading after the completion of budset is designed to increase seedling nutrient reserves to luxury consumption levels, thus increasing field performance potential (Dumroese 2003, Grossnickle 2012, Grossnickle and MacDonald 2018a, Hawkins 2011, Timmer 1997).

Question 2 – How Can Irrigation Management Be Used To Promote Stress Resistance?

Modifying irrigation practices to create water stress events at the end of the growing season affects plant development and can be used to increase stress resistance and hardening. These water stress events result in “physiological adjustments” in plants (Kozlowski and Pallardy 2002), increased drought resistance (Teskey et al. 1984), and induction of bud formation (Calme´ et al. 1993, Lavender and Cleary 1974, Macey and Arnott 1986, Timmis and Tanaka 1976, Young and Hanover 1978). Drought resistance is a combination of drought avoidance and drought tolerance (Abrams 1988, Teskey et al. 1984). Drought avoidance (i.e., postponement of plant dehydration through reduction in water loss) includes cuticular development (Grossnickle 2000), stomatal sensitivity (Folk and Grossnickle 1997, Timmis 1980), morphological balance (Mexal and Landis 1990, Thompson 1985), increased water absorption by roots (Carlson and Miller 1990), and improved root growth capacity (van den Driessche 1991). Drought tolerance (i.e., capacity to undergo dehydration without irreversible injury) includes osmotic and cell wall elasticity adjustment (Joly 1985, Lopushinsky 1990, Ritchie 1984, Timmis 1980) and chloroplast drought resistance (Timmis 1980).

Exposing seedlings to water stress, in combination with reduced photoperiod and fertilization, is used to harden seedlings (Landis et al. 1999). Successful implementation of this cultural practice requires an understanding of necessary water stress levels for the development of seedling drought resistance. For example, loblolly pine (*Pinus taeda* L.) seedlings developed drought resistance during a 5-week reduced irrigation regime (figure 2a) with a 50-percent increase in drought avoidance (cuticular transpiration declined from 3.8 to 2.3 percent water loss h-1 after stomatal closure) and a 100-percent increase in drought tolerance (osmotic potential at turgor loss point that declined from

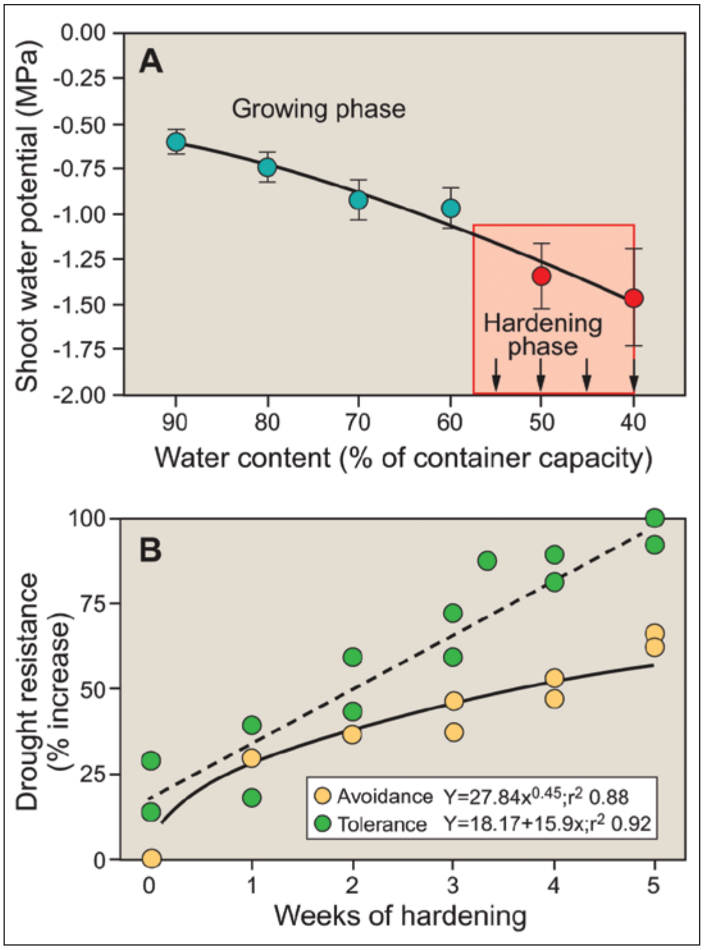

Figure 2. (a) Mid-day shoot water potential of loblolly pine (*Pinus taeda* L.) seedlings changes in relation to the water content container capacity percentage (CC%). The arrows along the X-axis are hardening targets to progressively lower the CC% to 40 percent over a series of weeks. (b) Drought resistance is measured by drought avoidance (cuticular transpiration that declined from 3.8- to 2.3-percent water loss per hour after stomatal closure) and drought tolerance (osmotic potential at turgor loss point that declined from -1.0 to -2.0 MPa) during nursery hardening (i.e., reduced fertilization and watering) (Grossnickle unpublished).

-1.0 to -2.0 MPa) (figure 2b). Other studies have also found that restricted watering hardens loblolly pine seedlings (Bongarten and Teskey 1986, Hennessey and Dougherty 1984, Seiler and Johnson 1985). As loblolly pine seedlings proceed through this drought-hardening event, their shoot and root systems stop growing, needle cuticular development occurs resulting in tactile changes from a feather-like to a stiff feel when moving one's hand across the foliage, needle color changes from lush green to light green, and root suberization occurs resulting in a color shift from white to brown (figure 3). These visual cues allow the nursery practitioner a means to track seedling changes during the drought hardening process.

For a water-stress cultural practice to be successful, one needs to increase water stress in a stepwise

