# Tree Planters' Notes

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# Dear TPN Reader

I'm pleased to share another issue of *Tree Planters' Notes* (TPN) with all of you!

This issue contains 12 articles and has something for everyone. The articles cover a diversity of topics and range from the Hawaiian Islands, to Morocco, and across the United States. Articles of interest to nursery managers and growers include a study to examine lift date and seed lot of Douglas-fir seedlings (Zhang et al., page 4), a review of iron fertilization in bareroot nurseries (South, page 90), a step-by-step propagation protocol for Hawaiian sandalwood (Speetjens et al., page 34), and an overview of plant hydraulic physiology for nursery applications (Sheridan and Nackley, page 72). Articles of interest to those who plant trees for reforestation and restoration include guidance for seed transfer of white spruce and jack pine (Pike, pages 19 and 26), a technique to plant two seedlings in the same planting hole to reduce browse damage (Murray, page 16), a review of fall-planting considerations for northern forests (Grossnickle and MacDonald, page 59), and a study to assess the use of inverted cups to shade seedling stems on hot, dry sites (Vetter and Haase, page 82). Additionally, Sedia et al. (page 51) describe results of a study to assess the effects of salinity of Atlantic white-cedar, a species threatened by rising sea levels, and Hibilik et al. (page 117) examine mycorrhizal colonization of *Casuarina* trees. Finally, this issue contains the annual report of seedling production in the United States (Haase et al., page 110).

As always, I encourage you to consider submitting an article to TPN. Many authors who have written articles for TPN had never published anything previously, but they had something interesting and worthwhile to share. As long as an article is relevant to the scope of this journal, I never reject it. Together, I work with authors to make necessary revisions such that the final article is clear, concise, and scientifically accurate. Given its applied focus, acceptance policy, assistance to authors, and free distribution, TPN is unique among forestry journals. Recently, an author submitted an article, and I told him it would be several months before it would be published. He replied, "I don't mind because our industry folks really want it to appear in the TPN. Their argument is that publishing somewhere else may not reach the real users; I agree." Feedback like this is what makes it so rewarding to be your editor!

Best wishes for a productive tree growing and planting season ~

Diane

Diane L. Haase

*You can't have the fruits*
*without the roots.*
— Stephen Covey

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

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**Editor: Diane L. Haase**

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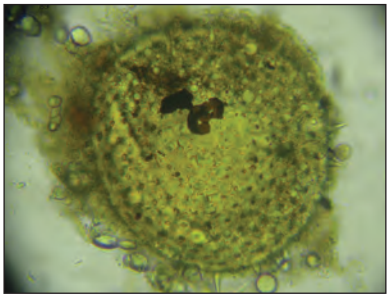

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# Effects of Lift Date and Seed Lot on Field Performance of Containerized Douglas-fir Seedlings

Jianwei Zhang, Thomas Jopson, and Mark Gray

*Research Forester, USDA Forest Service, Redding, CA; Nursery Owner and Manager, Cal-Forest Nurseries, Etna, CA; Reforestation Manager – Coast Cascade, Sierra Pacific Industries, Redding, CA*

## Abstract

To determine the effect of nursery lifting dates on field performance of containerized Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings, we lifted and stored seedlings from 2 seed lots on 9 dates from late October 2019 to early March 2020. We planted the seedlings in a field test and in large pots in April 2020. All seedlings broke bud and grew vigorously. No mortality occurred in the pots, and less than 6 percent mortality occurred in the field test. Bud burst was slightly faster for seedlings lifted after mid-January than for those lifted on earlier dates. We found consistent differences in phenology and growth between seed lots, representing seeds collected from the same seed orchard at different years. Seed lot effects were confounded, however, by differences of sowing time and dormancy induction and must be studied further.

## Introduction

In reforestation or afforestation programs, successful plantation establishment is not possible without high-quality seedlings. Delivering high-quality seedlings requires nursery managers to not only understand how to grow seedlings in nursery beds or in containers in the greenhouse, but also how to lift, pack, store, and deliver seedlings while maintaining quality. Although physiological and biological rationales are well established for these processes (Grossnickle et al. 2020, Haase et al. 2016, Ritchie 1984), very little research has been conducted to verify the relationship between chilling hours, subsequent seedling quality, and field performance (Haase et al. 2016). Transferring these rationales to nursery operations will empower nursery managers in making the best decisions for providing high-quality seedlings for forest regeneration programs.

Conifer seedlings require a period of chilling to complete dormancy before they resume growth and are exposed to favorable photoperiods and temperatures (Haase et al. 2016; van den Driessche 1975, 1977; Wommack 1960). Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) needs 1,200 to 2,000 chilling hours to break dormancy (van den Driessche 1977), which dictates the timeline for lifting or packing seedlings at the nursery. Although chilling can sometimes be partially achieved through storage (Lavender and Stafford 1985), Haase et al. (2016) found that seedlings lifted in early to mid-October performed poorly after outplanting. It appears that seedlings may have a threshold of natural chilling hours and photoperiod required before lifting and storage, after which seedlings are less susceptible to handling stresses. Natural chilling hours typically accrue gradually, thereby allowing seedlings to acclimate to cold temperatures. Because cold storage is an abrupt change to a uniformly cold temperature, seedlings may not have adequate time to acclimate to the sudden low temperature in the cooler. Haase et al. (2016) suggested that Douglas-fir requires a minimum of 300 to 400 hours of natural chilling before storage. Ideally, the lift date and the storage temperature are matched to avoid seedling damage before seedlings are cold hardy. Cold hardiness is defined as a minimum temperature at which a certain percentage of a random plant population will survive or sustain a given level of damage (Ritchie 1984). Seedling hardiness in the nursery relates to overall resistance to stresses associated with lifting, packing, storing, and outplanting and to survival and growth in the field (Haase et al. 2016, Landis et al. 2010).

Studies on Douglas-fir seedling quality in northwestern States and the Rocky Mountains identified seed source differences on chilling hours and cold hardiness (Landis et al. 2010, Tinus 1996). For example, most temperate conifer seedlings typically achieve peak dormancy in

October or November (Haase et al. 2016), although they do not reach maximum cold hardiness until January (Timmis et al. 1994). One guideline to determine the lifting window is the "F-date," which is 30 to 45 days after the average date of the first fall frost (Landis et al. 2010).

Considerable information is available for dormancy, cold hardiness, lifting, and storage (Landis et al. 2010), though data specific to California are limited. Because seedling characteristics vary with seed source and nurseries have varying geographic locations and associated daylength and temperature patterns, it is useful to study specific seed sources and nursery locations. The objective of this study was to evaluate survival and performance in northern California of seedlings from two Douglas-fir seed lots lifted on several dates. The main purpose was to find an effective and efficient way to deliver the best seedlings for reforestation programs.

## Materials and Methods

### Seedlings and Lift Dates

Douglas-fir seeds were collected from the Sierra Pacific Industries seed orchard near Trinity Lake, California (N 40.8791, W 122.8363; 3,465 ft [1,056 m] elevation). The orchard consists of families from California seed zones 331, 332, and 521 with elevations ranging from 3,000 to 4,000 ft (915 to 1,220 m) in the central interior of northern California. Open-pollinated seeds were collected in 2009 (seed lot S503) and in 2011 (seed lot S349). Seeds were stored in the freezer until they were sown at Cal-Forest Nurseries (Etna, CA; N 41.4746, W 122.8234; 2,845 ft [867 m] elevation). The S349 lot was sown on March 12, 2019 and the S503 was sown on April 29, 2019. These sow dates were based on the nursery's commercial operations. Seedlings were subjected to artificially reduced daylength ("blackout") to hasten the hardening process during the first week of July and during the second week of August in 2019 for S349 and S503, respectively.

Seedlings were grown in Styroblock™ containers (412B; 5.8 in$^3$ [95 cm$^3$]; Beaver Plastics, Alberta, Canada) under standard nursery operational practices. Seedlings were lifted and stored on 9 dates during fall-winter 2019-2020: October 23, November 21, December 4, December 18, January 2, January 15, January 29, February 12, and March 5. On each date, 50 seedlings from each seed lot (except December 18, 2019 when only 30 seedlings were lifted) were pulled from the containers, packed in plastic bags, placed in waxed corrugated boxes, and stored in a cooler with an average temperature of 33.3 °F (0.7 °C) until April 2020.

### Environmental Monitoring and Chilling Hours

Hourly temperatures inside the storage cooler, outside at the nursery, and inside the greenhouse were obtained from Argus weather stations (Argus Control, Surrey, BC, Canada). The accumulated chilling hours were calculated from hourly temperatures inside the greenhouse for natural chilling plus accumulated chilling hours inside the storage cooler based on temperatures between 32 and 41 °F (0 and 5 °C) (Bailey and Harrington 2006, Haase et al. 2016, van den Driessche 1975) and were also calculated using the Richardson method (Richardson et al. 1974). Daylength was estimated based on Campbell and Norman (1998).

Field site air temperature and precipitation were obtained from a Remote Automatic Weather Station at Sims, CA (https://raws.dri.edu/cgi-bin/rawMAIN.pl?caCSIM) located 9.3 mi (15 km) northeast of the field site. In addition, we recorded soil temperature and soil water content at a 4-in (10-cm) depth with a HOBO® datalogger (H21-USB; Onset Computer Corporation, Bourne, MA) at 60-minute intervals.

### Pot Trial

After storage, we planted 10 seedlings from each seed lot/lifting date combination in two 15-gal (57-L) nursery pots (5 seedlings per pot) on April 24, 2020 (figure 1). The growing medium consisted of 80:20 peat and sawdust and pots were irrigated with a continuous liquid feed irrigation regime with all necessary nutrients. Bud burst was evaluated on May 13, 2020 based on the first six developmental stages used by Malmqvist et al. (2017) who cited Krutzsch (1973): (1) buds slightly swollen; (2) buds swollen, green to grey–green in color, bud scales still closed; (3) burst of bud scales, tips of needles emerging; (4) needles elongated to about double the bud length; (5) first spread of needles, buds now have the appearance of a painter's brush; and (6) elongation of shoot, basal needles spread. On June 24, 2020, we harvested 5 seedlings from each seed lot/lift

**Figure 1.** (a) Douglas-fir seedlings growing in 15-gal (57-L) pots at Cal-Forest Nurseries (Etna, CA) on May 13, 2020. (b) Seedlings were removed from the pots, (c) washed, (d) measured, and prepared for dry mass measurements on June 24, 2020. (Photo A by Tom Jopson and Photos B, C, and D by Jianwei Zhang)

date combination, rinsed the growing medium off, and measured each for shoot height, root length, and root collar diameter (RCD). Then, root and shoot dry mass for each seedling were determined after drying them at 176 °F (80 °C) for a constant weight.

## Field Trial

On April 23, 2020, we planted 40 seedlings from each seed lot/lifting date combination (except for the December 18 that only had 20 seedlings available) to a field test site (figure 2). The site is located in Shasta County on ground salvaged after the 2018 Delta Fire (N 40.9465, W 122.4698; 2,525 ft [770 m] elevation), approximately 20 mi (32 km) east of the seed orchard. The previous stand was a mixed conifer forest with ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson), Douglas-fir, white fir (*Abies concolor* [Gordon] Lindley ex Hildebrand), and California black oak (*Quercus kelloggii* Newberry). During the post-fire salvage operation, logging debris and most slash were left on the ground. The soil is well-drained gravelly loam in a Marpa family based on USDA's Natural Resources Conservation Service (https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm).

We collected soil samples in June 2020 and found an average of 3.60 percent carbon (C) and 0.19 percent nitrogen (N) in the top 4 in (10 cm) soil layer and 2.72 percent C and 0.14 percent N in the 10 to 20 cm soil layer. Seedlings were planted at 6 by 6 ft (1.8 by 1.8 m) spacing. The herbicide GlyStar® (Albaugh, LLC, Ankeny, IA) was spot sprayed on April 27, 2020 at the 1.5 percent rate recommended on the label. Bud burst was evaluated on May 15, 21, and 29, 2020 using the 6 developing states previously described. Final seedling height and ground-level diameter (GLD, 1.5 in [4 cm] aboveground) were measured for all seedlings on December 2, 2020.

**Figure 2.** (a) Douglas-fir seedlings were outplanted to a field site following storage. Seedlings were from (b) seed lot S349 and (c) seed lot S503. (Photos by Jianwei Zhang, May 15, 2020)

## Experimental Design and Data Analyses

The design in the nursery, pot trial, and field trial was a split-plot randomized complete block design with lifting date as the main plot effect randomly assigned to each of two (pot trial) or four (field trial) blocks and two seed lots as the subplot effect assigned to the main plot. The analyses were performed using SAS PROC MIXED (SAS Institute Inc. Cary, NC). For each variable, residuals were examined to ensure that statistical assumptions of normality and homoscedasticity were met. If not, a natural log transformation was applied. Multiple comparisons among treatments were conducted for least squares means using the Tukey-Kramer test by controlling for the overall $\alpha = 0.05$. The full statistical model is:

$$y_{ijkl} = \mu + \alpha_i + \varepsilon_{1ik} + \beta_j + \alpha\beta_{ij} + \gamma_k + \varepsilon_{2ijkl} \qquad [1]$$

Where $y_{ijkl}$ is the dependent variable summarized for the *ith* lift date, *jth* seed lot, and the *kth* block, $\mu$ is the overall mean, $\alpha_i$ and $\beta_j$ are the fixed effect of the *ith* lift date ($i = 1, 2, \ldots, 9$) or *jth* seed lot ($j = 1$ and $2$), $\gamma_k$ is the random effect of the *kth* block ($k = 1, 2, 3,$ and $4$), $\gamma_k \sim N(0, \sigma_B^2)$ and $\varepsilon_{1ik}$ is an experimental error to test main plot effect, $\varepsilon_{2ijkl}$ is an experimental error to test subplot effect and other terms, $\varepsilon_{1ik} \sim iid\ N(0, \sigma_{e1}^2)$, and $\varepsilon_{2ijkl} \sim iid\ N(0, \sigma_{e2}^2)$.

For the seedling phenology of budburst, we calculated a bud developmental index for each experimental unit:

$$BDI = \sum n_i S_i \qquad [2]$$

where $n_i$ is a percentage of seedlings that are in developing Stage i ($S_i$). BDI values range between 0 and 6 matching the stage categories. We modelled developmental trends with conventional chilling hours prior to left date as the independent variables (linear and/or quadratic term) and BDI as a dependent variable using SAS GLM procedure. The statistical model is adapted from the full model [1] above.

## Results

### Environmental Conditions and Chilling Hours

From October 2019 through April 2020, air temperature outside the greenhouse averaged 44.2 °F (6.8 °C) and daylength ranging from 9.1 to 12.7 hours (figure 3). Chilling hours based on the conventional method reached 172 when the first seedlings were lifted on October 23, 2019 and 1,752 when the last seedlings were lifted on March 5, 2020 (figure 4). Except for the first two lifting dates, the Richardson's method yielded fewer chilling hours than the conventional method. By counting chilling hours completed in the storage cooler, seedlings from all lift dates achieved more than 2,500 hours.

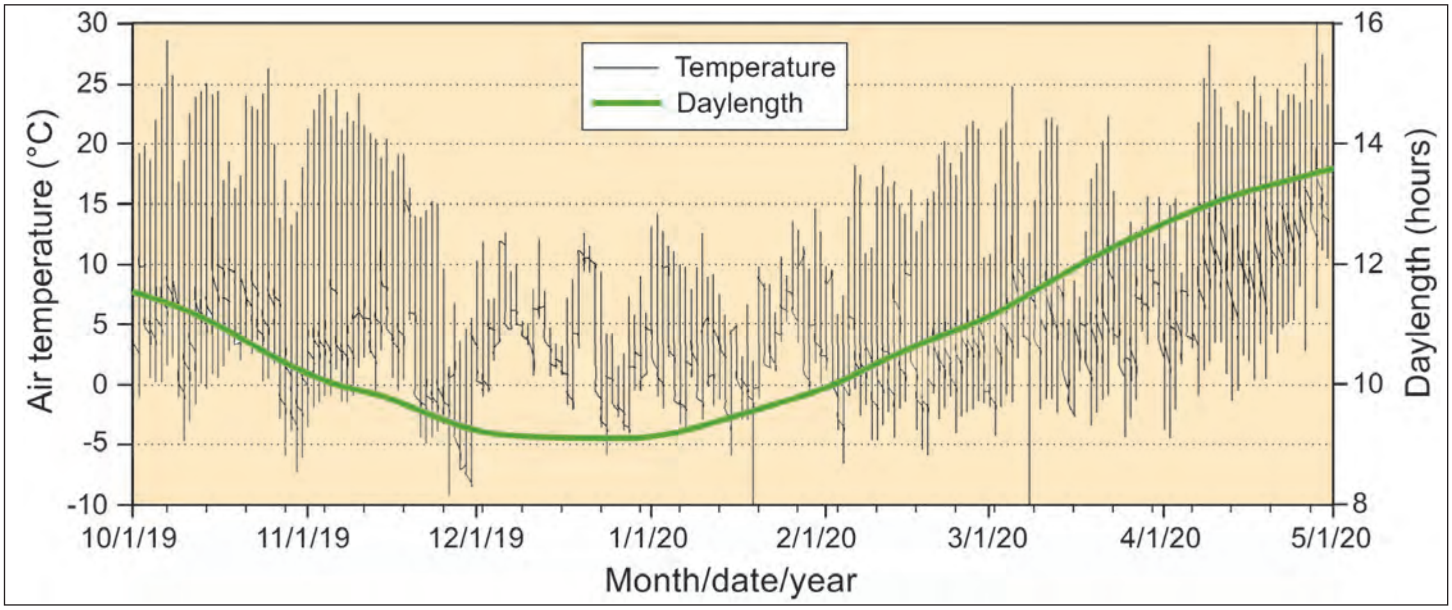

**Figure 3.** Hourly air temperature and estimated daylength (based on Campbell and Norman 1998) from October 1, 2019 to March 31, 2020 at the nursery.

## Pot Trial

Within about 3 weeks after seedlings were potted, most had broken bud and started elongation (figure 5). Using the chilling hours as a quantitative independent variable, we found that the natural chilling hours prior to lifting and storing seedlings significantly affected bud burst phenology, both linearly and quadratically (figure 6). The latest lifted seedlings with more natural chilling hours developed more quickly than the earlier lifted seedlings, especially for the S503 lot. The S349 lot showed a similar trend throughout all lift dates and developed significantly faster than the S503 lot (P<0.001) (figure 6).

All seedlings in the pot trial survived and grew vigorously (table 1). Except for root length and root mass, the S349 seedlings grew significantly larger than the S503 seedlings. All measured variables varied

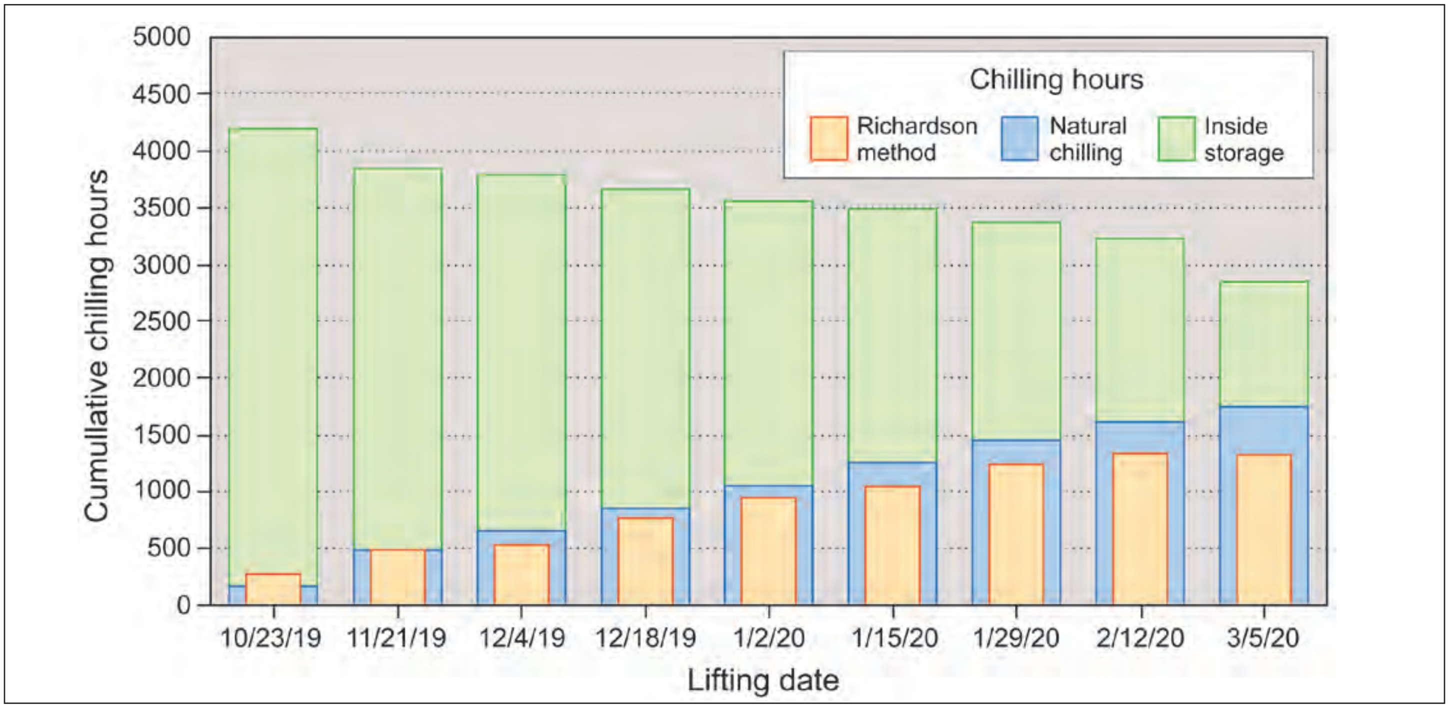

**Figure 4.** Accumulated natural chilling hours at each lift date calculated using the conventional method (32 to 41 °F [0 to 5 °C]) and the Richardson method (Richardson et al. 1974) plus hours in storage varied by lift date.

**Figure 5.** Douglas-fir seedlings bud burst varied between two seed lots (S349 and S503) and among lifting dates: (a) October 23, 2019, (b) November 21, 2019, (c) December 18, 2019, (d) January 29, 2020, and (e) March 5, 2020. (Photos by Tom Jopson, May 13, 2020).

significantly among lifting dates with best performance for those lifted in the last 3 dates. Seedling height and root length showed significant interactions between lifting date and seed lot. Regardless of seed lot or lift date, root and shoot mass were strongly correlated ($r^2=0.94$, $P<0.001$) with a R:S ratio of about 0.30 (figure 7).

## Field Experiment

Precipitation was low and temperatures were warm compared with normal for March and April 2020 (figure 8). After seedlings were planted on April 23, it rained about 5.35 in (136 mm) from May 10 to May 18 resulting in sufficient soil moisture by the middle

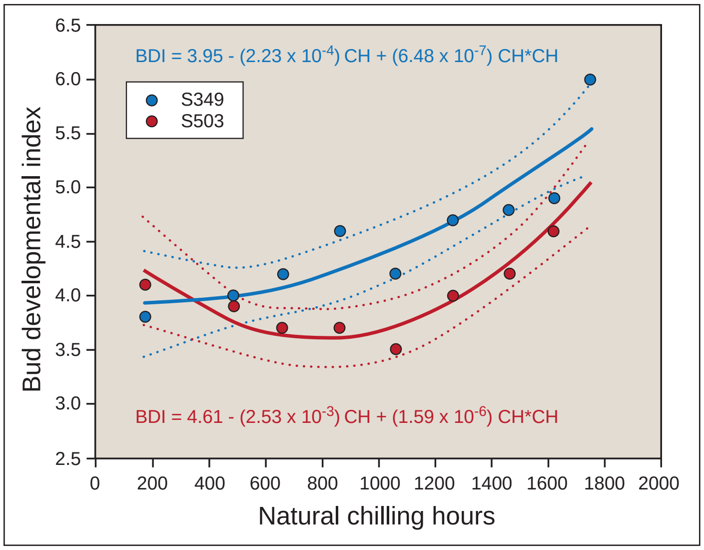

**Figure 6.** The relationship between bud developmental index (BDI) and chilling hours at the time of lift varied by seed lot for Douglas-fir seedlings grown in the pot trial at Cal-Forest Nurseries. Dashed lines are the 95% confidence intervals for their respective regression lines.

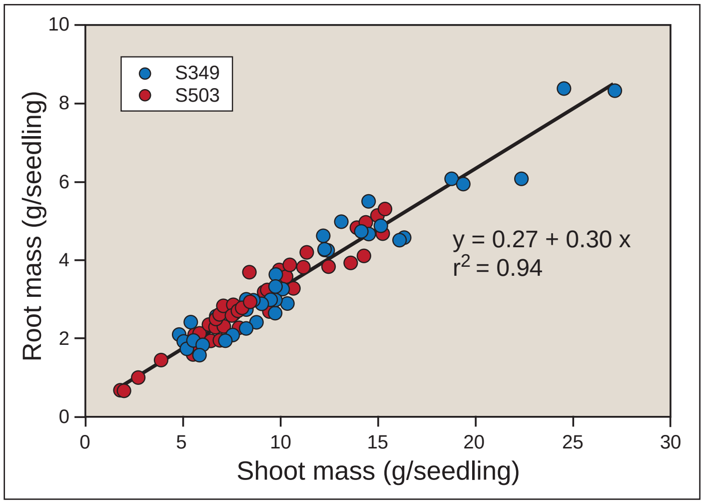

**Figure 7.** There was a strong correlation between root and shoot dry mass for Douglas-fir seedlings from two seed lots and 9 lift dates 2 months after transplanting into pots.

of June (figure 8). Bud burst differed significantly between seed lots ($P < 0.001$). Similar to the pot trial, the S349 seedlings developed faster than the S503 seedlings, especially for seedlings from the later lift dates that had had substantially more natural chilling hours (figure 9). Chilling hour significantly affected bud burst on May 15 and 21 (figures 9a and 9b ). By May 29, however, most seedlings had developed beyond our evaluation stages (figure 9c), when neither seed lot nor chilling hour continued to affect bud burst phenology.

Survival was 100 percent on May 29, 2020. By the final measurements, 3.2 percent of seedlings from the S503 lot and 5.3 percent seedlings from the S349 lot died across the lift dates. The difference

**Table 1.** Mean morphology of Douglas-fir seedlings grown in 15-gal (57-L) pots for 2 months. Seedlings were from two seed lots lifted on 9 different dates. (Note: Seedlings from S349 on January 2 lift date were not sampled.)

| | | Height (cm) | | Root collar diameter (mm) | | Root length (cm) | | Shoot mass (g) | | Root mass (g) | | Total mass (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lift date** | **Seed lot** | **S349** | **S503** | **S349** | **S503** | **S349** | **S503** | **S349** | **S503** | **S349** | **S503** | **S349** | **S503** |
| **Oct 23** | | 35.7 | 42.2 | 4.8 | 5.2 | 38.2 | 42.2 | 5.5 | 7.7 | 2.2 | 2.7 | 8.5 | 10.5 |
| **Nov 21** | | 44.2 | 34.0 | 6.2 | 4.2 | 39.4 | 36.6 | 10.3 | 5.2 | 3.9 | 2.0 | 14.2 | 7.2 |
| **Dec 04** | | 41.7 | 45.7 | 5.5 | 5.8 | 35.9 | 37.9 | 7.8 | 9.6 | 2.7 | 2.8 | 10.5 | 12.4 |
| **Dec 18** | | 50.3 | 41.4 | 5.7 | 4.9 | 36.9 | 36.1 | 11.2 | 6.7 | 3.1 | 2.3 | 14.3 | 9.0 |
| **Jan 02** | | - | 43.2 | - | 5.7 | - | 37.0 | - | 8.6 | - | 2.9 | - | 11.4 |
| **Jan 15** | | 49.8 | 39.6 | 6.1 | 5.4 | 35.6 | 34.4 | 11.2 | 7.4 | 3.1 | 2.7 | 14.4 | 10.2 |
| **Jan 29** | | 49.5 | 44.7 | 6.3 | 6.3 | 40.4 | 33.8 | 13.1 | 9.9 | 4.5 | 3.4 | 17.6 | 13.4 |
| **Feb 12** | | 52.8 | 41.4 | 6.8 | 5.7 | 41.0 | 38.9 | 13.8 | 9.7 | 4.2 | 3.4 | 18.1 | 13.1 |
| **Mar 05** | | 53.3 | 52.3 | 6.9 | 6.6 | 34.1 | 39.7 | 13.5 | 11.9 | 4.4 | 4.1 | 17.8 | 16.1 |
| | **Mean** | 47.2 | 42.7 | 6.0 | 5.5 | 37.7 | 37.4 | 10.8 | 8.5 | 3.5 | 2.9 | 14.4 | 11.5 |
| **Probabilities for treatment effects** | **Lifting date** | 0.001 | | 0.004 | | 0.029 | | 0.030 | | 0.034 | | 0.049 | |
| | **Seedlot** | 0.004 | | 0.025 | | 0.771 | | 0.016 | | 0.054 | | 0.018 | |
| | **LD*S** | 0.013 | | 0.120 | | 0.004 | | 0.273 | | 0.590 | | 0.451 | |

Conversions: 1 cm = 10 mm = 0.39 in; 1 g = 0.035 oz

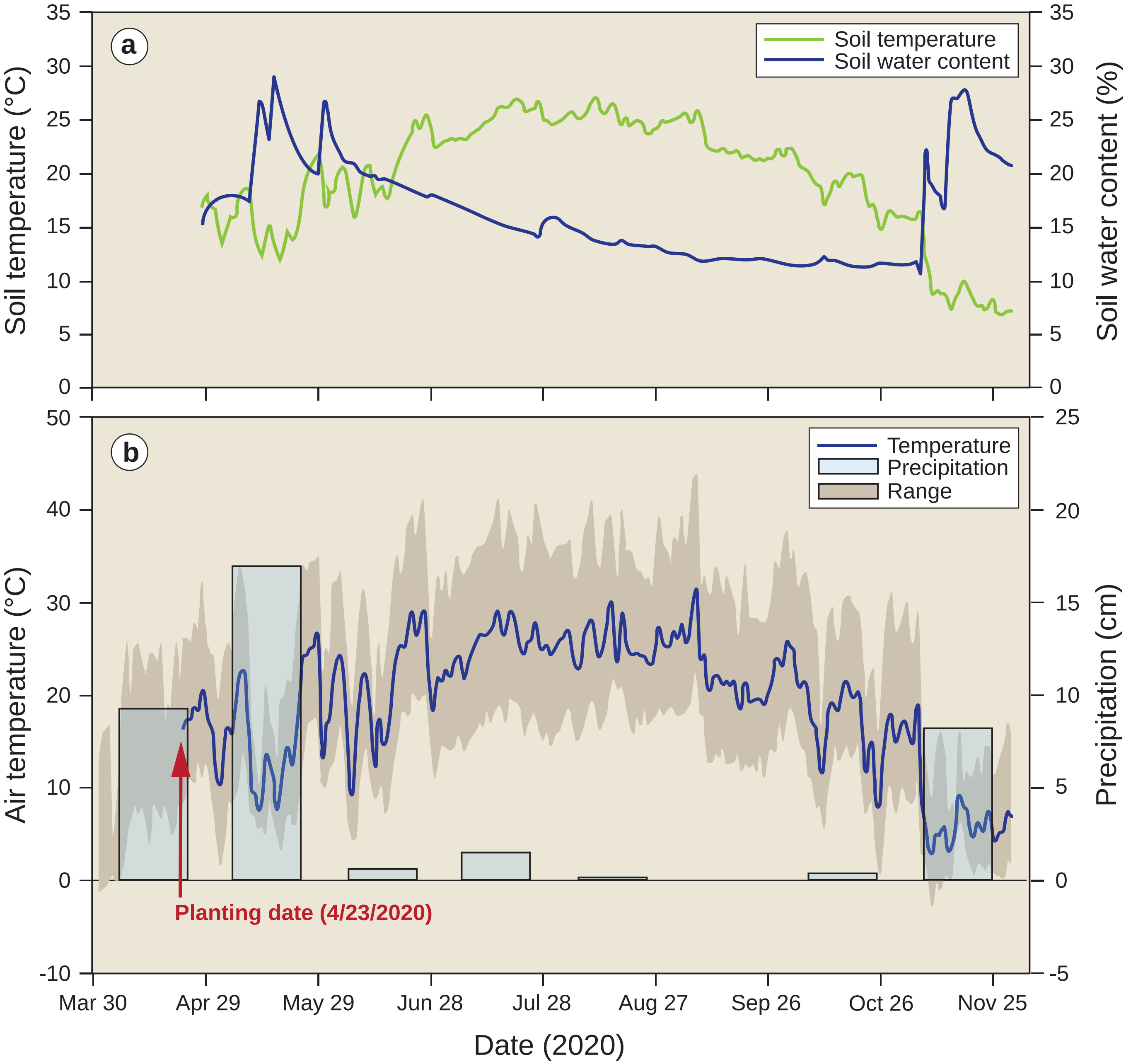

**Figure 8.** (a) Soil temperature and water content at the field trial site and (b) air temperature and precipitation at a nearby weather station were used to understand seedling responses during the study.

between the seed lots was significant (P < 0.001) but not between lift dates (P > 0.50).

There was a significant interaction on both height and GLD between seed lot and lift date (P = 0.004). Overall, seed lot S349 tended to have greater height and diameter growth with few exceptions (figure 10). Because lift date had little influence on height and GLD, we did not model the chilling hour effect on these growth variables.

## Discussion

The purpose of this study was to address some concerns from regeneration foresters on poor survival of Douglas-fir plantings in southeastern Oregon and northern California. Poor seedling quality may be one of possible causes, but the specific reasons for poor performance often cannot be determined. We focused on two factors that may influence seedling quality: (1) lifting date and associated effects on seedling physiology and (2) seed lot.

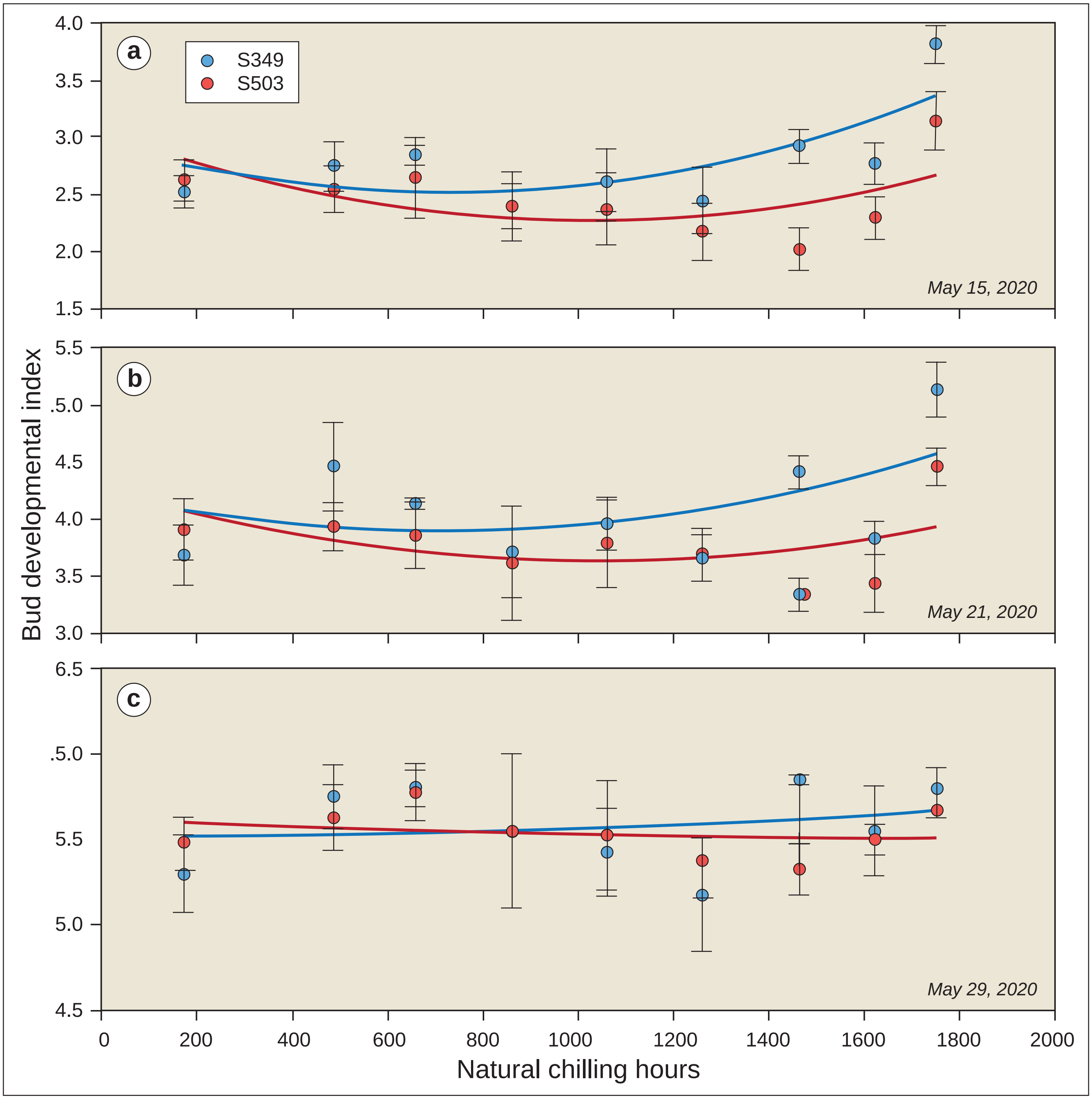

**Figure 9.** The relationship between bud developmental index (BDI) and chilling hours at the time of lift for Douglas-fir seedlings outplanted to a field site near Redding, CA varied between seedlots on (a) May 15 and (b) May 21 but was no longer evident on (c) May 29 when most seedling buds had developed beyond the index phases.

## Chilling Hour Effect

Results from this study indicate that lift dates from late October to early March did not affect seedling survival or growth in the field. Seedlings lifted in February or March, however, could be negatively affected during years with warmer winter conditions in the nursery that cause seedlings to break bud early.

Nursery location is an important factor because each site has its own photoperiod and temperature patterns which affect seedling physiology and phenology (Campbell and Sugano 1975, Ritchie 1989).

Haase et al. (2016) reported that Douglas-fir may benefit from a minimum of 300 to 400 natural chilling hours for optimal stress resistance before being

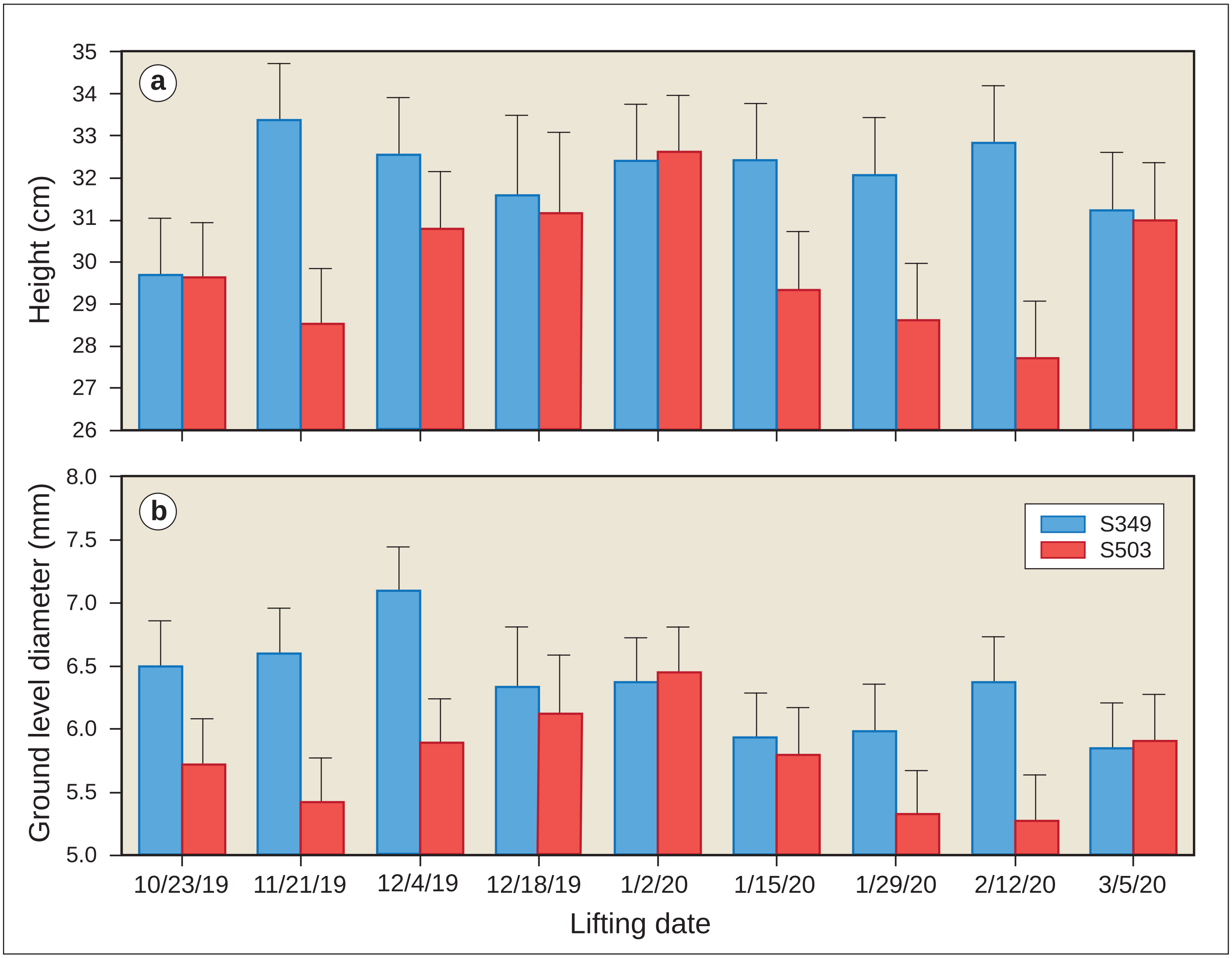

**Figure 10.** Average (a) height and (b) ground-level diameter of Douglas-fir seedlings after one growing season in the field trial site near Redding, CA. Seed lot S349 tended to have more growth across lift dates.

lifted and stored for completing a threshold of 1,200 chilling hours (Ritchie 1984; van den Driessche 1975, 1977). If these two minimum requirements are true, the first packing date in our study did not meet the former with only 172 chilling hours calculated with the conventional method and 185 with the Richardson method (figure 4). Nonetheless, all seedlings performed well in both the pot and field trials (figures 7, 9, and 10).