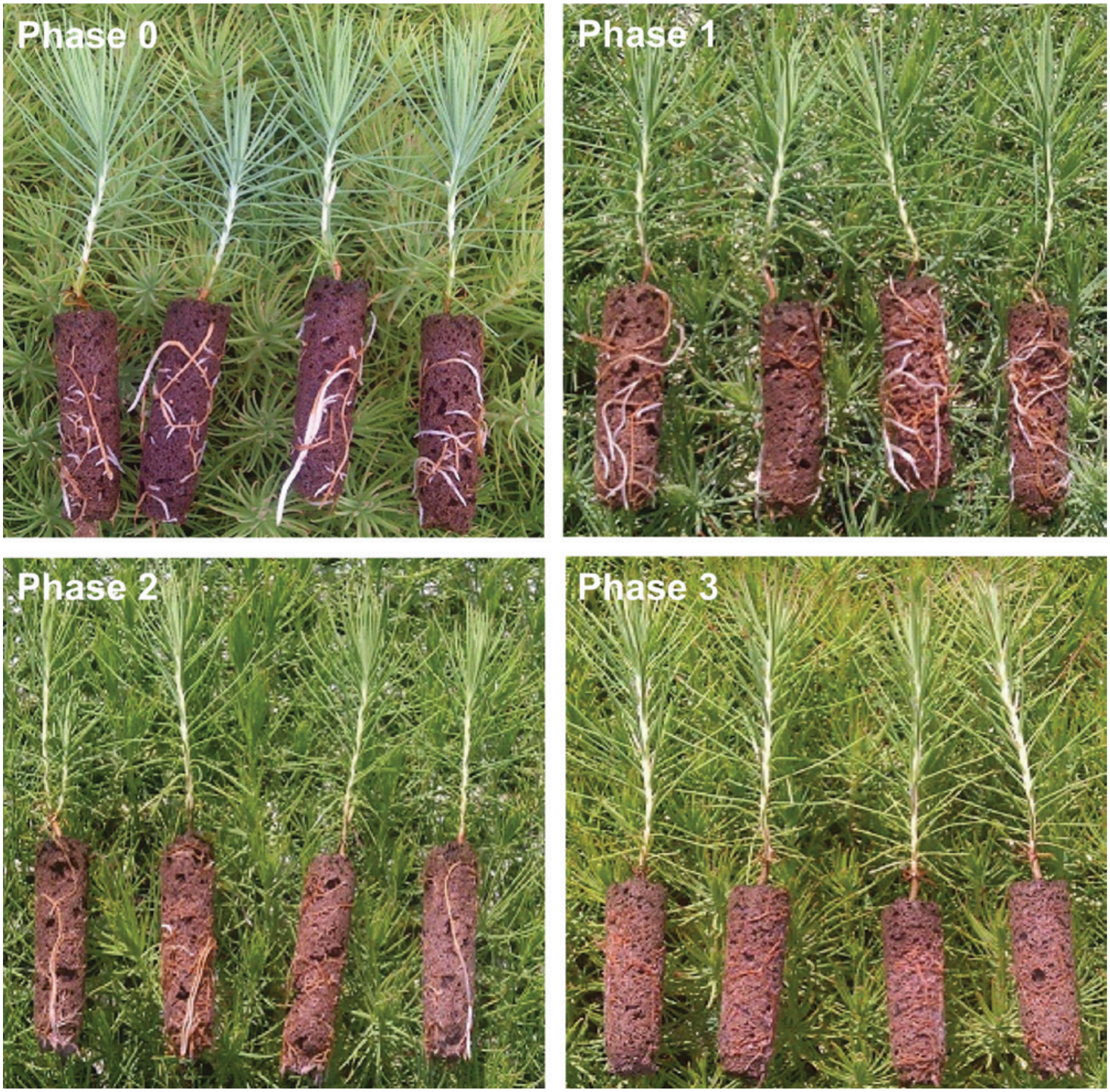

Figure 3. Loblolly pine (*Pinus taeda* L.) seedling morphological development during drought hardening. Phase 0 (onset of hardening, week 0) is an actively growing seedling with needles exhibiting a lush, green color, feather-like feel when moving one's hand across the foliage, and more than 50 percent of the root system is unsuberized with a white color. In Phase 1 (occurring by week 2), seedling needles start to lose their green luster and roots show initial stages of suberization on the upper portions of the plug. Phase 2 (occurring by week 3 to 4) is characterized by light green needles that exhibit initial cuticle development and have a slightly stiff feel; also, less than 25 percent of the root system shows an unsuberized white color. In Phase 3 (occurring by week 5), needles are light green and exhibit full cuticle development with a stiff feel, plus 100 percent of the root system shows brown, suberized roots. (Photos by Steven C. Grossnickle)

progression as seedlings transition from the growing phase into the hardening phase. For example, container-grown loblolly pine seedlings typically go through a series of drying cycles (i.e., watered to saturation and allowed to dry to a defined container weight) with an initial dry down to 60-percent container capacity, followed by progressively lower levels over 3 to 5 weeks until reaching 40-percent container capacity and a mid-day shoot water potential of -1.5 MPa (figure 2a). These drying cycles are intended to expose seedlings to drought stress that comes near, but does not exceed, the shoot wilting point (Landis et al. 1999). A standard operational monitoring practice for certain species is to wait until 10 percent (Kiiskila, personal communication), 25 percent (Grossnickle et al. 1991), or even up to 40 percent (Grossnickle, personal communication) of the crop has shoot tip wilting before rewatering. A minimum predawn water potential of -1.0 MPa (Lavender and Cleary 1974) or daytime readings between -1.2 and -1.5 MPa (Cleary 1978), or even as low as -1.5 to -1.7 MPa (Landis et al. 1999, Tinus 1982) over a series of stress events was sufficient to terminate shoot growth and develop stress resistance in conifer species. When seedlings are fully hardened, the crop will not show shoot system wilt during a drought event (Grossnickle, personal communication). If water stress is too severe or too rapid during these drying cycles, it impedes the physiological development of drought resistance (Cleary 1978). Avoiding rapid development of water stress is critical to ensure this is an effective cultural practice.

Vapor pressure deficit is another environmental variable related to the plant-water balance and can be used to harden seedlings. Seedlings harden with exposure to the combination of lower available soil water and higher vapor pressure deficit (Larcher 1995). These conditions will cause moderate plant water stress and reduced photosynthesis (Grossnickle 2000, Kozlowski et al. 1991) which can slow or stop seedling growth (Grossnickle 2000, Kozlowski 1982) and help harden seedlings for reforestation site conditions (Landis et al. 1999).

The use of water stress is not always successful in hardening seedlings within an operational nursery environment (Landis et al. 1989). First, there is difficulty in implementing a uniform drought treatment due to differences in irrigation coverage and variation in individual seedling water use. Second, when standard peat-based growing media dry, they can become hydrophobic, making it difficult to rewet and thereby causing uneven exposure to the drying regime. To avoid or overcome media becoming hydrophobic, it is important to overwater after a drought-stress treatment to ensure all cavities are fully saturated (Kiiskila, personal communication). Third, species differ in development of drought resistance (Abrams 1988), making it difficult to apply water stress as a universal hardening treatment across all species. Thus, it is important to monitor water stress treatments to ensure they are applied uniformly and result in successful hardening.

Question 3 – What Nursery Cultural Strategies Promote Vigorous Root Growth?

A well-developed, functional root system is critical for outplanting success (Grossnickle 2005, 2012; Grossnickle and MacDonald 2018a). Quality root systems readily uptake water and nutrients and give structural support to the seedling. Measures of root quality include mass, shoot-to-root ratio, form, length, fibrosity, root growth potential, and nutrient/carbohydrate content (Davis and Jacobs 2005, Haase 2011a). Although the root system is not easily observed compared with the shoot due to its belowground nature, attention to root morphology and physiology in the nursery are imperative to help ensure good field performance. When working with growers, Landis (2008) often referred to seedlings as a “root crop” to emphasize the importance of good-quality root systems.

For the most part, nursery strategies for developing vigorous seedling root systems are inextricably linked with strategies for promoting overall plant quality. For instance, root vigor is tied to the transfer of photosynthates from the shoots (Binder et al. 1990, Philipson 1988, van den Driessche 1987). To achieve target specifications, the grower must consider the phenological cycle for the species and seed source (figure 1), along with environmental patterns at the nursery. As such, growing regimes must be tailored to stocktype (i.e., container type, size, depth, and density, seedling age, and outplanting season) and its associated target specifications for the outplanting site conditions. For example, some species (e.g., pine) are strongly taprooted and tend to not generate lateral roots in the upper part of the root system. In a nursery setting, however, development of lateral roots and numerous root tips is a primary goal for ensuring root egress and vigor after outplanting (figure 4). In studies with overwintered spruce (*Picea* spp.) seedlings, root hydraulic conductivity increased with new root growth because newly developed roots have low root resistance and high water uptake capability during the first few weeks after thawing (Colombo and Asselstine 1989, Grossnickle 1988). Thus, alleviation of planting stress depends on the number of new roots a seedling develops just after planting (Grossnickle 2005).

To encourage a quality seedling with well-developed roots, the grower must sow seed into a well-drained container growing medium (or bareroot seedbed) with adequate aeration (Landis et al. 1990) during temperature and moisture conditions suitable for germination and rapid root elongation. Irrigation is one of the most useful culturing tools in any nursery and can make all the difference between the production of high-quality or low-quality plants. Irrigation based on the plant's transpirational demands, target water content, and seedling growth phase is far more effective and efficient than irrigation on a set schedule (Dumroese et al. 2015). The best irrigation programs always involve watering to saturation and then allowing a dry down sufficient to ensure good root aeration. High irrigation levels tend to result in higher shoot-to-root ratio (Moser et al. 2014) and proliferation of pathogens and other pests (Dumroese and Haase 2018). Similarly, excessive fertilizer, especially nitrogen, promotes excessive shoot growth and an unbalanced shoot-to-root ratio (Landis et al. 1989).

Figure 4. Good quality seedlings have vigorous roots that egress rapidly after outplanting, such as the Douglas-fir container seedling. (Photo by Diane L. Haase 2013)

Proper timing of nutrient and water deprivation to induce budset and hardening correlates with the push to generate stem diameter and root growth in the fall before temperatures drop and all growth ceases. This phase is critical for achieving target height-to-diameter and shoot-to-root ratios. Quality container-grown seedlings have root plugs with good integrity such that the plug is readily extractable and stays together during, lifting, handling, storage, and planting. Root development should be adequate to fill the plug and hold the growing medium, but care must be taken to not create a rootbound condition (South and Mitchell 2006). After outplanting, rootbound seedlings may have poor root egress, root deformation, slowed growth, instability, and/or reduced survival. This issue can be avoided with careful attention to sow date, container size, irrigation, and fertilization.

Root pruning is another tool to manipulate root architecture and function. For container seedling production, the use of containers with copper-coated walls chemically prunes elongating roots and increases the proliferation of a fibrous root system within the plug (Sword-Sayer et al. 2009, Tsakaldimi and Ganatsas 2006). For bareroot seedling production, nursery growers prune roots horizontally (i.e., undercutting or wrenching) or vertically (sidecutting) (Landis 2008, Riley and Steinfeld 2005). When applied and timed properly, bareroot root culturing results in a more compact, fibrous root system at the time of lifting for both conifer (Dierauf et al. 1995) and hardwood (Schultz and Thompson 1997) seedlings. This practice is also used to create a mild stress event to control height growth (Buse and Day 1989), induce bud formation (van Dorsser and Rook 1972), and mitigate soil compaction (Miller et al. 1985).

Question 4 – When and How Long Can Storage Be Used For "Hot-Lifted" Seedlings?

Hot-lifted seedlings used for summer or fall planting have usually developed a "hard bud" that will not break even if the seedlings are exposed to optimal environmental conditions (MacDonald and Owens 2006, 2010), although they are still growing roots (Ritchie and Dunlap 1980, Ritchie and

Tanaka 1990) and developing drought resistance (Abrams 1988, Teskey et al. 1984) and freezing tolerance (Weiser 1970). Thus, hot-lift seedlings are still physiologically active at planting and require unique handling procedures.

In Western Canada and the United States, hot-lifted seedlings are commonly planted in two distinct periods: the first being late June through July (summer planting), and the second being mid-August through early October (fall planting). Seedlings for both summer and fall planting programs are subject to the same cultural hardening practices at the nursery (see Question 1) and are in a similar phenological state at the time of planting. Thus, physiological hardiness is similar between summer- and fall-planted seedlings and any field performance differences are generally associated with environmental conditions during and after planting (Pikkarainen et al. 2020).

Handling and storage practices can affect quality of hot-lifted seedlings (Binder and Fielder 1995, DeYoe 1986, Landis et al. 2010). In particular, temperature conditions will influence maintenance respiration; each 10 °C (18 °F) increase approximately doubles the respiration rate (Kramer and Kozlowski 1979). The temperatures inside closed boxes can quickly increase, causing hot-lift seedlings to use more of their stored carbohydrates (Landis et al. 2010). Thus, hot-planted seedlings must be kept cool to maintain their vigor. After harvest, hot-lifted seedlings should be kept in a nursery cooler and/or refrigerated trailer at 2 to 10 °C (35 to 50 °F) prior to shipment, with 2° C (35 °F) being the ideal short-term storage temperature (Grossnickle personal communication). Depending on seed source, species, and nursery hardening regime, seedlings in both summer and fall planting programs develop some degree of cold hardiness after budset (Bigras et al. 2001) and can easily withstand cold storage temperature conditions.

Properly hardened summer/fall planted seedlings can be safely cold stored for approximately 4 weeks prior to outplanting without any chilling requirement (Jackson et al. 2012). Cultural practices to induce hardening (i.e., water stress and low N) resulted in container-grown loblolly pine seedlings being able to withstand 4 to 6 weeks of cold storage without prior chilling hours (Grossnickle and South 2014). While it is possible to safely hold hot-lifted seedlings for a maximum of 4-weeks, it is contingent on maintaining a 2 °C (35 °F) storage temperature; safe storage duration decreases with increasing storage temperature (Paterson et al. 2001).

Figure 5. Refrigerated trailers are required for transporting large quantities of hot-lift seedlings from the nursery and are ideal for short-term cool seedling storage prior to planting. (Photo by Steven B. Kiiskila 2010)

Shipping hot-lifted seedlings to the outplanting site occurs in refrigerated trailers with temperatures below 10 °C (50 °F) (Dunsworth 1997, Stjernberg 1997). Upon arrival at the planting site, seedlings may be kept in a refrigerated trailer (figure 5) or transferred to a field cache in a shady location and/or under a suspended tarp with boxes opened to prevent heat buildup (Kiiskila 1999, Landis et al. 2010). During summer and fall months, moisture stress might occur; therefore, seedlings need to be monitored and irrigated if required (Landis et al. 2010). Under these conditions, only enough seedlings for 1 day of planting should be transported to the site. Alternatively, hot-lifted seedlings have been stored at 4 to 21 °C (40 to 70 °F) in refrigerated trailers on the planting site for up to a week (Dumroese and Barnett 2004), although lower temperatures between 2 to 4 °C (36 to 39 °F) are recommended (Landis et al. 2010). The full storage duration for hot-planted seedlings includes time spent in the nursery's cold storage facility and time spent in storage away from the nursery (e.g., in refrigerated trucks or other off-site holding areas). The combined length and care for all of these handling and storage steps is critical to ensure quality seedlings are outplanted.

Question 5 – How Long Can Seedlings Be Overwintered in Refrigerated Storage?

Overwintered spring plant seedlings are harvested in the fall once dormant and most commonly held in refrigerated storage until shortly before planting. Refrigerated storage is differentiated by temperature into cooler (1 to 2 °C) or freezer (–2 to –4 °C) storage, with the storage practice dependent on species' tolerance to freezing temperatures, available facilities, and expected storage duration (Grossnickle and South 2014, Landis et al. 2010).

Properly hardened and dormant seedlings (see Question 1) can be lifted in late fall and early winter and stored well into the spring for planting (Camm et al. 1994, Ritchie 1987). A dark and cold or frozen environment, however, is an unnatural environment for seedlings. The lack of light in storage prevents seedlings from replenishing carbohydrates lost through respiration (Ritchie 1987) and interrupts the seedling's circadian rhythm (Camm et al. 1994, Lavender 1985). Seedlings lifted and stored correctly are rarely damaged by cold or frozen storage, though some plant deterioration can occur as storage time lengthens (McKay 1997). Both cold and frozen storage conditions, when managed properly, allow properly hardened seedlings to maintain their physiological integrity required for good seedling quality (Landis et al. 2010). This is critical because quality seedlings typically have vigorous rooting at planting, which is required to overcome planting stress (Grossnickle 2005), thus increasing chances for successful seedling establishment (Grossnickle 2012).

Cold storage is a cultural practice where seedlings are held at 1 to 2 °C (35 to 36 °F) for no longer than 2 months (Landis et al. 2010, Ritchie 2004). Increasing cold storage can result in decreases in days to bud break (DBB), root growth potential (RGP), freezing tolerance, and carbohydrates (Grossnickle and South 2014). Extended exposure to cold temperatures and high humidity during cold storage creates conditions for storage molds (Camm et al. 1994, Hocking 1971, Landis et al. 2010, Ritchie 2004). Treating seedlings with appropriate fungicides prior to storage can improve seedling storability (Barnett et al. 1988), though their beneficial effects diminish as cold storage lengths reach 2 or more months (Grossnickle personal communication). Managers holding seedlings in cold storage should monitor stored seedlings regularly to detect problems.

Frozen storage is a cultural practice where seedlings are held at -2 to -4 °C (25 to 28 °F). This below-freezing storage temperature further slows physiological changes in the seedlings, thereby allowing them to be stored longer compared with cold storage. Frozen storage temperatures should not drop below -5 °C (23 °F), however, because some species are susceptible root damage at lower temperatures (Bigras et al. 2001, Kooistra 2004). Seedlings harvested at the correct phenological stage, and thus in a state of maximum stress resistance, are usually freezer stored for 4 to 6 months (Grossnickle 2000, Kooistra 2004), though seedlings have been successfully freezer stored for up to 8 months (Helenius et al. 2005, Luoranen et al. 2012). Once planted, seedlings are in a state of ecodormancy whereby the chilling requirement has been met and buds will break after exposure to favorable temperatures and begin the yearly cycle of growth (Burr 1990, Haase 2011b, Lavender 1985).