In the pot trial, higher root mass was found in seedlings from the later lifting dates compared with those from the earlier dates although shoot-root ratios were unaffected (figure 7). These results differ from studies that found later lifted seedlings were exposed to higher temperatures which stimulated bud elongation and reduced root growth (Nadel et al. 2020, Ritchie and Dunlap 1980). We believe this discrepancy is due to our study being conducted in the comparatively cooler climate of Etna, CA where considerably more natural chilling hours were accumulated for seedlings lifted from late January to March (figure 4).

Douglas-fir seedlings grown under controlled conditions (Ritchie 1984, van den Driessche 1977) or in a field test with older saplings (Bailey and Harrington 2006) tend to have earlier bud burst with chilling hours beyond the minimum 1,200 hours. This phenomenon was observed in the current study if we only count the natural chilling hours, but not when we include the supplemental chilling hours in the storage cooler.

## Seed Lot Effect

Both seed lots were collected from the same seed orchard but in different years. Genetically, they should have been very similar. Douglas-fir is a monoecious species with both male and female cones occurring on the same tree and other pollens coming from surrounding trees. The two seed lots may have been influenced by climatic conditions during their parental reproductive periods. For the S503 lot (collected in 2009), precipitation in 2008 and 2009 was 435 mm and 628 mm, respectively. For the S349 lot (collected in 2011), precipitation in 2010 and 2011 was 1039 mm and 750 mm, respectively. Temperature conditions were similar. Seed sources or families from mesic habitats grow faster than those from xeric sites in common garden studies (Wright 1976). Thus, seeds produced in wetter years may yield larger seedlings than seeds produced in drier years which may explain the seed lot differences in our study.

Differences among seed lots may also be attributed to sowing dates and subsequent blackout timing in the nursery. The earlier sowing and blacking out for S349 seedlings affected photoperiod and dormancy induction. Unfortunately, these confounding effects could not be avoided. Notably, however, the earlier budbreak of S349 seedlings carries the risk of late frost damage (Malmqvist et al. 2018). Also, fast aboveground growth may deplete soil water and cause mortality in the late growing season (Darychuk et al. 2012) on droughty sites. Because our field test was planted relatively later than the usual spring planting time at this elevation, seedlings were not damaged by frost. During the late growing season when high temperatures and drought occurred, S349 seedlings had higher mortality (5.3 percent) than S503 seedlings (3.2 percent) in the field test, though these numbers are still quite low. In an adjacent site, an observational fall planting test (September 2019) had 63 percent mortality of S349 seedlings compared with 10 percent mortality of S503 seedlings. These results require additional research to better understand differences between the seed lots.

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# Planting Sitka Spruce and Western Redcedar in the Same Hole to Mitigate Browsing Damage

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## Abstract

This article describes an original approach to mitigating wildlife predation of young western redcedar (*Thuja plicata* Donn ex D. Don) seedlings in plantations in the Pacific Northwest and British Columbia. This method was developed by the author through direct observation while managing reforestation projects on the Olympic Peninsula in Washington State and British Columbia over many years. In the author's experience, this method has proven to be cost effective and environmentally sound.

## Background

Planting western redcedar (*Thuja plicata* Donn ex D. Don) and Sitka spruce (*Picea sitchensis* [Bong.] Carrière) seedlings in the same hole is an effective method to reduce deer and elk browse and increase western redcedar seedling survival. I first conceived of this silvicultural practice of planting double seedlings along the banks of the South Fork of the Pysht River (Olympic Peninsula, WA) while working on the Pysht South Fork Riparian Forest Restoration project. The goal of the project was to harvest red alder (*Alnus rubra* Bong.) and reforest the area with native conifer species including western redcedar. The forests in the Pysht South Fork drainage had been harvested in the 1920s, and a dense stand of conifer regenerated naturally. Unfortunately, a series of fires in the late 1930s destroyed the young conifer forests, and the ensuing forest became predominantly stocked with red alder.

The Pysht South Fork Riparian Forest Restoration project was initiated in June 1994. Five small alder stands were harvested along one side of the South Fork of the Pysht River. These stands were separated by a 1,000-foot (305-m) length of stream where the alder forest was left undisturbed. The average size of each harvested stand was less than 2 ac (0.8 ha), with an average linear distance parallel to the stream of 600 ft (183 m). All alder trees were cut, leaving scattered conifer trees. A 10-ft (3-m) tree buffer remained uncut along the stream to protect its bank integrity.

After the alder harvest, the site was prepared, and seedlings were planted the following winter. The mixture of seedlings planted included Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), Sitka spruce, and western redcedar. Field reviews the following winter showed high mortality in the planted western redcedar due to deer browse. It was then that I conceived the idea of planting doubles of one Sitka spruce seedling and one western redcedar seedling in the same hole. I knew that deer left the spruce seedlings alone because the very sharp, pointed needles hurt their noses. With some trepidation, I proceeded to plant spruce and cedar seedlings together in the same hole (doubles) the following winter. After the first growing season, 90 percent of the doubles survived.

## Recommendations

Planting Sitka spruce and western redcedar doubles is similar to planting any forest seedling with a few exceptions. The seedling stock of each species for an individual planting hole should be the same size whether they are bareroot or container seedlings. Each Sitka spruce and western redcedar double should occupy one planting microsite (figure 1).

**Figure 1.** Double Sitka spruce and western redcedar after planting. (Photo by Joseph Murray 2014)

**Figure 2.** Western redcedar forest after cutting out the double-planted Sitka spruce. (Photo by Joseph Murray 2016)

For example, if 430 planting spots per acre are prescribed for a stand, and doubles would inhabit all planting spots, then 860 trees per acre would be planted in the 430 planting spots with a Sitka spruce and a western redcedar in each hole. If other species are prescribed, then the densities would be adjusted accordingly.

Site preparation, vegetation control, and other animal control methods are similar to those used in routine plantation development and cultivation for a specific forest habitat. These silvicultural activities should be designed to assist the plantation in achieving a free-to-grow status in a cost-effective manner.

The double-planting practice does not completely eliminate deer and elk browse. It will, however, help young western redcedar seedlings survive and reach a free-to-grow condition in a timeframe similar to other conifer species on the same site (figure 2). As the trees grow together, the browse is limited to the margins of the double-plant complex, and both trees increase in size and height each growing season.

Within 7 to 10 years, depending on site quality and wildlife populations, the western redcedar seedling will attain a height where browse will no longer affect its survival. At this point, foresters should consider cutting the Sitka spruce out of the double-plant complex. The standard practice in much of the forest industry is to cut the Sitka spruce below the lowest green limb. This practice, however, has a high likelihood of damaging the western redcedar. Heavy-duty pruning shears can be used to remove the Sitka spruce, but this is very time consuming. I recommend cutting the Sitka spruce with a chain saw as high above the ground as possible where there is less opportunity to damage the western redcedar. Then, prune the lateral branches on the Sitka spruce stump growing on the side opposite from the western redcedar. The objective is to reduce the amount of live, green Sitka spruce branches, thus minimizing its potential to compete with the western redcedar.

Whether the intention is to grow western redcedar for economic return or for habitat diversity, planting doubles is a reasonable and cost-effective practice.

**Figure 3.** Western redcedar and Sitka spruce doubles grown in a single plug at the nursery. (Photo by Joseph Murray 2017)

New seedling products have been developed where western redcedar and Sitka spruce are grown in the same container at the nursery (figure 3). This production practice ensures uniformity and reduces planting cost.

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# White Spruce: Guidance for Seed Transfer Within the Eastern United States

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## Abstract

White spruce is a boreal conifer with a transcontinental range and intermediate shade tolerance that thrives in mixed stands. The species has high genetic variation, low population structure, and can tolerate moderate transfer distances with minimal maladaptation effects. White spruce has a tendency to break bud early in the spring and, as such, is susceptible to damage from early spring frosts. Spruce budworm is the most significant pest of white spruce. Seed collection areas should be developed from sources with a range of budbreak times and growth habits to maximize genetic diversity. White spruce is a good candidate for assisted migration because it is expected to experience a range shift, is generally unpalatable to browse from white-tailed deer, and can be transferred long distances with a low probability of maladaptation.

## Introduction

White spruce (*Picea glauca* Moench [Voss]) is a transcontinental, long-lived, boreal conifer that grows on a wide variety of sites exclusive of stagnant, wet, or excessively dry sites. Spruce trees provide habitat for small mammals and birds and are generally unpalatable to browse by white-tailed deer (*Odocoileus virginianus* [Zimmerman]). White spruce is valued in commercial forest markets for its use as pulpwood and sawlogs. In the United States, white spruce occurs across the Lake States (Michigan, Minnesota, and Wisconsin), northern portions of New York, Vermont, New Hampshire, and across Maine, but most of its range resides in Canada. White spruce likely had three glacial refugia (two in eastern North America and one in the west) based on evidence of genetic diversity and endemic haplotypes associated with each refugium (de Lafontaine et al. 2010). Two refugia based in eastern North America correspond to areas west and east of the Appalachian Mountains. White spruce from areas west of the Appalachian Mountains migrated northwards towards the Great Lakes, whereas populations east of the Appalachians migrated into New England and northwards into eastern Québec, Labrador, and the Atlantic Provinces (de Lafontaine et al. 2010).

White spruce is generally a minor component of northern forests and has low importance values. It rarely regenerates in an even-aged stand except when such conditions are created artificially through management. White spruce has intermediate shade tolerance and thrives in mixed stands, especially beneath an overstory composed of quaking aspen (*Populus tremuloides* Michx.) and/or paper birch (*Betula papyrifera* Marshall) (Gradowski et al. 2008, Man and Lieffers 1997). The overstory of these northern hardwoods may provide protection from radiational cooling on quiescent seedlings or seedlings that have broken bud in the spring (Groot and Carlson 1996) (figure 1). White spruce requires fewer growing-degree days to leaf out in the spring than other taxa (Lu and Man 2011, O'Reilly and Parker 1982, Rossi and Isabel 2017) rendering it more vulnerable to deleterious effects of early spring frost than trees with buds or flowers that emerge later in the season. In addition, female conelets emerge early in the spring which can increase frost risk to flowers and new shoots that leaf out early (figure 2). White spruce regenerates primarily from seed, but may regenerate by layering, in which lower branches that reach the soil form new roots (Katzman 1971, Stone and McKittrick 1976).

White spruce is intolerant to fire but regenerates well on disturbed sites with mechanically exposed mineral soil (Gärtner et al. 2011) or on sites immediately post-fire (Purdy et al. 2002). Additional details about this

**Figure 1.** A sapling of white spruce grows vigorously underneath a quaking aspen overstory. (Photo by C. Pike, 2004)

species may be found in the USDA Natural Resources Conservation Service plant guide (Nesom and Guala 2003). The Climate Change Atlas predicts that white spruce habitat will not change greatly, but additional warmth will likely stress the species, especially along its southern range edge (Peters et al. 2020).

## Genetics

White spruce seeds are lightweight, winged, and rapidly released when cones dehisce, usually in August (figure 3). Cones ripen and mature in one growing season as opposed to cones of *Pinus* species that require two years to mature. Mobile seeds and wind-dispersed pollen contribute to high rates of gene migration (O'Connell et al. 2006), resulting in high genetic diversity across the species' geographic range (Furnier et al. 1991). Genetic variation is low among populations (stands) and reflects high rates of migration: $F_{ST}$ values (a ratio of genetic variation between sub-populations and the total population) range from as low as 0.006 to 0.007 (Cheliak et al. 1988, Namroud et al. 2008) to as high as 0.113 along the northern range edge in Québec (Tremblay and Simon 1989). This high genetic diversity confers a strong capacity to adapt to local conditions. Provenance (geographic origin)

**Figure 2.** Spruce trees tend to leaf out earlier in the spring than other plants. Early spring frosts can damage female inflorescence (immature cones in photo) or developing shoots. (Photo by C. Pike, 2009)

**Figure 3.** Immature cones ripening on a tree at a seed orchard. Unlike cones of the *Pinus* genus, spruce cones only require one year to develop. A cut test of the cone is required to determine ripeness. Once the cone dries, the seed is released in late summer. (Photo by C. Pike, 2006)

effects are often insignificant and overshadowed by differences among trees within a provenance (Li et al. 1993). In other words, within any single provenance, trees with a variety of traits and habits can be found. White spruce is not known to hybridize with other *Picea* species in the wild. In summary, white spruce has high gene flow, high genetic variation, and greater differences among trees within a stand than among stands.

Clinal variation across the landscape is generally weak for white spruce, with steepest gradients occurring between eastern and western populations as observed in range-wide provenance trials (Khalil 1985, Sebastian-Azcona et al. 2019, Wilkinson et al. 1971). Sharp differences between eastern and western populations may be attributable to distinct refugia that were isolated during prior glaciation. In the eastern part of the range, differences among populations attributable to latitude of origin are generally weak but may be detected for some traits (Lesser and Parker 2004; Li et al. 1993, 1997; Lu and Man 2011; Lu et al. 2014).

White spruce trees have determinate growth and require a period of deep chilling (cold temperatures below freezing threshold) for shoot growth to resume after buds are set in the summer. Young seedlings may exhibit indeterminate growth, a habit that ceases by the fourth year (Nienstaedt 1966). Phenology traits (time to budbreak and budset) are important predictors for growth. After the chilling requirement has been met, warm temperatures in the spring (tabulated as growing degree days) lead to budbreak after a threshold is met (Lu and Man 2011, Nienstaedt 1966). The calendar date for budbreak timing varies annually by 1 or more months depending on spring temperatures (Pike et al. 2017). The amount of warming needed to induce budbreak is under strong genetic control (Lu and Man 2011, O'Reilly and Parker 1982). Even though budbreak time is highly adaptive, the trait exhibits weak clinal variation and no significant genotype-by-site interactions (Lesser and Parker 2004, Lu and Man 2011). For example, genotypes with a tendency to break bud early were not associated with any single provenance and were consistent for families across multiple sites (Lesser and Parker 2004, Lu and Man 2011). This paradox–an adaptive trait that is not associated with its native location–is best explained by the excessively high gene flow in white spruce that precludes isolation and local adaptation. Changes in daylength are the primary trigger for budset and the onset of winter dormancy in the fall (Hamilton et al. 2016). White spruce is generally not affected by fall frosts because the buds are set by mid-summer.

## Seed Transfer Considerations

White spruce is a good candidate for assisted migration because of its extensive genetic variation and its capacity to adapt (Lu et al. 2014). In addition, white spruce is highly tolerant of long-distance seed transfer with large optimal breeding zones of 3° latitude (approximately 200 mi [322 km]) and 10 to 12° longitude (Thomson et al. 2010). Mid- and northern populations grow in suboptimal conditions, and best seed sources generally originate from 1.0 to 1.5° latitude south of a site (Morgenstern et al. 2006, Prud'Homme et al. 2018, Thomson et al. 2010).

Southern sources moved north to a common garden are more likely to experience budbreak delays relative to northern sources because of the extra time required to accumulate degree days (Blum 1988, Lesser and Parker 2004, Prud'Homme et al. 2018). Migration of seed across short distances, however, is unlikely to strongly influence budbreak time (Lu and Man 2011). Seed collection areas should be developed from sources with a range of budbreak times and growth habits to maximize genetic diversity. Considerations for moving white spruce seed are summarized in table 1.

White spruce growth and survival can be correlated with weather conditions that occur during the active growing season. For example, tree growth (height and diameter) was related to maximum temperatures in May, June, and August across 6 sites in western Ontario (Thomson et al. 2010). Other studies determined that temperature and precipitation both contributed to growth (Andalo et al. 2005, Lesser and Parker 2004). White spruce is relatively insensitive to nadir winter temperatures (minimum temperatures in January, for example) (Lu et al. 2014) because it is hardy to -22 °F (-30 °C) by mid-fall and remains dormant until dormancy is released with spring warming (Sebastian-Azcona et al. 2019).

**Table 1.** Summary of considerations for moving white spruce seed.

| **White spruce, *Picea glauca* Moench** | |
|---|---|
| Genetics | • Genetic diversity: high<br>• Gene flow: high |
| Cone and seed traits | • Small, winged seeds<br>• 135,000 to 401,000 seeds per pound (297,000 to 882,200 per kg)<br>• Non-serotinous cones<br>• Seeds are released in late summer |
| Insect and disease | • Spruce budworm (major), sawfly (minor)<br>• Needlecasts can afflict spruce |
| Palatability to browse | • Low risk of herbivory from white-tailed deer |
| Maximum transfer distances | • White spruce can handle relatively long transfer distances relative to other taxa:<br>– Up to 200 mi (322 km) south to north<br>– Up to 300 mi (483 km) east to/from west<br>• Sources from 1.0 to 1.5° latitude south are generally superior to local or northern sources |
| Range-expansion potential | • Spruce is likely to experience a northward range-shift but may persist along its southern range edge because of high genetic variation and low deer palatability |

**Figure 4.** Spruce budworm is the most economically important pest of white spruce across North America. The adult form is shown in this photo, but most damage occurs from feeding by larvae. (Photo by J. Warren, USDA Forest Service, 2011)

## Insects and Diseases

Spruce budworm (*Choristoneura fumiferana* [Freeman]) is indigenous to North America and is a highly destructive pest of white spruce across its range (figure 4). Budworm serves an important successional role by accelerating the demise of decadent stands of spruce and fir (*Abies* sp.) in northern forests. Silvicultural practices that create monocultures of white spruce may help sustain populations of budworm and increase the vulnerability of managed forests to mortality (Blais 1983). Seed orchards that are tightly spaced can also be inundated with feeding during budworm outbreaks. Budworm outbreaks occur at approximately 40-year intervals (Blais 1983, Boulanger and Arseneault 2004), although intervals may be shorter if conditions favor the insects' proliferation. The intensity and extent of outbreaks depend on myriad site factors and can devastate timber resources (Gray and MacKinnon 2006).

Spruce budworm adults lay eggs in the summer on host trees, and larvae overwinter as second instars. Upon emergence in the early spring, larvae disperse and feed on shoots, favoring trees with buds that have recently emerged from their sheath. Larvae that emerge from winter hibernation before new shoots are available as a food source must find sustenance on sub-par sources, such as older needles. Thus, synchrony with new shoot growth in host trees is imperative (Blum 1988) to ensure the survival of newly emerged larvae. The movement of seed sources from southern to northern locales will likely interact with the budworm (i.e., if budbreak is delayed, then it may evade infestation barring any other adaptations by the insect).

Other insect pests that affect white spruce include yellow-headed spruce sawfly, (*Pikonema alaskensis* [Rohwer]) (figure 5) which can occasionally produce outbreaks (Katovich et al. 1995). Spruce budmoth (*Zeiraphera canadensis* Mutuura and Freeman) and spruce spider mites (*Oligonychus ununguis* [Jacobi])

**Figure 5.** Yellow-headed sawfly is an occasional pest on white spruce foliage. (Photo by J. Warren, USDA Forest Service, 2011)

are minor pests and associated with open grown trees in largely urban settings. Pathogens associated with white spruce affecting weakened hosts include *Rhizosphaera kalkhoffii* and *Stigmina lautii* needle cast (Walla and Bergdahl 2016), *Phomopsis* canker (*Phomopsis juniperovora*), and Diplodia tip blight (*Diplodia sapinea*) (Stanosz et al. 1997, Stanosz et al. 2007). *Rhizosphaera* and *Stigmina* are also likely important pathogens, especially in plantations and along the southern edge of white spruce's range.

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# Jack Pine: Guidance for Seed Transfer Within the Eastern United States

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## Abstract

Jack pine grows in boreal forests across the North American continent. Genetic diversity of this species is high and clinal, but populations exhibit genetic structure that is higher than other conifers with similar life-history traits. Cones are serotinous across most of its range but may be non-serotinous along the southern edge in the Lake States. The serotinous habit may limit seed dispersal and is likely the primary contributor to the genetic structure apparent in studies of mitochondrial DNA. Jack pine originating from southern sources tend to outgrow local or northern sources. Jack pine is likely to persist with climate change in its current range because of its tolerance to xeric conditions. Assisted migration should be well-tolerated by planting seed originating from 100 mi (160 km) to the south, but managers should avoid transferring seed more than 100 miles from origin and be aware of potential pests including jack pine budworm and eastern gall rust.

## Introduction

Jack pine (*Pinus banksiana* Lamb) grows across North America and is the most northerly occurring species of its genus, occurring predominantly in Canada. Its southern range edge dips into the Lake States (Michigan, Minnesota, and Wisconsin) with disjunct populations in parts of upstate New York, New Hampshire, and Maine. Modern jack pine populations in the eastern United States are likely derived from at least three glacial refugia: one in the Appalachian Highlands (Yeatman 1967), a second in the southeastern United States (Critchfield 1985), and a third along the Atlantic coast (Godbout et al. 2010).

Jack pine is shade-intolerant (requires full sunlight), indeterminate (capable of producing additional flushes of vertical growth after budset if weather conditions permit), and regenerates best on bare mineral soil in pure or mixed stands. Young, dense stands are critical habitats for the Kirtland warbler (*Setophaga kirtlandii* [Baird] [Parulidae]), a rare bird that was recently removed from the Endangered Species list (Parham and Golder 2019). Jack pine is highly drought tolerant and can survive on sandy, nutrient-poor soils along the prairie edge (figure 1) and across boreal forests (figure 2). This resilience to xeric conditions may allow populations of this species in its southern range edge to persist as the climate warms (Prasad et al. 2020), but provenance (geographic origin) trials have revealed that optimal temperature regimes for its growth may shift northward as the climate warms (Thomson and Parker 2008).

Commercial products derived from jack pine include pulp, boards, shipping crates, and posts (Rudolf 1985). Jack pine is usually associated with even-aged stands but also occurs in stands with more age-complexity along the southern range edge in Minnesota where cones are largely non-serotinous (Gill et al. 2015). Cones are generally closed (serotinous) across most of its range but non-serotinous (open) cones are common along the southern range edge in Minnesota (Schoenike 1976). Jack pine is moderately palatable to browse by white-tailed deer (*Odocoileus virginianus* [Zimmerman]) and often requires protection during the winter months. Additional details about this species can be found online in the USDA Natural Resources Conservation Service plant guide (Moore and Walker Wilson 2006) and at the Climate Change Atlas (Peters et al. 2020). The Climate Change atlas predicts a small decrease in the habitat suitability, but the species will likely be buffered by its abundance and inherent drought tolerance.

**Figure 1.**These young jack pine trees are growing on a xeric site in northwestern Minnesota, the southwestern edge of jack pine's range. (Photo by C. Pike, 2008)

**Figure 2.** Jack pine is common in the boreal forests of northeastern Minnesota where tree form is often tall and straight. (Photo by C. Pike, 2008)

## Genetics

Jack pine has high genetic diversity that is typical of other conifers but exhibits more population structure, phenotypically and genetically, than would be expected of a wind-pollinated tree (Cheliak et al. 1984, Godbout et al. 2010, Naydenov et al. 2005). Phenotypic differences among populations are manifest in traits such as cone serotiny and bark thickness, and to a lesser degree in needle morphology and cone curvature (Schoenike 1976). Foliage of northern seed sources tend to turn purple or bronze during the winter months, whereas southerly sources remain predominantly green, a finding confirmed to have a genetic basis in common garden studies (Sprackling and Read 1974, Stoeckeler and Rudolf 1956, van Niejenhuis and Parker 1996). The adaptive value of winter foliage color is not known, but the visibility of this trait may serve as a physical indicator of seed origin for seedlings growing in nurseries (Stoeckeler and Rudolf 1956). Jack pine is capable of hybridizing with lodgepole pine; introgressed populations are widespread in Alberta and the Northwest Territories of Canada (Wheeler and Guries 1987).

Genetic diversity in jack pine varies clinally across its range, but population sub-structure is evident from studies of neutral DNA (genes that are not associated with physical traits). In pines, chloroplasts are paternally inherited (via pollen). Chloroplast DNA (cpDNA) and allozymes (proteins with enough natural variation that they can be used as genetic markers) revealed moderate levels of gene flow among jack pine populations in southern Ontario, Quebec, and the Lake States (Godbout et al. 2010, Naydenov et al. 2005, Saenz-Romero et al. 2001, Xie and Knowles 1991). These results imply that pollen flows relatively unobstructed across populations. In contrast, mitochondrial DNA (mtDNA), which is maternally inherited, revealed pronounced separations among populations (Godbout et al. 2010, Godbout et al. 2005) implying that gene flow via seed is more restricted than that of pollen. The discrepancy in gene flow among populations between maternal and paternal sources of variation may be attributed, in part, to a lag time in seed dispersed from serotinous cones (Godbout et al. 2010, Ross and Hawkins 1986).

Fire has strongly influenced phenotypic and genetic variation of jack pine. This influence is especially evident in cone traits. Across its range, jack pine trees with serotinous cones are the predominant type, requiring high heat to open and release seeds (figure 3). Jack pine with non-serotinous cones that open and release seeds under ambient conditions are generally associated with southern range edge populations in the Lake States and New England (Hyun 1977, Rudolf et al. 1959, Schoenike 1976). Tree crowns may bear cones of one type (all serotinous or all non-serotinous) or contain a mix of both types (Gauthier et al. 1992, Rudolf et al. 1959) (figure 4). Serotiny appears to be under strong genetic control, with relatively simple inheritance (Rudolf et al.1959), so this trait is likely to evolve rapidly to environmental change. The presence of non-serotinous cones in the south may be favored by natural selection in areas where fire is absent (Gauthier et al. 1996). Bark thickness, a trait that influences tolerance to ground-level fires, also tends to be thicker for jack pine growing in warmer, drier climates where fires are more commonplace than in mesic regions such as the Maritimes (Schoenike 1976). Phenotypic traits associated with needle, bark, branch angle, and cone traits vary clinally across the range suggesting that gene flow, for the most part, is high in jack pine (Schoenike 1976). In Minnesota, natural stands of jack pine exhibit a sharp cline with distinct boundaries approximately 65 mi (100 km) wide (Critchfield 1985, Schoenike 1976) that do not coincide with other environmental gradients. Trees north of this line tend to have straight, closed cones while trees south of this line tend to exhibit curved cones that readily open and disperse seeds. This enigmatic population sub-structure has been attributed to different glacial refugia (Critchfield 1985) but underlying causes remain unresolved.

**Figure 3.** Serotinous (closed) cones, exhibited on this branch, are the most common type across most of jack pine's range. In addition, the cones are curled, a trait that also varies geographically, though the adaptive value is unknown. (Photo by C. Pike, 2010)

## Seed-Transfer Considerations

Jack pine has high genetic diversity but is more sensitive to seed transfer than other conifers in the eastern United States. In other words, long-distance transfer of jack pine seeds increases the likelihood of maladaptation compared with other conifers, such as white spruce (*Picea glauca* [Moench] Voss), where gene flow from seed and pollen are both relatively unobstructed. This sensitivity to transfer has been observed in common garden studies both in the United States (Lake States) and Canada (western Ontario) but was less obvious in Maine where jack pine sources from the Lake States performed above the mean (Carter and Canavera 1984). This finding, however, does not impose a blanket endorsement for seed transfer from Lake States to New England; seed sources significantly interacted with sites increasing the risk of failure without *a priori* testing. Furthermore, evidence suggests that some jack pine populations in the Northeast belong to unique, local genetic lineages (Godbout et al. 2010) that merit preservation. Seed source by site interactions are significant for jack pine across the Lake States, implying the importance of using local, rather than distant, sources (Bloese and Keathley 1998, Jeffers and Jensen 1980, King 1965, Morgenstern and Teich 1969). A summary of considerations for moving jack pine seed is contained in table 1.

Jack pine is relatively sensitive to seed transfer in the Lake States because of its heightened population structure. Seeds are not dispersed as ubiquitously as for other conifers, leading some populations to differentiate from others. Northern seed sources (relative to a common garden) were generally below the mean for tree height across the Lake States, Nebraska, Ontario, and Maine (Carter and Canavera 1984, Jeffers and Jensen 1980, Savva et al. 2007, Schantz-Hansen and Jensen 1952, Sprackling and Read 1974, Thomson

**Figure 4.** Non-serotinous cones (foreground) and serotinous cones (background) can sometimes occur on a single jack pine tree. (Photo by C. Pike, 2010)

and Parker 2008, van Niejenhuis and Parker 1996). Seed sources originating approximately 100 mi (160 km) (1 to 2° latitude) to the south are generally the tallest in provenance trials in Ontario and the Lake States (Jeffers and Jensen 1980, Morgenstern and Teich 1969, Thomson and Parker 2008). Studies of diameter growth, as measured by tree rings, recommend similar transfer limits of 1° latitude (Savva et al. 2007), from southern to northern locales. Long-distance transfers (greater than 250 mi [400 km]) of jack pine seed sources should generally be avoided across the northern United States.

For Lake States and Ontario seed sources, variability in jack pine provenance trials is more closely associated with temperature and photoperiod than with precipitation at the geographic origin (Matyas and Yeatman 1992). Specifically, jack pine growth is sensitive to mid-summer and winter temperatures (Thomson and Parker 2008, van Niejenhuis and Parker 1996) and precipitation to a lesser degree (van Niejenhuis and Parker 1996). Seed sources that are adapted to longer summer seasons may be genetically predisposed to late-season indeterminate growth in which multiple flushes in a season are possible under the right conditions. Northern sources exhibit more conservative growth patterns than other sources in common garden experiments,

**Table 1.** Summary of considerations for moving jack pine seed.

| **Jack pine, *Pinus banksiana* Lamb** | |
|---|---|
| Genetics | • Genetic diversity: high<br>• Gene flow: high (pollen); medium (seed) |
| Cone and seed traits | • Small, winged seeds<br>• 131,000 seeds per pound (288,200 per kg)<br>• Cones may be serotinous or non-serotinous<br>• Seed is released in late summer to early fall |
| Insect and disease | • Jack pine budworm, sawfly<br>• Eastern gall rust, western gall rust, Diplodia (young seedlings) |
| Palatability to browse | • Moderate to high browsing from white-tailed deer in the winter months |
| Maximum transfer distances | • Seed sources originating 70 to 140 miles south of the planting site (112 to 225 km; 1 to 2° latitude) display higher growth rates than local sources |
| Range-expansion potential | • Likely to shift range northward into Canada but southern range edge may persist in the United States due to its drought tolerance |

presumably because they are genetically adapted to shorter growing seasons and colder mid-winter temperatures (Thomson and Parker 2008). Efforts to conserve southern range edge populations are warranted as these populations are likely candidates for transfer to more northerly sites as the climate warms.

## Insects and Diseases

Jack pine budworm (*Choristoneura pinus* Freeman) is the most important insect on mature stands of jack pine in the Lake States (McCullough et al. 1994, McCullough 2000). Minor pests include white pine weevil (*Pissodes strobi* [Peck]) and eastern pine-shoot borer (*Eucosma gloriola* Heinrich), both of which damage or deform young trees, lowering future commercial value. Differences among seed sources for susceptibility to pine shoot borer were not significant in provenance trials (Hodson et al. 1982, King 1971). Pitch nodule maker (*Petrova albicapitana* [Busck]) is also a minor pest but may become problematic if outbreaks coincide with other pests (King 1971, McLeod and Tostowaryk 1971). Several sawfly species impact jack pine including red headed pine sawfly (*Neodiprion lecontei* [Fitch]) and jack pine sawfly (*Neodiprion Swainei* Midd.)(figure 5). Insects that feed on cones or seed can affect half or more of jack pine cones in a seed orchard (Rauf et al. 1985).

Several diseases affect jack pine across the Lake States. Two types of gall rusts occur in the Lake States and are largely allopatric: western gall rust (*Endocronartium harkensii* [J.P.Moore] Y. Hiratsuka) (Anderson 1970) and eastern gall rust (*Cronartium quercuum* [Berlc.] Miyabe ex Shirai) (Dietrich et al. 1985, Nighswander and Patton 1965) (figure 6). Eastern gall rust is more virulent and problematic on jack pine than western gall rust. The separation between their ranges is parallel to, and approximately 50 mi (80 km) west of, the divide between central and northern floristic regions in Minnesota (Aaseng et al. 2011). The northern edge of eastern gall rust corresponds with the same clinal break in jack pine illustrated in Schoenike (1976) and redrawn in Critchfield (1985). Susceptibility to eastern gall rust is strongly influenced by seed source: sources from northern Minnesota were significantly more susceptible at common gardens in lower Michigan and southern Wisconsin than local sources (King 1971). No other pests or insects studied demonstrated a similar association with latitude. Diplodia tip blight (*Diplodia sapinea* [Fries] Fuckel) has also become a major issue on young jack pine seedlings in the Lake States (Stanosz et al. 2007; Nicholls 1990). Needle cast, caused by *Hypodermella ampla* (Davis) Dearn has been reported in provenance trials (King and Nienstaedt 1965) but differences were not attributable to geographic origin of seed sources and this disease has not been problematic in recent years.

**Figure 5.** Sawfly larvae can defoliate large swaths of jack pine. (Photo by J. Warren, USDA Forest Service, 2011)

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**Figure 6.** Eastern gall rust is a devastating pathogen on jack pine in the Lake States. The globose galls (shown) can lead to windthrow and may dominate the canopy of highly susceptible trees. This sample was observed at a seed orchard in Minnesota. (Photo by C. Pike 2008)

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# Propagation of ‘Iliahi (*Santalum paniculatum* Hook. & Arn.), a Valuable Endemic Hawaiian Sandalwood Species

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## Abstract

Six *Santalum* species are endemic to the Hawaiian Islands where they are known locally as ‘iliahi. ‘Iliahi were once widespread throughout the islands, but most stands were harvested for the valuable heartwood, reducing the distribution by an estimated 90 percent. Limited natural regeneration and a burgeoning interest in ‘iliahi cultivation have prompted the need for reliable propagation systems. One species in particular, *Santalum paniculatum*, has been the focus of interest due to available planting area, relative abundance, and commercial-grade oil quality. We describe methods for seed harvesting, processing, germination, seedling transfer, growth, and field planting of *S. paniculatum*. These methods have successfully produced high-quality seedlings, although further research is needed.

## Introduction

Approximately 1 percent of all angiosperms are parasitic, and most are root hemiparasites (Heide-Jørgensen 2013, Matthies 2017). Hemiparasitic plants can photosynthesize yet rely on specialized root structures called haustoria to connect to hosts and extract resources (Bell and Adams 2011, Matthies 2017). Hemiparasites tap into the xylem tissue of host and plants and are capable of extracting water, mineral nutrients, amino acids, and carbon (Govier et al. 1966, Těšitel 2010, Westwood 2013). These species are generally able to parasitize a variety of plant taxa, although nitrogen-fixing legumes are commonly found to be superior hosts, producing greater growth in the parasite (Annapurna 2006, Ouyang 2016). There is evidence of a bi-directional flow of resources, although the dominant direction of flow is towards the parasite, often resulting in reduced growth of the host (Lu et al. 2020, Radomiljac and McComb 1998b, Westwood 2013). Of all hemiparasitic woody angiosperms, most belong to the Oleaceae and Santalaceae families (Veenendaal et al. 1996).

Within the Santalaceae family is the *Santalum* genus, which has species widely distributed from India to Australia and throughout the Pacific Ocean (Teixeira da Silva et al. 2016, Wagner et al. 1999). Members of the *Santalam* genus are collectively known as sandalwood. There are six *Santalum* species and several varieties endemic to the Hawaiian Islands (Harbaugh et al. 2010). The Hawaiian species comprise approximately one-third of the *Santalum* species worldwide (Harbaugh et al. 2010). The six species are derived from two separate founding events, one ancestral group with white flowers and the other ancestral group with red flowers (Harbaugh et al. 2010) (table 1). The five upland tree-like species in Hawaii are locally known as ‘iliahi and the one coastal-growing, shrubby species is known as ‘iliahialo‘e. Additional vernacular names for the ‘iliahi species include ‘a‘ahi, ‘aoa, lā‘au ‘ala, and wahie ‘ala (Wagner et al. 1999). Scientific nomenclature are *Santalum ellipticum* Gaudich (coastal sandalwood), *Santalum freycinetianum* Gaudich. (Freycinet sandalwood), *Santalum haleakalae* Hillebr. (Haleakalā sandalwood), *Santalum involutum* H. St. John, *Santalum paniculatum* Hook. & Arn. (mountain sandalwood), and *Santalum pyrularium* A. Gray (forest sandalwood) (figure 1).

**Table 1.** Six ʻiliahi species (*Santalum* sp.) and varieties are distributed throughout the Hawaiian Islands (based on Harbaugh et al. 2010).

| Scientific name[1] | Common names | Distribution | Flower group |
|---|---|---|---|
| *Santalum ellipticum* Gaudich | ʻiliahi, ʻiliahialoʻe, coastal sandalwood | Kauaʻi, Oʻahu, Maui, Lanaʻi, Molokaʻi, Kahoʻolawe, Hawaiʻi and Northwestern Hawaiian Islands | White |
| *Santalum freycinetianum* Gaudich | ʻiliahi, Freycinet sandalwood | Oʻahu | Red |
| *Santalum haleakalae* Hillebr | ʻiliahi, Haleakala sandalwood | Maui | Red |
| *Santalum involutum* H. St. John | ʻiliahi | Kauaʻi | White |
| *Santalum paniculatum* Hook. & Arn | ʻiliahi | Hawaiʻi Island | White |
| *Santalum pyrularium* A. Gray | ʻiliahi | Kauaʻi | Red |

[1] Harbaugh et al. (2010) identified species varieties for *S. ellipticum* and *S. haleakalae*. Additionally, some practitioners recognize *S. paniculatum* var. *pilgeri*.

ʻIliahi are broadleaf evergreen, hardwood tree species native to moderately wet to dry Hawaiian forests (Merlin et al. 2006, Wagner et al. 1999). All ʻiliahi species can form a single bole trunk and reach varying heights. *Santalum ellipticum* is most commonly found as a sprawling shrub but can occasionally be found as a short stature tree 3.3 to 16 ft (1 to 3 m) tall (Merlin et al. 2006). The largest species of ʻiliahi (*Santalum paniculatum* and *Santalum freycinetianum*) can reach heights of 43 to 66 ft (13 to 20 m). ʻIliahi have opposite and simple leaves that can be ovate, elliptic, orbicular, or obovate (Wagner et al. 1999). ʻIliahi and other *Santalum* species have been, and are still, coveted internationally as sources for the aromatic heartwood that is primarily used for carving, constructing fine furniture, burning in religious practices, and producing sandalwood essential oil (Teixeira da Silva et al. 2016, Thomson et al. 2011). As a result of this resource value, ʻiliahi and other *Santalum* species have been severely exploited throughout their ranges (Kepler 1983, Teixeira da Silva et al. 2016).

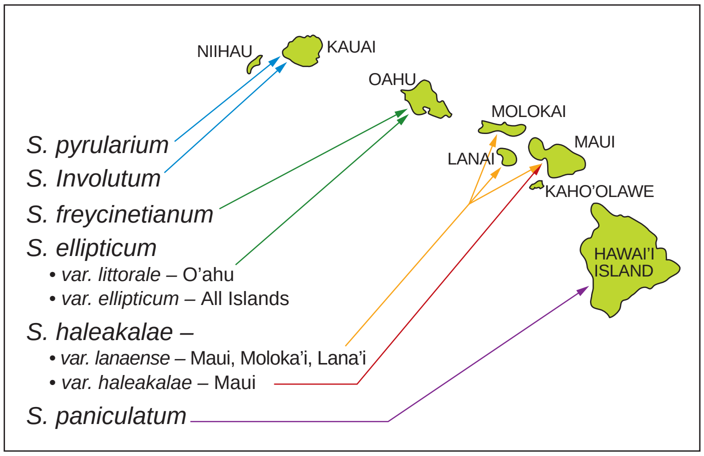

**Figure 1.** ʻIliahi species (*Santalum* sp.) and varieties are distributed throughout the Hawaiian Islands (based on Harbaugh et al. 2010).

Native Hawaiians used parts of ʻiliahi in a variety of applications including the construction of traditional stringed instruments (Buck 1964), perfume and preservation of traditional plant fiber cloths (kapa) (Kepler 1983, Kraus 1972), and medicinal treatments for dandruff, head lice, and reproductive ailments (Kraus 1972). Before western contact, native Hawaiians likely affected lowland ʻiliahi populations (below 1,500 ft [457 m] elevation) by burning and clearing to make way for agricultural fields (Kirch 1982). Following western contact, visiting merchants who were familiar with the valuable sandalwood tree being traded in China soon realized there was a substantial supply of these trees in Hawaiʻi.

The export of ʻiliahi from Hawai'i to Chinese markets began as early as 1790, after the Hawaiian monarchy became aware of the value of this natural resource (Merlin and VanRavenswaay 1990). Expansion of the ʻiliahi trade was catalyzed by predatory lending practices that involved foreign traders allowing monarchs to purchase foreign luxury items on the promise to be paid in exorbitant amounts of ʻiliahi. The monarchy incurred a substantial ʻiliahi debt and the burden of repayment fell on the shoulders of the common people who were forced to harvest ʻiliahi to repay the merchants. Indeed, the first written law in the Hawaiian kingdom was a sandalwood tax that stated "every man was required to deliver one half of a picul (133.3 lb [60.5 kg]) of sandalwood to the governor of the district to which he belonged, or to pay in lieu thereof four Spanish dollars, on or before September 1, 1827" (Merlin and VanRavenswaay 1990). When easily accessible, lowland ʻiliahi became depleted, native Hawaiians resorted to harvesting trees from distant upland forests, resulting in fatal exposure to the elements and the neglect of food crops. This ultimately contributed to famine and compounded the detrimental effect of alien diseases on native

Hawaiians (Merlin and VanRavenswaay 1990). At its peak, the sandalwood trade in Hawai‘i was so extensive that the islands were known in China as “Tahn Heung Sahn” or “the Sandalwood Mountains” (Kepler 1983). The Hawaiian sandalwood trade had all but ceased by 1940, with the decline attributed to dropping ‘iliahi prices associated with competition from Indian sandalwood sources and poor-quality material sourced from the increasingly depleted Hawaiian forests (Merlin and VanRavenswaay 1990).

By the end of the trade, an estimated 90 percent of the natural ‘iliahi stands were harvested (Rock 1974). The most extensive remnant portions are located in the upland mountains of the Kona district on Hawai‘i Island (Rock 1974). All ‘iliahi species are still extant but have smaller ranges due to the harvest history. Other threats to forest health, such as grazing, invasive species, and fire, have suppressed natural regeneration of ‘iliahi stands (Merlin et al. 2006). Poaching is an additional threat given the species’ high commercial value. The discrepancy between possible historic ranges, as modeled by Price et al. (2012), and currently known ranges for ‘iliahi suggest extensive areas for potential restoration of this species.