Two issues should be considered with regard to freezer storage effects on seedling quality. First, seedlings are still physiologically active (albeit at a low level), which is reflected in the decrease in DBB, RGP, freezing tolerance, and carbohydrates as the storage duration lengthens (Grossnickle and South 2014, Landis et al 2010). Second, the low humidity in freezer storage prevents storage molds (Haase and Taylor 2012, Hansen 1990, Trotter et al. 1991) but can desiccate seedlings with excessive storage duration, which may lead to reduced root growth potential (Deans et al. 1990). Packaging frozen-stored seedlings in a plastic bag or a poly-lined paper bag inside a waxed box minimizes seedling desiccation (Kooistra 2004), although seedlings may still lose up to 10 percent water content after 5 or more months in frozen storage (Lefevre et al. 1991).

The thawing process prior to planting should also be considered in conjunction with frozen storage duration (figure 6). While frozen seedlings were originally thawed slowly over a period of weeks, it has been shown that slow thawing causes seedling quality to decrease with increasing thawing duration (Silim and Guy 1997), and rapid thawing within a matter of days maintains seedling quality (Rose and Haase 1997). Thus, thawing seedlings as quickly as possible is now recommended (Landis et al. 2010). Rapid thawing is also preferred because it prevents

Figure 6. Rapid thawing of frozen seedlings in closed boxes can be done by spacing stacked boxes in a warm location without direct sunlight and rotating the boxes top to bottom. (Photo by Steven B. Kiiskila 2009)

storage molds (Rose and Haase 1997), minimizes depletion of carbohydrates (Silim and Guy 1997), and results in seedlings having later bud break and greater frost hardiness at time of planting (Camm et al. 1995). Seedlings can also be planted frozen without thawing, but must be individually wrapped at harvest such that seedling plugs can be separated from one another while frozen (figure 7). Eliminating the thawing process requires more effort in the nursery at lifting but offers operational flexibility during the busy planting window. Research to date has shown no deleterious physiological effects of planting frozen seedlings (Camm et al. 1995, Kooistra and Baaker 2005), although limiting site environmental conditions at the time of planting such as dry cold soils can have a negative effect (Helenius 2005).

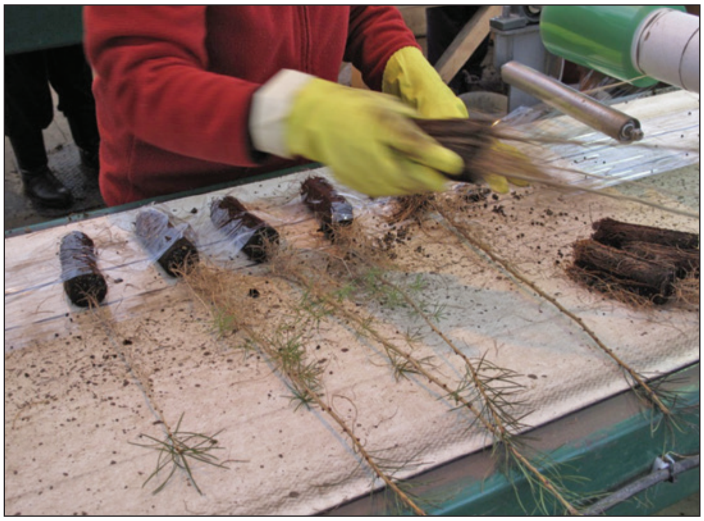

Figure 7. One method to enable separation of frozen seedlings from one another without damaging their roots is to place poly wrap around each seedling's root plug when bundled after lifting. (Photo by Steven B. Kiiskila 2010)

Long-term frozen storage for "late" spring planting may result in seedlings being initially out of sync with the annual growth rhythms of the planting site. That is, planted seedlings may have budbreak patterns that do not reflect that of natural seedlings on the planting site (Grossnickle 2000). Seedlings require sufficient time to complete the growth processes initiated with budflush and begin development of hardiness before the onset of fall frosts. The potential risk of fall frost damage to seedlings at different planting dates can be estimated through analysis of long-term climatic data (Hänninen et al. 2009). Delaying spring planting into early summer increases the likelihood of bud break and shoot elongation when the site environment has warm temperatures, high vapor pressure deficits, and dry soils (Grossnickle 2005, Mitchell et al. 1990). As such, the site may not be suitable for planting until late summer/fall and may be more appropriate for planting with hot-lifted seedlings (see Question 4).

Conclusions

Seedlings are not widgets; they are biological organisms that respond to their surrounding environment. Nursery cultural practices have a direct influence on the seedling environment, thereby influencing seedlings' physiological function and subsequent morphological development. This discussion shows that all nursery cultural decisions, from hardening practices, to strategies that promote vigorous root growth, to storage practices affect seedling development. Understanding how cultural practices affect seedling performance will ensure that the nursery practitioner develops sound practices that enhance seedling quality and subsequent success of forest restoration programs.

Address correspondence to—

Steve Grossnickle, Nursery*To*Forest Solutions, 1325 Readings Drive, North Saanich, BC, Canada, V8L 5K7; email: sgrossnickle@shaw.ca; phone: 250-655-9155.

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The Role of the Seedling Nursery in Helping Its Reforestation Clients

Dennis M. Farquharson

Registered Professional Forester, GRO TRZ Consulting Inc., Barriere, BC, Canada

Abstract

To be successful in their work, reforestation managers rely on a variety of information sources to acquire the information they need to understand seedling physiology and make good management decisions. Nursery managers and growers can be a great source of information and critical allies to the reforestation manager, helping them to achieve high rates of survival and optimum growth after field planting. This paper was presented at the 2019 Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Associations (Sidney, BC, September 30-October 2, 2019).

Managing a reforestation project may seem like a simple endeavor: bring together seedlings, planters, and a reforestation site, and voila… Right? Anyone who has ever managed a reforestation project is grinning and shaking his or her head in disagreement. Reforestation might look that easy from a distance, but it is not. A successful reforestation manager is a facilitator who brings together the necessary knowledge, partners, and resources in the correct time and space, so that each piece and phase of the project supports the next, ultimately resulting in the establishment of a well-growing, young forest (figure 1).

Reforestation managers can hire planting contractors and order seedlings, but where do they get all of their "necessary knowledge" to manage a successful reforestation program? They only get some of it from college. After college, the lucky ones start their real learning as an assistant to a reforestation manager, during which time they can benefit from the manager's experience. Others are tossed directly into the deep water as a reforestation manager and must learn as they go. There is a huge amount of information about reforestation practices available from government publications, university extension agents, and forestry research articles, but searching the web to find pertinent articles and then reading them takes a lot of time. Today's challenge is time, and the workday for any reforestation manager is loaded with a long list of must do's.

Reforestation managers can also gather information at workshops and conferences, and can turn to experienced reforestation partners such as nursery managers, seedling growers, and extension specialists to ask questions and increase their understanding about such concerns as why seedlings are not growing well, or if a certain species/stocktype combination would be appropriate for a particular reforestation situation. These professionals are the people that the reforestation manager can contact for a detailed understanding about seedling physiology and how to help seedlings grow well at the reforestation site. For the nursery manager/grower, it is important to remember that reforestation clients will usually continue to purchase seedlings from your nursery when they are successful with their

Figure 1. Reforestation managers must understand various impacts on seedling growth and survival to be able to be able to duplicate a favorable outcome and avoid the unfavorable outcome. For example, these 1-year-old lodgepole pine seedlings, both from the same seedlot and planted by the same planter, were planted in different microsites resulting in differing field performance. (Photo by Dennis Farquharson)

Figure 2. A high-quality seedling from the nursery planted in favorable conditions is a win-win for both nursery managers/growers and reforestation managers. This 2-year-old, nursery-grown Douglas-fir seedling is doing very well after outplanting. (Photo by Dennis Farquharson)

seedlings (figure 2). A positive relationship between nurseries and reforestation managers, characterized by good communication, is critical to reforestation success (Haase 2014).

In addition to seasonal questions and conversations between nursery and reforestation managers related to seedling cost, over-sow factors, seedling performance, crop status, seedling balance, hardiness, and overruns; ongoing questions can arise regarding broader plantation survival, establishment, and growth performance. Some important questions that reforestation managers want to discuss with nursery managers and other plant professionals with regard to reforestation opportunities are:

- Are there growth benefits from selective microsite planting? If so, what are the features of a preferred planting microsite and what sort of gain should be expected?
- Does prompt planting benefit the seedlings due to the population of mycorrhiza or other beneficial organisms in the soil? If so, what degree of benefit can be expected? Will that benefit decline over time and if so, over what duration will that occur?
- Due to climate change and seemingly more frequent drought events, should we be considering a different stock-type to improve the reforestation success? Also, would planting the same, or a different, stock-type at a different time of the year improve the reforestation success?

Other things nursery managers can do to support their client (the reforestation manager) are:

- Get to know them and become part of their professional network.
- Invite them to view their seedlings at the nursery.
- Contact them to discuss sowing dates, germination rates, and species stock-type selections.
- Offer to stop by their work for a visit and maybe a tour of some recently planted seedlings, or a reforestation challenge.
- Look for opportunities to streamline or reduce their workloads. This could be something as simple as reducing the number of invoices annually, modifying box labels to highlight relevant information, or helping to coordinate seedling transportation. It could also be something significant like individually wrapping frozen seedlings to increase the flexibility and reduce the management of thawing and shipping for planting.
- Make environmental adjustments that reduce the use of packaging, pesticides, etc.

Ultimately, the active participation of the nursery team as members of the reforestation team will support the reforestation manager and positively influence the reforestation project (figure 3).

Figure 3. Ultimately, good communication and an abundance of knowledge and experience results in a team of nursery and field professionals that can ensure reforestation success such as this healthy, 6-year-old Douglas-fir seedling. (Photo by Dennis Farquharson)

Address correspondence to—

GRO TRZ Consulting Inc., Attn: Dennis M. Farquharson, RPF, Barriere, BC, Canada; email: grotrz@telus.net

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Could DNA-Based Detection Technology Help Prevent Conifer Seed-Borne Pathogen Diseases?

Nicolas Feau, Joey B. Tanney, and Richard C. Hamelin

Research Associate, The University of British Columbia, Forest and Conservation Sciences, Vancouver, BC, Canada; Research Scientist, Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, Victoria, British Columbia, BC, Canada; Professor, The University of British Columbia, Forest and Conservation Sciences, Vancouver, BC, Canada and Faculté de foresterie et géomatique, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada.

Abstract

Pathogens, often carried in seeds, can cause substantial economic losses to forest nurseries and put at risk the large investment in genetically selected seeds, as well as endanger reforestation efforts and, therefore, future forests. Mitigating plant health problems relies on rapid detection and identification of causal agents. Traditional detection protocols, however, rely on symptom manifestation, but many fungal pathogens exhibit a prolonged asymptomatic phase within their hosts. DNA-based detection assays based on the real-time polymerase chain reaction (qPCR) are among the most accurate, rapid, and cost-effective methods for detecting pathogens at the species level. The development of a DNA detection system for seed-borne pathogens would increase accuracy and speed in determining if seedlots are contaminated above an acceptable level and would help forest nurseries to make cost-effective management decisions. This paper was presented at the 2019 Joint Annual Meeting of the Forest Nursery Association of British Columbia and the Western Forest and Conservation Nursery Associations (Sidney, BC, September 30-October 2, 2019).

Introduction

North American forests must meet the public's socio-ecological and economic needs while simultaneously overcoming contemporary health challenges. To answer reforestation demands, British Columbia (B.C.) annually produces more than 200 million conifer seeds and seedlings with improved growing performance. However, adverse climate, pests, and diseases represent major threats to the sustainable supply of forest tree products. Seeds and seedlings are especially susceptible to diseases, which can be exacerbated by their environment, such as extreme temperatures, and water and mechanical stresses. As a consequence, seeds and seedlings also constitute a pathogen source representing an inconspicuous, yet significant, phytosanitary risk. For example, nursery trade of asymptomatically infected white pine (*Pinus strobus* L.) seedlings resulted in the introduction of the white pine blister rust (*Cronartium ribicola*) at the beginning of the 20th century in North America (Geils et al. 2010). Similarly, the trade of pine and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seeds and planting stock is of high risk, as these could contribute to global spread of the pathogen *Fusarium circinatum*, the fungal agent responsible for pine pitch canker (Cleary et al. 2019, Evira-Recuenco et al. 2015, Storer et al. 1998;). This aggressive fungus, recommended for quarantine regulation in Europe (Vettraino et al. 2018), is present in the United States and is a potential threat to Canadian forests.

In the current context of trade globalization, the risk of disseminating non-native and potentially invasive pathogens via seed exchange is increased (Burgess and Wingfied 2002, Cleary et al. 2019, Elmer 2001, Franić et al. 2019). Replacing genetically improved seed trees can take years, and the high cost of producing these seeds makes even small losses due to disease unacceptable. Seed and seedling diseases can cause substantial economic losses to nurseries and endanger reforestation efforts, and, therefore, future forests. With increasing seed losses reported across B.C. forestry nurseries, it is critical that we improve our capacity for identifying, detecting, and mitigating seed and seedling diseases to secure the renewal of tomorrow's forests. Therefore, new approaches and tools are needed to prevent and mitigate seed and seedling losses that are emerging at critical phases within the forest renewal cycle.

Forest Seed Pathology: Battling the Unknown

A plant disease prevention program relying on pathogen detection requires, as an initial step, a priori knowledge of which organisms to target. Unfortunately, there are fundamental knowledge gaps in the current understanding of the microbial pathogens responsible for conifer seedling losses. Despite their historical and contemporary significance, the basic etiology and transmission processes of many important pre-emergent and post-emergent conifer seedling diseases remain poorly understood. Root rot and damping off of seedlings are among the most frequent diseases observed in tree nurseries, and are responsible for massive crop failure and economic losses. However, while root rot and damping off have been attributed to many common rhizosphere (root and soil) fungi (e.g., *Fusarium* and *Cylindrocarpon* spp.) and oomycetes (e.g., *Pythium* spp.), only a few species have been clearly associated with conifer diseases (Kope et al. 1996, Rossman et al. 2007).

Fungal species of the *Fusarium* genus are important causal agents of damping off and root rot in Douglas-fir seedlings. These species are known to be ubiquitous in most container and bareroot nurseries, occurring in soil, seeds, and roots and needles of asymptomatic and diseased Douglas-fir seedlings (Alexrood et al. 1995, Stewart et al. 2012). However, the biology of the diverse *Fusarium* species associated with Douglas-fir seeds and seedlings is still poorly explored. Particularly, the mode of infestation of Douglas-fir seeds remains uncertain. Two possibilities are that seed colonization occurs through systemic invasion from the mother plant vascular tissues to the embryo, or from seed-coat surface contamination from exterior cone parts. Historically, *F. oxysporum* was considered the most important cause of Fusarium root rot in Douglas-fir seedlings, but a direct relationship between this species and seedling mortality was never convincingly demonstrated. Moreover, specific strains of *F. oxysporum* are known to be benign to Douglas-fir seedlings and can even protect them from other virulent *Fusarium* species (Dumroese et al. 2012). Recent studies indicate that the highly virulent *F. commune*, a species closely related to, but distinct from, *F. oxysporum* is probably one of the major causes of disease in Douglas-fir seedlings (Kim et al. 2012, Stewart et al. 2012). That species, however, does not seem to be ubiquitous among conifers in forest nurseries, making it unlikely that it is the only problematic seed-borne *Fusarium* species in Douglas-fir. In a preliminary survey of 67 Douglas-fir seeds, we identified seven *Fusarium* species, including *F. proliferatum* (pathogenic on Douglas-fir seedlings) and *F. oxysporum*, but we never found *F. commune* on Douglas-fir or any of the four other conifer species included in the survey (figure 1).