The economic value of ‘iliahi lends to the potential for it to be a native hardwood forestry crop. Several small operations have reintroduced ‘iliahi products into the global market through salvage harvest operations of dead or dying trees from upland populations. While natural regeneration of ‘iliahi is often minimal, coppicing and root sucker growth are stimulated by salvage harvests. Harvesting the whole tree (trunk and root ball) while leaving lateral roots has resulted in replacement rates of 7 to 10 new coppices or suckers per harvested tree (Senock 2017) (figure 2). Coppice and root suckers produce flowers and seeds within 2 years of emergence compared with 4 years from a seed-planted individual. Coppicing and root suckering cannot, however, expand the current range to the estimated former range because new shoots can only occur in areas where ‘iliahi are already present. Considering this, artificial regeneration is a key strategy for restoring ‘iliahi species beyond the current range. Thus, reliable propagation systems for ‘iliahi must be developed to ensure the long-term success of restoration and commercial cultivation.

Of the ‘iliahi species native to Hawai‘i, *Santalum paniculatum* is an optimal candidate for cultivation due

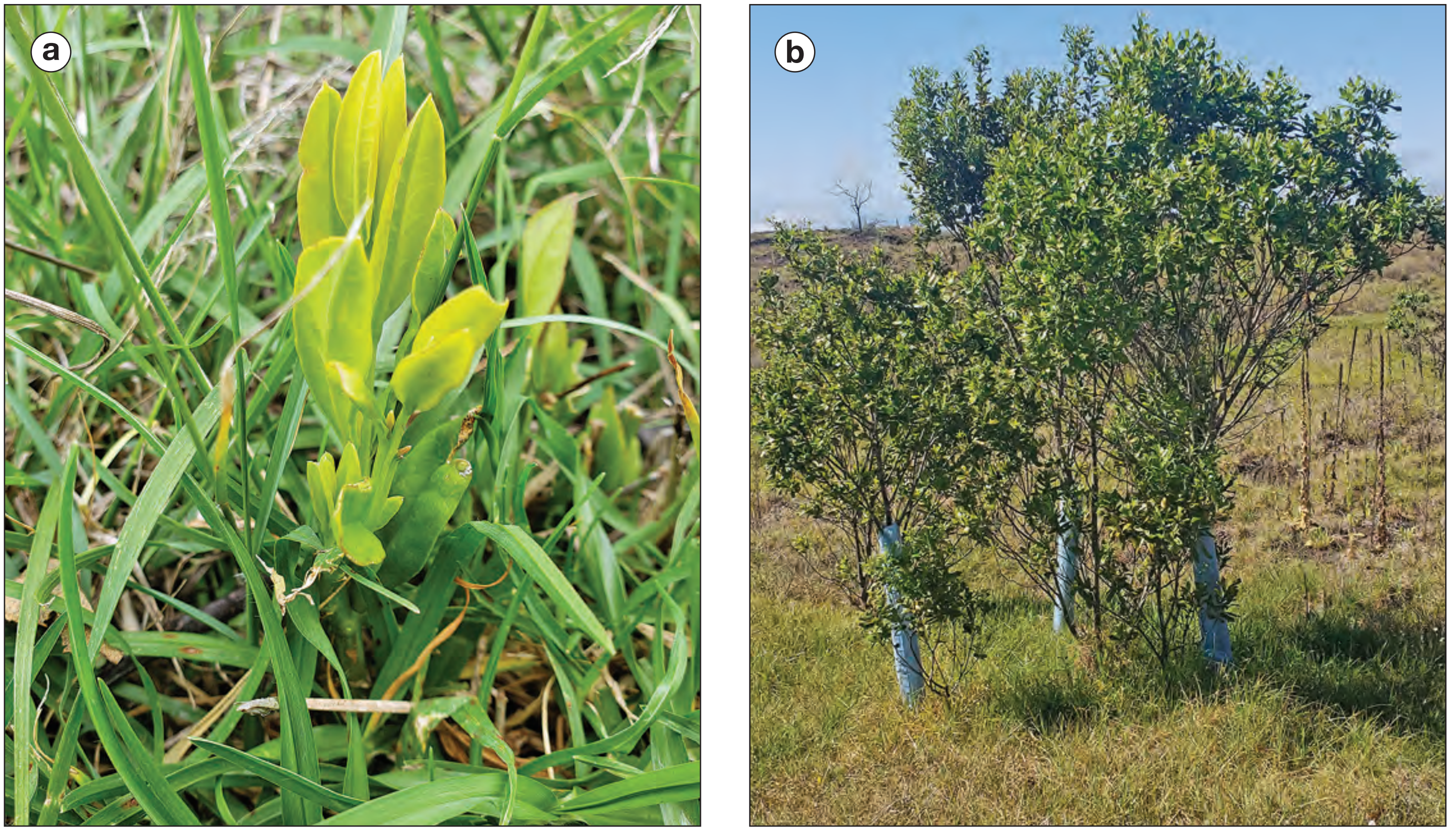

**Figure 2.** ‘Iliahi regenerates from root suckers such as this (a) 1-month-old ‘iliahi root sucker and this (b) group of 7-year-old root suckers that have regenerated from harvesting a single tree. (Photos by T. Speetjens, 2020)

to its large potential range for restoration, the greatest remnant abundance relative to other species, and its ability to produce commercial-grade oil (Braun et al. 2014, Price et al. 2012, Rock 1974). This article focuses on the propagation of *S. paniculatum*, hereafter referred to as ‘iliahi. Our objective is to provide a detailed and illustrated protocol on the proper care of ‘iliahi seeds and seedlings to help guide future propagation efforts. We provide our recommendations based upon our observations and successes. Each nursery location is different, however, and methods should be adjusted to suit local conditions. Propagating ‘iliahi is an evolving topic and several ongoing research projects currently strive to broaden the understanding of ‘iliahi regeneration to support efforts toward expanding ‘iliahi’s current range to be more representative of its former, more abundant distribution.

## Step 1: Seed Harvest and Processing

The ‘iliahi fruiting season is highly variable between and within populations. The timing of fruiting depends on the tree health and its geographic location. ‘Iliahi of Hawai‘i Island’s south Kona district typically begin to flower in November and will carry fruit into June. Each fruit (drupe) contains a single seed and ripens from green to a mature reddish-purple or black (Wagner et al. 1999, Wilkinson 2007) (figure 3). Fruits should be picked at peak ripeness before the pulp begins to dry. If no fresh fruits are available on the tree, fallen fruits may be viable and suitable for use (Isch 2021). Seeds should be processed immediately to reduce the likelihood of pulp rot and associated negative effects on the seed embryo. If immediate processing is not possible, fruit can be stored between 35 and 39 °F (2 and 4 °C) in a paper bag for up to 1 week.

The first step of seed processing is to remove the pulp from the seed. Seeds should be soaked in water to soften the pulp which aids in its removal (figure 4a). We recommend soaking fresh fruits for a minimum of 1 hour and older, dried fruits for a minimum of 3 to 4 hours. Some propagators have found success in soaking fruits for up to 4 days to aid in pulp removal (Isch 2021). Once soaked, the pulp can then be removed by rubbing fruits against a metal mesh screen with a gloved hand (figure 4). To process large quantities, the seeds can be enclosed in a wire mesh cage and power washed until the majority of pulp is removed (figure 5). After

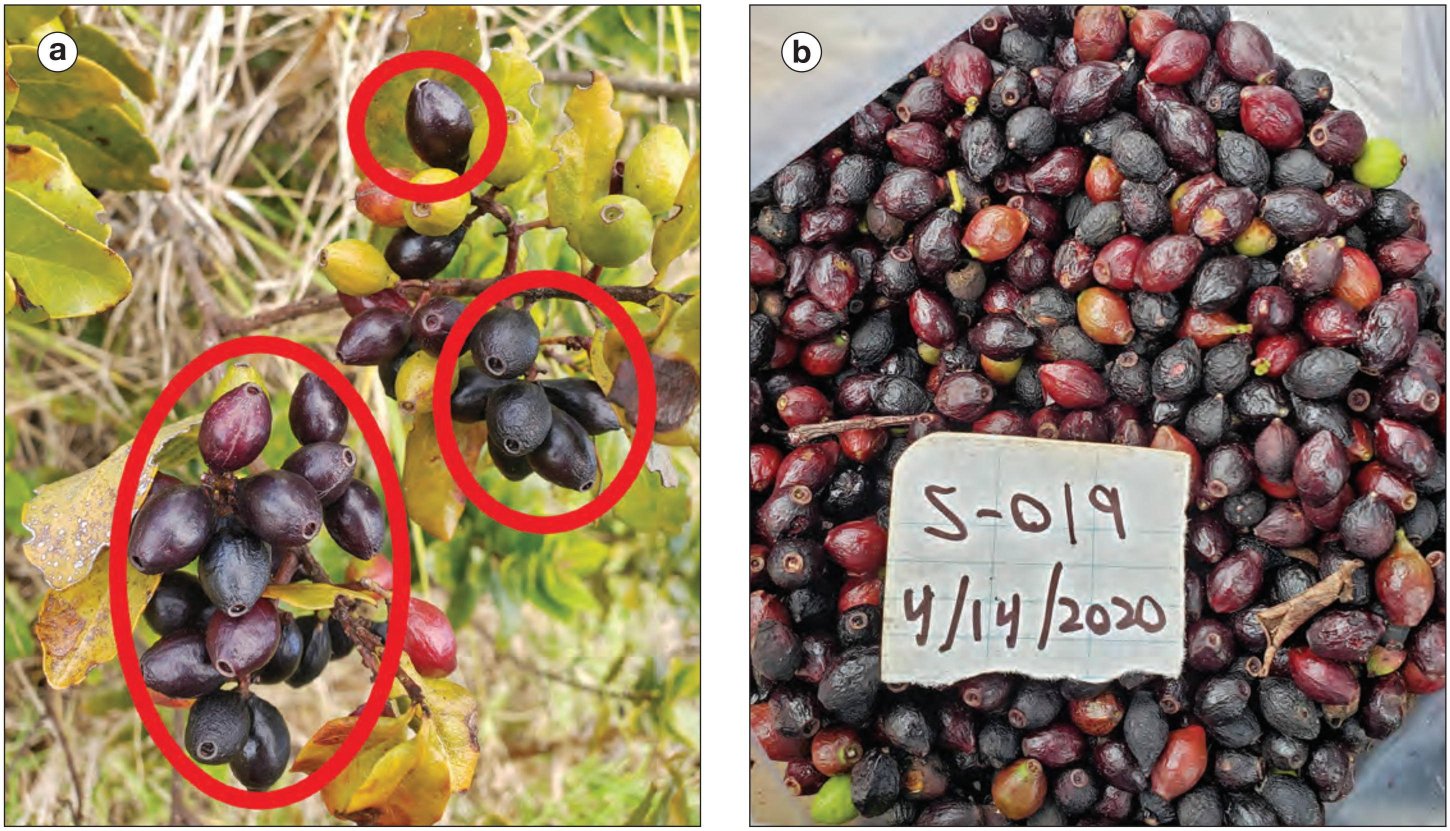

**Figure 3.** During seed collection season, ‘iliahi trees can have both (a) green unripe fruits and dark purple ripe fruits. (b) Fruits should be collected when they are ripe. (Photos by T. Speetjens, 2020).

**Figure 4.** The first step in processing ‘iliahi seeds, is to remove the pulp. (a) Fruits are soaked in water for 3 to 4 hours to soften pulp. (b) A wood box with a mesh screen bottom can then be used for pulp removal by (c) repeatedly rubbing fruits against the mesh screen and rinsing so the pulp falls through the screen. The process is continued until (d) all pulp is removed from the seed. Seeds should be sown immediately or (e) dried for storage by placing on a wire mesh or cloth weed mat to be air dried for 3 to 5 days. (Photos by T. Speetjens, 2020)

power washing, it is often necessary to remove any remaining pulp with the metal mesh screen method. For all methods, it is important not to crack the seed coat and expose the embryo. Some propagators have found success sowing seeds with pulp still on when the seed is fresh (Shigematsu 2021), although we recommend removing the pulp for storage preparation and to reduce the amount of material available for potential fungal growth.

Once the pulp has been removed, the seeds should be sown immediately or properly stored. Seed can be dried for 1 to 3 days (and up to 7 days) to 8 percent moisture (figure 4e) and stored at 39 °F (4 °C) in airtight containers or bags for several years (Elevitch and Wilkinson 2003, Wilkinson 2007). ‘Iliahi species have been identified as likely freeze sensitive (Chau et al., 2019), indicating that more research would be beneficial in guiding long-term storage and seed banking.

## Step 2: Seed Germination

We have observed variable germination rates from 10 to 90 percent and variable germination time from 4 months to 2 years depending on seed treatment (Elevitch and Wilkinson, 2003, Wilkinson, 2007).

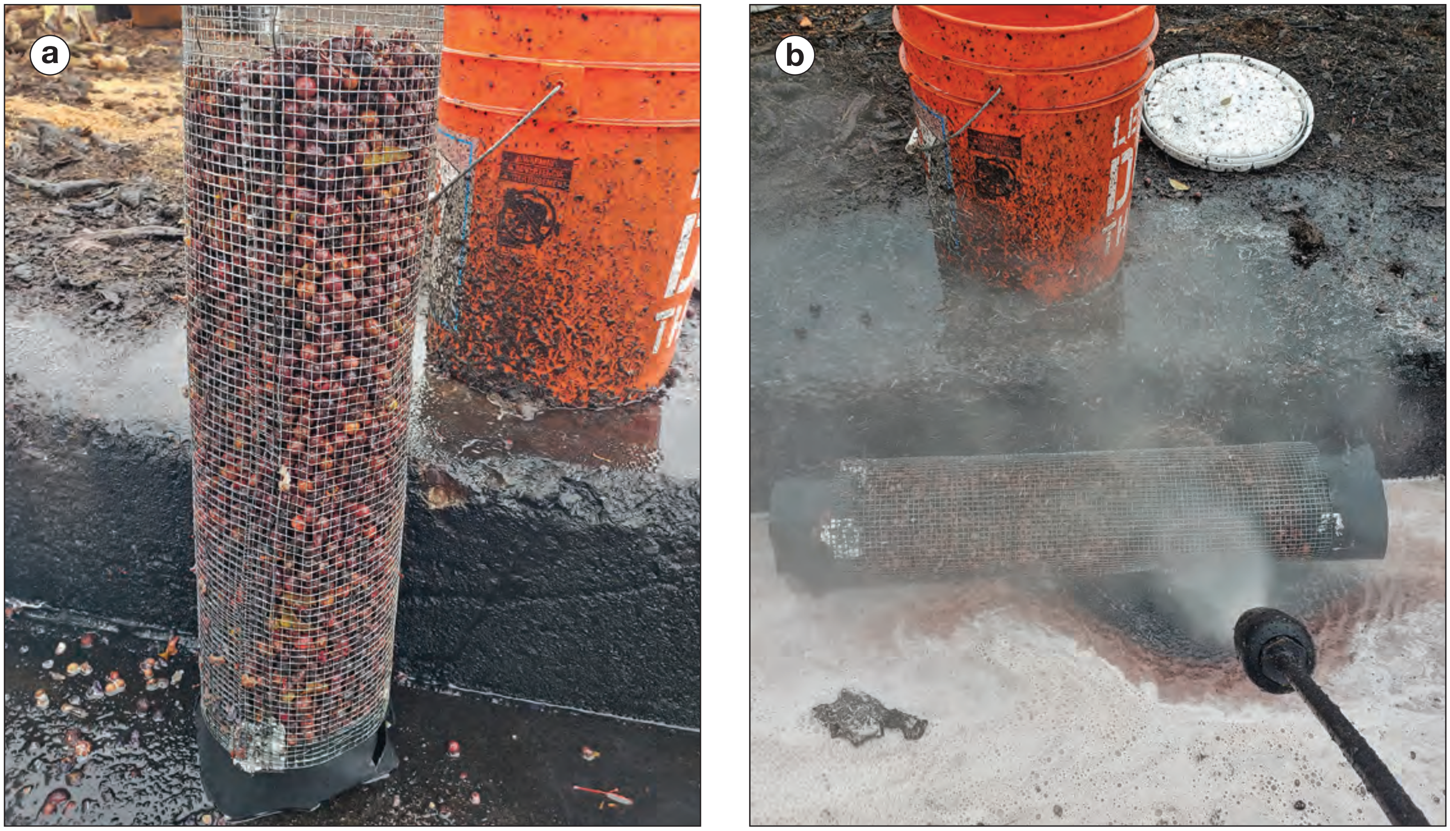

**Figure 5.** To remove large quantities of ʻiliahi seeds, fruit can be (a) placed in a mesh cage and then (b) washed with a power washer until the majority of pulp is removed. (Photos by T. Speetjens, 2019)

Sandalwood seeds have a hard, semi-permeable seed coat, a large endosperm, and a reduced embryo. Viable seeds will have a crisp, white-colored endosperm when split and the radicle will emerge at the seed apex when germinating (figure 6). Indian sandalwood (*Santalum album* L.) seeds exhibit morphophysiological dormancy, meaning seeds require treatment with the plant hormone gibberellic acid ($GA_3$) to overcome physiological aspects of dormancy (Jayawardena al. 2015). This type of dormancy appears to hold true

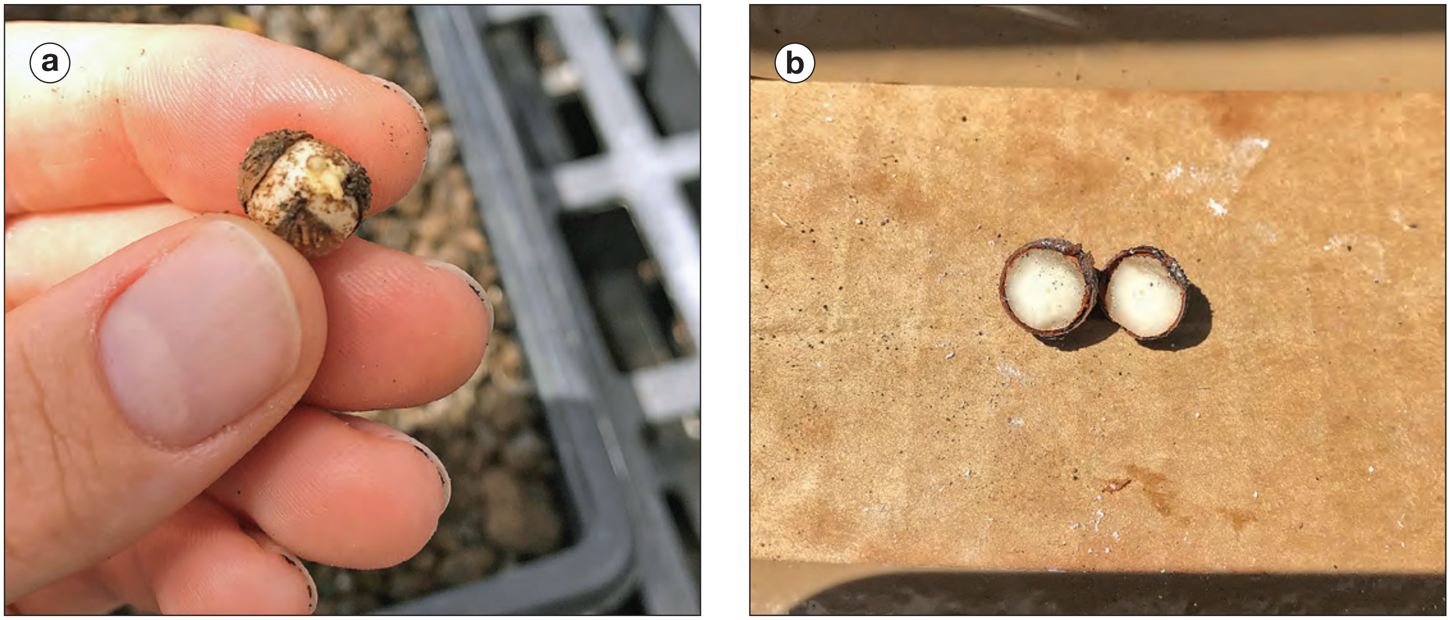

**Figure 6.** (a) A radicle will emerge from the large seed endosperm of a viable seed as it germinates. (b) Viable seeds have a crisp white embryo when split. (Photos by E. Thyroff, 2020)

for other *Santalum* species, including ‘iliahi, although further studies are needed to confirm this. Confirmation of morphophysiological dormancy would imply the seed coat is permeable to water and that scarification (e.g., clipping the tip of the seed coat or using sandpaper abrasion) is not necessary. Some Hawai‘i propagators have had variable success with physical scarification improving germination rates, although extreme care should be taken not to expose or damage the embryo. Exposing the embryo can potentially increase the chances of the seed rotting before germination.

Treating seeds with $GA_3$ is known to assist many plant species in overcoming physiological dormancy by altering the $GA_3$ and abscisic acid (ABA) ratio within the embryo (Kucera et. al, 2005). Many studies have shown that $GA_3$ reduces germination time and increases germination success for Indian and Australian sandalwood (*Santalum accuminata* A.DC.) (Das and Tah 2013, Jayawardena et al. 2015, Teixeira da Silva et al. 2016). $GA_3$ can be purchased in a powder or a pre-dissolved liquid form. The powder form must be dissolved in water (preferably deionized water) to reach the target concentration using a reference formula: (mg/ml)*1000 = ppm, or (ppm*ml)/1000 = mg, or (oz/fl oz)*1000 = ppm, or (ppm*fl oz)/1000 = oz. For example, to mix 500 ml (16.9 fl oz) of 500 ppm $GA_3$, use 250 mg (0.00882 oz) $GA_3$ powder. Mix the $GA_3$ powder and water in glassware using a magnetic mixer for 2 hours to ensure the powder is fully dissolved (figure 7).

Research with Indian and Australian sandalwood species found 100 to 500 ppm $GA_3$ was effective for stimulating germination (Lu et al. 2014, Ouyang et al. 2015, Radomiljac, 1998). A study at Lyon Arboretum with *Santalum ellipticum* found comparable germination success between 200 and 600 ppm (Kroessig and Chau 2021). We have found that ‘iliahi seeds treated with a 24-hour soak at 400 to 500 ppm $GA_3$ begin to germinate within 30 to 45 days, and most viable seeds germinate within 180 days. After treating with $GA_3$, we observed that two ‘iliahi species (*S. ellipticum* and *S. freycinetianum*) appear to have similar germination timelines to *S. paniculatum*, whereas another ‘iliahi species (*S. haleakalae*) appears to take longer. Further studies are needed to confirm and compare germination rates within ‘iliahi species in response to $GA_3$. After $GA_3$ treatment, seeds should be sterilized by soaking for 1 minute in a 10-percent bleach solution, rinsed, then coated with powdered sulfur and/or Captan® fungicide to reduce the risk of embryo rot (Elevitch and Wilkinson 2003, Hirano 1990, Wilkinson 2007, personal observations).

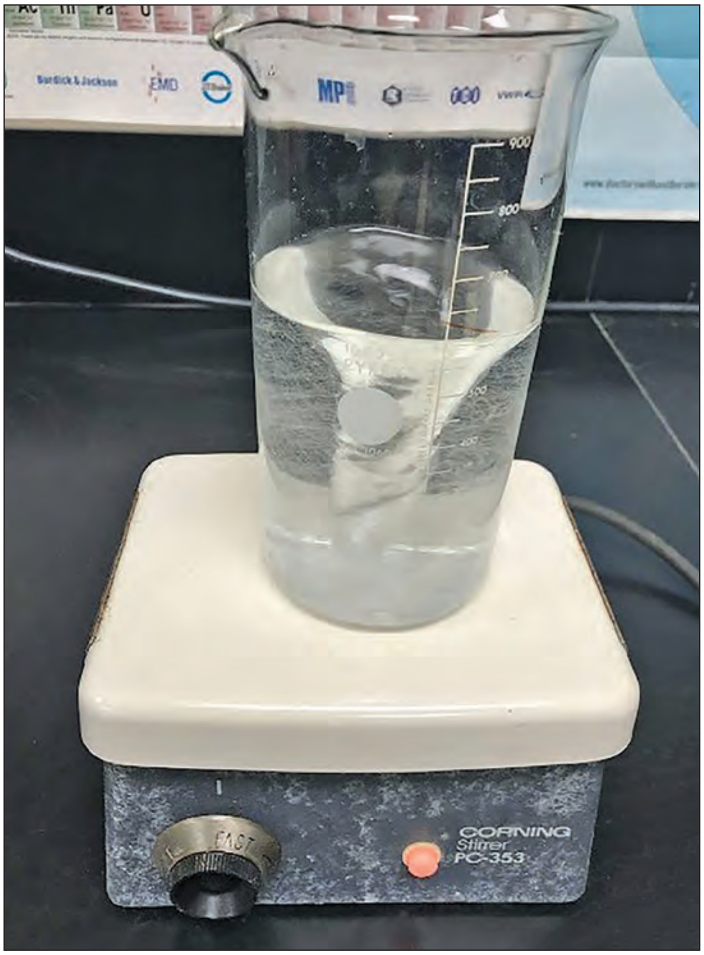

**Figure 7.** A magnetic mixer can be used to stir deionized water and gibberellic ($GA_3$) powder into solution. (Photo by E. Thyroff, 2020)

Seeds are sown in germination trays onto the surface of the germination medium at approximately 2 to 4 seeds per $in^2$ (2 to 4 per 6.5 $cm^2$) in a single layer (figure 8a). The size of ‘iliahi seeds may vary, which will affect sowing density (figure 8b). A layer of black cinder (0.5 to 0.75 in [1.3 to 1.9 cm]) should be applied over the seeds to minimize weeds (figure 8c).

We have used a variety of media for germinating ‘iliahi seeds including: 1) 5:1:1 ratio of perlite:coconut coir:vermiculite topped with black cinder; 2) 5:1 ratio of perlite:vermiculite, 3) 1:1 ratio of perlite:potting soil topped with black cinder; 4) all vermiculite; and 5) 1:1 ratio of perlite or vermiculite: *Sphagnum* peat moss. Perlite used in soil mixtures should have a particle size of 0.25 to 0.38 in (6.4 to 9.5 mm; i.e., #2, classic super coarse). Further studies are required to

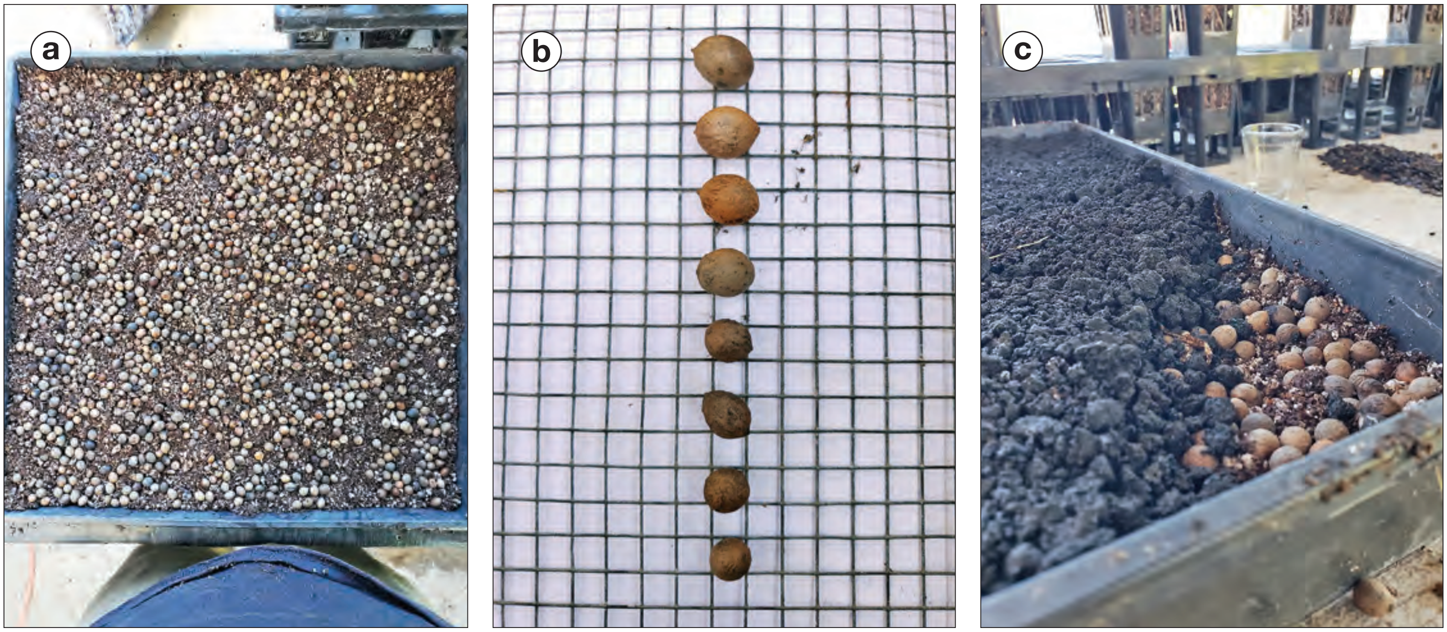

**Figure 8.** (a) ʻIliahi seeds should be sown in a single layer on medium composed of equal parts perlite and potting soil. (b) Sowing density will be affected by the variable seed sizes. (c) Seeds can be covered with a layer of fine, black cinder to prevent establishment of weeds. (Photos by T. Speetjens, 2019)

determine an ideal germination medium. Regardless of the medium used, it is critical to maintain proper moisture to aid in seed imbibition while also minimizing fungal infection and fungus gnat infestations. The medium should not remain completely saturated for multiple days and should be allowed to partially dry before rewatering. We recommend irrigation trials to determine the best frequency and duration for a given medium based on the nursery or greenhouse conditions (e.g., sunlight and temperature) where the germination trays will be located.

## Step 3: Seedling Transfer and Growth

Young seedlings should be transplanted out of germination trays within 1 to 2 months of emergence when germinants have reached the 2 to 6 true-leaf stage (figure 9). Transplanting at this stage ensures the seedling will have sufficient roots although not enough to tangle with neighboring seedlings in the germination tray. Transplanting seedlings too early could result in compromised survival, while transplanting seedlings too late increases the risk of root stress and transplant shock.

Similar to the germination tray media, there are several recommendations for container media. A few examples include: 1) 1:1:1 perlite:coconut coir:vermiculite; 2) 1:1:1 perlite:fine black cinder:potting soil; and 3) 1:1 perlite:potting soil. Wilkinson (2007) recommended a 14-14-14 (N-P-K) controlled-release fertilizer, dolomite, and gypsum integrated into the growing medium. We recommend the medium and fertilizer regime used by the Hāloa ʻĀina nursery (Kealakekua, Hawaiʻi) which consists of equal parts perlite, potting soil, and fine black cinder with controlled-release fertilizer (Osmocote® Plus 15-9-12 + micros) and organic fertilizer (Espoma® Organic Plant-Tone 5-3-3) incorporated into the medium at a rate of 10.54 lbs per yd$^3$ (6.2 kg per m$^3$). We also recommend applying a 0.4 oz (1.2 ml) ethylenediamine (EDDHA) chelated iron powder to the surface of each container at least 30 days after transplanting (applying earlier has been observed to kill some seedlings). The chelated iron powder should be watered in immediately and reapplied every 2 to 3 months. The chelated iron treatments appear to be necessary for sustained seedling growth (Hirano 1990, Wilkinson 2007, personal observations). Trials are currently being conducted to explore potential interactions of chelated iron and controlled-release fertilizer on ʻiliahi seedling quality. In addition to nutrient applications, it is good practice to inoculate ʻiliahi seedlings with local mycorrhizae to help improve growth rates, increase stress resistance, and improve nutrient uptake, especially with phosphorus (Binu et al. 2015, Davies 2000, Miyasaki 1993).

As a hemiparasite, ʻiliahi seedlings will ultimately need to attach to a host, but pairing with a host

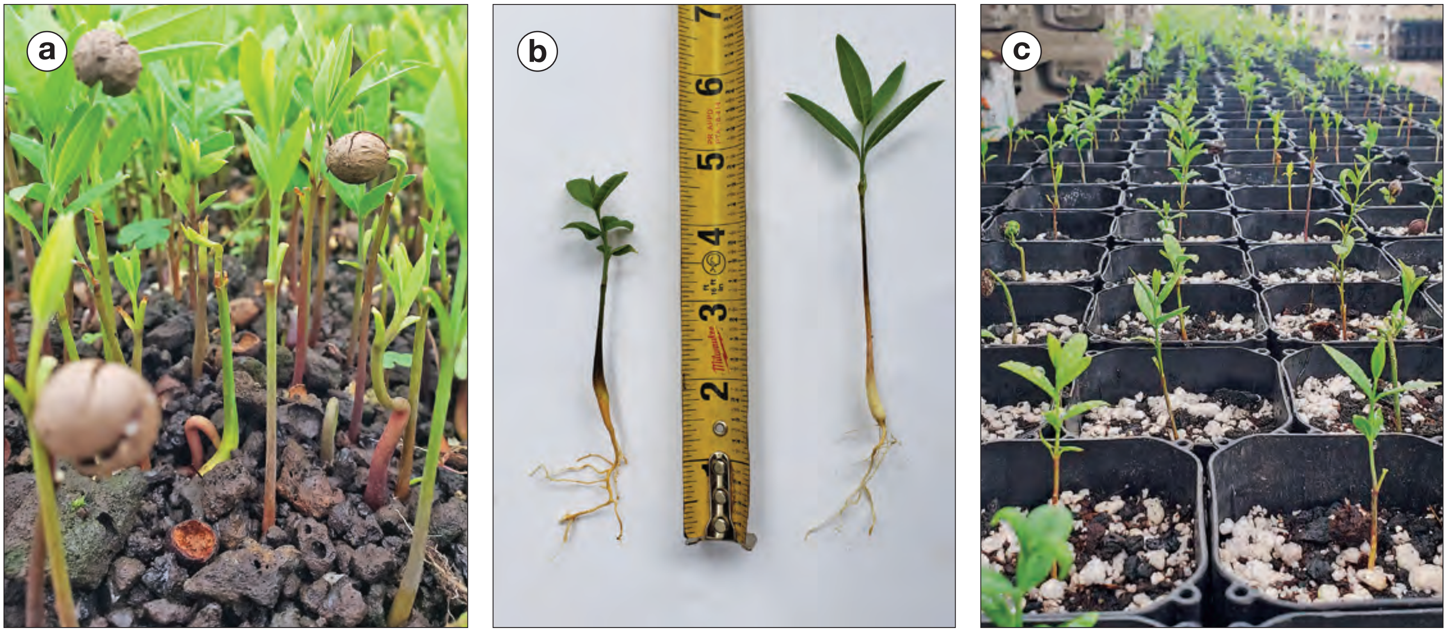

**Figure 9.** (a) ʻIliahi seeds treated with $GA_3$ will germinate in a uniform flush over 2 to 6 months and are ready to transplant when (b) seedlings have 2 to 6 true leaves. (c) ʻIliahi seedlings can be transplanted into individual containers. (Photos by T. Speetjens, 2019)

during nursery cultivation is not necessary to produce healthy growth if seedlings are fertilized sufficiently. The effect of growing ʻiliahi with a container host, in combination with fertilizer treatments, however, is not well understood. While ʻiliahi can survive without a container host, the presence of a host may help with haustoria development and long-term outplanting success. A study on Indian sandalwood showed that a non-leguminous host did not provide any benefit to growth in the nursery but significantly affected outplanting success (Radomiljac and McComb 1998a). Growing ʻiliahi with container hosts is complicated by the potential for the container host to out-compete the ʻiliahi seedling if the parasitic connection is not formed. We have observed that ʻiliahi seedlings without container hosts have good survival (more than 90 percent) when planted in areas with pre-established, 4-year-old, koa (*Acacia koa* A. Gray) host trees. We recommend not using a container host if the intended field planting area has long-term hosts established. When planting in barren areas such as a pasture, we recommend using a container host due to the likelihood of improved outplanting success. If container hosts will be used, ʻiliahi seedlings should be grown for a minimum of 3 months before introducing a host. This 3-month period will allow the ʻiliahi seedling to become established and help it compete against hosts which typically grow more vigorously. Container hosts can be introduced by sowing host seeds or by transplanting host germinants. Introducing a host via seed sowing is preferred because it reduces the risk of damaging existing ʻiliahi root structure. Seed sowing is most effective for host species with high seed viability such as koa and ʻaʻaliʻi (*Dodonaea viscosa* Jacq.). Host seeds should be treated in the manner appropriate to the species to maximize germination rates. If the seed viability of the host is poor or unknown, then we recommend germinating host seedlings in a separate flat and transplanting into ʻiliahi containers as soon as it is appropriate for the given species. When transplanting host seedlings, it is important to minimize the impact to ʻiliahi roots by using a fine point instrument to create the transplant holes.

A host suitability study on Indian sandalwood found that low-lying, prostrate legumes are the most effective at improving parasite growth during nursery propagation due to limited light competition and increased nitrogen availability (Annapurna et al. 2006). Several studies have shown that a leguminous host species usually will provide better overall growth than non-leguminous hosts (Ouyang et al. 2015, Radomiljac and McComb 1998b). We recommend using koa as a container and field host, although it may need to be top pruned in either application to reduce light competition. Although koa is not a low-lying species, it is leguminous and commonly found in association with ʻiliahi on

Hawai'i Island. Trials are underway to explore the host suitability of other native forest species and to examine the effect of a container host in nursery propagation and outplanting performance.

Depending on growing medium composition and nursery climate, 'iliahi plants should be watered 1 to 3 times per week. Similar to germination trays, medium should be saturated evenly, then allowed to partially dry before rewatering. Excessive soil moisture can lead to root rot and stunt seedling growth. Environmental conditions vary among plant nurseries. Again, we recommend irrigation trials to determine the ideal watering regimen during this propagation stage. For lighting, we recommend 60 to 80 percent shade during the active growth period. Indian sandalwood seedlings grown under 80 percent shade developed favorable characteristics while those grown in full sun had the lowest survival rates (Barrett and Fox 1994).

Containers should be large enough to allow sufficient root development during the 10-to-12-month growth period in the nursery. The Hāloa 'Āina nursery uses 46 $in^3$ (760 $cm^3$) containers with root pruning air-holes (028PIFD, Proptek Inc.; 3-in [7.6-cm] diameter by 8-in [21.6-cm] depth) to grow seedlings to maturity within 1 year (figure 10a). The air pruning holes reduce root bunching and circling at the bottom of the container and can improve new root growth formation after outplanting (Marler and Willis 1996). Indian sandalwood grown in 37 $in^3$ (600 $cm^3$) containers produced larger seedlings, compared with seedlings grown in polybags of equal or greater volume (Annapurna et al. 2004). An 'iliahi stocktype trial is currently underway to evaluate other container types and sizes (figure 10b). When growing 'iliahi with a container host, a larger container may be beneficial to accommodate the host's root mass and to reduce light competition by placing seedlings farther apart.

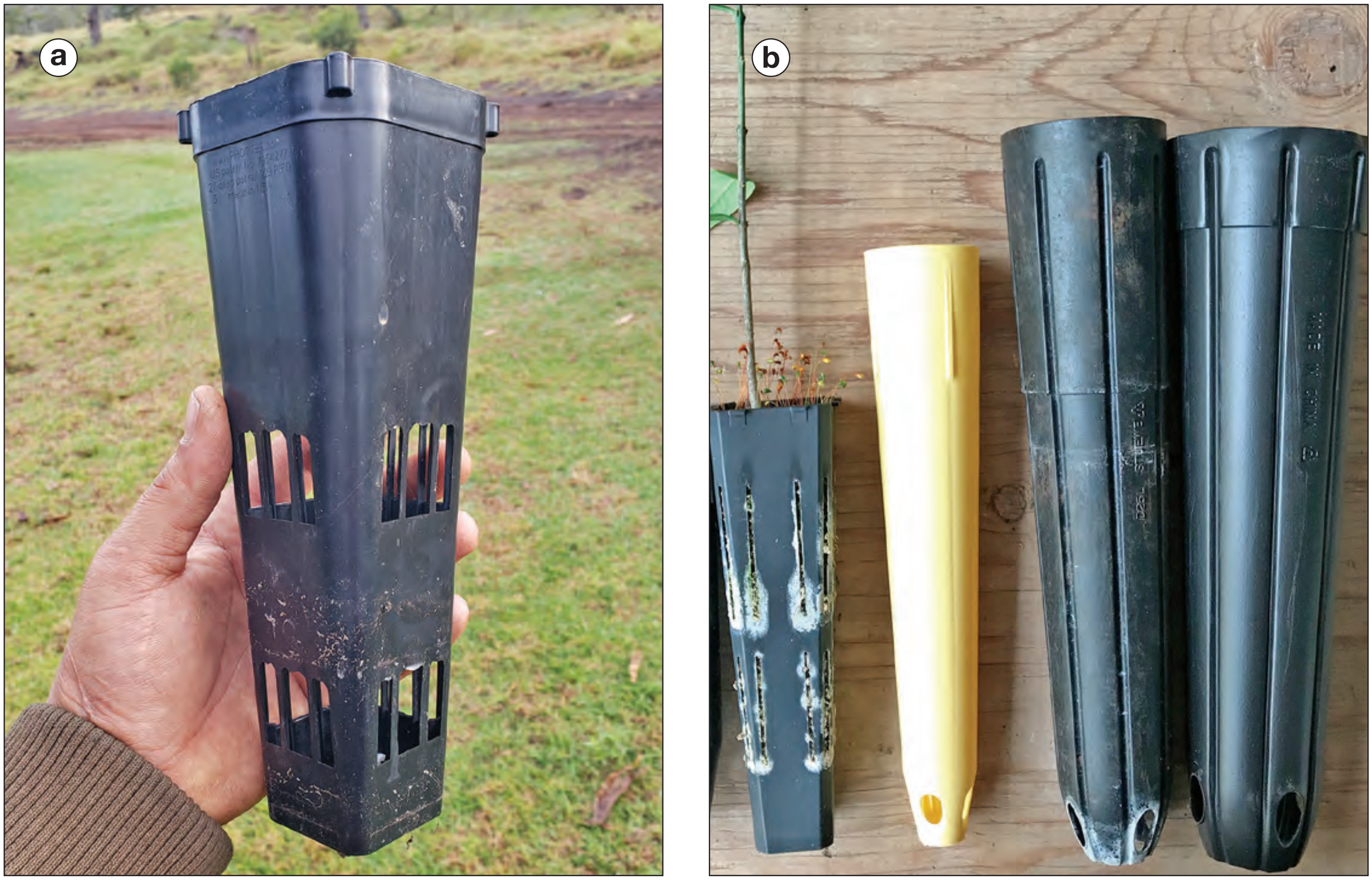

**Figure 10.** (a) Proptek 028PIFD containers (46 $in^3$ [760 $cm^3$]) have root pruning air holes that reduce root circling and bunching and have been used to grow 'iliahi seedlings successfully. (b) Iliahi has also been successfully grown in other containers (left to right: Side Slit 150, Ray Leach SC10U, Deepot 25L, and Deepot 40L; Stuewe & Sons, Inc.; container specifications available at https://stuewe.com) and additional research is underway to further evaluate 'iliahi growth in these containers (Photo a by T. Speetjens, 2019; photo b by E. Thyroff, 2020)

**Figure 11.** (a) ʻIliahi seedlings can be grown to outplanting size without a pot host in 1 year if fertilized appropriately. (b) ʻIliahi seedlings grown under suitable conditions for 1 year are ready to move to a hardening area where they will remain for at least 1 month before outplanting. (Photos by T. Speetjens, 2020)

## Step 4: Field Planting

ʻIliahi seedlings are generally ready for outplanting 1 year after transplanting from the germination flat to the individual containers (figure 11). Ideally, seedlings ready for outplanting will have new shoot growth, root systems that fully occupy the container, adequate hardening (at least 1 month), and active haustoria development before outplanting (figure 12). Hardening should consist of moving seedlings from partial shade to full sun and reducing irrigation frequency without subjecting seedlings to harmful desiccation.

The target plant concept can be used to improve seedling survival and growth by matching seedling characteristics to the outplanting site (Dumrose et al. 2016). The ideal outplanting window is determined by expected precipitation timing for the specific outplanting site. Given ʻiliahi's hemiparasitic nature, it will ultimately require a host in the field to survive and thrive. Unfortunately, there is limited data on ʻiliahi field planting and interaction with hosts after outplanting. For Indian sandalwood, a field host provides supplemental water and nutrients, resulting in higher carbon assimilation rates (Rocha et al. 2014). Additionally, hosts of Indian sandalwood seedlings appear to aid in reduced drought stress as indicated by higher pre-dawn water potential for hosted sandalwood seedlings (Rocha et al. 2014).

Ideally, ʻiliahi should be planted next to an established host, but if no established hosts are present, then a container host should be used, and additional hosts should be planted concurrently (figure 13). The container host alone will not sufficiently support the parasitic need of ʻiliahi, so additional hosts will be needed to support long-term ʻiliahi seedling growth (Wilkinson 2007). Australian planting guidelines recommend planting at least 3 hosts per sandalwood (Brand 2005). As a general rule, distance between ʻiliahi and field hosts should not exceed the height of the host plant to optimize the chance ʻiliahi roots will

**Figure 12.** (a and b) Active haustoria formation, as shown on this 1-year-old ʻiliahi paired with mamane (*Sophora chrysophylla*), is desired at the time of outplanting and indicates the parasitic connection has been formed. (Photos by T. Speetjens, 2019)

encounter host roots. Additionally, ʻiliahi should also be planted on the south side of the host to reduce shading during dawn and dusk. In a plantation cultivation setting, Indian sandalwood has often been planted at a 10- to 20-ft (3- to 6-m) spacing with host plants interspersed (Page et al. 2012, Wilkinson 2007). Exploring the suitability of the large number of native plant species that could serve as hosts to ʻiliahi may be beneficial to long-term ʻiliahi restoration efforts (figure 13d). Several potential host species identified by anecdotal observations of nursery propagation, planting trials, and plant communities within remnant ʻiliahi stands. These potential hosts species include koa, koaiʻa, (*Acacia koaia* Hillebr.), ʻaʻaliʻi, ʻōhiʻa lehua (*Metrosideros polymorpha* Gaudich.), and māmane (*Sophora chrysophylla* [Salisb.] Seem.).

In Hawaiʻi, there are many limitations to restoration work including the need to protect seedlings from herbivory (Friday et al. 2015). This herbivory concern necessitates the protection of both ʻiliahi and its host. Given the opportunities for forest restoration in Hawaiʻi, and considering ʻiliahi's once expansive range, there is great potential for ʻiliahi to be incorporated into many restoration projects and to be cultivated commercially. Various publications and guides for other sandalwood species worldwide may be complementary to efforts with ʻiliahi (Lu et al. 2020, Neil 1990, Noordwijk et al. 2001, Surata and Butarbutar 2008).

## Pests of Concern

Several pests present major challenges to ʻiliahi cultivation. Rats (*Rattus* sp.) eat ʻiliahi seeds and can also kill seedlings by gnawing on the stem. Seeds in trees should be protected from rat predation using metal tree bands installed around the trunk of the tree to prevent rats from climbing up the tree trunk to reach seeds (figure 14a). Rats should also be excluded from germination trays using wire mesh cages (figure 14b). If left unchecked, a single rat can eat dozens of seeds per night.

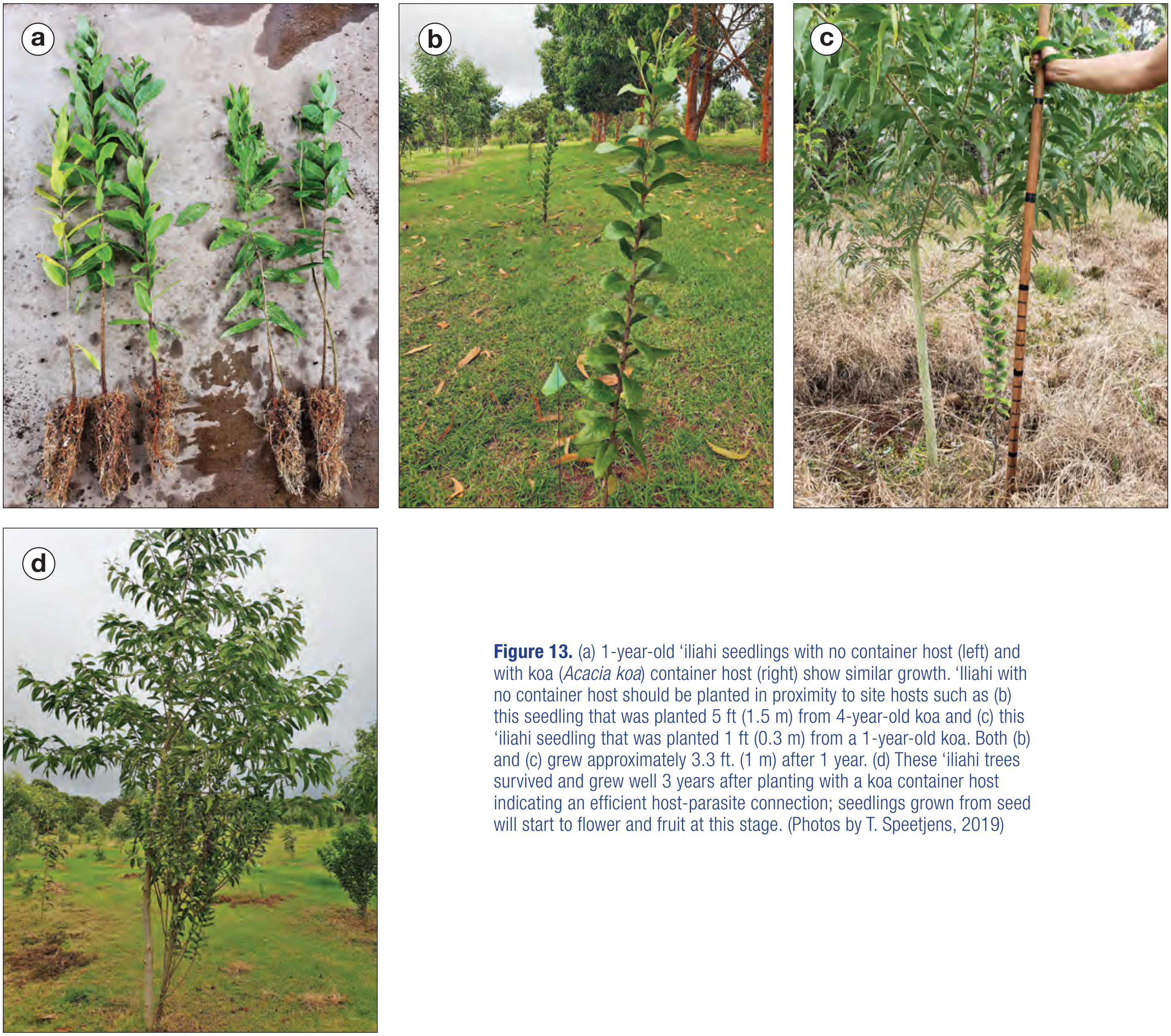

**Figure 13.** (a) 1-year-old ‘iliahi seedlings with no container host (left) and with koa (*Acacia koa*) container host (right) show similar growth. ‘Iliahi with no container host should be planted in proximity to site hosts such as (b) this seedling that was planted 5 ft (1.5 m) from 4-year-old koa and (c) this ‘iliahi seedling that was planted 1 ft (0.3 m) from a 1-year-old koa. Both (b) and (c) grew approximately 3.3 ft. (1 m) after 1 year. (d) These ‘iliahi trees survived and grew well 3 years after planting with a koa container host indicating an efficient host-parasite connection; seedlings grown from seed will start to flower and fruit at this stage. (Photos by T. Speetjens, 2019)

Fungus gnats are a major contributor to embryo and cotyledon rot for young seedlings (figure 15). Fungus gnats (*Bradysia* sp.) thrive in excessively wet media containing organic matter. We observed less damage from fungus gnats in winter months at the high elevation Hāloa ‘Āina nursery at (4,500 ft [1,370 m]), suggesting fungus gnats may be negatively affected by colder temperatures (40 to 50 °F [4.4 to 10 °C]). Preventative measures against fungus gnats include controlling media moisture, top dressing with Growstone® gnat nix™, treating germination media with azadirachtin (AzaMax™), applying *Bacillus thuringiensis* var. *israelensis* to growing media, and placing a fine-mesh weed mat at the bottom of the germination flat. Gnat nix™ is a silica-based granule applied over the surface of the germination tray in place of gravel. This product acts as a mechanical control by creating an inhospitable physical barrier between the fungus gnats and the germination media. The fine mesh weed mat deters fungus gnat adults from entering the underside of germination flats. When using the weed mat, seedlings must be transplanted before the main root grows into the weed mat to avoid root damage upon extraction. *Bacillus thuringiensis* var. *israelensis* is an organic larvicide biocontrol available in pellet and powder form. This product kills fungus gnat larvae and is applied to the surface of media and watered in. The azadirachtin is applied as a drench at a concentration

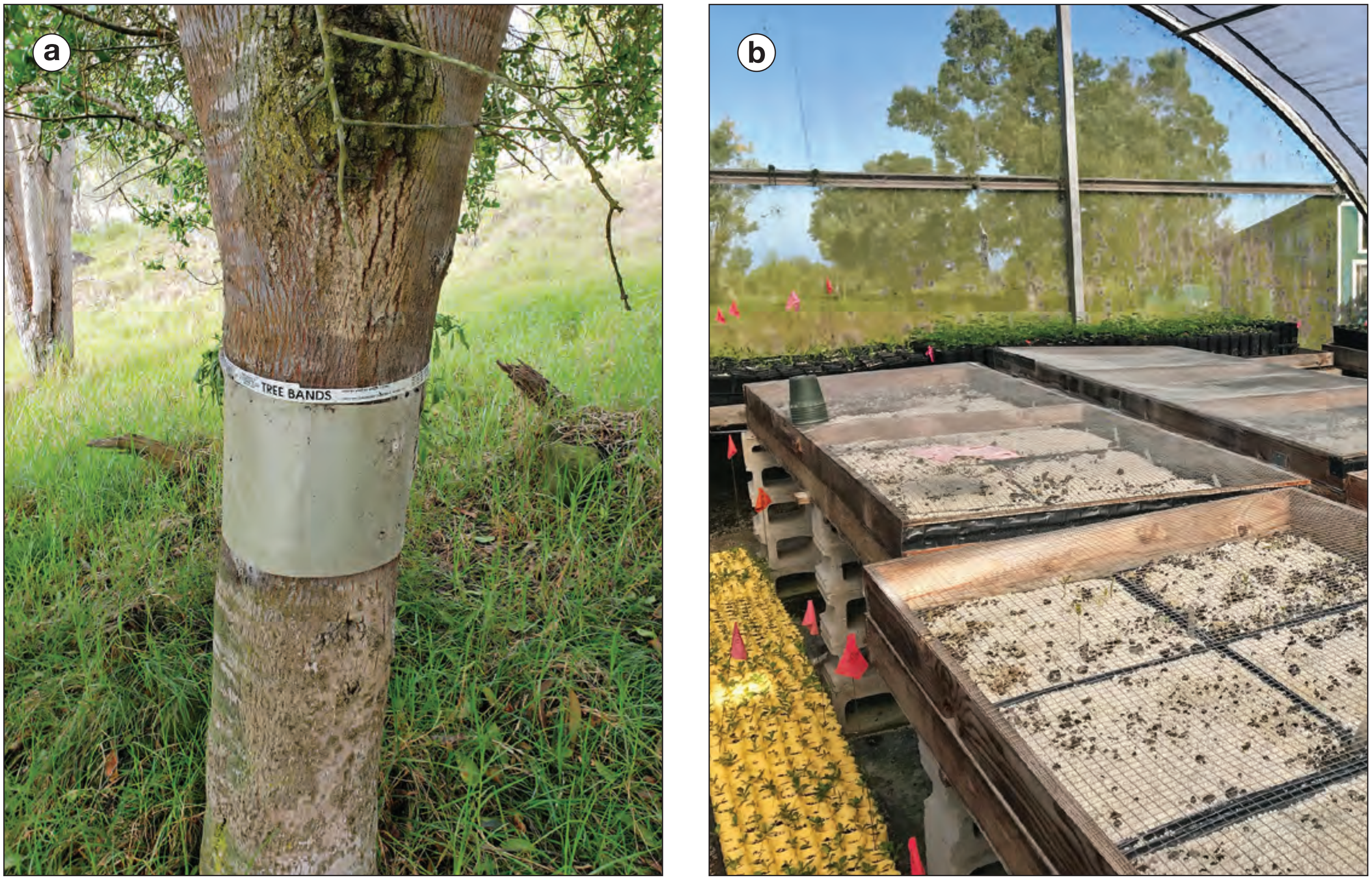

**Figure 14.** (a) Productive ʻiliahi (*Santalum paniculatum*) seed trees are fitted with metal bands around the trunk to prevent rats from climbing into the canopy and feeding on seeds. To protect ʻiliahi seeds from rodent predation in the nursery, (b) germination flats should be enclosed in a box with wire mesh screen covers. (Photos by E. Thyroff, 2020)

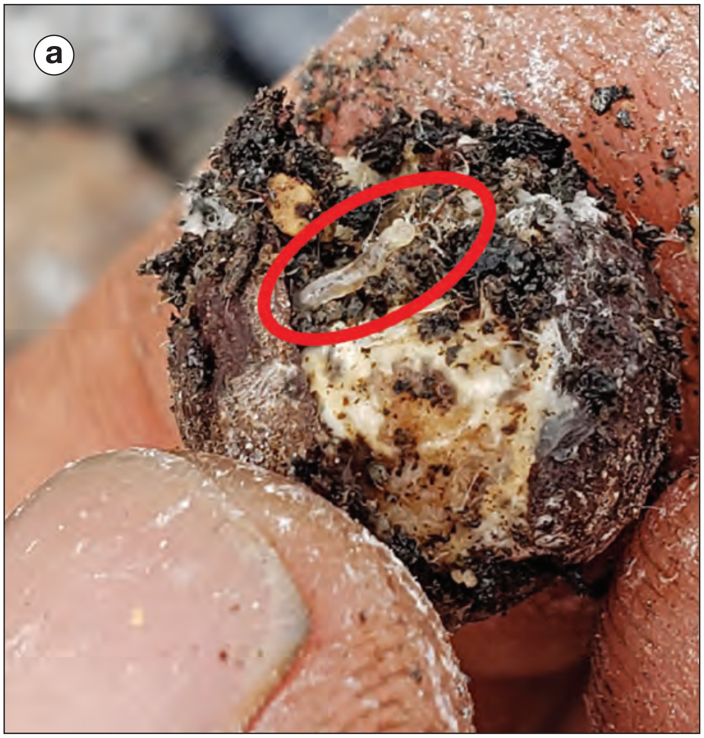

**Figure 15.** (a) Fungus gnat larvae will feed on ʻiliahi seed embryos, especially in association with embryo rot. (b) Seedling meristem rot is a common occurrence in germination flats infested with fungus gnat. (Photos by T. Speetjens, 2020)

of 0.04 to 0.08 percent every 2 to 3 weeks and can be used instead of a watering day. High concentrations of azadirachtin can burn radicals. Be sure to read and follow the label directions for all products.

## Future Work

While foundational information exists for other sandalwood species within the *Santalum* genus, there is still a need for improved knowledge for ‘iliahi. The methods described in this article are the best recommendations at this time for producing healthy *Santalum paniculatum* seedlings and may be applicable to other ‘iliahi species. Some of these methods are anecdotal, however, and require further research. Propagators are encouraged to determine what methods work for specific ‘iliahi species, environmental conditions, and other factors (e.g., host species, climate, elevation, and light intensity).

Future research is much needed on ‘iliahi species to better understand seed dormancy, germination treatments, fertilizer response, vegetative propagation, stand management, host suitability, oil quality, genetics, and biocultural importance. Currently, the Tropical Hardwood Tree Improvement and Regeneration Center (https://www.trophtirc.org/) has several ongoing research projects that will expand our knowledge and recommendations for propagation and management of this valuable native species.

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# Salinity and Flooding Affect Mortality of Atlantic White-Cedar (*Chamaecyparis thyoides*) Seedlings

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## Abstract

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] Britton, Sterns & Poggenb.) seedlings in pots were subjected to four levels of salinity in irrigation water (0.0, 0.1, 0.2, or 0.4 percent) with either continuous flooding or irrigation as needed. Mortality of continuously flooded seedlings with 0.4, 0.2, and 0.1 percent saltwater was 100 percent after 2, 4, and 8 months, respectively, compared with 20 percent mortality for seedlings flooded 17 months with fresh water. For seedlings irrigated as needed, mortality after 17 months was 100, 85, and 40 percent for 0.4, 0.2, and 0.1 percent saltwater, respectively, compared with 5 percent for seedlings that received fresh water. Results have implications for site selection for regeneration or restoration with Atlantic white-cedar in areas affected by rising sea level.

## Introduction

Atlantic white-cedar, (*Chamaecyparis thyoides* [L.] Britton, Sterns & Poggenb.) (AWC) is a wetland tree species (Reed 1988) that occurs in a narrow band along the Atlantic coast from Maine to Georgia with a separate population on the Gulf of Mexico coast from Florida to Mississippi (Laderman 1989, Little and Garrett 1990). AWC is an early succession species that usually occurs in dense, natural stands (figure 1). A new AWC stand often arises when an existing stand is destroyed by fire, logging, or weather-related blowdown (Frost 1987). When new stands develop, the seed source is 1) the seed bank that accumulated in the surface layers of the soil beneath the previous AWC stand, or 2) seed from an adjacent stand. AWC grows best on organic soils (Histosols) underlain by sandy substrate (Little and Garrett 1990). The species occupies a narrow hydrologic position intermediately between deciduous swamp forest and evergreen pocosin (Frost 1987). Historically, AWC was favored by fire return intervals of 50 to 250 years (Frost 1987, 1995; Motzkin et al. 1993). Fires that are too frequent or too severe, however, will eliminate AWC. In addition, repeated logging in the absence of fire reduces the area occupied by AWC and eventually leads to extirpation (Frost 1987).

AWC wood is mechanically and chemically similar to western red cedar (*Thuja plicata* Donn ex D. Don) which was historically prized for siding, shingles, pilings, posts, lumber, shallow-draft boats, and waterfowl decoys. Early in the 20th century, stumpage prices for AWC were 2 to 5 times greater than prices for other swamp species like baldcypress (*Taxodium distichum* L. [Rich]) and blackgum (*Nyssa sylvatica* Marshall), so loggers sought it anywhere they could find it (Krinbill 1956).

An estimated 500,000 ac (202,000 ha) of the AWC forest type existed in pre-settlement times (Kuser and Zimmermann 1995). The greatest concentrations were in the Great Dismal Swamp in the coastal plain region of southeastern Virginia and northeastern North Carolina, the Albemarle/Pamlico peninsula of eastern North Carolina, and southern New Jersey (Ackerman 1931, Kuser and Zimmerman 1995, Pinchot and Ashe 1897). Logging of AWC accelerated after 1880 and rapidly depleted stands in North Carolina and the Great Dismal Swamp during the following 40 years. In addition, massive drainage projects permanently altered the landscape, mostly for conversion of swamp land to agriculture (Frost 1987, Lilly 1981). The frequency of destructive wildfires also increased in the 20th century, making it more difficult to permanently maintain AWC stands (Frost 1987, 1995).

**Figure 1.** Atlantic white-cedar (AWC) occurs in a patchy distribution in freshwater wetlands within a narrow coastal belt from southern Maine to northern Florida and west to southern Mississippi. (a) Historical records indicate maximum potential height and diameter of 120 ft (37 m) and 60 in (152 cm), respectively. In present-day stands on good sites, typical heights are 70 to 80 ft (21 to 24 m) and maximum diameters are 24 to 26 in (60 to 66 cm). (b) Mature AWC stands tend to maintain high stem density and basal area. (c) AWC is an early succession species that usually occurs in dense stands such as this small pole stand in Gloucester County, NJ that was thinned from 230 to 190 $ft^2$ basal area per acre (53 to 44 $m^2$ per hectare). (Photos a and c by Robert Williams, Pine Creek Forestry, Clementon, NJ and photo b by George Zimmermann)

In 1998, the estimated land area of AWC stands was 108,000 ac (44,000 ha) (Sheffley et al. 1998). Hurricane Isabelle (2003) destroyed 2,000 ac (810 ha) of mature AWC (figure 2) in the Great Dismal Swamp in northeastern North Carolina (Laing et al. 2003). Hurricane Sandy (2012) caused significant losses of AWC in southern New Jersey as a result of blowdown (figure 3) and saltwater flooding (figures 4 and 5). Today, less than 10,000 ac (4,050 ha) of AWC remain in eastern North Carolina, mostly in coastal Dare County. New Jersey currently has less than 30,000 ac (12,000 ha) (Widman 2005) of AWC, possibly as low as 13,000 ac (5,240 ha) (Williams 2021). In addition, the threat of catastrophic wildfire is high owing to accumulated debris from Hurricane Sandy (figure 3). Additional losses have occurred in some locations as a result of prolonged flooding by beavers (*Castor canadensis*) (figure 6).

**Figure 3.** Vast areas of Atlantic white cedar were blown down during Hurricane Sandy in 2012 such as this stand in Burlington County, New Jersey. Most of that timber was not salvaged, thus creating a serious wildfire threat in southeastern New Jersey. (Photo by Robert Williams, Pine Creek Forestry, Clementon, NJ)

**Figure 2.** This large tract of peatland in the Great Dismal Swamp National Wildlife Refuge was destroyed by wildfire in the aftermath of a salvage operation to remove residue from 2,000 ac (810 ha) of mature Atlantic white-cedar blown down by Hurricane Isabelle in 2006. Owing to a low water table, the fire burned deep into the peat and killed the dense natural regeneration of AWC already on the site. The fires also eliminated the remaining seed bank from the previous AWC stand. The resulting landscape is mostly open water unsuitable for forest vegetation, probably for hundreds of years. (Photo by Bill Pickens, North Carolina Forest Service, retired)

The AWC forest type is also facing a threat from accelerating rates of rising sea level. The New Jersey Meadowlands and other parts of the East Coast have been experiencing submergence and influx of salt water since historical records have been recorded (Zimmermann and Mylecraine 2003) not only in coastal habitats but also in many previously freshwater marshes farther inland. Dead stands of AWC (ghost forests) are becoming a familiar landscape feature of the New Jersey Pinelands. In coastal Dare County (North Carolina), satellite imagery indicates that 11 percent of the forests in Alligator River National Wildlife Refuge have transitioned to ghost forests in the last 35 years, with a pronounced peak following flooding by Hurricane Irene in 2011 (Ury et al. 2021). Most remaining AWC stands in eastern North Carolina are in Dare County and will become ghost forests if sea level rises 3.2 ft (1 m) as projected in the 21st century (Bhattachan et al. 2018).

The effects of flooding and increasing salinity on forests depend on the species and can vary among families within a species (Allen et al. 1996, Pezeshki et al. 1990, Ruter 2017). Coastal habitats have species that can withstand prolonged flooding and increased salinity, but many inland species are unable to cope. Frost (1995) and Ruter and Pennisi (2017) broadly categorized AWC as intolerant of salt water but included no experimental data. The objective of this study was to determine the effects of flooding and salinity on survival and growth of AWC seedlings.

**Figure 4.** This aerial view (January 2013) shows dead and dying Atlantic white-cedar (red foliage) in southern New Jersey after Hurricane Sandy in October 2012. (Photo by James Dunn, New Jersey Department of Environmental Protection)

## Methods

In December 2003, 200 1-year-old AWC seedlings were transplanted into 2-gal (7.8-L) pots filled with *Sphagnum* peat substrate and placed in a greenhouse. Seedlings were uniform in size and came from a single seed source. Temperatures in the greenhouse ranged from a minimum of 65 °F (18 °C) in the winter to 90 °F (32 °C) in the summer.

Commercially available sea salt (Instant Ocean Synthetic Sea Salt, Aquarium Systems Inc., Mentor, OH) was used to prepare solutions representative of salinity ranges in the lower watersheds of the Mullica, Egg Harbor, and Tuckahoe rivers (figure 7) in southeastern New Jersey. Seedlings were treated with four saline solutions (0.1, 0.2, or 0.4 percent salt plus a tap-water control) applied with two water regimes (as needed with periodic irrigation or with continuous flooding) for a total of 8 treatments. Treatments began in July 2004. For each treatment, 20 seedlings were randomly assigned and randomized on the greenhouse benches. Pots assigned to flooding treatments were placed inside 3-gal (10.4-L) pots lined with plastic bags to retain irrigation water (figure 8). Water levels in flooded pots were maintained near the substrate surface. Non-flooded pots were allowed to drain following watering.

Seedling mortality was observed weekly or biweekly until February 2006 (17 months). Ratings were based on foliage color and branch pliability.

The study used a completely randomized design with a 2 by 4 factorial (water regime and salt level) arrangement of treatments and 20 replications of each treatment (160 seedlings total). Survival percentages were transformed (arcsin) to meet ANOVA assumptions and subjected to analysis of variance (ANOVA) using SAS (SAS Institute 2003). Treatment means were compared using Dunnett's Test.

## Results

Analyses found a significant interaction ($p \leq 0.001$) for salinity and flooding treatments (figure 9). Mortality of control seedlings (no flooding, no salt) was 5 percent, whereas continuous flooding with fresh water (no salt) resulted in 20 percent mortality. Seedlings continuously flooded with 0.4 percent or 0.2 percent salt concentrations all died within 2 to 4 months after initiation of treatments, and those flooded with 0.1 percent salt water reached 100 percent mortality after 8 months. The same salt treatments, when applied only in irrigation water, caused 100, 85, and 40 percent mortality, respectively, after 17 months (figure 9).

**Figure 5.** Atlantic white-cedar near the Mullica River in Atlantic County, New Jersey in January 2013. Dying Atlantic white-cedar on the left side of highway resulted from a surge of brackish water that did not drain back into the Mullica River after Hurricane Sandy (October 2012). In contrast, the storm surge retreated on the right side of the highway. (Photo by George Zimmermann)

## Discussion

AWC stands often experience shallow flooding (fresh water) during the winter months when trees are dormant and evapotranspiration is low. Water regimes can fluctuate widely during the growing season, including short-term flooding from rain, but AWC tends to grow best in wetlands where the water table is 5 to 8 in (10 to 20 cm) below the surface during much of the growing season (Atkinson et al. 2003, Little 1950). In the current study, 80 percent of seedlings survived 17 months of continuous flooding with fresh water, but those flooded with salt water all died (figure 9).

**Figure 6.** Beavers can kill stands of Atlantic white-cedar. This site is in Atlantic County, New Jersey near the Great Egg Harbor River was flooded due to the beaver dam (foreground). Land managers should monitor beaver activity to avoid excessive losses of valuable timber from prolonged flooding. (Photo by Robert Williams, Pine Creek Forestry, Clementon, NJ)

The lowest salt concentration (0.1 percent) caused significant mortality of AWC seedlings although at a slower rate in non-flooded treatments (figure 9). This suggests that AWC might have a threshold salt tolerance between 0.0 and 0.1 percent. Follow-up observations in rivers in southeastern New Jersey, however, noted up to 50 percent mortality of mature AWC where salinity was only 0.03 percent, and healthy stands only occurred where salinity was 0.0 percent further upriver. Therefore, based on greenhouse results and observations of river salinity, it seems reasonable to conclude that AWC has no tolerance of salt water.

The sensitivity of AWC to low salt concentrations, especially when combined with continuous flooding, helps explain AWC mortality following inland flooding by Hurricane Sandy (2012) in New Jersey (figures 4 and 5) and Hurricane Irene (2011) in eastern North Carolina. Field observations suggest that mature AWC can survive longer than seedlings when

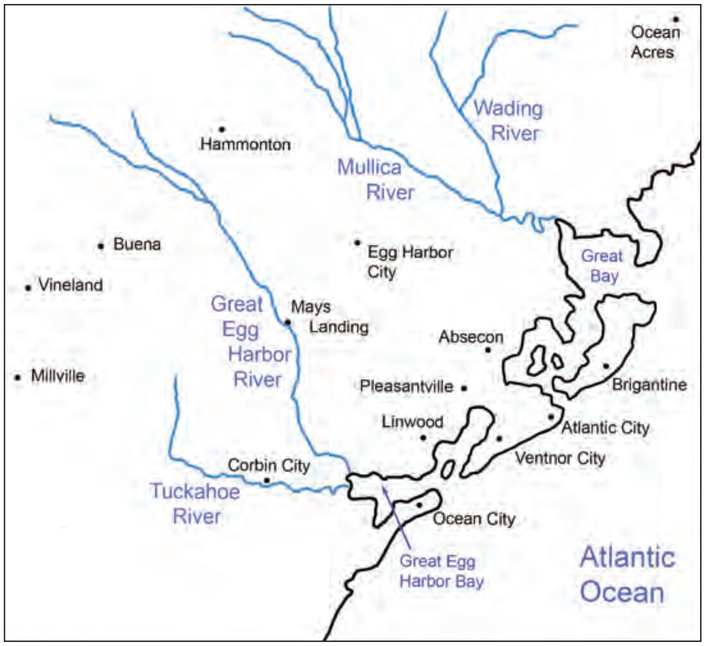

**Figure 7.** Atlantic white-cedar is abundant in river watersheds (Mullica, Great Egg Harbor, and Tuckahoe) in southeastern New Jersey. (Adapted from a portion of a New Jersey road map, https://www.new-jersey-map.org/road-map.htm)

**Figure 8.** Potted Atlantic white-cedar seedlings were subjected to various levels of salinity (0 to 0.4 percent) and two flooding regimes (flooded or not flooded). Pots designated for flooding treatments were placed inside larger pots lined with plastic. The two plants in the center of the image (second row) were flooded and the one on the right has died from higher salinity. (Photo by George Zimmermann)

subjected to prolonged flooding, but the overall health and longevity of the stands will be affected by factors such as river salinity, distance from rivers, severity and frequency of storms, and rates of salt dissipation from soils following flooding.

Results of this study have important implications for future regeneration and restoration of AWC. Significant acreage of AWC, especially in eastern North Carolina and southern New Jersey, will be lost if sea level rises as projected. In addition, hurricanes pose an ongoing threat of blowdown and/or flooding (Lang et al. 2011, McCoy and Keeland 2009, Ury 2021). Potential impacts are likely to become more extreme as sea level rises. Future AWC restoration plantings should be established on suitable sites farther inland at elevations high enough to allow sufficient time for stands to complete one or more life cycles without the risk of salt water inundation.

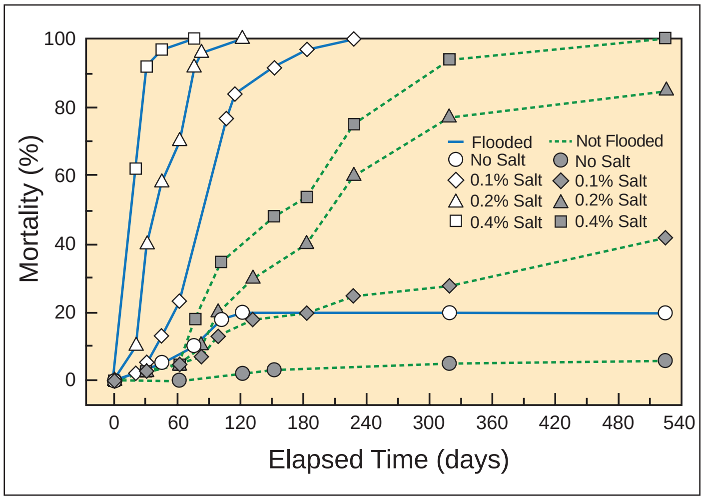

**Figure 9.** Atlantic white-cedar seedlings subjected to factorial combinations of two water regimes (flooded vs. not flooded) and four concentrations of salt water (0 percent, 0.1 percent, 0.2 percent, and 0.4 percent) had varying mortality rates during a 17-month greenhouse experiment. Each mean is based on 20 plants.

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# Fall Planting in Northern Forests as a Reforestation Option: Rewards, Risks, and Biological Considerations

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## Abstract

This paper examines the option for fall planting in northern forests to help foresters make informed silvicultural decisions regarding plant date. A literature review determined that 75 percent of fall-planting trials conducted in northern forests had field survival and/or growth that was comparable with, or higher than, spring- or summer-planted seedlings. Nonetheless, 25 percent of trials did not show fall planting to be effective, thus illustrating risks associated with this planting option. Reasons for an unsuccessful fall-planting program were related to nursery hardening practices and planting into stressful environmental conditions. The annual phenological cycle must be considered for developing hardened seedlings suitable for fall planting. This information allows foresters and nursery managers to determine when and where fall planting is a viable option for northern reforestation programs.

## Introduction

Silviculturists have long considered fall planting as an option for reforestation programs (Toumey 1916). Currently, its use in reforestation programs is dictated by regional climatic conditions. In regions where late spring and summer are hot and/or dry, fall planting is a standard operational practice. For example, 60 percent of all containerized seedlings are planted from October through December in the southeastern United States, with the remainder planted during winter (Starkey et al. 2015). Fall planting of oak (*Quercus*) species in Mediterranean ecosystems is also a recommended practice (Sánchez-González et al. 2016). Furthermore, in southern Europe, approximately 66 percent of seedlings are planted during October and November (Ivetić 2021), with a multiple site survey showing comparable survival between fall- and spring-planting programs (Ivetić 2015). With increasing latitude, however, the use of fall planting decreases. In central Europe, fall planting occurs, but it is not a primary reforestation practice (Repáč et al. 2017). In the Pacific Northwest, 10 to 20 percent of seedlings are fall planted in Oregon and Washington (Swain 2021) and in British Columbia (Anonymous 2020). In Finland, 10 to 20 percent of seedlings are fall planted before onset of colder fall conditions (Riikonen 2021). Recent surveys in Nordic countries reported fall planting into October as viable for Norway spruce (*Picea abies* [L.] H. Karst.), but not Scots pine (*Pinus sylvestris* L.) (Luoranen et al. 2018, Pikkarainen et al. 2020). Overall, these observations indicate that fall planting at northern latitudes is an option, though regional climate and species performance determines whether it can be successfully used in reforestation programs.

When deciding whether to fall plant, each reforestation manager needs to clearly understand why they want a fall-planting program. The most common operational reasons for considering fall planting in a northern reforestation program are limited access to sites during the preferred spring-planting window and too many seedlings for the available workforce to properly plant during the spring- and summer-planting windows (Farquharson 2020). The reforestation site environmental conditions that lead silviculturists to consider fall planting are the exposure of spring-plant seedlings to frost or drought, or summer-plant seedlings to drought (Grossnickle 2000). Furthermore, fall planting provides an environmental window that gives seedlings an opportunity to grow roots and become established before onset of winter (Krumlik 1984, Mitchell et al. 1990, Rose 1992, Toumey 1916).

Silvicultural decisions are based on a risk/reward decision process. Foresters need to understand the risks and rewards of fall planting so they can make effective management decisions when deciding whether to incorporate this practice into their reforestation program. This article presents an introduction to the physiological capability of fall-planted seedlings and their response to field site climatic conditions. This information will help foresters to make sound, biologically based decisions on whether to implement this planting practice into northern reforestation programs.

## Literature Review

We reviewed articles covering fall planting for multiple species and field conditions at northern forest sites (tables 1 and 2). When examined as a whole, 75 percent of trials found fall-planted seedlings had field survival and/or growth that was comparable with, or higher than, spring- or summer-planted seedlings. In northern latitude forests (table 1), montane forests (table 2), and coastal forests (table 2), 81, 60, and 83 percent, respectively, of trials found fall-planting field performance to be comparable with, or better than, spring, or summer planting. This finding shows that, depending on local environmental conditions and program objectives, fall planting can be considered as an option for northern reforestation programs.

### Rewards Related to Fall Planting

One benefit of fall planting at northern reforestation sites is that seedlings are planted in the window between hot, dry summer and cold, late-fall environmental conditions. During this period, milder edaphic conditions typically prevail at the planting site and are conducive to root growth and thus seedling establishment. Root growth reaches its maximum at soil temperatures between 10 and 20 °C, decreases at temperatures below 10 °C, and stops at temperatures below 5 °C (Grossnickle 2000). Soil water near field capacity is optimal for root growth (Grossnickle 2000), but soil water less than 35 percent field capacity decreases root growth (Spittlehouse and Stathers 1990). White spruce (*Picea glauca* [Moench] Voss) seedlings fall planted into soils near field capacity, initiated root growth within 10 days after outplanting and continued growing during a 40-day trial (Day and MacGillivary 1975). Other studies have also shown mild edaphic conditions during late summer and early fall are favorable for root growth of recently planted seedlings before onset of colder edaphic conditions (Folk et al. 1994, Folk et al. 1996, Luoranen et al. 2006, Luoranen 2018). Just after spring snowmelt, soil temperatures in the rooting zone can quickly rise above 5 °C (Spittlehouse and Stathers 1990) allowing root growth to resume.

The combination of fall root growth and subsequent early spring root growth can result in well-established seedlings on the reforestation site (figures 1 and 2). Sufficient root growth is critical for newly planted seedlings to avoid planting stress by coupling them into the site hydrologic cycle (Grossnickle 2005). Due to greater root development, fall-planted seedlings can have lower levels of daytime water stress compared with spring-planted seedlings (figure 3), thus improving their transition into the establishment phase during their first full growing season after outplanting (Grossnickle 2000).

Unlike root growth, subsequent shoot growth has no consistent trend for better performance of spring- or fall-planted seedlings. Many studies show improved shoot growth in spring-planted seedlings (e.g., Miller 1981 a,b; Luoranen and Rikala 2013; Narimatsu et al. 2016), other studies show greater shoot growth in fall-planted seedlings (e.g., Ellington 1984; Barber 1989, 1995; Luoranen 2018), and some studies show equal shoot growth in both spring- and fall-planted seedlings (e.g., Folk et al. 1994, Folk et al. 1996, Luoranen and Rikala 2015, Suwa et al. 2016). Shoot growth of fall-planted seedlings is determined by seedling quality at planting in response to field site conditions (Grossnickle and MacDonald 2018).

### Risks Related to Fall Planting

Our literature review found 25 percent of fall-planting trials were not successful in northern forest reforestation programs (tables 1 and 2). By understanding reasons for unsuccessful fall planting, foresters can better manage risks.