Usually, a contamination level by *Fusaria* of greater than 5 percent within any conifer seedlot is considered to be significant for disease potential, and may consequently provoke pest-management actions (Peterson 2008). Systemic infestation of conifer seed, as well as surface contamination during seed development and management, by pathogenic *Fusarium* species makes testing for their presence a relevant step for managing it as a seed-borne organism (Peterson 2008). The presence of *Fusarium* on seedling roots in the absence of any disease symptoms is generally insufficient grounds for rejecting seedlings scheduled for outplanting. Potentially pathogenic fungi can, however, rapidly spread from seedling to seedling, as well as intensify within the roots of infected seedlings (Kope et al. 1996). When outplanted, systemically infected seedlings can have reduced performance and quickly succumb to planting shock and, if exposed to a subsequent heat or drought stress, will often die.

Management of Fusarium disease in forest nurseries could be greatly enhanced by accurate identification of the *Fusarium* species. Given their diversity and their functional variability, a clear identification of problematic species and establishment of causality between the presence of seed-borne species and seedling disease is required. Being able to differentiate pathogenic species from innocuous ones would enhance early-stage testing, thereby allowing rejection of seed lots contaminated with truly pathogenic species.

Opportunities for Development of a Pathogen-Detection System

A traditional approach to the complex problem of identifying and detecting fungal pathogens uses classical phytopathological concepts that rely on the combination of culture-based surveys and microscopy techniques. Although reliable, such approaches require high standards of knowledge in mycology and plant pathology, and are usually time-consuming, necessitating, in some cases, several weeks before being able to establish a proper diagnosis. So far, testing for conifer seed health in B.C. is carried out by the Plant Health Laboratory of the B.C. Ministry of Agriculture (Abbotsford, BC) using a culture-based method. The assay consists of plating and incubating subsamples of seeds on fungal-specific media and is successful in testing seedlots for the presence and rate of infection of three major seed-borne pathogens, including *Fusaria*. Identification of the cultures, however, relies only on morphological characters of spores, thus limiting the identification of *Fusarium* pathogens to the genus level only. Another limitation of cultural approaches is that they target fungi

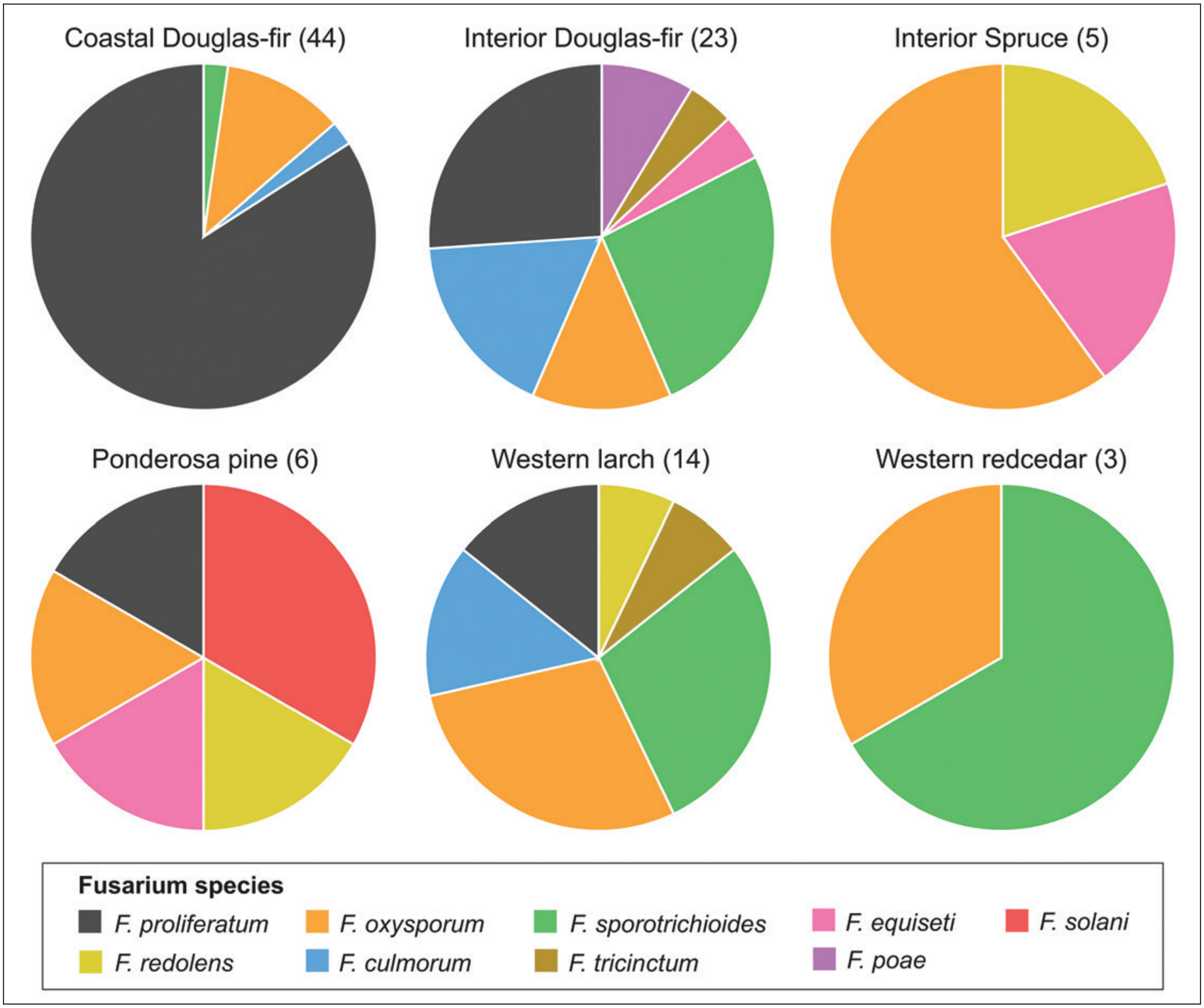

Figure 1. *Fusarium* species isolated from a preliminary survey of conifer seed-associated fungi in B.C. seedlots. *Fusarium* cultures were obtained by plating seeds on *Fusarium*-specific media at the Plant Health Laboratory of the B.C. Ministry of Agriculture (Abbotsford, BC) and cultures were identified using a DNA-barcoding approach. Numbers between brackets indicate the number of seeds tested for each conifer species.

that produce fruiting bodies or can be easily cultured on synthetic and semi-synthetic growth media. Many fungi (particularly endophytes; e.g., White and Cole 1986) do not sporulate in culture, making visual identification challenging. Many other fungi are notorious for being difficult or impossible to cultivate on culture media; good examples are the powdery mildew and smut fungi.

Over the last few decades, several new approaches have been developed for plant pathogen identification and detection. The use of monoclonal antibodies and enzyme-linked immunosorbent assays (ELISA) drastically increased the speed in which pathogen antigens could be detected in vivo. For example, this technique has been used for routine detection of the seed-borne fungal pathogen *Sirococcus conigenus* in spruce (*Picea* spp.) seedlots (Mitchell and Sutherland, 1986). However, ELISA assays have three major limitations: (1) immunological tests require the availability of an antibody that properly responds to a target pathogen; if it is not available, this requires extensive work to develop such an antibody; (2) the antibodies used to recognize proteins that are supposed to be unique to the targeted organism can sometimes cross-react with other species, resulting in false positives and therefore a lack of specificity (Kox et al. 2007, Luchi et al. 2020, Martinelli et al. 2015); and (3) antibody-based tests often lack sensitivity, which is frequently a problem when dealing with plant pathogens.

Innovations in genomics and molecular biology have provided a new toolbox that can address pathogen identification and detection challenges. The polymerase chain reaction (PCR) generates in a single in vitro reaction several million copies of “diagnostic” DNA region(s) located on the pathogen’s genome. This method has the advantage of being sensitive, specific, and quick. PCR-based tests can be conducted by a broad range of users because they require less knowledge and expertise in mycology than classical culture-dependent approaches. In the context of plant-pathogen diagnostics, PCR-related methods can be used in two ways, i.e., for pathogen identification and for pathogen detection. The DNA-barcoding method uses a short, standardized DNA marker providing a high, interspecific variability (i.e., the sequence is different from those found in individuals from other species) and low intraspecific differences (i.e., the sequence is identical in individuals of the same species) that enables the identification of organisms at the species level (Hebert et al. 2003). Usually, the selected marker is present in several copies (e.g., about 100 tandem repeats per nucleus for the nuclear ribosomal internal transcribed spacer locus [ITS], formally selected as the universal DNA-barcode marker for fungi; Schoch et al. 2012) in the genome of the targeted organism, allowing a high sensitivity of the PCR amplification. DNA-barcoding has proved to be effective in identification and surveys of forest pathogens (Feau et al. 2009, 2011; Hidayati et al. 2014; Shestibratov et al. 2018).