In early trials, insufficiently hardened fall-planted seedlings had reduced ability to tolerate stressful field site environmental conditions resulting in lower survival compared with spring-planted seedlings (Cram and Thompson 1981, Miller 1982, Sinclair and Boyd 1973). At that time, nursery cultural practices were not refined enough to adequately harden seedlings for fall planting. In recent decades, improved cultural practices have been developed to properly harden

**Table 1.** Field performance of seedlings in fall-planting reforestation programs in northern latitude forests globally. Performance was defined by comparing first-year survival (and growth if presented) of fall-planted (FA) seedlings with spring- (SP) or summer- (SU) planted seedlings in the same trial. Where only fall-planted seedlings were identified in the trial, first-year survival greater than 75 percent was classified as good field performance. Stocktypes are defined when both bareroot (BR) and container-grown (CON) were planted in the trial.

| Species | Fall program | | Comment | Reference |
|---|---|---|---|---|
| | Good | Poor | | |
| **Northern latitude forests** | | | | |
| *Pinus banksiana* Lamb. | √ | | SP and FA survival equal with SU lower survival | Bunting and Mullin 1967 |
| *Picea glauca* (Moench) Voss & *Picea mariana* (Mill.) B.S.P. | √ | | SP and FA survival equal | Mullin 1968 |
| *Pinus resinosa* Sol. ex Aiton & *Pinus strobus* L. | | √ | SP survival higher than FA due to lack of hardening | |
| *Picea glauca* (Moench) Voss | | √ | SP survival higher than FA due to lack of hardening | Cram and Thompson 1981 |
| *Picea pungens* Engelm. | √ | | SP and FA survival equal | |
| *Pinus sylvestris* L. | √ | | SP and FA survival equal | |
| *Picea mariana* (Mill.) B.S.P. | √ | | FA survival equal to, or better than, SP | Alm 1983 |
| *Picea glauca* (Moench) Voss | √ | | | |
| *Pinus sylvestris* L. | √ | | SP, SU, and FA survival equal | Valtenan et al. 1986 |
| *Larix sibirica* Ledeb. | √ | | | |
| *Pinus strobus* L. | √ | | SP and FA survival equal | Dierauf 1989 |
| *Pinus sylvestris* L. | √ | | SP and FA survival equal | Kinnunen 1989 |
| *Picea abies* L. Karst. | √ | | | |
| *Picea abies* L. Karst. | √ | | SP and FA had comparable survival; SU had lower survival due to nonhardened seedling frost damage; SU and FA had greater height growth than SP | Luoranen et al. 2006 |
| *Picea abies* L. Karst. | √ | | SP, SU, and FA survival equal; multiple trials found >70% survival | Luoranen et al. 2011 |
| *Picea abies* L. Karst. | √ | √ | SP and FA survival equal for BR; SP survival higher than FA for CON | Repác et al. 2011 |
| *Fagus sylvatica* L. | √ | √ | | |
| *Pinus sylvestris* L. | √ | | SP and FA survival comparable for both BR and CON | |
| *Larix decidua* Mill. | √ | | | |
| *Acer pseudoplatanus* L. | √ | | | |
| *Pinus sylvestris L.* | √ | | SP, SU, and FA survival equal; shorter FA height resulted in shorter seedlings at year 5 | Luoranen and Rikala 2013 |
| *Pinus sylvestris* L. | √ | | SP, SU, and FA survival equal; SP and FA had shorter seedlings at year 3 | Luoranen and Rikala 2015 |
| *Larix kaempferi* (Lamb.) Carr. | √ | | FA survival higher than SU due to greater drought tolerance and summer drought | Harayama et al. 2016 |
| *Larix kaempferi* (Lamb.) Carr. | √ | | SP, SU, and FA survival equal; FA lower root growth due to low soil temperature | Narimatsu et al. 2016 |
| *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. | √ | | SP, SU, and FA survival equal; comparable height growth for SP and FA | Suwa et al. 2016 |
| *Picea abies* L. Karst. | √ | √ | Early FA (September) comparable to SU (August), but late FA (November) lower due to cold temperatures | Wallertz et al. 2016 |
| *Pinus sylvestris L.* | √ | | SP, SU, and FA survival equal; FA lower initial root growth, but better shoot growth at year 2 | Luoranen 2018 |
| *Picea abies* L. Karst. | √ | | | |
| *Pinus sylvestris* L. | | √ | FA seedlings sensitive to harsh winter conditions | Luoranen et al. 2018 |
| *Picea abies* L. Karst. | √ | | FA planted in October when suitable sites are selected | |
| *Pinus sylvestris* L. | | √ | FA had lower survival than SP and SU, though all planting dates had low survival (40-55%) | Pikkarainen et al. 2020 |
| *Picea abies* L. Karst. | √ | | SP and FA had equal survival and were greater than SU | |
| *Pinus sylvestris* L. | √ | | SP and FA had equal survival for both BR and CON | Repác et al. 2021 |
| *Picea abies* L. Karst. | √ | | SP and FA had equal for BR, whereas SP CON had higher survival | |

**Table 2.** Field performance of seedlings planted in fall-planting reforestation programs in western North American montane and coastal forests. Performance was defined by comparing first-year survival (and growth if presented) of fall-planted (FA) seedlings with spring- (SP) or summer- (SU) planted seedlings in the same trial. Where only fall planted-seedlings were identified in the trial, first-year survival greater than 75 percent was classified as good field performance.

| Species | Fall program | | Comment | Reference |
|---|---|---|---|---|
| | Good | Poor | | |
| | | | **Montane forests** | |
| *Abies grandis* (Dougl.) Lindl. | | √ | SP survival higher than FA due to lack of hardening | Sinclair and Boyd 1973 |
| *Larix occidentalis* Nutt. | | √ | | |
| *Picea engelmannii* Parry | | √ | | |
| *Abies grandis* (Dougl.) Lindl. | | √ | | |
| *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco | | √ | SP survival higher than FA due to lack of hardening | |
| *Pinus monticola* Dougl. | √ | | SP and FA survival equal; FA had lower height growth | Miller 1981a |
| *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco | √ | | SP and FA survival equal; FA had lower height growth | Miller 1981b |
| *Thuja plicata* Donn | | √ | FA poor survival due to poor hardening | Miller 1982 |
| *Picea engelmannii* Parry | √ | | SP and FA survival and growth equal | |
| *Abies magnifica* A. Murray | √ | | FA survival and growth higher than SP | Ellington 1985 |
| *Larix occidentalis* Nutt. | √ | | FA survival and growth higher than SP | Barber 1989 |
| *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco | √ | | Early FA had high survival and good growth | Adams et al. 1991 |
| *Pinus monticola* Dougl. | √ | | | |
| *Pinus ponderosa* Laws. | √ | | | |
| *Larix occidentalis* Nutt. | √ | | FA survival and growth higher than SP | Barber 1995 |
| *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco | √ | √ | Later FA survival was high due to drought avoidance | Taylor et al. 2009 |
| *Larix occidentalis* Nutt. | √ | √ | | |
| *Populus tremuloides* Michx. | √ | | SP, SU, and FA survival equal; hardening reducing shoot dieback due to frost | Landhäusser et al. 2012 |
| | | | **Coastal forests** | |
| *Pseudotsuga menziesii* (Mirb.) Franco | √ | | SP and FA survival equal | Winjum 1963 |
| *Abies procera* Rehd. | √ | | | |
| *Pseudotsuga menziesii* (Mirb.) Franco | √ | | 5-year SP and FA survival was comparable; *T. heterophylla* survival was lower due to drought | Arnott 1975 |
| *Tsuga heterophylla* (Raf.) Sarg. | √ | | | |
| *Thuja plicata* Donn | √ | | SP and FA had comparable survival; FA had greater initial root growth and end of season diameter growth; SP had greater height | Folk et al. 1994 |
| *Chamaecyparis nootkatensis* (D. Don) Spach | | √ | SP higher survival than FA due to fall drought; FA greater initial root growth; SP and FA equal shoot growth | Folk et al. 1996 |

seedlings for fall-planting programs (see Nursery Cultural Practices section).

A survey of over 100 fall-planted sites in Finland reported approximately 10 percent of poor seedling performance was due to drought and/or frost (Pikkarainen et al. 2020). Stressful environmental conditions (i.e., unfavorable soil moisture and soil temperature conditions, plus frosts) after outplanting are factors that can affect field performance of fall-planted seedlings (Grossnickle 2000, Margolis and Brand 1990).

Fall-planting programs can fail even when hardened seedlings are planted into droughty soils (Folk et al. 1996, Taylor et al. 2009), resulting in water stress and potential mortality, especially if new root growth is inadequate (Grossnickle 2005). Recent fall-plant-

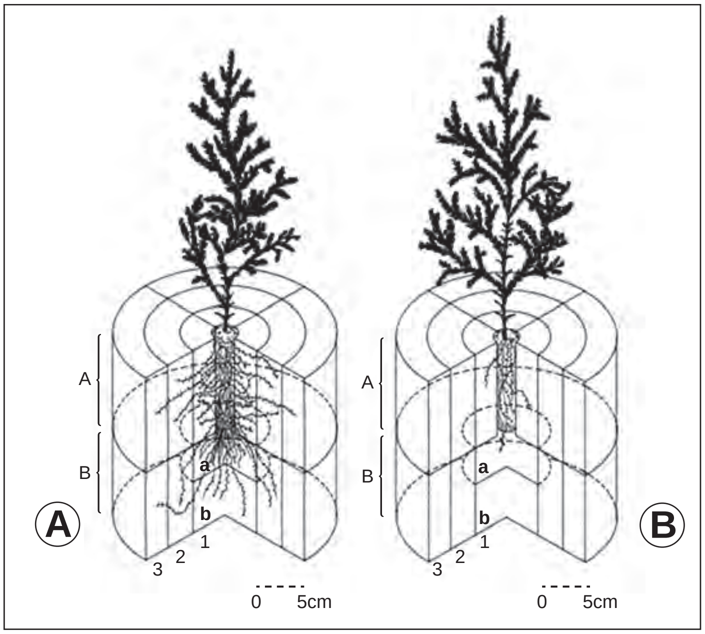


**Figure 1.** Diagrammatic representation (n= 20) of morphological development in western redcedar (*Thuja plicata* Donn ex D. Don) seedlings that were (a) fall planted (mid-September) or (b) spring planted (mid-April) on an afforestation site. Seedlings from both planting dates were assessed in mid-May. New root growth out of the container plug into the surrounding soil was significantly greater (t-test, $\alpha = 0.05$) in fall-planted seedlings (400 mg +/- 25) than spring planted seedlings (70 +/- 12 mg). (Adapted from Folk et al. 1994 and Grossnickle unpublished data)

**Figure 2.** Root development of a western redcedar (*Thuja plicata* Donn ex D. Don) seedling that was fall planted (mid-September) and excavated in early May. (Photo by Dennis Farquharson 2020)

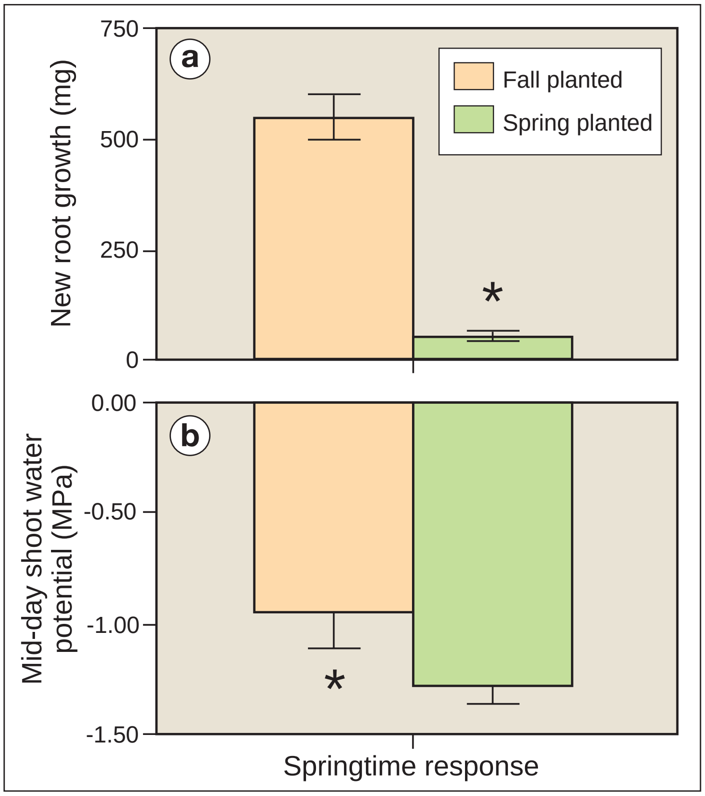


**Figure 3.** Fall-planted and spring-planted yellow cypress (*Cupressus nootkatensis* D. Don) seedlings differed significantly (t-test, $\alpha = 0.05$) for (a) end of spring new root dry weight (mean +/- standard error) and (b) mid-day shoot water potential (mean and standard error). Shoot water potential means are based on 6 measurement dates from mid April (just after spring planting) through June. (Adapted from Folk et al. 1996)

ing recommendations suggest planting into loamy soil rather than sandy soil, when there is sufficient soil water for root growth (Luoranen et al. 2018). Sub-optimal soil temperatures (below 10°C) can be a late growing-season stress in cool, temperate conifer forests (Niinemets 2010) because they limit root growth and water uptake (Grossnickle 2000, Luoranen 2018, Wallertz et al. 2016).

Fall-planting programs can fail when seedlings are planted into frosty sites (Landhäusser et al. 2012, Luoranean et al. 2006, Pikkarainen et al. 2020). Properly hardened seedlings can handle minor, but not severe, frost events (Bigras 1996, Sakai and Larcher 1987). After fall-planted seedlings are exposed to cold temperatures at the planting site, they develop freezing tolerance at a sufficient level to handle freezing temperatures of mid- to late fall and winter (Bigras 1996, Grossnickle 2000, Sakai and Larcher 1987).

Frost heaving is a concern after fall planting when the planting date does not allow for adequate root development before winter (Krumlik 1984). Frost heaving occurs on planting sites with fine-textured soils, high soil water content, and no snow cover (Grossnickle 2000). When air temperatures are just below freezing, temperatures in the upper soil layer fluctuate around 0 °C, resulting in ice-lens formation. These ice lenses cause seedlings to frost heave if there is inadequate root growth to anchor seedlings into the surrounding soil (Goulet 1995, Örlander et al. 1990). In a recent survey of 93 fall-planted sites in Finland, however, frost heaving accounted for only 1 percent of reported losses (Luoranen et al. 2018), indicating it was only a minor concern. Frost heaving can be minimized by mulching exposed mineral soil, creating microsites that have an overlying organic layer (Grossnickle 2000, Luoranen et al. 2018), and planting seedlings deeply, if appropriate for the species (Luoranen 2018).

Winter desiccation is a common phenomenon in conifer trees (Sakai and Larcher 1987) and occurs under conditions of frozen, snow-covered soils, bright sun, and dry air. On northern reforestation sites, winter desiccation can occur where snow does not consistently cover recently planted seedlings (Krasowski et al. 1993). Winter desiccation depends on the depth to which the soil is frozen, the amount of shoot system exposed to atmospheric conditions (i.e., freezing air temperature, low humidity, and wind) (Grossnickle 2000), and the extent of new root growth. Fall-planted Scots pine seedlings can be at risk of winter desiccation because they are typically planted in coarse-textured soils resulting in poorly rooted seedlings (Luoranen et al. 2018). In contrast, seedlings planted in fine-textured soils with readily available soil water had minimal winter desiccation (Luoranen and Rikala 2013; Luoranen 2018). Field site conditions that cause winter desiccation damage in fall-planted seedlings can also occur for spring- and summer-planted seedlings (Grossnickle 2000, Krasowski et al. 1993).

## Nursery Cultural Practices to Support A Successful Fall-Planting Program

In nature, northern tree species undergo an annual cycle of morphological and physiological changes that have

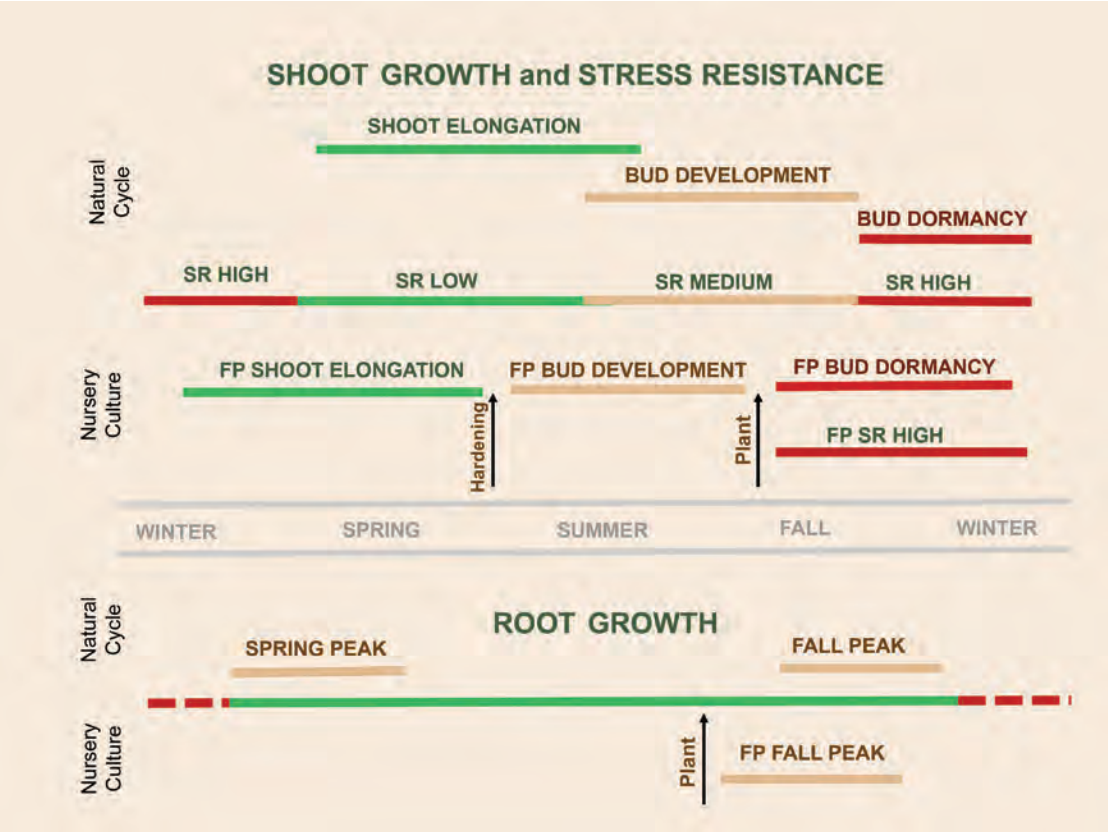


**Figure 4.** This chart illustrates the phenology of growth (roots and shoots), dormancy (shoots), and stress resistance (SR) of northern conifers in response to their natural cycle compared with nursery cultural practices to produce containerized fall-planted seedlings (FP). Green lines represent periods of growth and low stress resistance, tan lines represent periods of bud development and increasing stress resistance, and red lines represent periods of inactivity and high stress resistance.

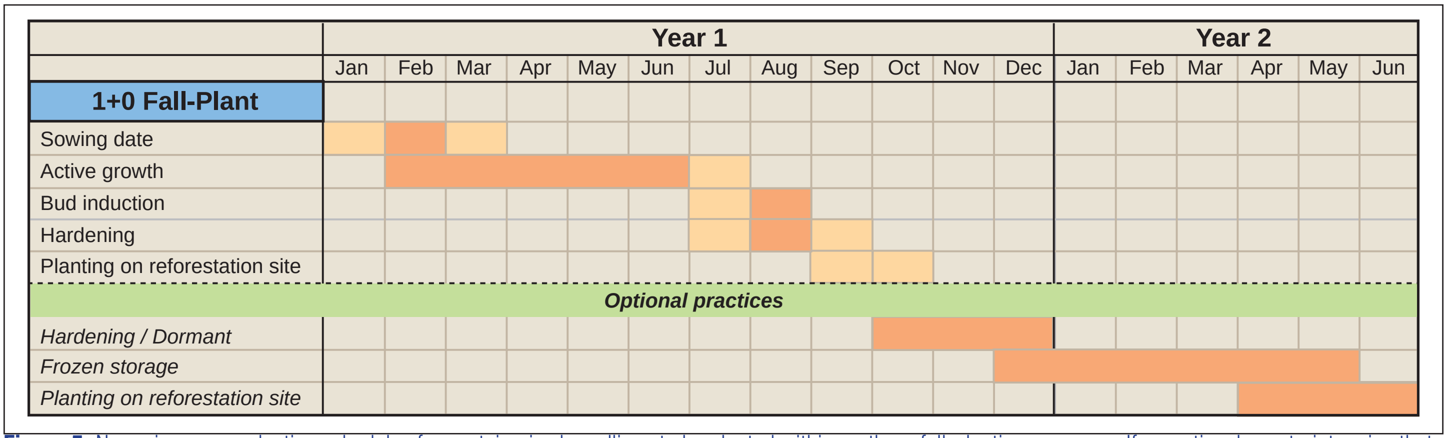
evolved in response to seasonal environmental conditions to ensure species survival (Fuchigami et al. 1982, Lavender 1985). Thus, northern conifers at different latitudes and elevations have distinctive seasonal phenologies (figure 4). These seasonal shoot (Fuchigami et al. 1982) and root (Ritchie and Dunlap 1980) growth cycles overlap with seasonal cycles of stress resistance (i.e., freezing [Fuchigami et al. 1982, Sakai and Larcher 1987] and drought [Teskey et al. 1984]). Nursery cultural practices have been designed to account for these phenological cycles (Burr 1990, Ritchie and Tanaka 1990). Nursery hardening practices cue the start of multiple morphological and physiological processes. Thus, nursery practices can be used to shift the phenological cycle to earlier in the year, resulting in properly hardened seedlings for a fall-planting program (figure 4).

Containerized cultural practices that improve seedling quality have been developed over the past 40 years (Tinus 1974). Growing containerized seedlings allows one to dramatically shift the nursery cultural schedule to accommodate the timely completion of the crop cycle (figure 5), which is why the containerized stocktype is preferred for fall-planting programs in northern forests. Nursery production schedules must allow seedlings to complete morphological and physiological development before lifting. This development is critical because higher quality seedlings have increased survival (Grossnickle 2012) and growth (Grossnickle and MacDonald 2018) just after outplanting. The forester and nursery manager need to develop a partnership marked by excellent communication so that seedlings for fall planting are grown with sufficient time to develop seedling quality attributes that are matched to the outplanting site (Dumroese et al. 2016).

Containerized seedlings for fall planting are sown from early January through early April (figure 5), with timing dependent on species and stocktype size. The active growth phase for shoot elongation is maintained through spring into early or mid- summer to ensure seedlings achieve the desired target height before budset (Landis et al 1989, 1992; Tinus and McDonald 1979). For fall-planting stock, the active growth phase is adjusted to end in July when hardening begins (figure 5).

Hardening involves manipulating morphological and physiological processes within seedlings that, when completed, prepare seedlings for winter stresses. Seedling stress resistance is the ability to withstand stresses associated with the reforestation process, ranging from lifting through storage to planting (Duryea 1985, Ritchie 1984), and is closely correlated with bud dormancy (Lavender 1985). Frost hardiness (Colombo et al. 1989) and drought tolerance (Grossnickle 1989) have been related to completion of bud development in northern conifers, with greater freezing- and drought-stress resistance being cued by cold temperature events (Bigras 1996, Grossnickle 2000).

Hardening begins with a dormancy-induction treatment that stops seedling height growth and starts terminal bud development (Dormling et al. 1968, Lavender et al. 1968) (figure 4). Stem diameter growth continues during and after budset (Grossnickle 2000). During hardening, photosynthates are reallocated towards woody and non-woody root growth and the initial stage of stress resistance is cued (Grossnickle 2000). Nursery cultural practices such as artificially shortened days,



| | Year 1 | | | | | | | | | | | | Year 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun |
| **1+0 Fall-Plant** | | | | | | | | | | | | | | | | | | |
| Sowing date | 🟨 | 🟧 | 🟨 | | | | | | | | | | | | | | | |
| Active growth | | 🟧 | 🟧 | 🟧 | 🟧 | 🟧 | 🟨 | | | | | | | | | | | |
| Bud induction | | | | | | | 🟨 | 🟧 | | | | | | | | | | |
| Hardening | | | | | | | 🟨 | 🟧 | 🟨 | | | | | | | | | |
| Planting on reforestation site | | | | | | | | | 🟨 | 🟨 | | | | | | | | |
| ***Optional practices*** | | | | | | | | | | | | | | | | | | |
| *Hardening / Dormant* | | | | | | | | | | 🟧 | 🟧 | 🟧 | | | | | | |
| *Frozen storage* | | | | | | | | | | | | 🟧 | 🟧 | 🟧 | 🟧 | 🟧 | 🟧 | |
| *Planting on reforestation site* | | | | | | | | | | | | | | | | 🟧 | 🟧 | 🟧 |

**Figure 5.** Nurseries use production schedules for containerized seedlings to be planted within northern fall-planting programs. If operational constraints arise that preclude fall planting, the manager can shift to optional practices to hold seedlings over for a spring-planting program. Dark orange indicate the cultural practice occurs during that month. Light orange bars indicate that the start/stop date is variable within that month depending upon species, seed lot, stock type, or field planting schedule (Swain 2021).

reduced irrigation, and reduced fertilization, alone or in combination, are used for dormancy induction in conifer species (Landis et al. 1999, Landis 2013, Tinus and McDonald 1979). The timing, combination, and intensity of these practices are dictated by species, seedlot (i.e., genetic source), and stocktype (Swain 2021) (figure 5). For example, when applied to interior spruce (*Picea glauca* [Moench] Voss x *Picea engelmannii* Parry ex Engelm.) seedlings, hardening practices increased needle-primordia number within terminal buds and seedling stress resistance while only slightly decreasing root growth potential to a level that was still sufficient for seedling establishment (Grossnickle and Folk 2003).

Hardening practices must be of sufficient duration for seedlings to respond morphologically and physiologically (Kozlowski and Pallardy 2002). Forming the full complement of needle primordia within terminal buds takes many weeks after the start of a dormancy-induction treatment and must be completed before bud dormancy onset (MacDonald and Owens 2006, Owens and Molder 1973). As mentioned, bud dormancy is correlated with seedling stress resistance during the reforestation process, but root apical meristems must remain active after fall planting until temperatures become unfavorable for root growth. After seedlings have reached the desired level of seedling quality to optimize seedling survival (Grossnickle 2012) and growth (Grossnickle and MacDonald 2018) after outplanting, they are biologically ready to ship during the fall-planting window (See Planting Windows and Seedling Field Performance section).

If there is a mid-to-late summer decision not to fall plant due to unfavorable site conditions or operational issues, then the forester needs to let the nursery manager know as soon as possible, ideally by early August (figure 4), so that a storage option can be implemented to ensure quality seedlings are available for a spring carry-over planting program (Landis et al. 2010). The nursery manager needs sufficient time to modify cultural practices to reduce the active-growth phase for roots, thereby minimizing the potential for root-bound plugs while achieving sufficient frost hardiness for frozen storage. Properly hardened seedlings can be lifted and stored with a level of high quality (Grossnickle and South 2014), and have high survival (Simpson 1990) and growth (L'Hirondelle et al. 2006) during the next growing season.

## Planting Windows and Seedling Field Performance

Primary risks for spring-, summer-, or fall-planting windows are related to seedling stress resistance and environmental conditions at the reforestation site. Environmental conditions of the reforestation site in northern forests can be generalized as having some combination of the following: (1) moderate to high light intensity, (2) high soil water availability in spring and fall with potential for low soil water availability in summer and fall, (3) low to medium soil temperatures in spring and fall, (4) medium to high soil surface temperatures in summer, (5) medium vapor pressure deficits (VPD) in spring and fall and high VPD in summer, (6) incidence of spring and fall frost, (7) high wind speeds, and (8) high nutrient availability in the soil solution (Margolis and Brand 1990). These conditions broadly reflect the regional climate, but microclimatic conditions vary considerably by elevation, topography, and aspect. Site disturbance also has a direct effect upon site microclimate, thereby affecting site energy, hydrologic cycles, and nutrient cycles (Spittlehouse and Stathers 1990). In addition to potential planting site environmental conditions, timing of planting within the fall-planting window for northern forests (i.e., September through mid-October) is also dictated by forecasted weather conditions.

Seedlings can be exposed to a wide range of environmental conditions within any planting window. Ideal environmental conditions allow an optimum physiological response by seedlings, while extreme conditions can exceed their ability to withstand stresses (Grossnickle 2000). An example of the expected biological response of seedlings planted across the spring-, summer- or fall-planting windows is defined for northern spruce species based on their known ecophysiological performance capabilities relative to seasonal reforestation site climate conditions (table 3). These ecophysiological patterns, in general, fit other northern conifer species, thus providing a perspective on what to consider when choosing a planting window. Knowing the risks of fall planting, in comparison with other planting windows, allows foresters to make an informed decision on whether this window is suited to their reforestation program.

**Table 3.** Potential for spring-, summer- and fall-planted northern spruce (*Picea*) seedlings to be negatively affected by typical climatic environmental stresses that can occur at the reforestation site with additional details regarding stress-resistance status of fall-planted seedlings (from Grossnickle 2000).

| Environmental Stress | Spring planting | Summer planting | Fall planting | Stress resistance status of fall-planted seedlings |
|---|---|---|---|---|
| **Atmospheric** | | | | |
| Air temperature (frost) | High | Low | Moderate | Freezing tolerance from -10 to -15 °C |
| Air temperature (heat) | Low | High | Moderate | Heat tolerance to 40 °C |
| Vapor pressure deficit | Low | High | Moderate | Good photosynthesis and water status capability at VPD < 2 kPa |
| **Edaphic** | | | | |
| Drought | Moderate | High | Moderate | Fall values at 90% of the maximum yearly level of drought tolerance for spruce species |
| Flooding | Moderate | Low | Low | Dormant seedlings can temporarily withstand flooded soil conditions |
| Low soil-root temperature | High | Low | Moderate | Root growth declines between 3 to 5 °C , but increases when > 10 °C |
| Soil surface temperature | Low | High | Moderate | Stem girdling occurs above 45 °C |
| Frost heaving | Moderate | Low | Moderate | Minimized by planting when soil temperature is > 5 °C |

## Recommendations

Research and operational experience from around the world have found that fall-planting programs can be successful, though challenges must be recognized and addressed for each site. The following are recommended operational steps to consider in maximizing the likelihood of a successful fall-planting program.

- Plan ahead to select sites with suitable environmental conditions and to determine appropriate species and stocktypes for each site.
- Nursery managers and foresters need to work together to plan the crop so there is sufficient time to grow seedlings to proper size and still have adequate time for the required hardening process before outplanting.
- Prepare sites in advance for fall planting, but also develop contingency plans (e.g., alternative sites, short-term storage for lifted seedlings, etc.) in case the plant date must be adjusted due to forecasted, adverse weather conditions.
- Develop a contingency plan with the nursery for overwinter storage and spring planting if stressful site conditions or other operational constraints arise and seedlings cannot be planted within the fall-planting window.

Foresters need to understand the rewards and risks for fall planting in northern forests. By considering these recommended steps, they can make informed decisions on whether to implement fall planting within their reforestation program.

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# A Primer on Plant Hydraulic Physiology for Nursery Professionals

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## Abstract

Plant hydraulic physiology, the study of water movement through plants, offers mechanistic explanations for better understanding connections among nursery production regimes, seedling growth, and outplanting performance. Applying plant hydraulic physiology methods and concepts starts with an understanding of flow rates, conductance, and conductivity. Water flow and hydraulic failure in woody plants are affected by the size and arrangement of the xylem, the morphology and allometry of the plant, and the environmental conditions in which the plant is growing. Nursery professionals can predict and manipulate the complex and dynamic responses of seedlings to water stress with knowledge of the plant hydraulic traits. The application of plant hydraulic physiology will help with the efficient and sustainable production of seedlings that can survive in challenging outplanting environments.

## Introduction

Plant hydraulic physiology is the study of water movement through plants (McCulloh et al. 2019). From research outcomes in the field of plant hydraulic physiology, we can build mechanistic explanations of how woody plants respond to water availability and limitations (Venturas et al. 2017). This information can help explain the impacts of nursery production regimes on plant physiology and growth. The field's relevance to seedling nursery production has long been recognized (Carlson and Miller 1990). Advances in the last few decades make the potential takeaways and applications from plant hydraulic physiology into nursery science even more enticing.

Though relevant to nursery science, plant hydraulic physiology can be a complex topic to understand without first being introduced to the basic concepts and research methods. In this article, we explain the definitions and foundational ideas of water flow through plants and the intersection with plant anatomy, morphology, and physiology. We also provide examples of how a nuanced understanding of plant hydraulic physiology can lead to better decision making and planning in nursery production and outplanting (Lauri et al. 2011). With this improved understanding, researchers, nursery professionals, and foresters can leverage what is known through plant hydraulic physiology to ensure that high-quality, stress-resistant seedlings are grown in the nursery and planted in the field.

## Quantifying Water Movement

We will begin by describing the basics of water flow through plants and the units used to quantify water movement. For nursery professionals, the parallel to water flow through irrigation systems is a useful starting point. More technical definitions for water flow through plants and related equations can be found in plant physiology textbooks (e.g., Lambers et al. 2008, Tyree and Zimmermann 2002).

### Flow Rate

Flow rate, Q, is a volume of water per amount of time.

1) $Q = V/t$; e.g., gal $\text{min}^{-1}$ or L $\text{min}^{-1}$

In the nursery, we often consider flow rates such as the capacity of a well in gallons (or liters) per minute. Sprinkler emitters and hose nozzles are also typically classified by their flow rates (e.g., a mist head with a flow rate of 0.25 gal/min [1 L/min]). If the flow rate of a hose or sprinkler head is unknown, it could be approximated by timing how long it takes for the hose or sprinkler to fill a container of a known volume (figure 1).

The flow rate through a system is affected by the conduits through which the water flows, whether pipes or xylem. Much more water can flow through a 1-in (2.5-cm) pipe than a 0.25-in (0.6-cm) pipe at the same pressure. For very small conduits, like xylem, that have non-turbulent water flow, the flow rate is proportional to the fourth power of the radius of the conduit. Thus, a single xylem conduit with a diameter of 40 µm can move as much water as 256 xylem conduits with a diameter of 10 µm (Tyree and Zimmermann 2002). Small differences in xylem size can lead to large differences in the flow rate of water through the xylem.

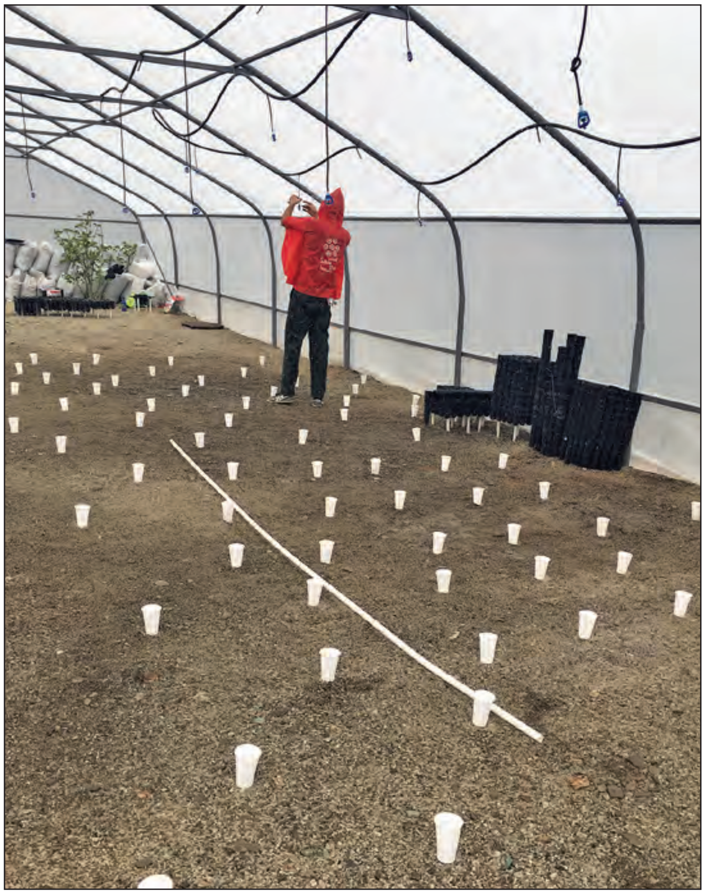


**Figure 1.** A cup test measures the amount of irrigation water that fills cups in a set amount of time. This test is used to determine the application rate of the irrigation system and accounts for the cumulative flow rate of multiple sprinkler heads across the growing area. (Photo by Rebecca Sheridan 2017)

## Conductance

In most nursery systems, pressure regulators are used to keep the water pressure constant, so we do not usually need to consider how pressure affects flow on a day-to-day basis for irrigation. With a pressure regulator, we assume the irrigation system is under a consistent pressure over time, allowing for even and predictable watering. When flow is considered in terms of the pressure gradient that drives water movement, this is conductance. Conductance, k, is given in units of water per time per pressure gradient.

2) $k = Q/\Delta\psi$; e.g., g $s^{-1}$ $MPa^{-1}$

In the next section, we will describe in more detail how conductance through a plant is impacted by xylem shape and arrangement.

## Conductivity

Conductivity, *K*, scales conductance to a length or an area.

3) $K = Q/(\Delta\psi \bullet area)$; e.g., g $m^{-1}$ $s^{-1}$ $MPa^{-1}$

This calculation allows us to compare capacity of different parts of the water movement system or water movement at different scales. Conductivity is not often used to describe irrigation systems, but in plants, we might be interested in comparing water flow through stems with different stem sizes and would therefore scale conductance by sapwood area to obtain a measurement of conductivity (Melcher et al. 2012). Across the breadth of work in plant hydraulic physiology, hydraulic conductivity is scaled in various ways such as sapwood area ($K_S$), leaf area ($K_L$), stem length ($K_l$), root mass, and so on. When reviewing a reported value of plant hydraulic conductivity, be sure to check how conductance is normalized to the plant's dimensions.

## Water Movement Through Plants

The metaphorical parallels between irrigation and water movement through plants start to break down when we consider the force of water movement in plants. Instead of being pushed by a pump or falling with gravity from a cistern, water in a plant is pulled by tension created when water evaporates from the leaf (evapotranspiration).

To explain this further, we can start by defining water potential. Water potential, ψ, is the potential energy of water within the plant's xylem or cells. Water potential is reported relative to the potential of pure water (ψ = 0). In plants, water potential is negative due to solutes, such as sugars, in sap and the tension created by evapotranspiration. While negative pressure is nonsensical for gases (the lower limit of pressure for a gas is zero, or a vacuum), liquids, such as water, can withstand tension, or negative pressure. The most often reported unit of pressure in plant hydraulic physiology is megapascals (MPa). For context, 1 MPa equals 9.85 atmosphere (atm) or 145 pounds per square inch (PSI). Plant water potential in leaves and stems is commonly measured using a Scholander-type pressure chamber (figure 2) (Tyree and Hammel 1972).

When water potential varies among different parts of the plant or between the plant and the growing environment, this results in a water potential gradient. Water moves from less negative to more negative water potentials, such as from a root in wet soil to the leaf surface, where water is evaporating into the ambient air. The pathway of water movement from the soil (or growing medium) through the plant and into the atmosphere is called the Soil-Plant-Atmosphere Continuum or SPAC. The water potential gradient created by evapotranspiration moves water against the gravitation gradient, allowing water to move up into even the tallest tree canopies.

## Xylem Function and Dysfunction

The bulk of water flow in a plant is through the plant's xylem. As with an irrigation system, the constraints and capacity of water movement are dictated by the size of the conduits through which water moves (xylem anatomy) and the arrangement of the system (plant morphology). Xylem is basically a modular system (figure 3). In conifers, xylem conduits are tracheids, and in angiosperms the conduits are vessel elements and tracheids. Water flow through an individual xylem conduit is determined by the inner diameter of the conduit (Hacke et al. 2017). The conduits are stacked end to end and connected via intervessel pits (Wason et al. 2019). The thickness of the xylem's cell walls, as well as the shape, size, and arrangement of vessel-to-vessel connections, also impact water movement. The width, length, and arrangement of the xylem conduits vary by species (Schenk et al. 2018). Classifications within wood as latewood and earlywood or ring-porous and

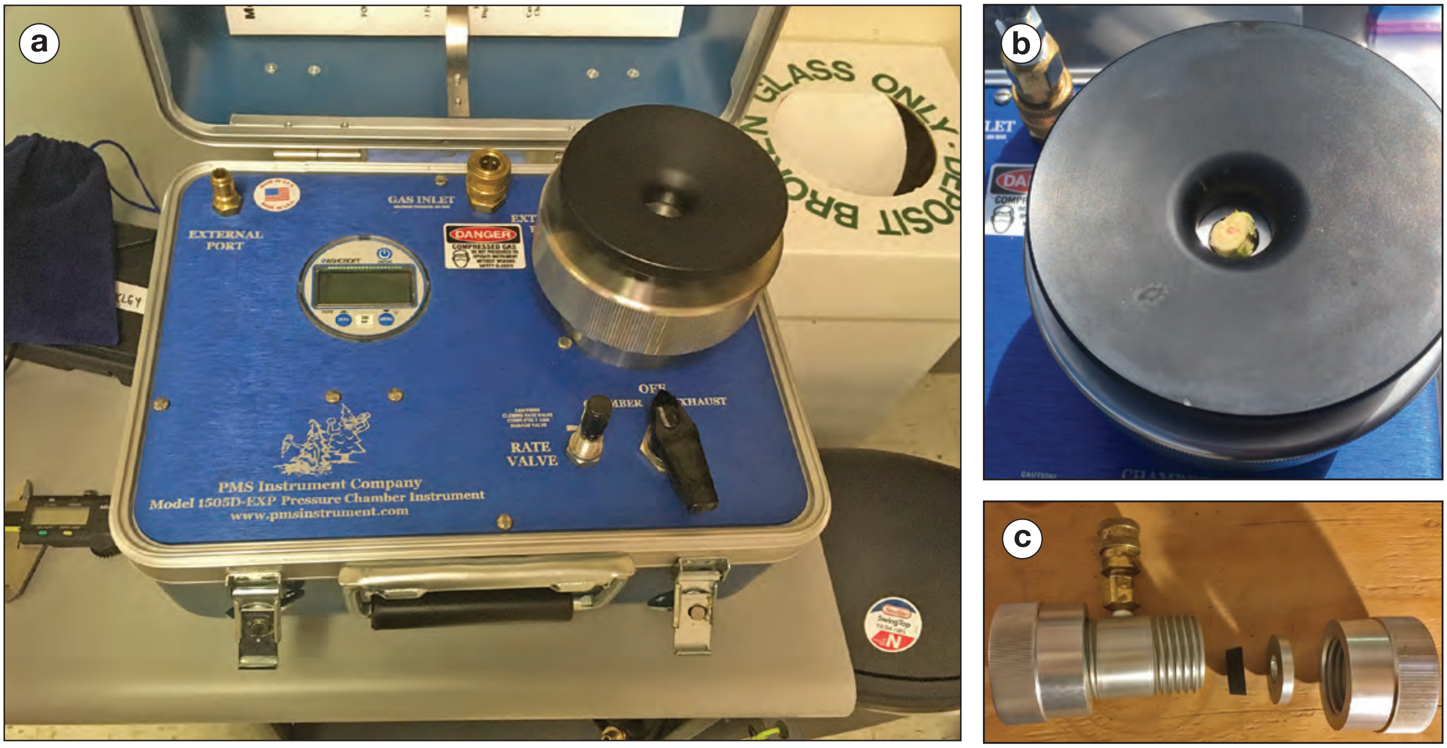


**Figure 2.** (a) A pressure chamber with a digital gauge to read pressure measurements is a useful instrument for plant hydraulic physiology measurements. (b) The plant stem segment is placed in the compression gland and pressure is applied to force water through the cut end. (c) A cavitation chamber is used to pressurize stem segments. Not pictured are the portable compressed gas tank and the pressure-resistant hoses that connect the tank, chamber, and accessories. (Photos by Rebecca Sheridan 2020)

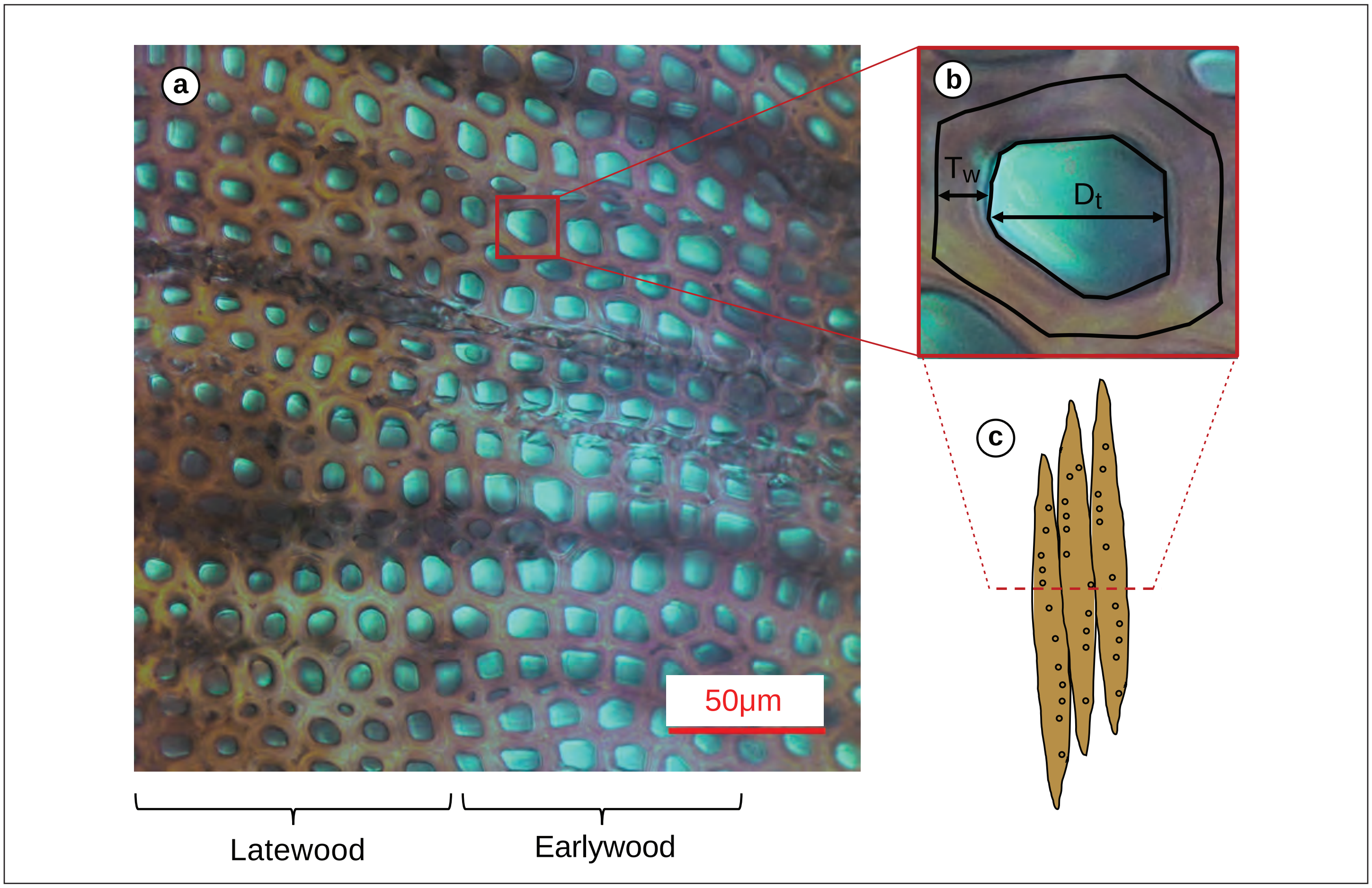


**Figure 3.** A cross-section of Douglas-fir xylem shows (a) individual xylem tracheids arranged with a notable transition in tracheid size from earlywood to latewood. (b) A close-up of an individual xylem element shows dimensions of inner diameter ($D_t$) and wall thickness ($T_w$) that impact water flow. (c) An illustration of tracheids with bordered pits (not to scale). (Photo by Rebecca Sheridan 2017)

diffuse-porous describe the way xylem elements of different sizes are arranged within the stem. This arrangement of xylem elements affects the efficiency of water movement through the stem (McCulloh et al. 2010). Dimensions of xylem conduits can change in response to environmental factors such as drought (Eilmann et al. 2009, Eldhuset et al. 2013, Fonti et al. 2013). Predictably, this variability impacts conductance at the xylem, organ, and whole-plant scales. In this way, plant structure and allometric relationships are also connected to plant hydraulic physiology (Mirabel et al. 2019).