The genetic variation within DNA barcodes has also been widely translated into taxon-specific rapid and sensitive detection assays using PCR and has been applied to forest pathogen detection (Vincelli and Tisserat 2008). Specifically, real-time PCR using TaqMan probes has become the gold standard in forest pathogen detection. The principle of this technology relies on a fluorescently labeled probe designed so that it hybridizes only to its target DNA sequence and releases a fluorescent signal (detected by the PCR machine) when the target site is amplified during PCR. TaqMan real-time PCR constitutes the most sensitive, specific, and rapid method available, and has been used to detect many forest pathogens in quantities as low as one single fungal spore (Bergeron et al. 2019, Feau et al. 2019, Lamarche et al. 2015).

Using real-time, PCR TaqMan technology has several benefits. The increase in genomic resources brought by next-generation sequencing makes it possible to mine entire genomes of plant pathogens to identify genes or genomic regions of higher discriminatory power than the conserved genes traditionally used to develop real-time PCR assays. Once identified, these unique genes can be translated into TaqMan probes of high specificity, reducing the risk of false positives (Feau et al. 2018). Another advantage of TaqMan-based detection is the possibility of combining (i.e., “multiplexing”) several probes in the same PCR reaction. Probes can be labeled with different distinguishable fluorophores, which allows amplification and detection of two to four distinct sequences in one reaction tube. Probes targeting different taxonomy levels (e.g., *Fusarium proliferatum* species; *Fusarium* genus; Nectriaceae

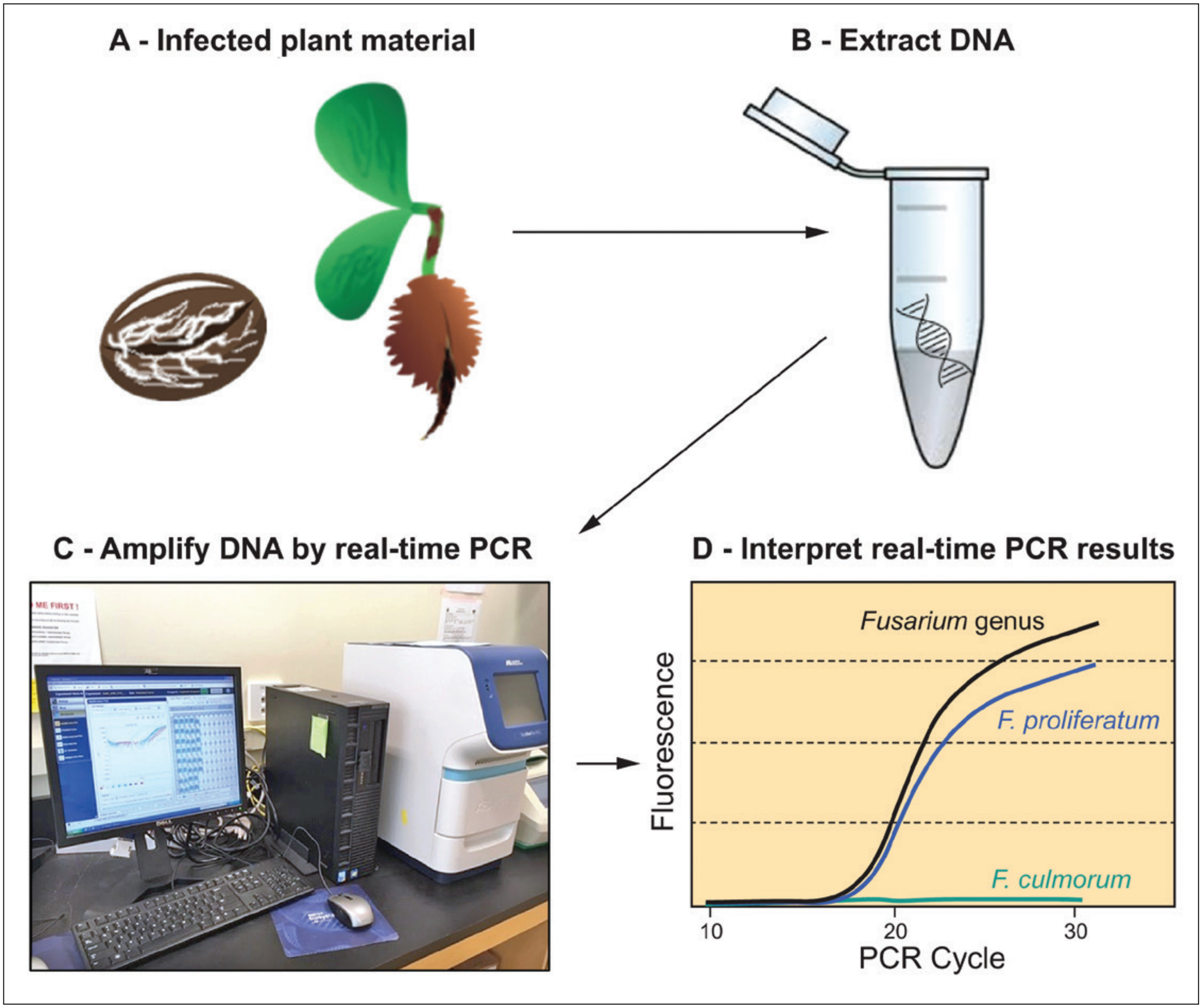

Figure 2. Pathogen detection theoretical workflow. (a) Infected material such as germinated and non-germinated conifer seeds is identified and collected, then (b) DNA is extracted from the infected material. (c) The extracted DNA is amplified by PCR in a real-time PCR machine. (d) TaqMan fluorescence curves produced in real-time PCR for three multiplexed detection assays tested on a DNA sample extracted from seeds. Each curve represents the accumulation of a PCR product (for a targeted gene) over the duration of the real-time PCR experiment. The test is positive for the *Fusarium* genus and *F. proliferatum* with an exponential accumulation of PCR products starting after 17 PCR cycles; for *F. culmorum*, no PCR product is accumulated, meaning that DNA of this species was not present in the tested sample. This illustrates the advantages of using multiplexed detection assays: three detection assays are combined in one reaction tube; two *Fusarium* species are targeted at the same time; two taxonomical levels are targeted (genus and species). (Photo by Nicolas Feau 2019)

family) can be combined together in a hierarchical way, providing vertical redundancy (figure 2). Another advantage of multiplexing is to increase redundancy by combining probes with different discriminatory powers. Confidence and reliability of the detection will increase by multiplexing probes of high sensitivity (by targeting a multicopy locus such as the ITS region) with species-specific genes providing a high-detection specificity (Feau et al. 2018, 2019). Multiplexing also allows querying different genome regions with different discriminative power. In the same reaction tube, sensitivity can be increased by targeting a multicopy gene, such as the ITS locus, while specificity of the detection can be achieved by targeting a species-unique gene.

Making a DNA-Based Detection System Operational for Seed-Borne Pathogens

A detection assay targeting seed-borne pathogens should be sensitive, specific, rapid, robust, inexpensive, and simple to implement and interpret (Maddox 1998, Walcott 2003). Depending on the probes designed, real-time, PCR TaqMan technology can provide a high degree of specificity, sensitivity, or both combined. The detection accuracy, however, will still depend on conditions that are inherent to the type of material tested (in this case, conifer seeds and the quality of the fungal DNA purified from these seeds). DNA quality is critical for the overall success of the detection test. Real-time PCR can suffer from the interference with inhibitory compounds found in seed extracts (Demeke and Jenkins 2010). Particularly, the yield and quality of the fungal DNA purified from conifer seeds can be limited by a high content of secondary metabolites. These compounds either impede DNA extraction or limit DNA polymerase activity (Bashalkhanov et al. 2008, Wilson et al. 1997), leading to false negative results. To overcome this problem, several protocols have been developed for plant and conifer tissues to separate pathogen DNA from inhibitory compounds and optimize PCR reaction conditions. For example, the combination of a DNA-enrichment procedure with quantitative PCR (qPCR) facilitated the sensitive detection of *Fusarium circinatum* from pine seeds (Ioos et al. 2009). Other solutions to this problem have been proposed for detection of *F. circinatum* (Dreaden et al. 2012), *Lophodermium seditiosum* (Bentele et al. 2014), and *Diplodia sapinea* (Decourcelle et al. 2015) in seeds. False-negative results caused by PCR inhibition can also be prevented by using a PCR internal control (e.g., a heterologous DNA template with priming sites identical to one of the primer pairs and probe used for the amplification) (Decourcelle et al. 2015, Ioos et al. 2005).