So far, we have introduced concepts of water flow through plants when functioning in the absence of stress. When a plant is in water-limited conditions, such as drying soil, the plant experiences increasingly negative water potentials. With increasing stress on the water transport system through the SPAC, plants are at risk of xylem embolism or cavitation. Cavitation is the introduction of air into the water-filled xylem, which results in embolism, air-filled cavities within the xylem. As with xylem conductance, the mechanics of xylem embolism are related to xylem conduit size, arrangement, and interconnection (Gleason et al. 2016). Just as there is variety in the xylem system's function across organs, species, and environment conditions, there are also variable responses to water stress (Kavanagh et al. 1999, Stout and Sala 2003). Hydraulic failure due to water stress can reduce growth or cause dieback; the most severe consequence of water limitation to a plant is mortality (Hammond et al. 2019).

## Measuring Stem Conductance

Plant conductance and related hydraulic traits are measured in a wide variety of ways. Many techniques can be accomplished with relatively low-cost or lab-made equipment. Recent advances in x-ray microCT imaging technology allow water movement within plants to be measured *in vivo* in real time (Miller et al. 2020). We will not go into describing all the methods by which plant conductance can be measured, but several articles provide good starting points (e.g., Kolb et al. 1996, Li et al. 2008, Melcher et al. 2012). For purposes of this paper, we will focus on one commonly used metric within plant hydraulic physiology: stem conductance (figure 4). Stem conductance is the flow through a stem segment across a known pressure gradient and can be measured relatively easily in a lab setting using an elevated reservoir of ultra-pure, filtered water, tubing, and a pipette or microbalance to track output of water (Sperry et al. 1988). Most often, stem conductance measurements are scaled to the stem's length and diameter and reported as stem conductivity.

One way to quantify the impact of water stress on a plant is with the metric of stem conductivity. The relationship between water stress and conductivity is presented as a vulnerability curve (Tyree and Zimmermann 2002). Measurements made on a stem segment directly removed from a plant capture the conductivity of the stem under ambient conditions and the history of conditions the stem has encountered. If xylem conduits within the stem segment had embolized under ambient conditions, the embolism can be removed by putting the stem segment under water in a vacuum and pulling the air out. Stem conductivity measured after embolisms have been removed gives the maximum stem conductivity. Pressure can also be artificially imposed on a stem segment using a pressure chamber to replicate the stress a segment would face under drought or other water stress (figure 2) (Cochard

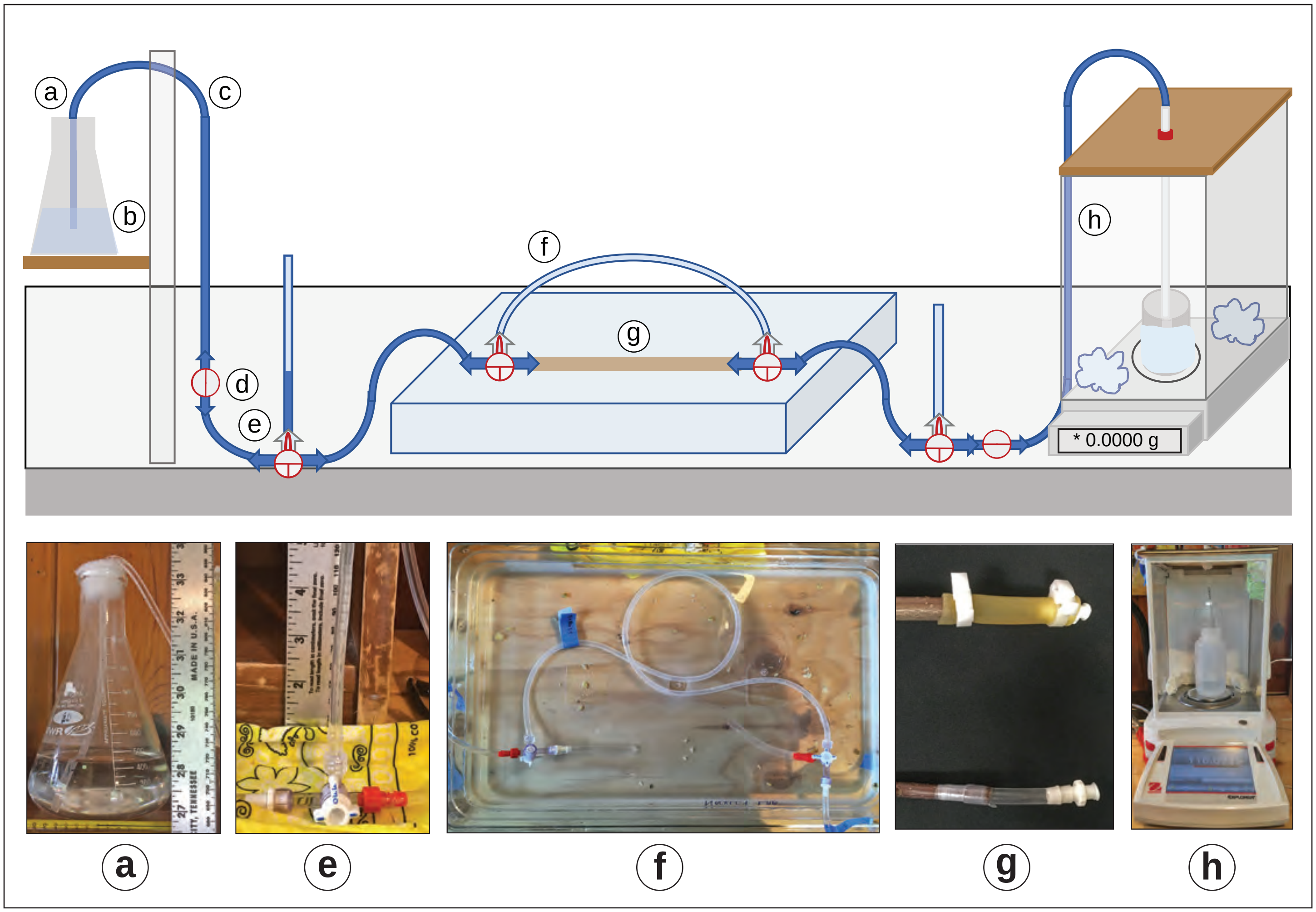


**Figure 4.** A stem conductivity apparatus consists of: (a) an elevated reservoir of water to provide a pressure gradient to induce flow through the stem, (b) a meter stick to measure height of the hydraulic head, (c) tubing through which water flows from the reservoir through the stem segment and onto the balance, (d) stopcocks that direct flow of water to the stem or to bypass tubing depending on the step within the method, (e) standpipe that allows pressure gradient to be equilibrated with the stem segment height, (f) bypass tubing, (g) stem segment plumbed into the system with rubber or tubing gaskets, and (h) an analytical balance that measures flow over time. With the flow rate onto the balance, the pressure gradient, and the length and diameter of the stem segment, stem conductance and conductivity can be calculated. The balance can be connected to a computer to automate the calculations. (Photos by Rebecca Sheridan 2020)

et al. 1992). The same stem segment can be measured multiple times with stepwise increases in water stress to create a response curve between water potential and conductivity (figure 5). Most often, changes in conductivity are reported relative to maximum conductivity, and subsequent values of conductivity are given in terms of the percent loss of conductivity (PLC).

Maximum conductivity and vulnerability curve parameters vary by organ within a plant, environmental conditions, and species. Within a plant, it is thought that more expendable organs such as leaves and fine roots will experience hydraulic failure first, to protect the rest of the plant under drought conditions (Johnson et al. 2016). To compare among populations of interest, plant physiologists often report the water potentials at which 50 percent of conductivity is lost ($P_{50}$); $P_{12}$ and $P_{88}$ are also used to summarize key points on vulnerability curves. Thresholds for mortality have been identified for conifers at 50 percent stem PLC and for angiosperms at 60 to 88 percent stem PLC (Adams et al. 2017, Brodribb and Cochard 2009, Urli et al. 2012). The water potentials at which mortality-inducing losses of stem conductivity occur should obviously be avoided in nursery production.

## Other Physiological Responses

In the preceding sections, we have focused on stem hydraulic conductivity. However, plants draw on a suite of physiological traits and dynamic responses to avoid or mitigate water stress (Choat et al. 2018). In the context of seedling production, it is useful to also understand the physiological responses to water stress that occur before stem hydraulic failure and plant mortality. In the same way that a vulnerability curve is built for stem hydraulic conductivity, other hydraulic traits such as stomatal closure, leaf hydraulic conductivity, and leaf wilting can be mapped to changes in water potential. The response curves of multiple traits can be layered together, showing the sequence of physiological responses to increasing water stress (figure 6). For example, a plant may first respond to water stress by adjusting stomatal conductance, then leaf conductivity, before stem conductivity is impacted at more extreme water potentials. Sequential physiological responses have been mapped for individual species such as grapevine (*Vitis vinifera* L.) (Gambetta et al. 2020) as well as summarized for hundreds of species (Bartlett et al. 2016). Identifying, testing, and modeling sequences of physiological events may be useful for nursery professionals in operational contexts, such as identifying thresholds for irrigation.

## Application to Nursery Production

In nursery production, the goal is to produce seedlings with morphological and physiological attributes that are suitable for specific outplanting conditions, thereby

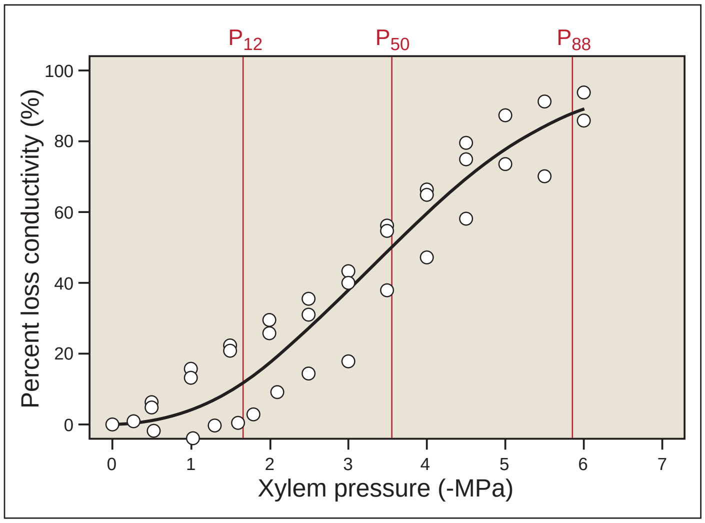
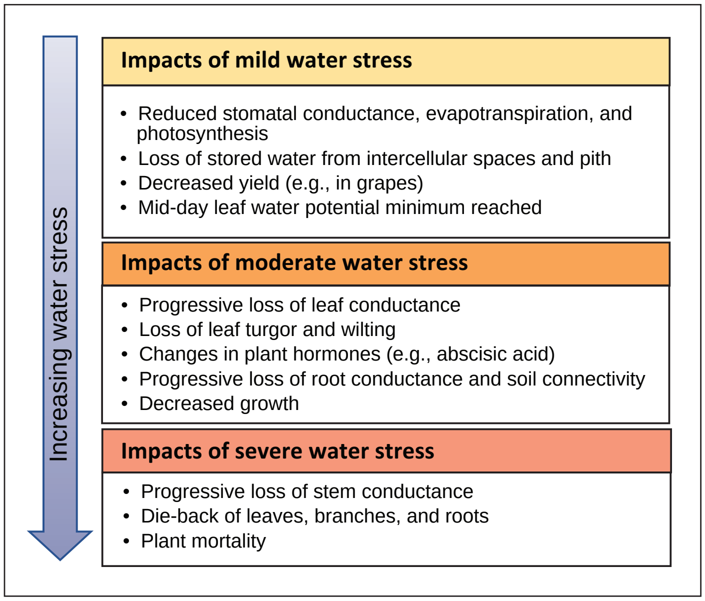


**Figure 5.** A vulnerability curve for *Malus* var. 'Prairifire' shows the percent loss of conductivity in response to more negative water potentials (n = 3). The vulnerability curve was built using measurements of stem conductivity from a stem conductivity apparatus with stepwise increases in stem water potential induced in the cavitation chamber. Vertical lines indicate 12 percent, 50 percent, and 88 percent loss of conductivity relative to maximum conductivity. (Adapted from unpublished data from Sheridan and Nackley)



**Impacts of mild water stress**

- Reduced stomatal conductance, evapotranspiration, and photosynthesis
- Loss of stored water from intercellular spaces and pith
- Decreased yield (e.g., in grapes)
- Mid-day leaf water potential minimum reached

**Impacts of moderate water stress**

- Progressive loss of leaf conductance
- Loss of leaf turgor and wilting
- Changes in plant hormones (e.g., abscisic acid)
- Progressive loss of root conductance and soil connectivity
- Decreased growth

**Impacts of severe water stress**

- Progressive loss of stem conductance
- Die-back of leaves, branches, and roots
- Plant mortality

**Figure 6.** Plant hydraulic physiology traits respond to increasing water stress in a predictable sequence. The precise water potentials at which these responses will be triggered depends on the species and population. (Adapted from Bartlett et al. 2016, Gambetta et al. 2020, and Miller et al. 2020)

optimizing survival and growth potential after planting, as stated in the Target Plant Concept (Dumroese et al. 2016). Plant hydraulic physiology can be a useful tool to develop nursery regimes to produce high-quality seedlings.

We often use proxy measurements to estimate water potentials within the SPAC for making decisions about when and how much to irrigate. This may be as simple as watching for visual signs of plant water stress, such as wilting (figure 7). A more precise method of irrigation scheduling is to track container weights, then irrigate at a target weight (Dumroese et al. 2015). Deciding to irrigate at a target container weight links the weight to the growing medium's water content which is linked to the growing medium's water potential and the assumed physiological response of the crop. To make a more direct connection between the decision to irrigate and the plant's physiological response, some investigators build response curves of photosynthesis to changes in water availability whereby irrigation is triggered before water limitations reduce photosynthesis (Fulcher et al. 2012, Nambuthiri et al. 2017). Controlling the irrigation in this way resulted in greatest plant growth under mild water deficits, more so than when containers were maintained at full saturation or when more severe water deficits occurred. Producing seedlings of similar or better quality with less water is important for improved water conservation in nursery production (Fulcher et al. 2016). This type of irrigation management system could be expanded to include the sequence of physiological responses to water stress described above, thereby shifting the framework from a focus on changes in container weights to a focus on plant hydraulic and physiological responses to water limitations.

**Figure 7.** Wilting is a sign that a plant has experienced moderate to severe water stress. Waiting to irrigate until wilting is observed can be a risky strategy for some species. (Photo by Rebecca Sheridan 2020)

Beyond irrigation scheduling, plant hydraulic traits can be used to proactively guide desirable plant attributes in nursery production. At an ecological scale, water deficits in one season have been found to impact plant growth in the following season (Kannenberg et al. 2019). Likewise, drought conditioning during nursery production exposes plants to mild water stress to prepare the seedlings for stressful outplanting conditions. Measurements of stem conductance, photosynthesis, and stomatal conductance, and imaging of stem segments identify which plant parts are impacted by drought conditioning, the amount of change, and how long the impacts persist. These measurements can help nursery growers tailor when, and to what degree, crops should be exposed to water stress for drought conditioning (Sloan et al. 2020). Further research is needed to explore how nursery practices, such as hardening off crops with water stress, can be guided by plant hydraulic traits.

As a final example, plant hydraulic traits can inform the day-to-day risk assessment that occurs during nursery production regimes. During the establishment phase of a nursery crop, there is a trade-off between frequent irrigation of the crop to avoid stressing very small seedlings and increased risk of root disease with saturated growing media (Dumroese and James 2005). However, even conifer seedlings less than 10 weeks past germination can withstand some risk of embolism, as determined using confocal microscopy to image xylem anatomy and calculate theoretical conductance and resistance to embolism (Miller and Johnson 2017). With precise estimates of water-stress levels that will reduce xylem function in young seedlings, irrigation and disease management can be balanced with more nuance.

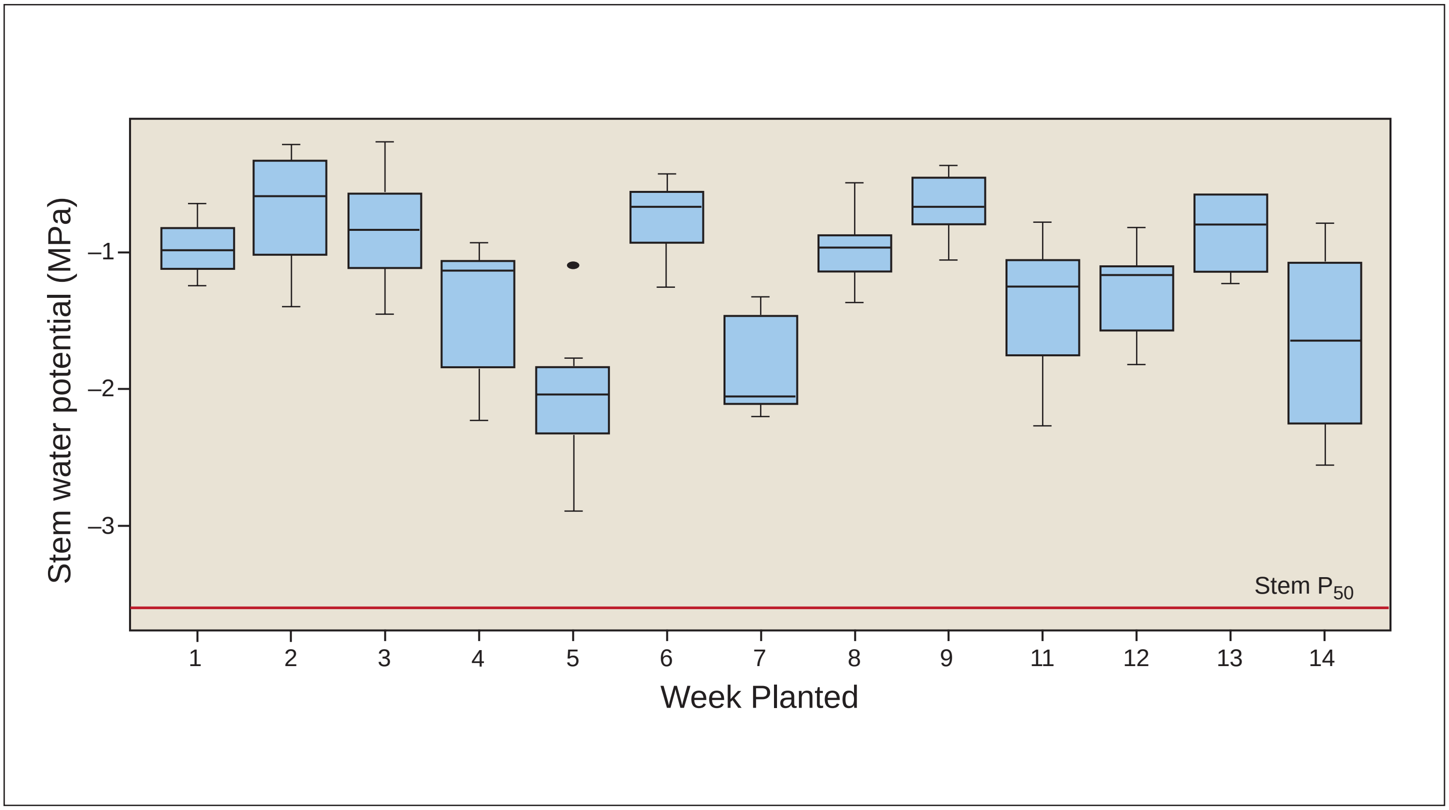


**Figure 8.** Stem water potential of *Malus* 'Prairifire' seedlings at the time they were removed from cold storage for outplanting (n = 7). Week 1 is the first planting date on March 19, 2020; the seedlings had been in cold storage since late 2019. The water potential at which stem $P_{50}$ (water potential at which 50 percent of conductivity is lost) occurs is indicated with a red line. Given that none of the stem water potentials were near stem $P_{50}$, the seedlings were not likely to suffer from hydraulic failure through the stem after extended cold storage. (Adapted from unpublished data from Sheridan and Nackley)

At the other end of the production cycle, seedlings kept in cold storage could be at risk of desiccation if stored too long, yet the timeframe for planting is often constrained by environmental conditions at the outplanting site. When nursery-grown seedlings were outplanted from storage each week through the spring season, however, the seedlings kept in storage longer did not show increasing symptoms of hydraulic failure as the season progressed, indicating seedling quality can be maintained for a time even if planting is delayed (figure 8). At many points within a production cycle, nursery professionals often face less-than-ideal circumstances in which information about plant hydraulic traits can help guide decisionmaking.

## Conclusion

Nursery production is a logical application of plant hydraulic physiology, though there is still much work to be done to leverage the science more widely into practice. Still to be bridged are the differences between nursery environments and the natural systems in which most foundational hydraulic physiology work has been done. In the nursery, we control plant water status through irrigation, medium type, and container choices, and we control water demand by adjusting air flow, temperature, humidity, and shade. Additionally, seedlings and saplings respond to water stress differently than mature individuals (Augustine and Reinhardt 2019, Medeiros and Pockman 2010, Sperry and Saliendra 1994). Despite these challenges, the current research and potential advances in plant hydraulic physiology can be useful to nursery production. The field offers tools and knowledge to ensure that we can efficiently and sustainably produce seedlings that are well suited to survive in challenging outplanting environments.

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# Effects of Basal Stem Shading on Field Performance of Bareroot and Container Douglas-fir Seedlings

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## Abstract

Reforestation on hot, dry sites can be challenging. One technique to protect seedlings from heat damage is to install shade around the basal stem. This study examined field performance of bareroot and container seedlings planted on north- and south-facing aspects in southwestern Oregon with and without basal stem shading using inverted peat cups. After two seasons, seedlings planted on the north-facing plot had greater survival and lower diameter growth than those on the south-facing plot and container seedlings had more growth and higher survival than bareroot seedlings. The shade treatment did not significantly effect growth or survival. During both seasons, temperature was above normal and precipitation was below normal.

## Introduction

In southwestern Oregon, survival and growth of outplanted seedlings can be a major challenge on some sites, especially in conjunction with even-age silvicultural systems. Abiotic factors, such as excessive heat and low soil moisture, can limit reforestation success by killing, suppressing, or damaging seedlings (Cleary et al. 1978). High soil-surface temperatures can irreversibly damage stem tissue near the soil line. On south-facing slopes, lesions can develop on the south side of seedling stems. These lesions can interrupt cambium function and can be an opening for secondary damage from disease or insects. Heat damage can also occur on north-facing slopes but the mortality rate is usually half that of south-facing slopes (Helgerson et al. 1992). Adequate soil moisture is important not only for plant use but also to mitigate temperature extremes and reduce excessive heating in the root zone (Flint and Childs 1987).

Treatments and planting strategies that lower soil temperature, reduce soil-surface evaporation, and reduce vegetative competition can protect newly planted seedlings (Flint and Childs 1987). Planting in favorable microsites or artificial shading can help reduce moisture stress and increase seedling survival (Cleary et al. 1978, Helgerson et al. 1992), especially on south-facing slopes (Helgerson 1986). Shade cards can be effective but are costly and difficult to install (Helgerson 1986). Current shade card costs for material, installation, and later removal total approximately $0.90 per seedling. Another option for artificial shading is to use inverted cups with the bottoms removed. In an early study, shading the basal portion of seedlings with inverted Styrofoam™ cups around the base of Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings was as effective as shade cards (Helgerson 1990). Pots made with pressed peat moss are also commercially available and have shown to be effective (Helgerson et al. 1992).

Over the last few years, the USDI Bureau of Land Management (BLM), Medford District in Oregon has been using peat cups to increase seedling growth and survival on harsh sites. These cups cost much less than shade cards ($0.31 per seedling) because the material is relatively inexpensive, the cups are easier to install, and no removal is required since peat cups are biodegradable within a few years. Furthermore, peat cups are an environmentally friendly option compared with Styrofoam™.

Although shading seedlings with cups has been studied previously, most studies were published 30 or more years ago. Since then, stock types, seedling quality, nursery practices, and customer expectations have evolved significantly. Thus, it is worthwhile to revisit this practice and test its efficacy. The purpose of this study was to evaluate the cup-shading technique on survival and growth of modern Douglas-fir stock types over two growing seasons.

## Materials and Methods

### Study Location

The study was established at two adjacent plot locations (north and south aspects) within the 2018 Miles Fire perimeter (Butte Falls Field Office, BLM Medford District, 42°48'49.1"N 122°45'26.0"W) (figure 1). Average elevation of the study area is 3,500 ft (1,067 m), and annual precipitation ranges 35 to 55 in (90 to 140 cm) with most of the precipitation occurring in winter through early summer. The mean annual temperature is 45 to 52 °F (7 to 11 °C), and the average frost-free period is 100 to 160 days. Soils in the area are Straight-Shippa, shallow, well-drained gravelly loams with bedrock occurring between 12 and 40 in (30 and 100 cm) deep. Slopes average 25 to 35 percent at both locations. The BLM Land Use Allocation for the area is considered Harvest Land Base (USDI BLM 2016), and the Timber Production Capability Classification is designated as commercial forest land with reforestation problems due to high temperatures (USDI BLM 1986)(figure 1).

The two plot locations are approximately 130 ft (40 m) apart and are separated by a mid-sloped ridge (figure 1). The north-aspect plot (figure 2) burned at a moderately low severity in the 2018 Miles fire and was established under a canopy of mature Douglas-fir. The basal area was estimated at 100 ft$^2$ (9.3 m$^2$) live and 60 ft$^2$ (5.6 m$^2$) of standing dead. Crown cover and average diameter-at-breast-height (4.5 ft [1.4 m]; DBH) are approximately 40 percent and 24 in (61 cm), respectively. The understory was predominately Oregon grape (*Mahonia aquifolium* [Pursh] Nutt.) and canyon live oak (*Quercus chrysolepis* Liebm.). The south aspect plot (figure 3) burned at high severity in the 2018 Miles Fire and evidence indicates that it likely burned 19 years prior in the Timbered Rock Fire. The south-aspect plot is open without an overstory and approximately 50 percent of the site was covered with Pacific madrone (*Arbutus menziesii* Pursh), canyon live oak, and deerbrush (*Ceanothus integerrimus* Hook. & Am.) skeletons that have resprouted since the fire to a height of 2 to 6 ft (0.6 to 1.5 m).

### Seedlings

Two Douglas-fir stock types were used in this study. Bareroot Q-plug +1.5 (seed zone JV2) were grown at the U.S. Department of Agriculture, Forest Service, J. Herbert Stone Nursery (Central Point, OR) and 1-year-old 515A container stock (seed zone EE2) were grown at Pacific Regeneration Technologies Nursery (Hubbard, OR).

The Q-plug+1.5 was developed in the early 2000s (Landis 2007, Steinfeld 2004), and the J. Herbert Stone Nursery is the only forestry nursery in the country currently growing this stock type. The process starts at International Horticultural Technologies, LLC (Hollister CA) where Styroblock® containers (2 in$^2$ [33 cm$^3$] cavities; Beaver Plastics, Alberta, Canada) are filled with a proprietary stabilized growing medium. The pre-filled containers are then shipped to the Cal Forest nursery (Etna, CA) where they are sown in January and placed in a greenhouse. Seeds germinate and grow to about 1-in (2.5-cm) tall by April when they are transported to the J.H. Stone Nursery and transplanted into bareroot beds. During the growing season in the bareroot beds, the seedlings are root pruned 2 to 3 times and laterally root pruned once. After budset, seedlings are lifted and packed in the fall. Although the production cost for this stock type is higher than conventional 2-year-old bareroot stock types, seedlings are easier to space in the nursery beds and height is more manageable. These advantages result in a favorable root-shoot ratio.

The Styroblock® 515A (2 in [5.1 cm] diameter by 6-in [15.2-cm] depth cavities; Beaver Plastics, Alberta, Canada) were designed in 1991 to produce "Plants de Fortes Dimensions" in the cold frame system (O'Neill 2021). This container size is now the most demanded size in the Pacific Northwest and is also a popular container type throughout much of North America.

All seedlings were grown from improved seed collected from BLM's Provolt Orchard (15 mi [24 km] south of Grants Pass, OR). The JV2 and EE2 seed zones used in the study for bareroot and container seedlings, respectively, are from first-generation seed orchards that were established in 1994. The orchards were developed from phenotypic selec-

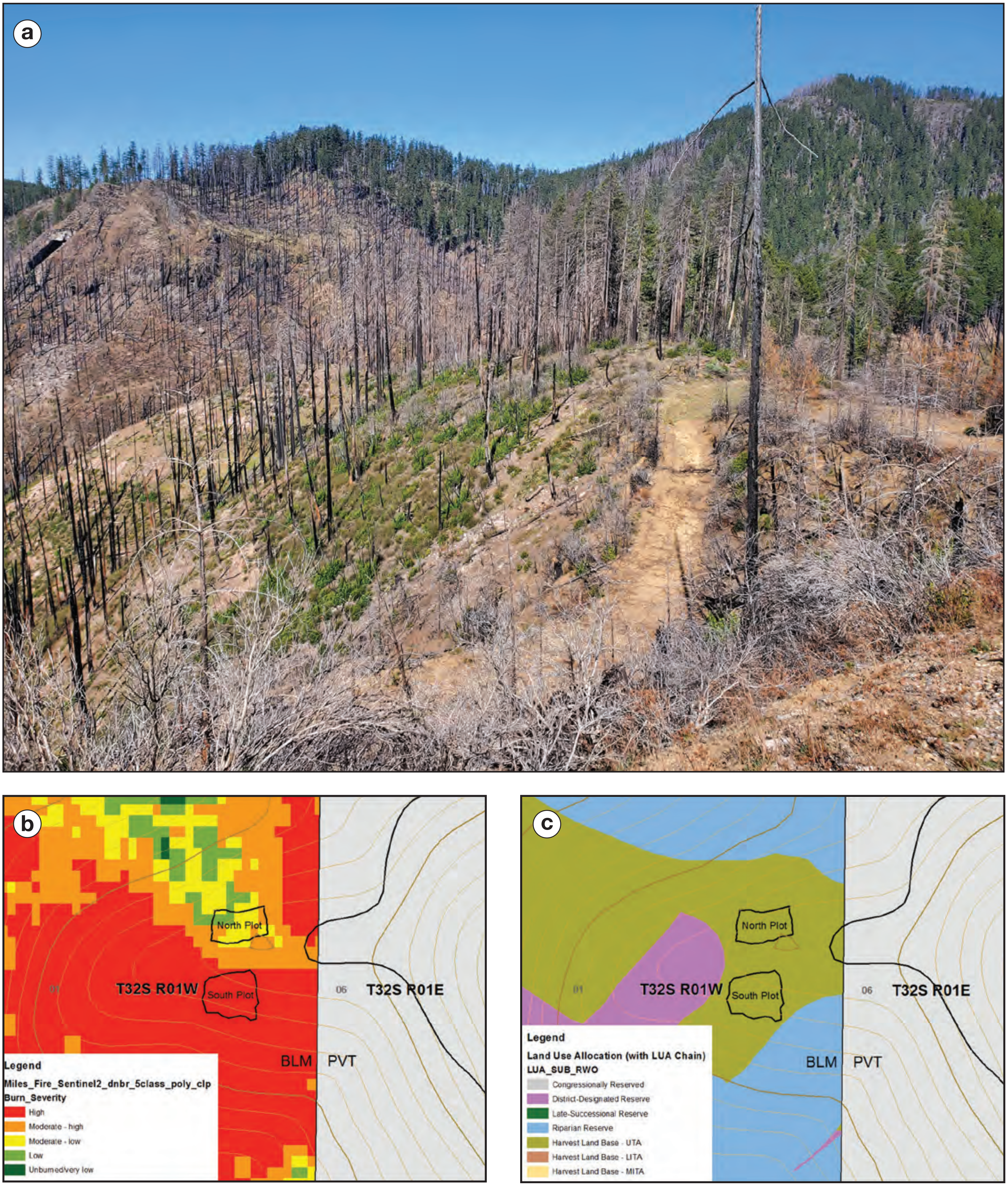

**Figure 1.** (a) The north and south plots were located (b) within the 2018 Miles Fire perimeter and (c) are considered Harvest Land Base by the USDI Bureau of Land Management. (Maps generated with ARC GIS, photo by Chad Vetter 2021)

**Figure 2.** The north-aspect plot location was installed under a canopy of mature Douglas-fir trees that were moderately burned in the 2018 Miles fire. (Photo by Chad Vetter 2021)

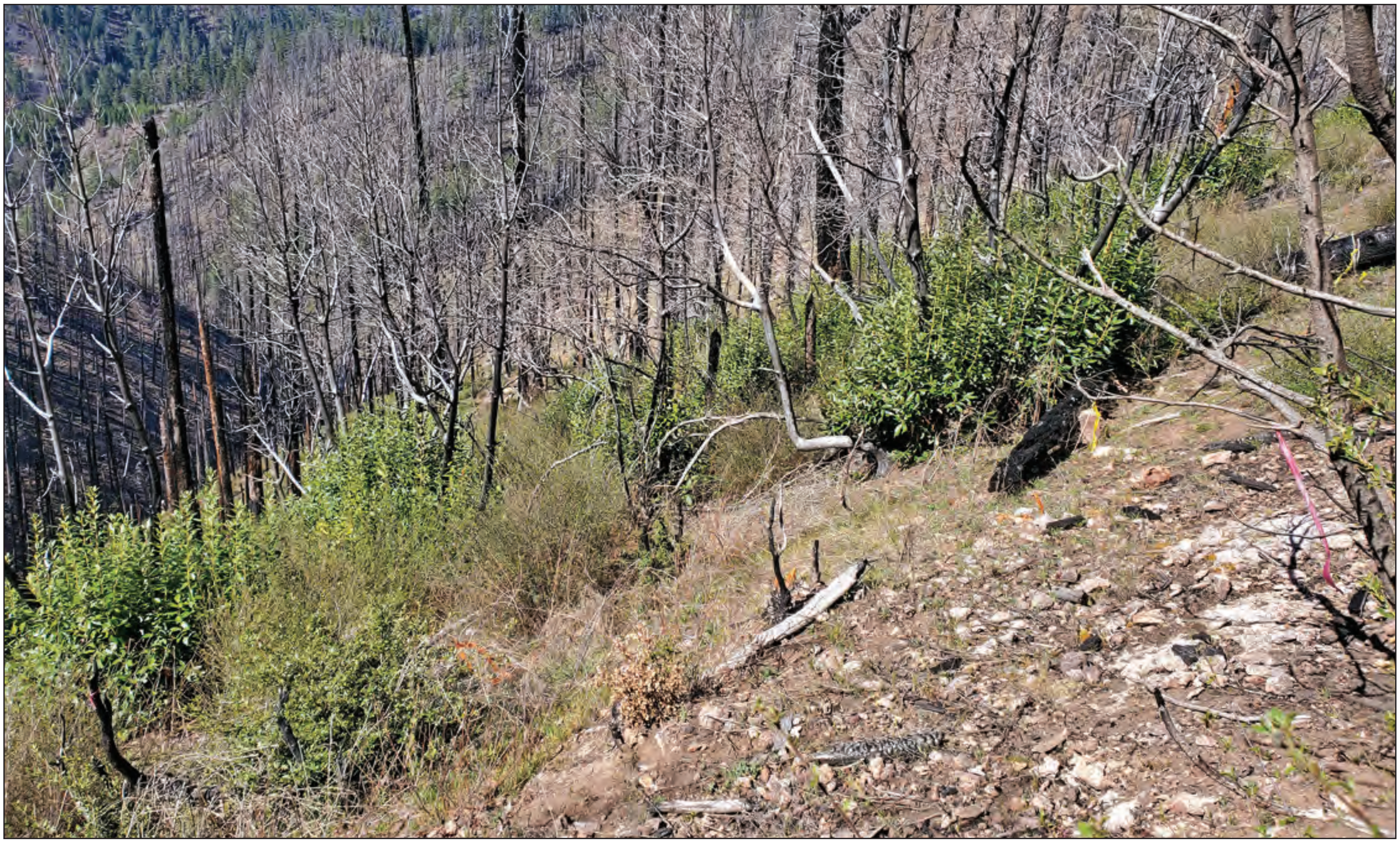

**Figure 3.** The south-aspect plot location was severely burned during the 2018 Miles fire and is an open area with no overstory. (Photo by Chad Vetter 2021)

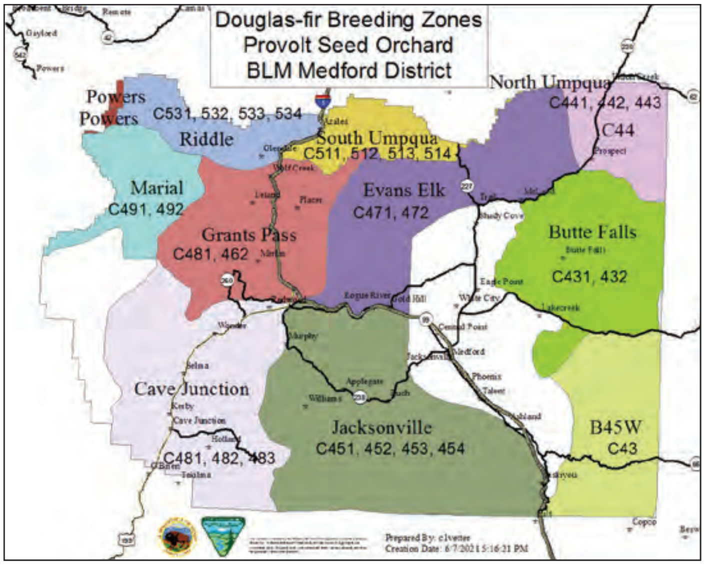

**Figure 4.** Seedlings for this study were from the Jacksonville 452 breeding zone (bareroot) and the Evans Elk 472 breeding zone (container). (Map generated by Chad Vetter using ARC GIS 2021)

tions collected from elevation bands in breeding units 472 and 452 (figure 4) and tested for growth and yield. Scion collected from the best parent trees were then field grafted to create the Evans Elk 2 (EE2) and Jacksonville 2 (JV2) orchards (table 1) (Crawford 2021).

## Treatments

Both plot locations were planted on the same day in late April 2020. All seedlings were planted at a spacing of 8 by 8 ft (2.4 by 2.4 m). Bareroot seedling roots were dipped in water prior to planting. Shade treatments were installed on half of the seedlings for each stock type. Treatments consisted of round Jiffy® peat pots (4-in [10-cm] diameter and 4-in [10-cm] depth; Jiffy Products of America Inc., Lorain, OH) inverted around the base of each seedling (figures 5 and 6). Jiffy pots are made from peat moss and wood pulp.

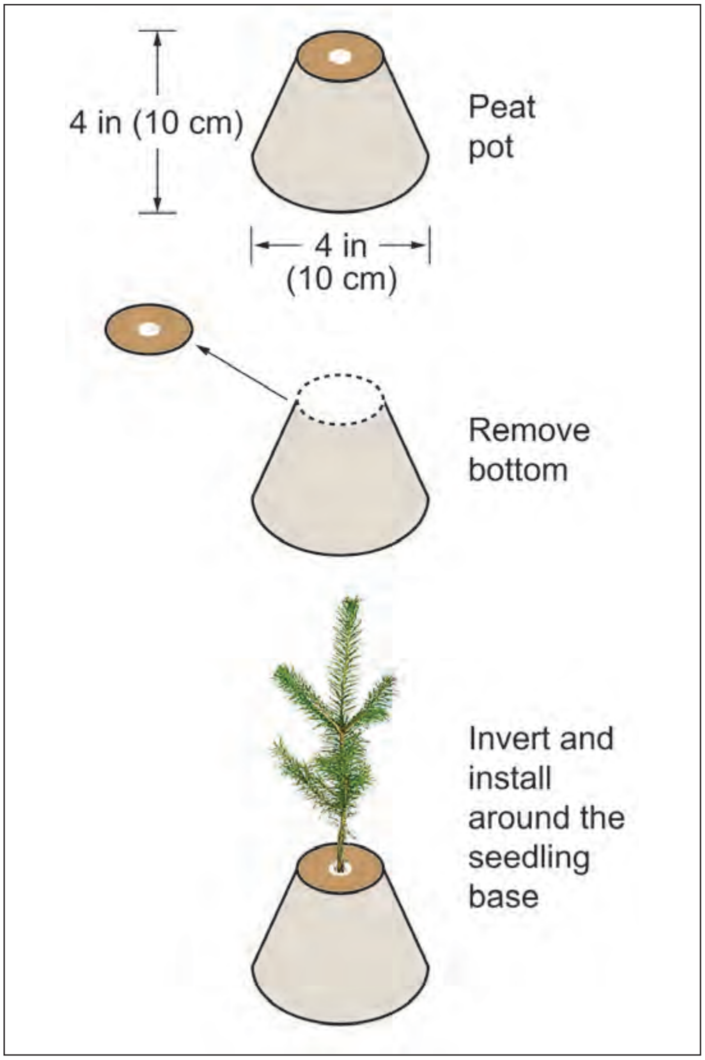

**Figure 5.** Peat pots were installed on each seedling designated for treatment by inverting the pot, removing the bottom, and placing carefully around the base of the seedling stem. (Adapted from Illustration by Chad Vetter 2019)

## Measurements

Initial heights and stem diameter were measured on all seedlings immediately after planting. Height was measured with a carpenter's tape on the uphill side from the ground to the tip of the leader. Diameter was measured at the ground line with a caliper. At the end

**Table 1.** Seed for the study was from two orchards at the USDI Bureau of Land Management Provolt Seed Orchard (near Grants Pass, OR). Bareroot seed was from the JV2 orchard and container seed was from the EE2 orchard.

| Orchard Name | Abbreviation | Breeding Unit | Elevation (ft) | 1966 Oregon seed zone | 1996 Oregon seed zone |
|---|---|---|---|---|---|
| Evans Elk 2 | EE2 | C472 | 3,000 to 4,000 | 501 / 502 | 15 / 16 |
| Jacksonville 2 | JV2 | C452 | 2,500 to 3,500 | 511 / 512 | 3 / 16 |

**Figure 6.** On each of the plot locations, half of the seedlings had (a) peat pots installed to shade their stems and (b) the other half were left unshaded. (Photos by Chad Vetter 2021)

of the first and second growing seasons (September 2020 and 2021), height and diameter were measured again along with an assessment of survival. Growth was calculated by subtracting initial values.

### Experimental Design and Data Analyses

The experimental design consisted of a 2 by 2 factorial (two stock types by two shade treatments) in a split plot. Within each whole plot (north and south aspects) there were 5 replications of each stock type and shade treatment combination. Replications were rows of 6 seedlings. Data were analyzed using SAS® software (SAS Institute 2013). Shaded seedlings that had missing cups were excluded from the analyses. Significant differences among treatments were determined using Tukey's multiple comparison test.

## Results and Discussion

Peat cups were easy to install and required no special tools. The bottoms of the cups were easy to manually tear out. The cups appear to be attractive to wildlife and insects, however. On the south plot, we suspect that wood rats removed nearly 20 percent of cups from seedlings. Also, some cups seemed to disappear slowly which may be due to wasps using the material as a resource to construct their nests (Hadley 2019).

At the end of both growing seasons, there were no interactions among aspect, stock type, or shade treatment. Seedlings growing on the north aspect plot had significantly higher survival and tended to have less diameter growth than those growing on the south aspect plot (figure 7). This result is not surprising given that south-facing aspects have hotter, drier environments compared with north-facing aspects (Redmond 1992, USDA Forest Service 2002). The increased diameter growth likely indicates greater root growth on the drier site which would be necessary for increased water uptake (Grossnickle 2005). Temperature and precipitation patterns based on a nearby weather station (Trail Creek, OR) showed that precipitation was below normal and temperature was above normal during both seasons (figure 8).

Container seedlings had significantly higher survival and greater height and stem diameter growth than bareroot seedlings. Often, container seedlings will have less transplant stress and better early growth than bareroot seedlings because their roots are protected and undamaged in the plug. This stock type difference can disappear in subsequent seasons (Haase et al. 2012, Rose and Haase 2005). The shading treatment was not statistically significant although there was an overall trend toward greater average growth for seedlings with the shading.

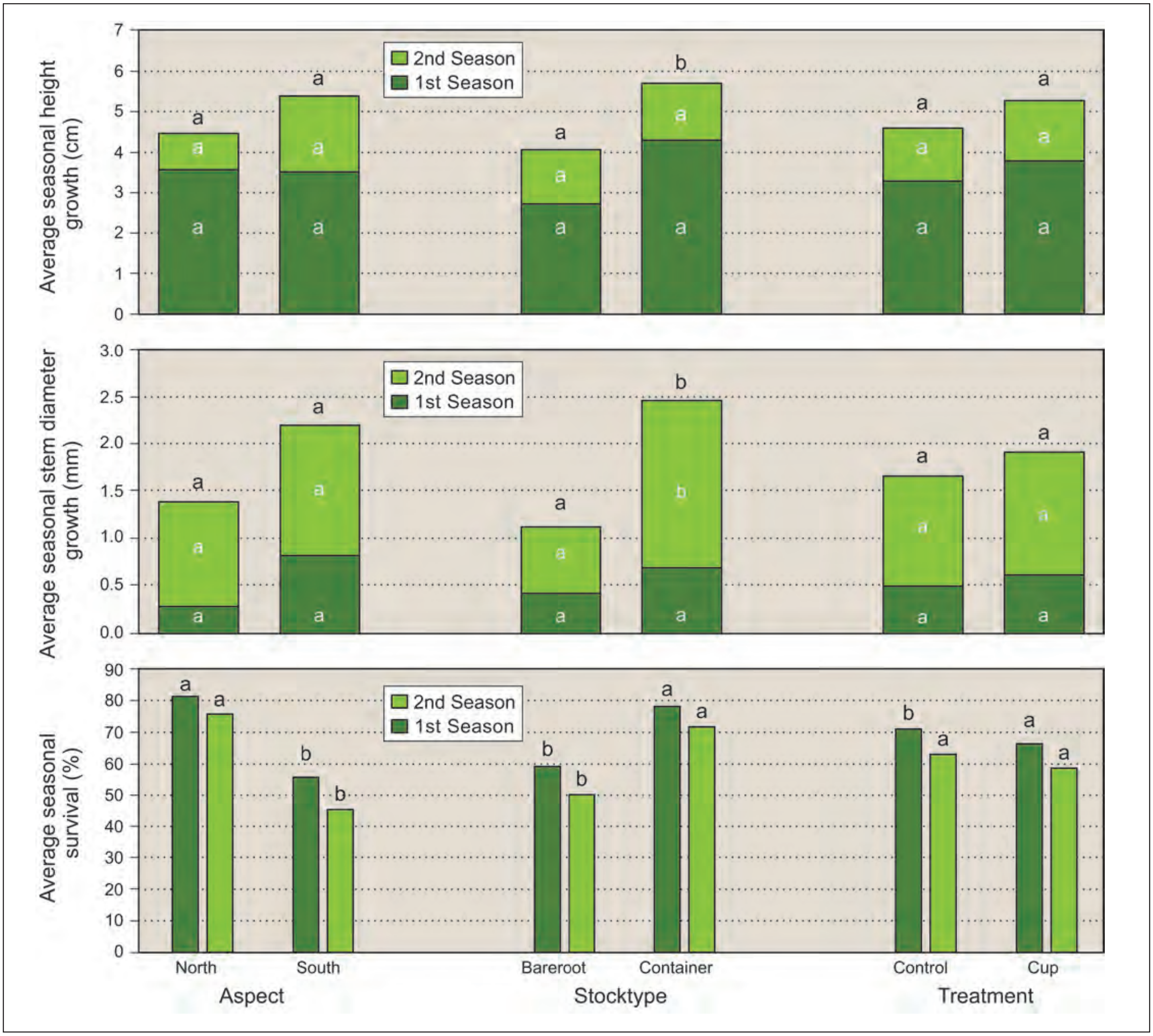

**Figure 7.** Seedling (a) height growth, (b) stem diameter growth, and (c) survival varied among treatments at the end of the two growing seasons. For each variable in each season, bars with different letters differed significantly at $\alpha \leq 0.05$ for aspect, stocktype, or treatment.

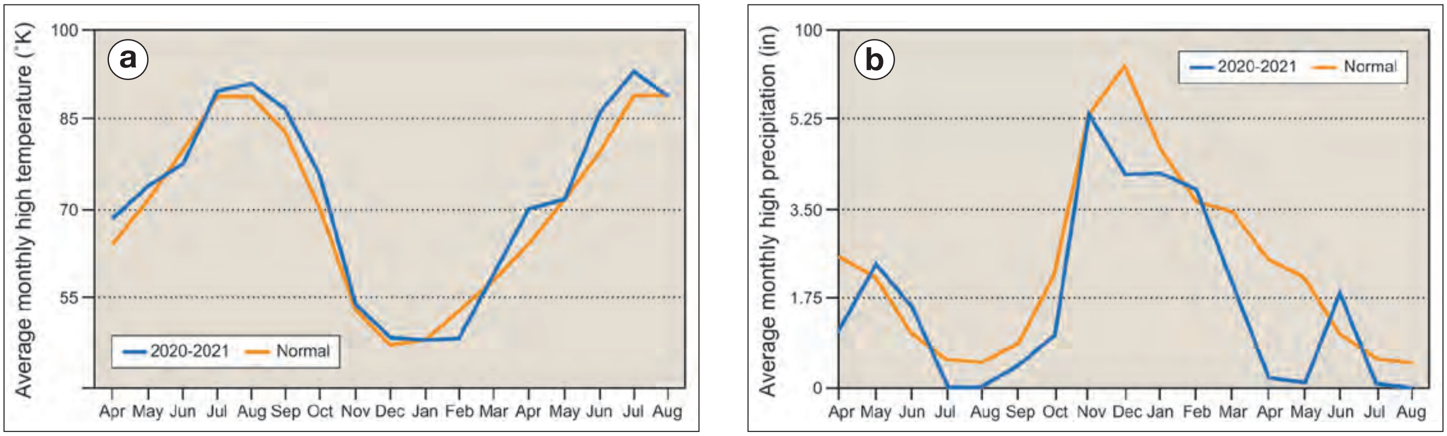

**Figure 8.** Data from a nearby weather station showed above-normal temperatures and below-normal precipitation during the two seasons after planting.

Overall, the study results were somewhat surprising given previous operational observations and earlier published studies with cups or shading that indicate increased survival or growth. In early summer, it is common to observe moisture under the cups while the adjacent soil is dry. There was quite a bit of variability in site conditions within each plot caused by microsites and browsing which may have reduced the treatment effect, though these factors did not interfere with aspect or stocktype effects. Additional research may yield a positive response depending on site conditions.

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# Iron Fertilization in Bareroot Nursery Seedbeds

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## Abstract

With adequate fertilization, bareroot conifers grow well at pH 4.5 to 5.5. Because some guidelines suggest that seedlings grow best at pH 5.5 to 6.5, lime is sometimes applied when soil drops below pH 5.5. Liming, however, can result in "lime-induced" chlorosis which may be attributed to an iron (Fe) deficiency. Fe deficiency is often associated with "summer chlorosis" or "nitrate-induced" chlorosis. With no treatment, however, seedlings may return to a green color when temperatures cool. At many bareroot nurseries, Fe deficiency has declined because: (1) soil acidity of seedbeds has been lowered to below pH 5.6; (2) nitrate application has decreased by more than 55 percent; and (3) irrigation is used to cool seedbeds in July. Now, about one-third of bareroot nurseries in the southern United States do not fertilize with Fe. This article gives an overview of publications and observations regarding the use of Fe in bareroot nurseries and associated products, challenges, and misconceptions.

## Introduction

Iron (Fe) is the most common element in the Earth and the fourth most common element in the Earth's crust. Although various soils may contain more than 6 percent Fe, sometimes soil tests extract less than 10 ppm (Mexal and Fisher 1987, Solan et al. 1979, Van Lear and Smith 1972). When growing in neutral or alkaline soils, bareroot seedlings can exhibit Fe deficiency since the ion occurs mostly in the oxidized form ($Fe^{+++}$) which some consider biologically inactive. In very acid soils, Fe deficiency is rare since Fe occurs in the ferrous ($Fe^{++}$) form. Although seedlings deficient in Fe have lower chlorophyl concentrations, chlorotic needles typically have high concentrations of inactive Fe. Details about the role of Fe in plants and the role of soil pH in Fe solubility have been reviewed previously (Abadía 1992, Barker and Stratton 2015, Brown 1961, Chen and Barak 1982, Korcak 1987, Landis 1988, Mortvedt 1991, Wallace 1962). Visual deficiency symptoms include chlorosis of newly formed needles or leaves. For hardwoods, the veins in the leaves often remain green. Photos illustrating Fe deficiency symptoms on various species have been published (table 1).

Fe has been applied to chlorotic bareroot seedlings for more than 100 years (Korstian et al. 1921). Some nursery managers routinely apply Fe to seedlings while others have not applied any Fe to bareroot seedlings this century (figure 1).

Although greenhouse trials demonstrate that Fe is an essential element (Howell 1932, Lyle 1969, Nelson and Selby 1974, Pessin 1937), the information is often of no practical use for bareroot nursery managers. For example, a 0.072 millimolar Fe solution applied to seedlings in a greenhouse does not inform growers if an Fe chelate product will reduce chlorosis in bareroot seedbeds (El-Jendoubi et al. 2011, Whittier 2018). Unfortunately, empirical trials with Fe in bareroot nurseries are rare. Although many questions remain unanswered, the goal of this paper is to provide some practical information and observations.

[Abbreviations: Al = aluminum. AN = ammonium nitrate. AS = ammonium sulfate. ATS = ammonium thiosulfate. Ca = calcium. Cl = chloride. Cu = copper. EDTA = ethylenediaminetetraacetic acid. EDDHA = ethylendiaminedi (o-hydroxyphenylacetic) acid. DTPA = Diethylenetriamine pentaacetic acid. Fe = iron. $FeSO_4$ = ferrous sulfate. HEDTA = N-(2-Hydroxyethyl) ethylenediaminetriacetic acid. K = potassium. $LSD_{05}$ = Least significant difference, $\alpha$= 0.05. Mg = magnesium. Mn = manganese. N = nitrogen. P = phosphorus. ppm = parts per million. S = sulfur. UAN = urea ammonium nitrate. Soil pH was measured in water.]

**Table 1.** Scientific and common names of selected species mentioned in this article. References listed provide photographs of Fe deficiencies.

| Species | Common name | Photo reference |
|---|---|---|
| *Acer rubrum* L. | Red maple | Starkey 2012 |
| *Acer saccharinum* L. | Silver maple | Koenig and Kuhnes 2002 |
| *Citrus unshiu* Marc. | Satsuma tangerine | No photo |
| *Eucalyptus maculate* Hook. | Spotted iron gum | Dell and Robinson 1993 |
| *Juglans nigra* L. | Black Walnut | Hacskaylo et al. 1969 |
| *Liquidambar styraciflua* L. | Sweetgum | Hacskaylo et al. 1969 |
| *Liquidambar styraciflua* L. | Sweetgum | Goldberg and French 2017 |
| *Pinus banksiana* Lamb. | Jack pine | Landis et al. 1989 |
| *Pinus contorta* Dougl. | Lodgepole pine | Majid 1984 |
| *Pinus elliottii* Engelm. | Slash pine | No photo |
| *Pinus palustris* Mill. | Longleaf pine | www.ipmimages.org/browse/detail.cfm?imgnum=1611152 |
| *Pinus pinea* L. | Stone pine | Lamhamedi et al. 2009 |
| *Pinus radiata* D. Don | Monterey pine | Will 1985 |
| *Pinus resinosa* Ait. | Red pine | Armson and Sadreika 1979 |
| *Pinus sylvestris* L. | Scots pine | Goslin 1959<br>Hacskaylo et al. 1969 |
| *Pinus taeda* L. | Loblolly pine | Bengtson 1968 |
| *Populus tremula × P. tremuloides* | Hybrid poplar | Masuda et al. 2018 |
| *Prunus persica* (L.) Batsch | Peach | Fernández et al. 2008 |
| *Pseudotsuga menziesii* Mirb. Franco | Douglas-fir | van den Driessche 1989 |
| *Quercus palustris* Münchh. | Pin oak | Harrell and Andrews 1986<br>Hoch 2015 |
| *Quercus suber* L. | Cork oak | Gogorcena et al. 2001 |
| *Robinia pseudoacacia* L. | Black locust | Hacskaylo et al. 1969 |
| *Tectona grandis* L.f. | Teak | Whittier 2005 |

## 20th Century Practices

Chlorotic seedlings are caused by a variety of factors: nematodes, too much organic matter, too much N fertilizer, too much soil alkalinity, not enough S, too much carbonates in irrigation water, too much *Pythium*, too much heat, anaerobic conditions in the rhizosphere, etc. Since foliar tests are not useful for proving an Fe deficiency, it was easier just to apply an Fe product and hope for the best. In some cases, untreated seedlings turned green in August with the onset of cooler weather.

Initially, $FeSO_4$ was the primary fertilizer used to correct an Fe deficiency (Cossitt 1938, Korstian et al. 1921) because it was available and could also be used to lower soil pH. At some nurseries, $FeSO_4$ (4 kg/ha/application) was sprayed on seedlings on a weekly basis to reduce chlorosis. At other nurseries, 700 kg/ha was applied before sowing to lower soil pH. Until 1950, chelates were rarely used in nurseries (Stoeckeler and Slabaugh 1965) because they were more expensive than $FeSO_4$. In some cases, $FeSO_4$ will penetrate leaves better than Fe chelates (Chakraborty et al. 2014).

Fe deficiencies were not common at nurseries in the Pacific Northwest (Anderson 1968, van den Driessche 1984). For 2-0 bareroot Douglas-fir seedlings, foliar Fe values for four nurseries ranged from 67 to 225 ppm (Krueger 1967). Approximately 2 out of 13 nurseries in New Zealand applied $FeSO_4$ to pine seedlings in 1977. At the FRI Nursery at Rotorua, chlorosis occasionally occurred on young needles during the cold, wet periods of spring weather (Knight 1978). This symptom was corrected by foliar applications of $FeSO_4$ (5 percent weight/volume) at about 465 L/ha: “One to two repeat applications made at 7- to 10-day intervals have generally been necessary to restore good colour” (Knight 1978).

At several pine nurseries, a rust fungicide (containing 13 percent Fe) was applied twice weekly at 0.3 kg/ha of Fe/application (Marx et al. 1984, Snow et al. 1979). At this rate, 18 applications would total 5.4 kg/ha of Fe which might explain why several managers did not apply Fe chelates to pine seedbeds (Marx et al. 1984). In addition, Bengtson (1968) tested several Fe products and he “never did get any definite response to the application of iron elements” (Rowan 1969). Likewise, several nursery managers saw no need to apply Fe when pine foliage was green (e.g., figure 1).

**Figure 1.** These green, bareroot loblolly pine seedlings are growing in an acid soil that has never received an application of Fe. (Photo by Tom Anderson)

Sometimes applying Fe chelate was ineffective because chlorosis was due to a S deficiency. In some cases, S can improve growth as much as Fe (Meena et al. 2013) or more than Fe (Browder et al. 2005, Dale et al. 1955, Lyle and Pierce 1968, van den Driessche 1989).

In the 1970s, fertilizer tests with hardwoods showed improved growth when the N source was switched from AN to AS (Stone 1980, Williams and Phares 1972). Likewise, when grown in containers (pH < 5.5), oak and maple seedlings respond to fertilization with S, but Fe-EDTA did not increase growth (Browder et al. 2005). Nursery managers in Virginia also noticed a lack of summer chlorosis when pine seedlings were fertilized with AS. When growers added ATS to liquid UAN, summer chlorosis did not occur. As a result, Fe fertilizers have not been applied to bareroot pines in state nurseries in Virginia in more than 25 years.

During the last century, many said that Fe was "immobile" in seedlings. Although Fe does not retranslocate easily away from older tissue, Fe is partially mobile (Bukovac and Wittwer 1957). When foliar applied, some Fe will retranslocate from a leaf surface towards the leaf petiole (Fernández et al. 2008, Hüve et al 2003, Zhang et al. 1995).

## Fe Fertilization

There are six approaches to fertilizing bareroot seedlings with Fe: (1) keep soil pH below 5.6 and apply no Fe (Bonner and Broadfoot 1964, Landis 1997, Mizell 1980, Young 1938); (2) apply Fe before sowing on alkaline seedbeds (Landis 1988, Martian 1989, Maxwell 1988); (3) wait until foliage starts to show deficiency symptoms and then apply Fe (Shoulders and Czabator 1965); (4) apply Fe chelate soon after the first N top-dressing (Carter 1964); (5) apply multiple Fe chelate treatments to green foliage (Jacobs and Landis 2014, Will et al. 2013); or (6) apply a mixture of micronutrients to soil or foliage (Flinn et al. 1980, Iyer and Wilde 1974, Marx et al. 1984). Although seedling growth may be correlated with soil Fe (South et al. 2018b), Fe fertilization is not based on routine soil tests (Davey 2002, Hardy et al. 2013, Hochmuth 2011, Horneck et al. 2011), in part, because soil Fe tests can vary widely. For example, when analyzing the same soil, one laboratory estimated 34 ppm of available Fe while another estimated 120 ppm (South 2019). Similarly, routine foliar Fe tests have little meaning for determining when to apply Fe. Furthermore, foliage Fe tests can fluctuate wildly from month to month due to contamination of epicuticular wax.

Soil pH, bicarbonates, and leaf color are the main factors used to determine when to apply Fe to seedlings. When soil pH and bicarbonates are high enough to lower chlorophyl concentrations, a visual ranking (Messenger 1990, Mexal et al. 2004), a "greenness" meter (Chieppa et al. 2017, Loh et al. 2002), or a simple color chart (figure 2) can be used to determine when to fertilize with Fe. In contrast, some recommend never using visual symptoms as a guide for Fe fertilization (Baer 1984, Jacobs and Landis 2014).

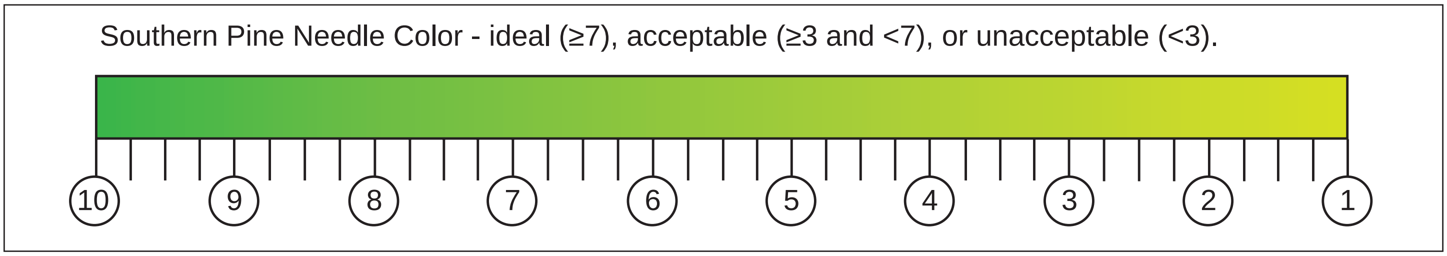

**Figure 2.** Steve Grossnickle (NurseryToForest Solutions, North Saanich, BC) developed this color chart used by CellFor in a quality control program for loblolly pine clonal stock (Grossnickle 2011). When bicarbonates in irrigation water increased pH levels, needle color decreased. A value of 3 was used to determine when to apply a chelated Fe product.

Examples of commercial products are provided in table 2. Granular products are typically applied before sowing as a prophylactic treatment while liquid products are primarily applied after seedlings have true leaves. Due to uniformity, many growers prefer to apply fertilizers as a liquid spray. Several products include urea which aids in the uptake of Fe (Hochmuth 2011). Frequent use of some wettable powder products can wear out sprayer parts (e.g., roller pumps) and increase application costs. Products applied before sowing include $FeSO_4$ (Landis 1988, Maxwell 1988) and Milorganite® (Landis and Dumroese 2012, Marx et al. 1984) while $FeSO_4$ and chelated products can be topdressed (Chakraborty et al. 2014, Landis 1997). Recommended rates vary. For example, DTPA (10 percent Fe) might be recommended at 3.7 to 10 kg/ha of Fe (Davey 1984, Danielson 1966, Shoulders and Czabator 1965), though nursery managers tend to use lower rates (0.40 to 0.56 kg/ha/application of Fe). Three weekly applications might total 1.7 kg/ha of Fe.

Fe is sometimes applied with every N application using a N/Fe ratio of 220 (Will et al. 2013). When bareroot seedlings receive 200 kg/ha of N, the total amount of Fe applied can vary from zero (Donald 1990, Marx 1984) to 3 kg/ha (Martin 1989). For container-grown pine seedlings, the Fe rate applied per 200 kg N can vary from zero (Dumroese and Wenny 1997), to 0.8 kg (Fu et al. 2017), to 4 (Landis et al. 1989), to 8 kg (Carlson 1979). Some container nurseries apply 8 applications over a 4-month period (June to September).

## Bicarbonates in Well Water

Bicarbonates ($HCO_3^-$) in irrigation water can produce chlorotic seedlings (Nelson and Switzer 1969, Wang et al. 2020). Bicarbonates are typically low in lakes and rivers, and therefore chlorosis is not a problem when nursery managers keep soil pH below 5.6 and irrigate using surface waters with less than 7 ppm Ca. Nursery well water, however, can contain high Ca and high bicarbonates which can cause problems. Well water containing 2 ppm Ca may have 30 ppm of bicarbonates while water with 38 ppm Ca may have 180 ppm of bicarbonates. In Florida, deep wells tend to have alkaline water with more than 100 ppm bicarbonates, while shallow wells have acid water and less than 40 ppm bicarbonates (Morgan and Graham 2019). Water with less than 40 ppm bicarbonates is desired while 60 ppm is considered marginal (Landis et al. 1989). In one survey, water samples from 8 nurseries (Alabama, Florida, Georgia and North Carolina) contained more than 30 ppm Ca (McNabb and Heidbreder-Olson 1998) and therefore several managers applied Fe to reduce summer chlorosis.

## Alkaline and Basic Soils

For conifers, Fe deficiencies are common at nurseries with alkaline soils where the soil solution contains mostly the oxidized form ($Fe^{+++}$). Due to high soil Ca and salinity, several bareroot nurseries on alkaline soils have closed. Some hardwood species also become chlorotic in alkaline soil (McComb and Kapel 1942, Smith and Mitchell 1977) while others grow well without any Fe fertilization (Fisher and Fancher 1984). The preferred chelate used at alkaline nurseries (figure 3) is Fe-EDDHA (Landis 1997) and one bareroot nursery applied this product 9 times (Mexal and Fisher 1987).

Several studies on alkaline soils indicate hardwoods and conifers respond favorably to applications of Fe (figures 3, 4, and 5). At one nursery, shoot mass of pine seedlings increased by 50 percent when soil (pH 7.5) was treated with Fe-EDDHA (Fisher and

**Figure 3.** The alkaline soil at the Albuquerque Nursery ranged from pH 7.2 to pH 8.4 with a $CaCO_3$ content from 2 to 12 percent (Mexal and Fisher 1987, Hooks et al. 1988). This photo demonstrates the difference between Fe-deficient seedlings (foreground) and those treated with a foliar application of Fe-EDDHA (background). A 1982 study at the Albuquerque Nursery (Fisher and Chan 1985) indicated a single soil application of Fe-EDDHA could increase shoot growth of 1-0 pine seedlings by 50 percent. Other forms of Fe chelate were ineffective at this nursery. (Photo by Tom Landis 1988).

**Table 2.** A partial list of iron (Fe) fertilizer products which are sold as liquids (L), wettable powders (WP), or granules (G).

| Tradename | Common name | Form | % Fe | % N | % S | Formula |
|---|---|---|---|---|---|---|
| Sucra Min® | Iron sucrate | G | 50 | | | $Fe_2O_3$ - organic complex |
| Brant® | Ferrous sulfate | G | 30 | | 18 | $FeSO_4 \bullet H_2O$ |
| Nu-iron™ | Iron malonate | G | 30 | | | $FeC_3H_4O_4$ |
| Ironite® | Ferric oxide | G | 20 | 1 | 5 | FeO |
| Extreme Green® | Ferrous sulfate | WP | 20 | | 12 | $FeSO_4 \bullet 7H_2O$ |
| Ferrous sulfate | Ferrous sulfate | G | 20 | | 11 | $FeSO_4 \bullet 7H_2O$ |
| Hi-Yield® | Ferrous sulfate | G | 19 | | 11 | $FeSO_4 \bullet 7H_2O$ |
| Micromax® | Ferrous sulfate | G | 17 | | 12 | $FeSO_4 \bullet 7H_2O$ |
| Osmocote® iron | Ferric sulfate | G | 17 | | 15 | $F_e2O_{12}S_3$ |
| Frit™ 503 G | Ferric oxide | G | 14 | | | FeO - crushed glass |
| Axilo® | EDTA | WP | 13 | 6 | | $Fe(C_{10}H_{12}N2O_8Na) \bullet 3H_2O$ |
| 13% Iron EDTA | EDTA | WP | 13 | 6 | | $Fe(C_{10}H_{12}N_2O_8Na) \bullet 3H_2O$ |
| Sequestrene® Na | EDTA | WP | 12 | 6 | | $Fe(C_{10}H_{12}N_2O_8Na) \bullet 3H_2O$ |
| Sequestrene® 330 | DTPA | WP | 10 | 7 | | $Fe(C_{14}H_{23}O_{10}N_3)$ |
| Sprint® 330 | DTPA | WP | 10 | 7 | | $Fe(C_{14}H_{23}O_{10}N_3)$ |
| Brant® EDTA | EDTA | L | 7 | 3 | | $Fe(C_{10}H_{12}N_2O_8Na) \bullet 3H_2O$ |
| Ferromec® AC | Ferrous sulfate | L | 6 | 15 | 3 | $FeSO_4 \bullet 7H_2O$ |
| Six Iron™ | Ferrous sulfate | L | 6 | 12 | 3 | $FeSO_4 \bullet 7H_2O$ |
| Sequestrene® 138 | EDDHA | WP | 6 | 4 | | $Fe(C_{18}H_{20}O_6N_2)$ |
| Sprint® 138 | EDDHA | WP | 6 | 4 | | $Fe(C_{18}H_{20}O_6N_2)$ |
| Greenol™ | EDTA | L | 5 | 2 | 3 | $Fe(C_{10}H_{12}N2O_8Na) \bullet 3H_2O$ |
| Green-T® | Iron glucoheptonate | L | 5 | | | $Fe(C_7H_{13}O_8)$ |
| Tracite® iron 5% | Ferrous citrate | L | 5 | | 3 | $FeC_6H_6O_7$ |
| Nutrite™ Iron Plus | Ferrous citrate | L | 5 | 5 | 4 | $FeC_6H_6O_7$ |
| Versenol™ | HEDTA | L | 4 | 2 | | $Fe(C_{10}H_{18}O_7N_2Na_2)$ |
| MaxiGreen II® | Iron glucoheptonate | L | 2 | | 2 | $Fe(C_7H_{13}O_8)$ |
| Milorganite® | Biosolid | G | 2 | 6 | 2 | — |

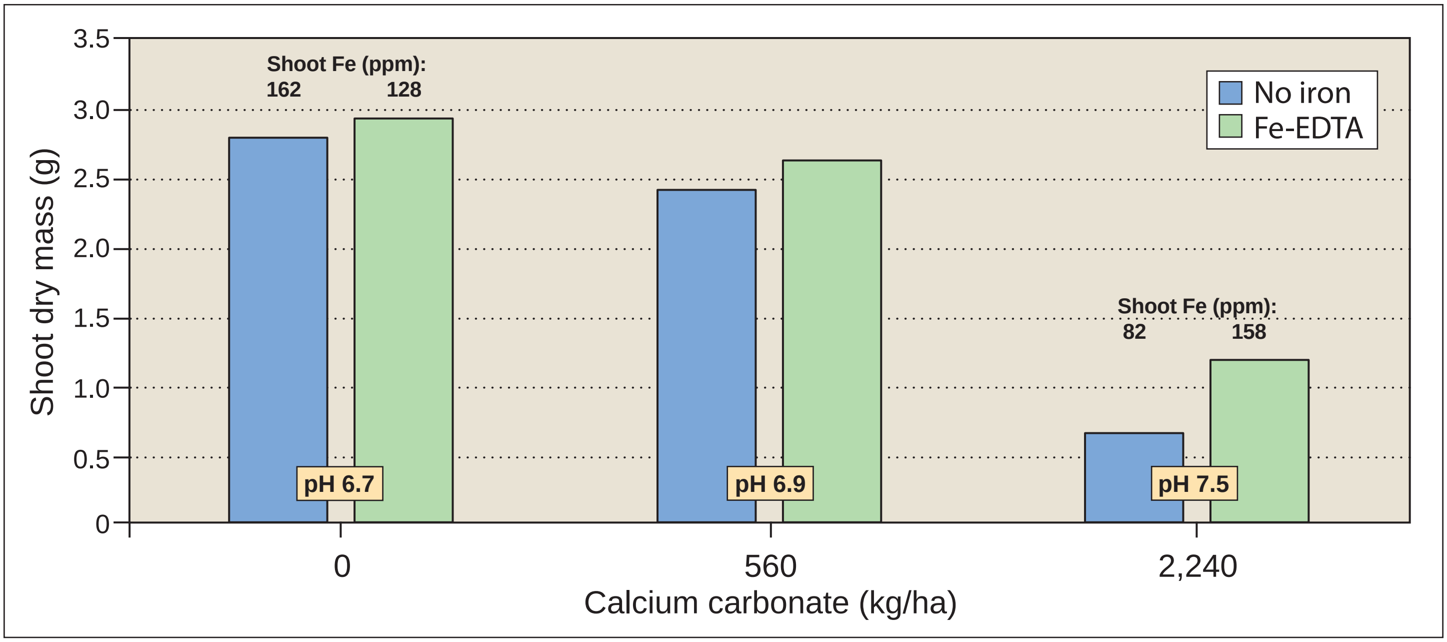

**Figure 4.** Applying lime increased soil pH, reduced loblolly pine seedling growth (5 months old), and increased seedling response to an application of Fe-EDTA (3.1 kg Fe/ha) (Richards 1961). Fe levels in shoots are provided above bars. Seeds were sown in pots on December 23 and chlorosis was noticed in early February (pH 7.5). An application of Fe-EDTA (0.25 percent solution) was applied on March 13 and the treated seedlings were green by March 28. The $LSD_{05}$ = 0.37 g.

Chan 1985). When soil pH is less than 5.5, however, pines are less likely to respond to Fe fertilization.

In a greenhouse trial with slash pine (pH 7.3), an application of Fe-tartrate reduced chlorosis in July and increased height growth (Steinbeck 1962). In another trial at pH 7.6, applying $FeSO_4$ increased the chlorophyll content of citrus leaves more than Fe citrate (figure 5). In greenhouses, it is relatively easy to produce deficiencies by growing pine seedlings in

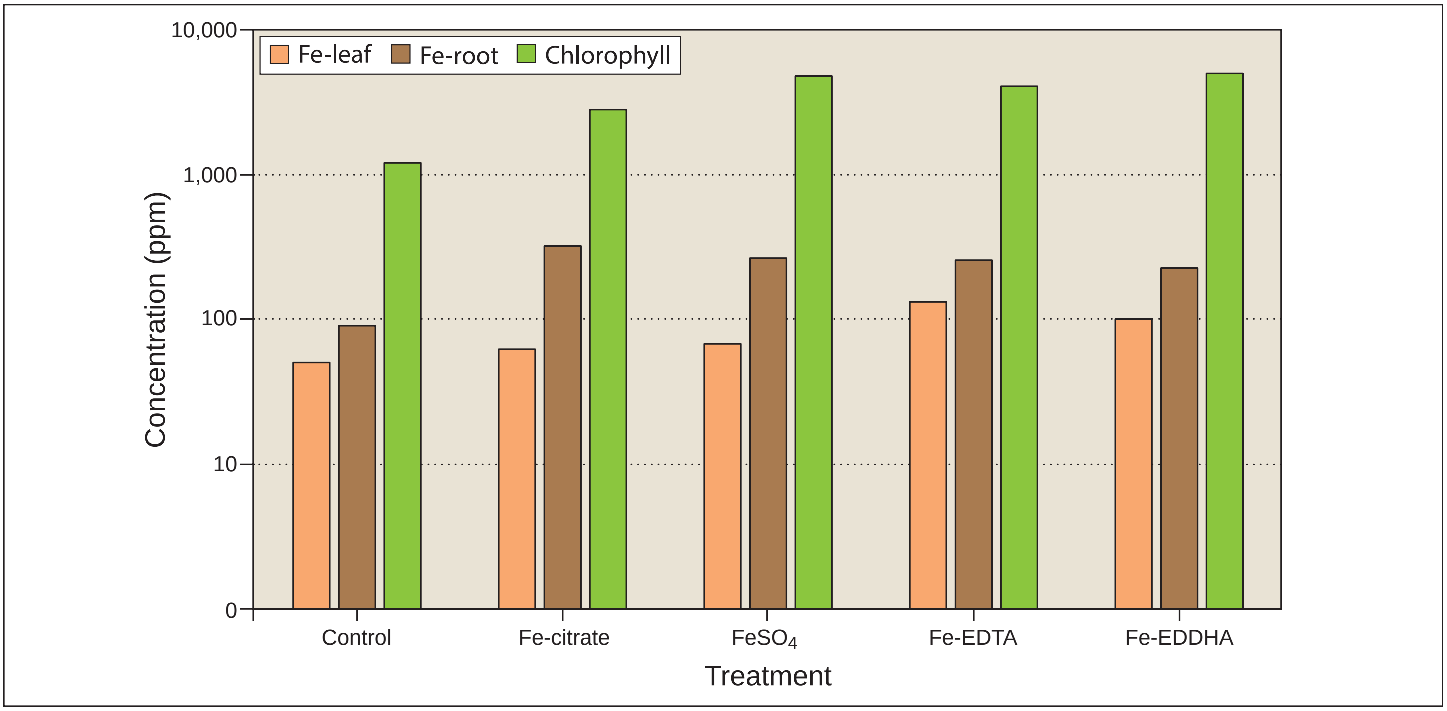

**Figure 5.** Iron applications (0.6 g of Fe per pot: equivalent to 60 ppm Fe) to Satsuma tangerine plants affected Fe concentrations in leaves and roots and chlorophyll concentration in leaves (Mordogan et al. 2013). The untreated sandy clay soil had a pH of 7.6 with 0.08 ppm Fe, and each pot was treated 5 times during the growing season. The $LSD_{01}$ values are 13 ppm (leaf), 54 ppm (root), and 930 ppm (chlorophyll).

deionized water or sand (Blackmon 1969, Goslin 1959, Steinbeck et al. 1966).

## Acid Soils

Low soil pH is critical for keeping $Fe^{++}$ from being oxidized into $Fe^{+++}$. Therefore, some bareroot nursery managers maintain soil pH below 5.6. At some locations, increasing soil acidity with sulfuric acid will increase growth of pines and eliminate the need to apply Fe (Dale et al. 1955). Fe chlorosis of conifers is rare when soil acidity is pH 3.7 to 5.5, and, so far, studies to demonstrate that Fe application will increase seedling growth on these soils have not been published (Lynch et al. 1943, Maki and Henry 1951). In fact, certain types of Fe fertilizers may even reduce growth (figure 6). Although applying Fe-phosphate increased growth of pines at a nursery in Indiana (pH 6), the phosphate, not the Fe, likely increased the growth of non-mycorrhizal seedlings (Auten 1945).

Fe deficiencies have purportedly occurred on mildly acid soils (Danielson 1966, Shoulders and Czabator 1965) due to applying too much lime (figure 4) or too much nitrate-N (Landis 1976). At these nurseries, seedlings typically turn green with the onset of cooler weather. If irrigation water contains 60 ppm (or more) bicarbonates, however, then applying Fe chelates may reduce the chance of chlorosis. In very strongly acid soils (pH 4.5 to 5.0), chlorotic seedlings might be due to a deficiency in Mg (Voigt et al. 1958) or Ca, or due to antagonism from Mn (South 2017). Some alkaline nursery soils have less than 25 ppm soluble Fe (Mehlich 3) but most acid nursery soils contain enough available Fe so that pine seedlings lifted in November are not Fe deficient (Baer 1984, Boyer and South 1985).