Poor PCR-based detection sensitivity also can result from low sampling intensity. Sample size and sampling methods used for other seed health tests are not necessarily appropriate for PCR-based tests as they might affect the quality and quantity of DNA extracted from seeds and, consequently, the result of the PCR test. One way to address this issue is to first determine the level of tolerance for the target pathogen in a seedlot at which the seedlot is considered to be significantly contaminated (Peterson 2008). Direct testing on seedlots can be done by extracting DNA and testing several individual seeds to determine if this threshold is reached. However, large numbers of seeds need to be tested to reduce the probability of having a Type I statistical error (accepting a seedlot with an actual greater level of contamination than the threshold) or a Type II statistical error (rejecting a seedlot with an actual smaller level of contamination than the threshold), making this approach economically unrealistic. Alternatively, indirect testing on sample units with a determined number of seeds can be carried out to determine if the contamination threshold is reached (Geng et al. 1983). Indirect testing minimizes the number of samples tested (by grouping seed samples in sample units) without affecting the efficiency of the DNA-based detection test. DNA is extracted from several sample units, each having a predetermined number of seeds, and the DNA extract of each sample is tested with the DNA-based detection assay. Several studies showed that fungal DNA can be efficiently extracted and detected from batches of 300 to 400 conifer seeds (Decourcelles et al. 2015, Dreaden et al. 2012). Geng et al. (1983) developed a statistical model providing the size and number of seed units needed as a function of an expected contamination threshold and the sensitivity of the test used (figure 3). For example, supposing that the tolerable disease rate threshold is 5 percent and that the sensitivity of the DNA-based test is 95 percent, we would need to test 6 sample units of 35 seeds each to be 99.99 percent confident that at least one test will result in a positive detection. With a sensitivity of 99 percent (which is more realistic for a DNA-based test), the number would drop to 5 sample units of 30 seeds each to reach the same confidence. Assuming an approximate cost of US$8 per PCR test (including the DNA extraction), testing one seedlot would cost less than US$50.

Conclusion and Perspectives

Traditional laboratory methods for tree pathogen diagnostics are accurate but slow and labor- intensive, requiring specialized personnel with mycological and plant pathology skills. Unfortunately, the availability of such trained staff to perform traditional techniques is in decline worldwide. DNA-based

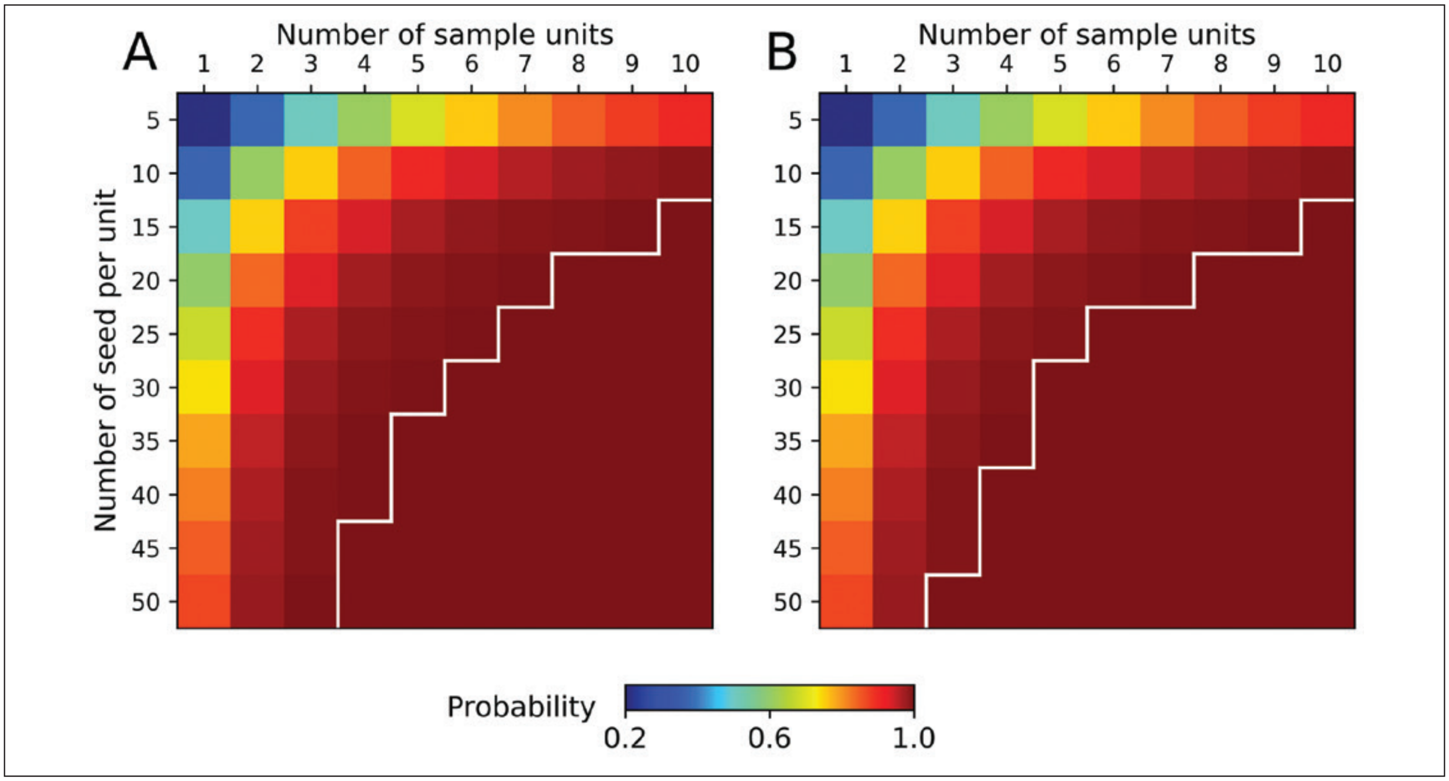

Figure 3. Probability of having at least one test resulting in a positive detection for different combinations of sample units and seeds per sample unit with an assay having a detection sensitivity of (a) 95 percent or (b) 99 percent. Combinations on the right of the white line have probabilities greater than or equal to 99.99 percent of resulting in at least one positive test. (Adapted from Geng et al. 1983)

technology has proven its utility in the rapid identification and detection of plant and forest tree pathogens. Major research progress has been made since the first development of DNA-based diagnostic tests to improve confidence in their results. Real-time PCR has become an established technique for the detection of known target pathogens due to its robustness and accessibility in high-throughput format. This accessibility and the popularity of this technology has driven down costs; real-time PCR is now a generic platform technology in plant diagnostic laboratories, usually exploited as a front-line diagnostic tool in plant health. We envision that this technology holds great potential for improving pathogen detection in conifer seeds, as it embodies many of the key characteristics including rapidity, specificity, sensitivity, and ease of implementation for routine testing on a diagnostic platform. With the implementation of PCR-based seed health testing in the seed and seedling industry, we can expect that this technology will eventually replace the seed detection assays currently employed, providing superior detection capabilities necessary for healthy seedling establishment.

Address correspondence to—

Nicolas Feau, The University of British Columbia, Forest and Conservation Sciences, Forest Sciences Centre - 2424 Main Mall, Vancouver, BC, Canada V6T 1Z4; email: nicolas.feau@ubc.ca

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