## Summer Chlorosis

In some soils (> pH 5.5), high temperatures in summer will temporarily turn conifer needles yellow (Foster 1959, Marks et al. 1985, Ronco 1970). This phenomenon is known as summer chlorosis (Carter 1964). When nursery beds are oriented east-west, chlorosis appears on the southside of the bed. Although

**Table 3.** Soil (Mehlich 3) and foliage data (July 23) from non-replicated plots that were sown on April 20, 2018 at the Arborgen Nursery at Bullard, Texas. Dolomitic lime (6,776 kg/ha) and gypsum (6,776 kg/ha) were applied prior to sowing. On July 9, loblolly pine needles on lime-treated plots were chlorotic but the chlorosis was gone by August 13. Soil and foliar samples were taken July 22. Highest air temperatures for June, July, and August were 35.5 °C, 41.1 °C, and 37.8 °C, respectively, and the highest seedbed temperatures (7.6 cm depth) were 35.0 °C, 40.6 °C, and 37.3 °C, respectively. Seedlings were not fertilized with Fe or N.

| Variable | No lime | Gypsum | Lime | Lime + gypsum |
|---|---|---|---|---|
| pH | 4.6 | 5.4 | 7.0 | 6.7 |
| Soil Ca (ppm) | 318 | 847 | 881 | 1251 |
| Soil Fe (ppm) | 291 | 279 | 262 | 325 |
| Soil Mg (ppm) | 49 | 69 | 168 | 124 |
| Soil P (ppm) | 145 | 136 | 131 | 153 |
| Foliar B (ppm) | 21 | 16 | 17 | 18 |
| Foliar Fe (ppm) | 90 | 69 | 68 | 65 |
| Foliar Ca (ppm) | 2,300 | 3,100 | 3,500 | 2,800 |
| Foliar Mg (ppm) | 1,100 | 1,100 | 1,200 | 1,100 |
| Foliar Mn (ppm) | 788 | 448 | 391 | 616 |
| Fe/Mn ratio | 0.11 | 0.15 | 0.17 | 0.11 |
| Height (cm) | 27.8 | 23.8 | 23.8 | 25.9 |
| Chlorosis (21 May) | no | no | no | no |
| Chlorosis (9 July) | no | no | some | yes |
| Chlorosis (24 Aug) | no | no | no | no |

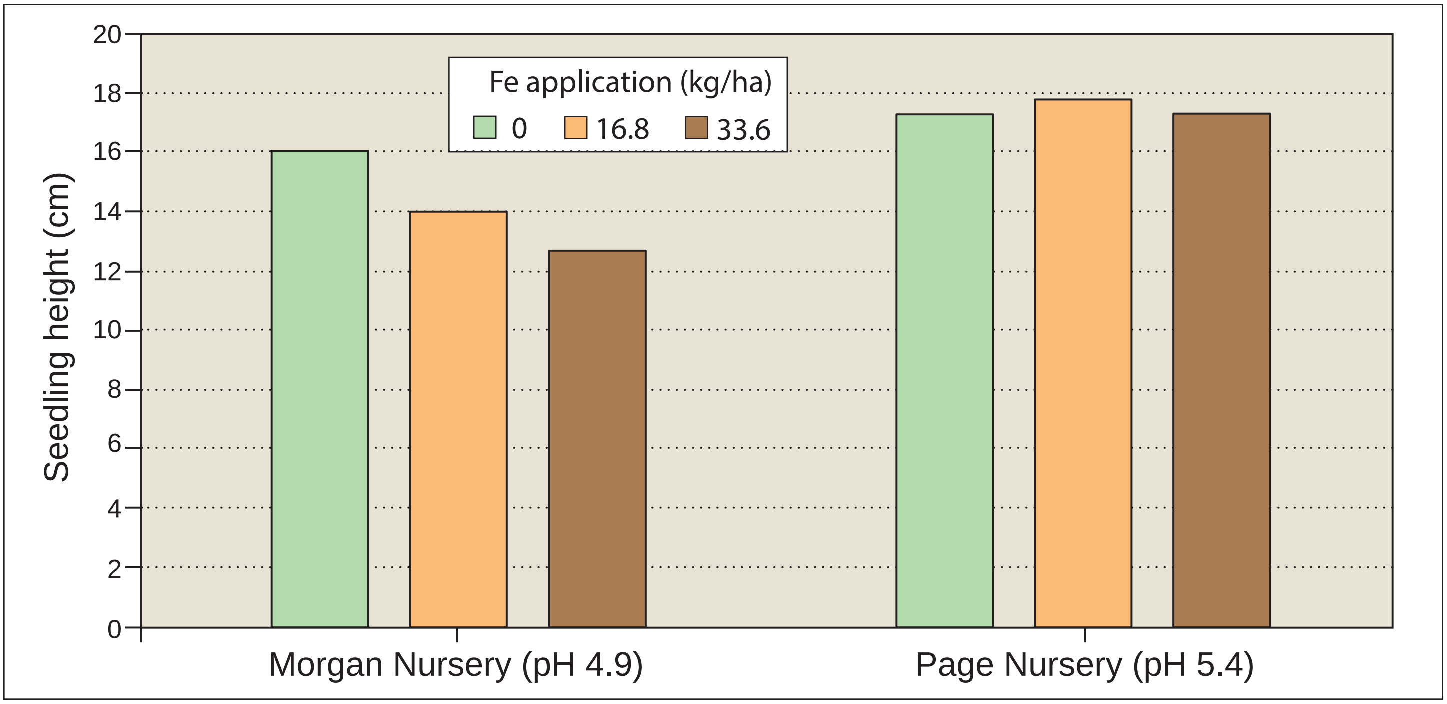

**Figure 6.** Presow applications of Fe-malonate (Nu-Iron™; Tenn Corp.) affected slash pine seedling height differently at two Georgia nurseries (Steinbeck 1962). Prior to sowing, all plots were treated with 118 kg N/ha, 240 kg P/ha, and 186 kg K/ha. Seed were sown on April 17, 1961 (Morgan Nursery) and April 12 (Page Nursery). Seedlings were treated with 224 kg N/ha in July. Seedlings were measured 149 days after sowing. $LSD_{05}$ = 3.3 cm.

summer chlorosis might have several causes (Hendrix and Campbell 1968, Lewis 1960, Nelson and Switzer 1969), high soil temperatures can reduce $Fe^{++}$ availability and limit both new root growth and soil moisture. Some growers believe they can prevent the occurrence of chlorosis with irrigation (Cloud 1969). In some cases, chlorotic needles occur when soil temperatures exceed 40 °C (table 3).

Summer chlorosis can also result from N fertilization. Several N sources can produce summer chlorosis, but nitrates likely play a key role (Carter 1961, Fisher and Chan 1985, Khalil et al. 1989, Nelson and Selby 1974, van den Driessche 1978). To reduce or prevent summer chlorosis, some managers have decreased

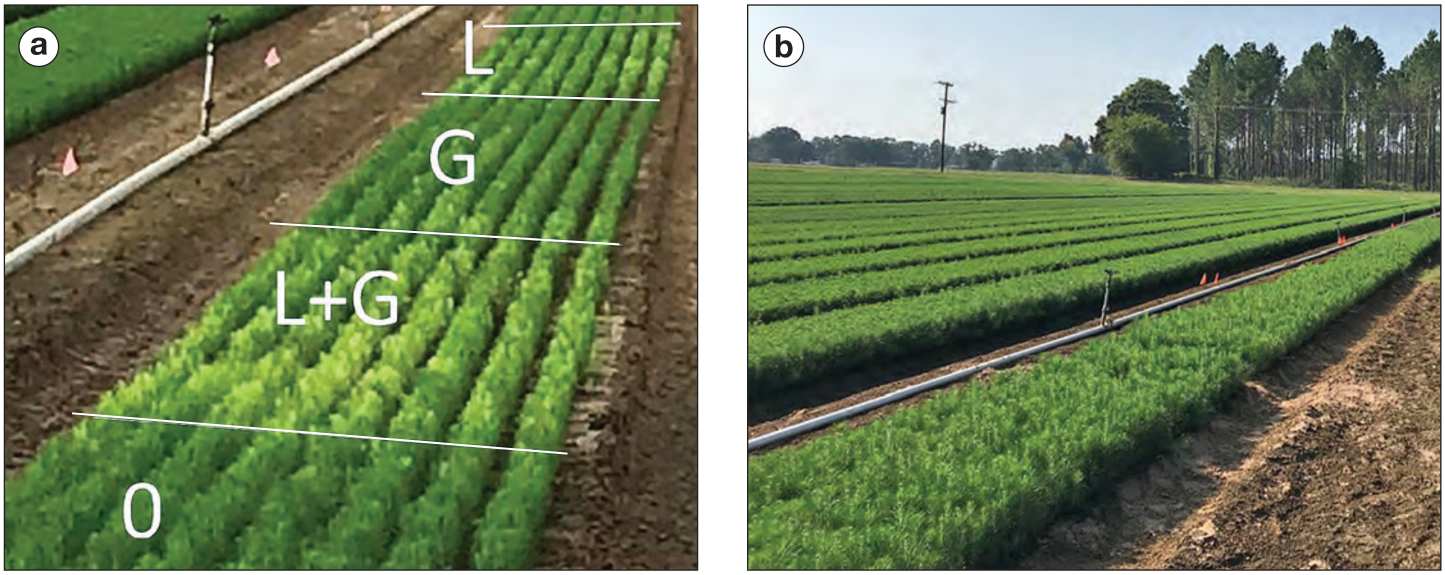

**Figure 7.** (a) Summer chlorosis (July 9, 2018) was observed for loblolly pine seedlings (L+G; pH 6.7) when growing in soil treated with dolomitic lime and gypsum (see table 3). Slightly chlorotic seedlings (L) were growing in soil treated with only dolomitic lime (pH 7.0). Untreated seedlings (0; pH 4.6) and seedlings growing in soil treated with gypsum (G; pH 5.4) remained green. (b) Without any treatment, all chlorotic seedlings turned green by August 24, 2018. (Photos by Gene Bickerstaff, 2018)

the use of AN (Nelson and Switzer 1969) and have increased application of ATS and UAN.

Although chlorotic needles in summer (figure 7) may initially cause some alarm, many seedlings turn green with cooler weather with no obvious damage (Foster 1959, Steinbeck et al. 1966). For this reason, nursery managers should always leave untreated check plots when they fertilize chlorotic seedlings with Fe. Otherwise, they may never know if the Fe treatment was the true cause of greener needles. Knowing chlorosis lasted about a month, one manager applied a Christmas tree colorant in July to keep loblolly pine seedlings green.

## Autumn Chlorosis

After the fall equinox, N concentration in pine needles declines (Irwin et al. 1998, Sung et al. 1997) and can result in some seedlings being yellow at lifting. Growth after transplanting to the field can be positively related to N concentration (Barker 2010, Irwin et al. 1998, Jackson et al. 2012, Larsen et al. 1988) which is related to foliar greenness. When lifted from strongly acid nursery beds, yellow needles in November are typically not deficient in Fe. As a result, field performance of seedlings is positively related to N status but is not positively related to total foliar Fe levels (Boling et al. 2006, Grossnickle and MacDonald 2018, Larsen et al. 1988, van den Driessche 1991). Thus, Fe fertilization is typically unnecessary to address autumn chlorosis.

## Soil and Foliage Tests

When using a Mehlich 1 soil test, nursery soils (pH 4.5 to 6.1) can range from 13 to 217 ppm Fe (South and Davey 1983). The Mehlich 3 method extracts more Fe from soils than Mehlich 1 (Sistani et al. 1995) and therefore nursery seedbeds may average 160 ppm Fe (Mehlich 3) instead of 45 ppm Fe (Mehlich 1). Less than 25 ppm Fe (Mehlich 3) is considered very low and 20 ppm Fe is considered a threshold value for deficiency (Davey and McNabb 2019). Likewise, 51 to 100 ppm is considered a medium level. Silt loam soils in Arkansas can average 149 ppm (Mehlich 3) with a range of 67 to 521 ppm (Bhandari and Ficklin 2009).

Foliar analysis can be confusing because total Fe concentration levels ($Fe^{++}$ plus $Fe^{+++}$) can be higher in stunted seedlings than in healthy foliage (Carter 1980, Landis 1988, Lewis 1960, Mitchell et al. 1990, MacFall et al. 1991, Potvin et al. 2014, South et al. 2018a, Zhang 2015). This effect is referred to as the “chlorosis paradox” (El-Jendoubi et al. 2011, Römheld 2000) and occurs when Fe concentration is increased by a slower growth rate.

Although reports suggest that the standard range for bareroot seedling foliar Fe levels should be different than for container-grown seedlings (Hawkins et al. 2011, Landis 1997), these differences are confounded with stock type and management practices. The cited range for container-grown conifers (40 to 200 ppm) was obtained from a fertilizer company (Landis 1985) while the range for bareroot conifers (50 to 100 ppm) was derived from needles sampled from trees in the forest (Powers 1974). Furthermore, the upper limit of 200 ppm Fe for forest trees is of no practical value for nursery managers who occasionally report values greater than 300 ppm (Baer 1984, Boyer and South 1985, Danielson 1966, Landis 1976). For example, when sampled in July, 45 percent of bareroot loblolly pine samples contained more than 300 ppm Fe, and one sample collected in February had 1,161 ppm Fe (figure 8).

## High Soil Phosphorus

In theory, phosphates within the seedling will combine with Fe to make both elements unavailable (Barker and Stratton 2015, Landis 1997). Therefore, it has been suggested that high soil P in nurseries can induce Fe chlorosis (Auten 1945, Foster 1964, Nelson and Switzer 1969, Wallace and Wallace 1986). Research shows, however, that 30 ppm P (Mehlich 3) does not result in Fe chlorosis (Dale et al. 1955, Teng and Timmer 1990, Yawney et al. 1982). In fact, some authors recommend seedbeds have 150 to 300 ppm P (Landis 1988, Solan et al. 1979, Teng and Timmer 1990). Applying 400 ppm P to slash pine growing in sand did not cause an Fe deficiency (van Lear and Smith 1972) and 1,152 kg/ha of P did not cause an Fe deficiency to hybrid poplar cuttings (Teng and Timmer 1990).

## Toxicity

Billions of nursery seedlings have been sprayed with Fe with no injury symptoms. Some species were

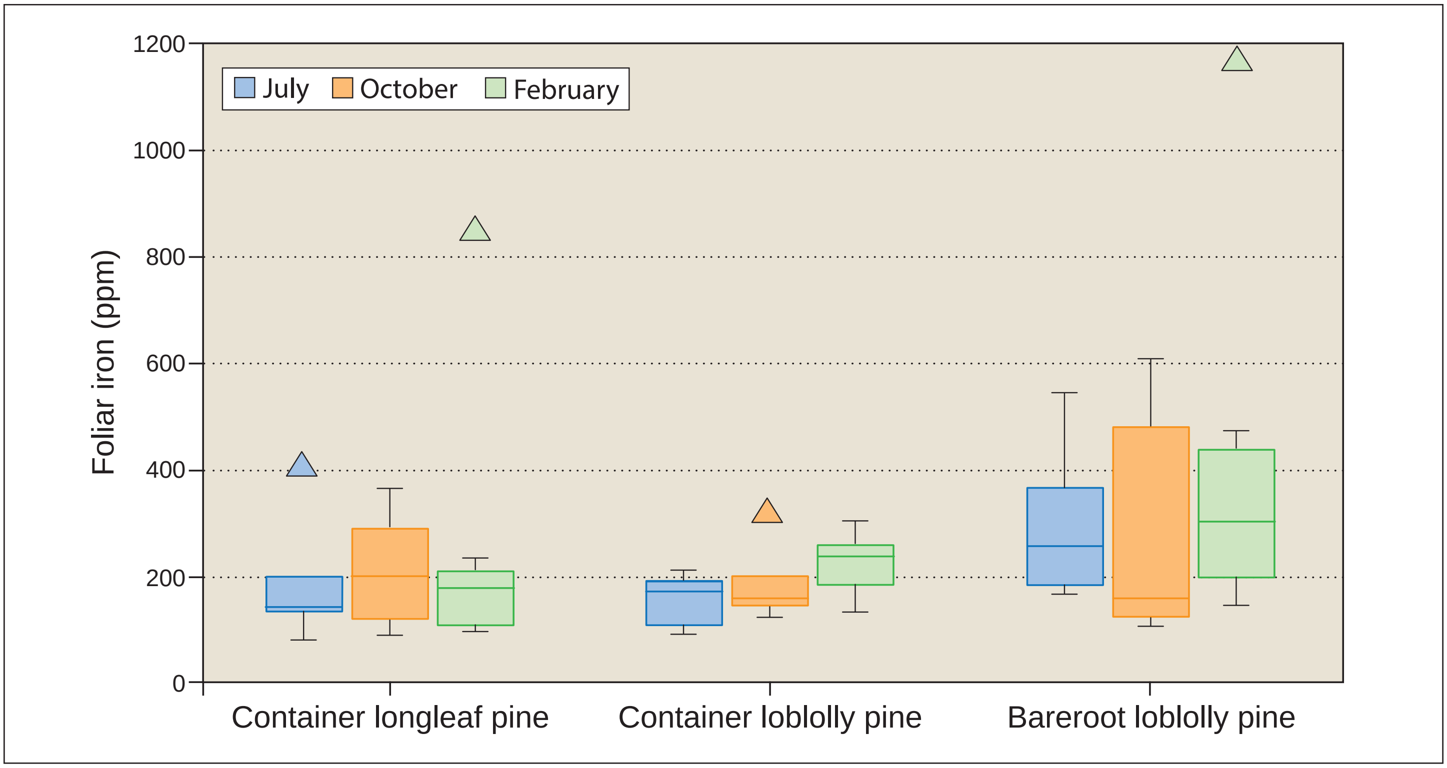

**Figure 8.** Foliar Fe can vary by sampling month (2010-2011), species, and stock type (Starkey and Enebek 2012). Data are based on 20 bareroot nurseries and 6 to 7 container nurseries with box plots for foliar Fe data (box represents the 25th and 75th percentiles, horizontal line within the box shows the median, and triangles indicate outliers). The lowest Fe values for each month were greater than the 25 to 75 ppm range considered adequate for conifers (Davey 1995). Sampling different species, different nurseries, or in different years will produce different trends.

treated with DTPA containing up to 18 kg/ha of Fe during the growing season (Davey 2002). Under specific circumstances, however, toxicity can occur (Majid 1984). Therefore, some managers lightly irrigate seedlings to reduce the chance of leaf burn (Davey and McNabb 2019). Delaying irrigation for 8 hours after application is likely not important since chelates will wash into the soil where they can be taken up by roots (Landis 1988, Lucena 2006). One HEDTA product is registered for use as an herbicide and applying too much might stunt seedlings (Landis 1997). Likewise, applying a high rate of EDTA can stunt growth of some container-grown conifers (Allen and Hallett 1987, van den Driessche 1989). James (1979) speculated that 614 ppm Fe in pine needles could cause chlorosis, but he was unaware that soil imbedded in epicuticular wax could elevate foliar Fe test results. Likewise, no chlorosis was reported when foliage samples from the Mount Sopris Nursery contained 610 ppm Fe (Landis 1976).

When compared to a foliar application, applying Fe to soil reduces the chance of toxicity. In some soils, high rates of $FeSO_4$ did not reduce pine germination (Cockerill 1957, Davis 1941) but did reduce growth at pH 6.1 (figure 9). Likewise, in a slash pine greenhouse trial, growth reductions may have resulted when soil was treated with 60 kg/ha of $FeSO_4$ (van Lear and Smith 1972).

## Soil Fumigation

Methyl bromide fumigation can reduce chlorosis because it reduces nematodes (Hodges 1962), increases the amount of Fe in soil solution (Fraedrich and Dwinell 2003), and sometimes increases Fe concentration in shoots (Danielson 1966). Soil fumigation in the spring may delay ectomycorrhizal formation resulting in P deficient seedlings while Fe in foliage is either increased (Danielson 1966) or unaffected (figure 10).

In non-fumigated soil, nematodes can stunt roots and cause chlorosis in acid nursery soils (Cram and Fraedrich 2012, Hodges 1962, Korstian et al. 1921, Marks et al. 1985). Without close examination, nematode injury may be confused with Fe deficiency. Although it is easy to claim that yellow seedlings in a bareroot nursery are Fe deficient, it is hard to prove by analyzing foliage.

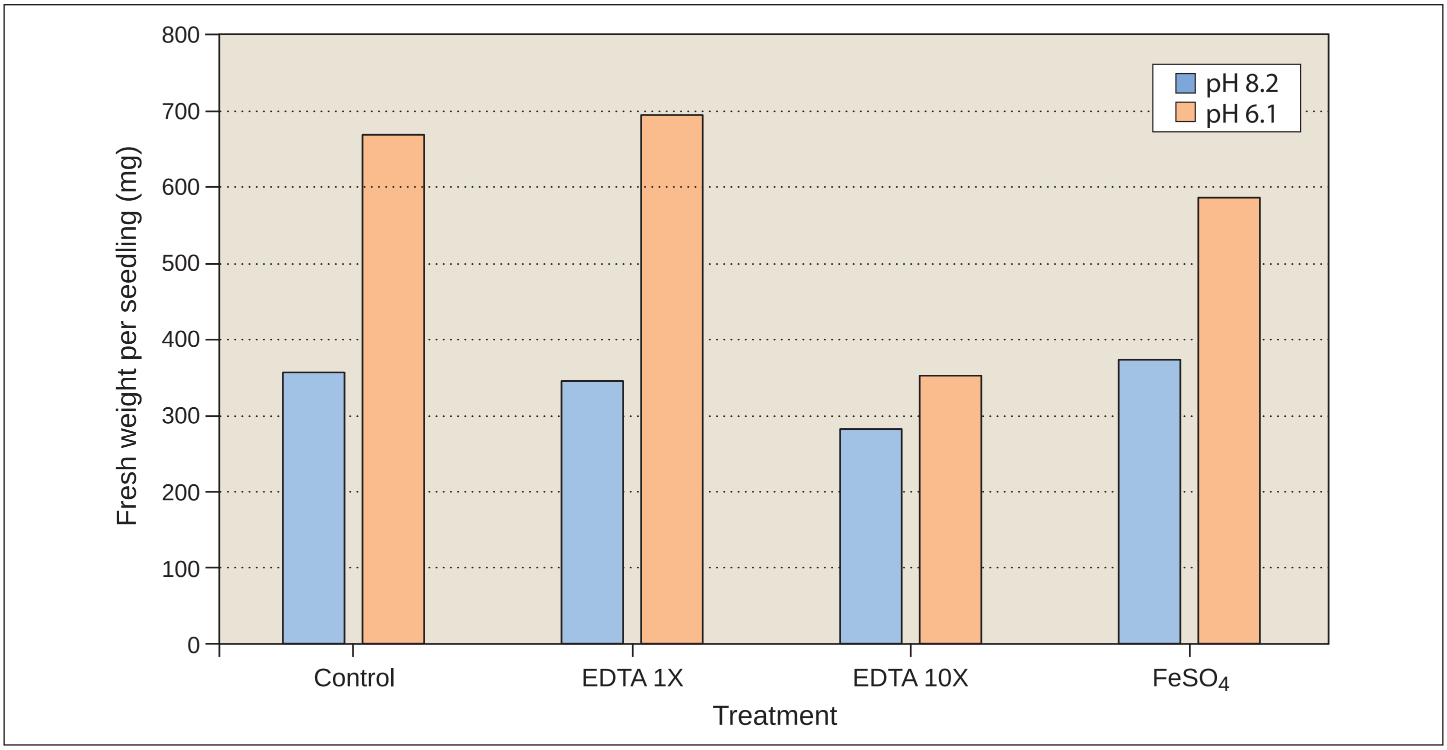

**Figure 9.** Acidifying an alkaline soil increased growth of small, non-mycorrhizal jack pine (end of first year). A 10X rate of Fe-EDTA (224 kg/ha or 29 kg/ha of Fe) reduced growth ($LSD_{05}$ = 65 mg) (Dale et al. 1955). In the acid soil, an application of $FeSO_4$ (224 kg/ha or 44.8 kg/ha of Fe) also reduced growth. None of the Fe treatments prevented chlorosis in the alkaline soil where non-mycorrhizal seedlings were pale green with purple needle tips and were near death.

## Nutrient Removal

The amount of Fe removed when a crop of seedlings is harvested depends on the overall mass of seedlings. Harvesting 1-0 pine seedlings from a nursery can remove 1.0 to 3.5 kg/ha of Fe (Boyer and South 1985, Hopmans and Flinn 1983, Knight 1978, Pritchett and Fisher 1987). Due to soil dynamics, irrigation, atmospheric deposition (Przybysz et al. 2014), and impurities in lime and fertilizers (Dillard et al. 1982, Fan et al. 2012, MikosSzymańska et al. 2019), most nursery managers need not worry about depleting Fe

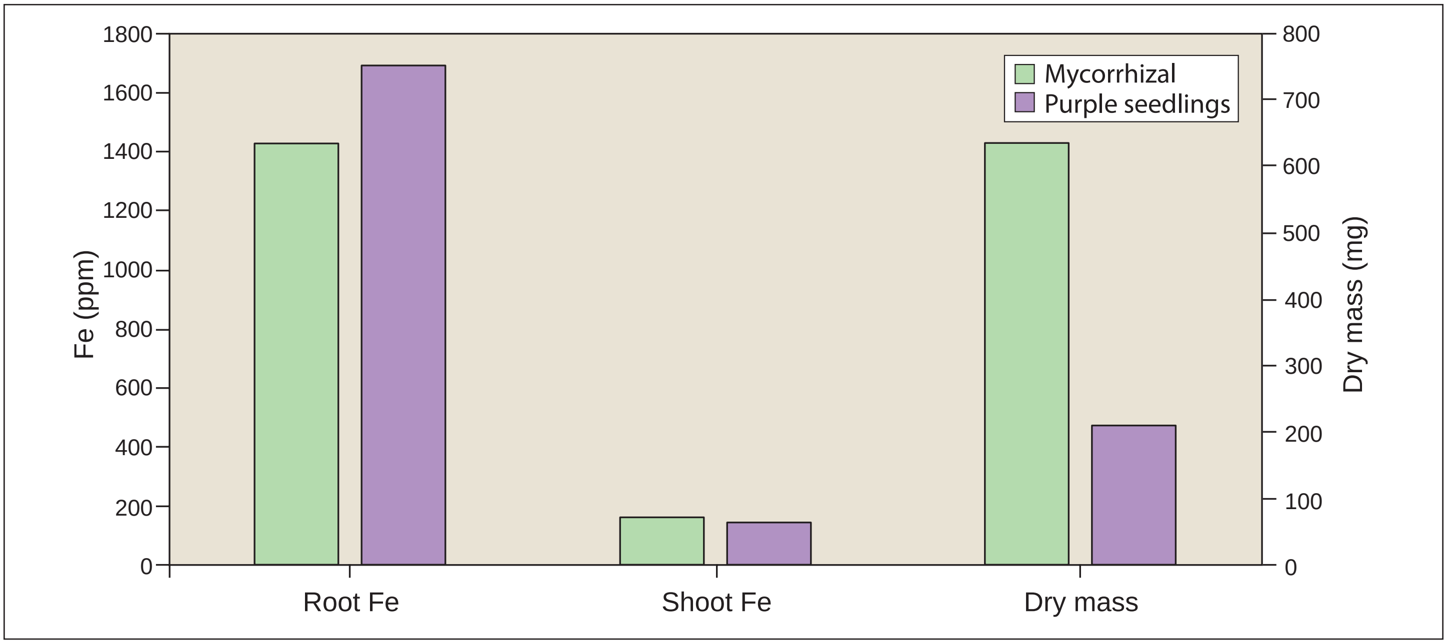

**Figure 10.** Mycorrhiza increased P uptake and seedling mass (P<0.003) but reduced root Fe concentrations (P=0.029) at the Union Camp Nursery in Alabama (July 1986). Shoot Fe concentrations were unaffected (P=0.16). The $LSD_{05}$ was 29 ppm Fe for shoots and 214 ppm Fe for roots. Prior to sowing, the soil was fumigated with 439 kg/ha of methyl bromide plus 9 kg/ha of chloropicrin (South et al. 1988) and the soil contained 16 ppm Fe (DPTA extraction).

levels. About one-third of bareroot nurseries in the South did not apply Fe fertilizers in 2020. When irrigation water contains 0.2 ppm Fe, 1,000 mm of irrigation will add 2 kg/ha of Fe to the soil.

Although some nursery managers apply organic matter to replace nutrients, this practice is not always effective in increasing soluble Fe levels. Applying 6.7 tonnes/ha of pine bark added 10 kg of Fe at one alkaline nursery but did not increase either soil solution Fe or foliar Fe (Mexal and Fisher 1987). At one nursery in Tennessee, mulch applied after sowing added 18 to 30 kg/ha of Fe (dos Santos 2006).

## Hidden Hunger

A hidden hunger exists when there are no visual deficiency symptoms (Landis et al. 2005), but addition of fertilizer increases growth (figure 11). Foliar Fe is adequate in cases where adding more Fe does not increase growth. A hidden hunger does not exist when a treatment increases the chlorophyll concentration but does not increase subsequent growth. The critical point (Landis et al. 2005) is the concentration value that separates the deficient zone from the adequate zone. In one greenhouse trial, 28 ppm Fe in the shoot was deficient and 31 ppm was adequate (van den Driessche 1989). Unfortunately, too few Fe trials exist to accurately identify a critical point. Thus, estimates for bareroot seedlings are quite variable. For example, estimates of adequate Fe in pine needles vary from 15 ppm (Jokela 2004), to 25 ppm (Aldhous and Mason 1994, Davey 1995), to 50 ppm (Ballard and Carter 1986, Garrison-Johnston et al. 2005, Hawkins et al. 2011). As a precaution, some managers apply Fe chelate when foliage Fe tests drop below 100 ppm. It is a misconception that loblolly pine hedges should contain 400 ppm Fe. For example, cuttings taken from hedges ranged from 36 to 57 ppm Fe (Rowe 1996).

Without using check plots, some managers may assume seedlings have a hidden hunger and routinely apply Fe chelates. The only way to prove a hidden hunger exists for Fe is to apply an effective Fe chelate and compare the biomass of treated and non-treated seedlings. Use of $FeSO_4$ is not recommended when testing for hidden hunger for Fe because a growth response could be due to a hidden hunger for S (Browder et al. 2005, Lyle and Pierce 1968).

## Soil Imbedded in Epicuticular Wax

Foliar Fe in bareroot pine seedlings has varied from 40 to 2,638 ppm in the USA (Baer 1984, Boyer and South 1985, Iyer and Wilde 1974, Landis 1976, Landis 1988), 104 to 483 ppm in New Zealand (Knight 1978), 36 to 382 ppm in Finland (Jalkanen and Rikala 1995), and 85 to 440 ppm in Australia (Flinn et al 1980, Hopmans and Flinn 1983). Leaves or needles with Fe values above 300 ppm are likely contaminated with soil, dust, or fertilizer residues (Jacobson 1945, Przbysz et al. 2014).

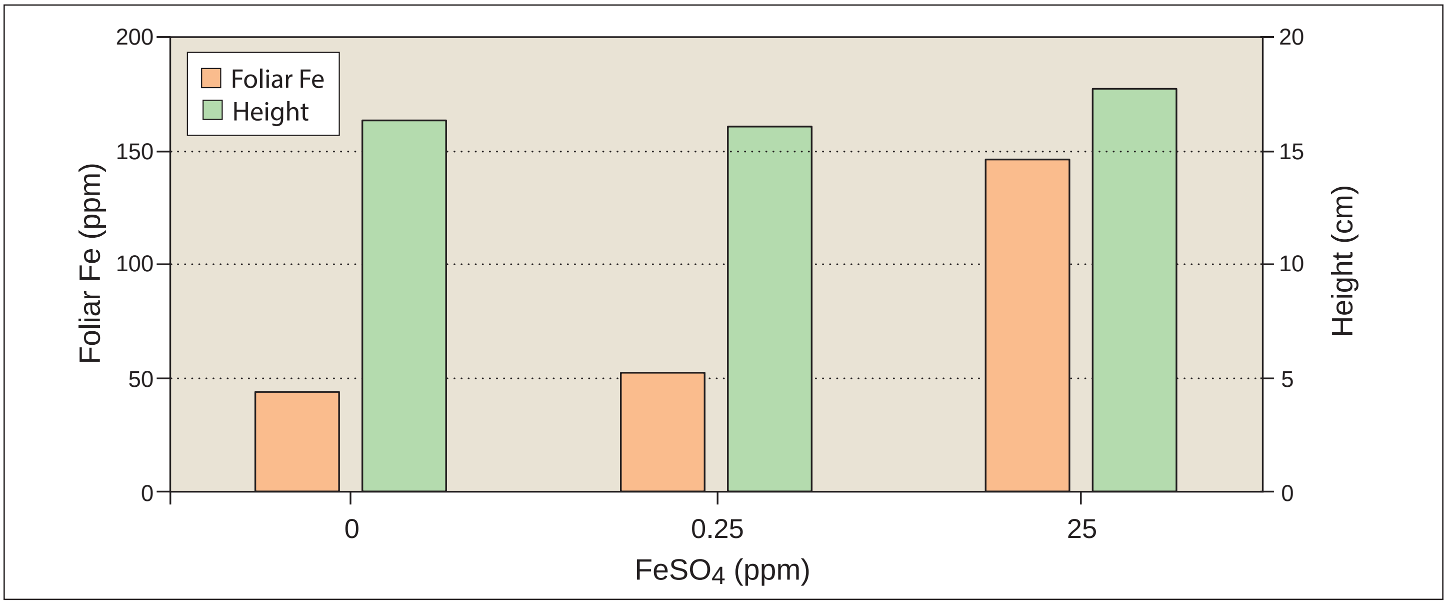

**Figure 11.** Soil applications of $FeSO_4$ applied every 2 days affected height growth and nutrient concentrations of lodgepole pine foliage in a greenhouse (Majid 1984). Seedlings were grown in sand (pH < 5.6) and were fertilized with 100 ppm N and 69 ppm S. The 25-ppm $FeSO_4$ rate increased height growth and foliar Fe concentrations ($\alpha$= 0.05) and lowered foliar S concentration.

At one nursery, container-grown loblolly pine foliage averaged 110 ppm Fe (range 95 to 120 ppm) which was similar to bareroot seedlings grown in an adjacent field (average 93 ppm; range 78 to 117 ppm). Values from a more distant bareroot field, however, averaged 2,131 ppm Fe (range 1,461 to 2,638 ppm). Apparently, wind-blown soil and dust can become imbedded in the epicuticular wax (Boyer and South 1985, Oserkowsky 1933, Przybysz et al. 2014, Weyttenbach et al. 1985) and will elevate foliar values for both Fe and Al.

Foliar values for Fe are typically not useful for diagnostic purposes (Landis et al. 2005) unless the wax is removed prior to sample digestion. After washing needles for 30 seconds in chloroform, green needles may contain 24 to 29 ppm Fe and deficient needles may contain 14 ppm (Van Dijk and Bienfait 1993). Values this low are almost never reported for conifer needles with wax. A light washing of samples using a colander and distilled water can be used (Krueger 1967), but this practice is not as effective as washing with a solution of HCl (Jacobson 1945). For North America, it appears the “adequate” Fe ranges for pine were estimated without first removing epicuticular wax with chloroform.

Typically, the concentration of N and K in pine needles declines after fertilization ceases while the concentration of Fe increases (Baer 1984, Baer 1985, Rowe 1996, Starkey and Enebak 2012, Sung et al. 2019). Foliar Fe concentration in loblolly pine needles can increase from October to January (figure 8) which may be due to accumulation of soil in the epicuticular wax. At one nursery, Fe in bareroot loblolly pine needles averaged 55, 199, 66, and 118 ppm in June, July, August, and November, respectively ($LSD_{05}$ = 88 ppm). The 66 percent drop from July to August was likely due to sampling new foliar growth with less soil contamination (Nelson and Selby 1974). At some nurseries, foliar Fe concentration can double after the second growing season (1-0 = 185 ppm; 2-0 = 380 ppm; Landis 1976).

## Fe/Mn Ratio in Foliage

Too much Mn can interfere with uptake or translocation of Fe (Lafond and Laflamme 1970, Morrison and Armson 1968). Therefore, some laboratories calculate a foliar Fe/Mn ratio. In laboratory reports, the expected ratio varies from 0.3 to 0.7. The ratios for good-quality seedlings (July) can range from 0.11 to 4.8 for loblolly pine, from 0.28 to 5.8 for ponderosa pine (Baer 1984, Starkey and Enebak 2012), and from 0.18 to 0.36 for loblolly pine cuttings (Rowe 1996). High Fe/Mn ratios are not a problem since they are likely due to soil imbedded in epicuticular wax. Thus, it is a misconception that the desired Fe/Mn ratio for loblolly pine seedlings should range from 1.3 to 1.7. Chlorotic needles and stunted pine seedlings may occur when foliage contains too much Mn relative to Fe (e.g., 86 ppm Fe and 1,895 ppm Mn). For jack pine, the foliar Mn concentration alone is more operationally meaningful than the Fe/Mn ratio (Morrison and Armson 1968).

## Cost

Some managers assume a hidden hunger for Fe exists and proactively apply DTPA several times during the growing season. The cost for these applications can exceed $800/ha/crop. At many nurseries, there is no need to apply Fe chelates (Landis 1997), so some managers have not purchased DTPA in more than 2 decades. Because of the expense, Fe chelates should not be used unless there is a clear need (Davey 1984). Since EDTA products can degrade when tank mixed with certain fertilizers, it might be wise to treat seedbeds soon after mixing to improve the cost effectiveness of the treatment (Albano and Miller 2001).

The cost (2020 USD) of 3 kg of Fe can vary from $20 (solid $FeSO_4$), to $115 (liquid $FeSO_4$), to $240 (EDTA), to $885 (DTPA), to $1,760 (EDDHA). When EDDHA products are applied, the cost typically exceeds 10 percent of the total fertilizer cost (Fisher and Chan 1985, Martian 1989, Mexal and Fisher 1987). Even so, for many managers, efficacy is more important than price (Mexal et al. 2004). For example, when an effective treatment increases nursery production by 3,600 seedlings/ha, the increase in revenue can equal $1,800 (when seedlings are sold for 50 cents each). Therefore, at some nurseries with alkaline soils, an increase of 0.3 percent in plantable seedlings could justify applying EDDHA chelates.

In some studies, applying $FeSO_4$ was more cost effective than applying either EDTA (figure 5) or EDDHA (Chakraborty et al. 2014, Ferrarezi et al. 2007, Mexal et al. 2004). Although comparison trials are not available for bareroot nurseries, some managers choose to apply the less expensive liquid $FeSO_4$ products (table 2).

## Conclusions

There is no need to apply Fe chelates to bareroot pine seedlings when unlimed soil (pH 4.0 to 5.5) is irrigated with water low in bicarbonates. To improve usefulness of foliar tests, researchers should first remove epicuticular wax from pine needles before analyzing for Fe.

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# Forest Nursery Seedling Production in the United States—Fiscal Year 2020

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## Abstract

Forest nursery production for the 2020 planting season was more than 1.27 billion tree seedlings (including about 18.5 million container seedlings imported from Canada). Approximately 73 percent of seedlings were produced as bareroot stock. Only a small portion (3 percent) of seedlings were hardwood species. Seedling production in some States was affected by the coronavirus pandemic which left some nurseries unable to lift and ship their stock. Based on this total number of seedlings and estimated planting densities in each State, more than 2.4 million ac (977,542 ha) were planted. Approximately 84 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. 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 estimate of planted acres for each State was calculated using FIA estimates of planting densities. In addition, FIA average annual estimates of tree planting area based on ground-plot data that States collected during 5-, 7-, or 10-year periods is included. 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 estimate of planted acreage. Data collected are reported for both hardwood and conifer species by bareroot and container seedlings produced (table 1) and by estimated acreage planted of each (table 2).

Table 1. Hardwood and conifer tree seedling production for each State and each region during the 2020 planting year.

| State | Bareroot hardwood | Container hardwood | Total hardwood | Bareroot conifer | Container conifer | Imported container conifer | Total conifer | Total seedlings produced |
|---|---|---|---|---|---|---|---|---|
| **Southeast** | | | | | | | | |
| Florida | 1,547,000 | 174,000 | 1,721,000 | 47,614,000 | 3,368,000 | — | 50,982,000 | 52,703,000 |
| Georgia | 3,927,000 | — | 3,927,000 | 199,645,000 | 133,732,000 | — | 333,377,000 | 337,304,000 |
| North Carolina | 231,000 | — | 231,000 | 54,310,000 | 12,204,000 | — | 66,514,000 | 66,745,000 |
| South Carolina | — | — | — | 148,944,000 | — | — | 148,944,000 | 148,944,000 |
| Virginia | 1,206,000 | — | 1,206,000 | 29,115,000 | 427,000 | — | 29,542,000 | 30,748,000 |
| **Regional Totals** | **6,911,000** | **174,000** | **7,085,000** | **479,628,000** | **149,731,000** | **0** | **629,359,000** | **636,444,000** |
| **South Central** | | | | | | | | |
| Alabama | 5,051,000 | 10,000 | 5,061,000 | 87,845,000 | 28,455,000 | — | 116,300,000 | 121,361,000 |
| Arkansas | 9,149,000 | — | 9,149,000 | 84,245,000 | — | — | 84,245,000 | 93,394,000 |
| Kentucky | 546,240 | — | 546,240 | 7,683,000 | — | — | 7,683,000 | 8,229,240 |
| Louisiana | — | — | — | — | 44,904,000 | — | 44,904,000 | 44,904,000 |
| Mississippi | 1,000,000 | 154,000 | 1,154,000 | 73,718,000 | 11,000,000 | — | 84,718,000 | 85,872,000 |
| Oklahoma | 451,000 | 3,000 | 454,000 | 2,396,000 | 160,000 | — | 2,556,000 | 3,010,000 |
| Tennessee | 1,750,000 | — | 1,750,000 | 3,400,000 | — | — | 3,400,000 | 5,150,000 |
| Texas | — | — | — | 76,781,000 | — | — | 76,781,000 | 76,781,000 |
| **Regional Totals** | **17,947,240** | **167,000** | **18,114,240** | **336,068,000** | **84,519,000** | **0** | **420,587,000** | **438,701,240** |
| **Northeast** | | | | | | | | |
| Connecticut | — | 2,000 | 2,000 | — | 500 | — | 500 | 2,500 |
| Delaware | — | — | — | — | — | — | — | — |
| Maine[1] | — | 2,055 | 2,055 | 2,000 | 1,100 | 3,600,000 | 3,603,100 | 3,605,155 |
| Maryland | 1,006,420 | 50,500 | 1,056,920 | 639,775 | 400 | — | 640,175 | 1,697,095 |
| Massachusetts | 5,001 | 50,010 | 55,011 | — | 4,000 | — | 4,000 | 59,011 |
| New Hampshire | 16,850 | — | 16,850 | 184,910 | — | — | 184,910 | 201,760 |
| New Jersey | 35,550 | — | 35,550 | 17,375 | — | — | 17,375 | 52,925 |
| New York | 90,900 | 20,678 | 111,578 | 445,000 | 25,140 | — | 470,140 | 581,718 |
| Pennsylvania | 54,333 | 25,500 | 79,833 | 10,826 | 100 | — | 10,926 | 90,759 |
| Rhode Island | — | — | — | — | — | — | — | — |
| Vermont | 500 | 100 | 600 | 100 | 150 | — | 250 | 850 |
| West Virginia | 82,908 | — | 82,908 | 45,535 | — | — | 45,535 | 128,443 |
| **Regional Totals** | **1,292,462** | **150,843** | **1,443,305** | **1,345,521** | **31,390** | **3,600,000** | **4,976,911** | **6,420,216** |
| **North Central** | | | | | | | | |
| Illinois | 590,000 | 7,025 | 597,025 | 175,000 | 2,040 | — | 177,040 | 774,065 |
| Indiana | 1,905,465 | 810 | 1,906,275 | 757,800 | 450 | — | 758,250 | 2,664,525 |
| Iowa | 315,620 | 500 | 316,120 | 10,125 | 2,500 | — | 12,625 | 328,745 |
| Michigan[1] | 4,792,593 | 500 | 4,793,093 | 3,716,203 | 20,000 | 7,920,888 | 11,657,091 | 16,450,184 |
| Minnesota[1] | 23,725 | 2,250 | 25,975 | 931,725 | 60,015 | 91,720 | 1,083,460 | 1,109,435 |
| Missouri | 1,035,770 | — | 1,035,770 | 498,900 | — | — | 498,900 | 1,534,670 |

| State | Bareroot hardwood | Container hardwood | Total hardwood | Bareroot conifer | Container conifer | Imported container conifer | Total conifer | Total seedlings produced |
|---|---|---|---|---|---|---|---|---|
| Ohio | 2,000 | 4,000 | 6,000 | — | — | — | — | 6,000 |
| Wisconsin[1] | 173,595 | 114,120 | 287,715 | 592,915 | 546,550 | 131,660 | 1,271,125 | 1,558,840 |
| **Regional Totals** | **8,838,768** | **129,205** | **8,967,973** | **6,682,668** | **631,555** | **8,144,268** | **15,458,491** | **24,426,464** |
| **Great Plains** | | | | | | | | |
| Kansas | — | 25,000 | 25,000 | — | 50,000 | — | 50,000 | 75000 |
| Nebraska | 402,545 | 4,000 | 406,545 | 360,000 | 850,000 | — | 1,210,000 | 1,616,545 |
| North Dakota | 19,575 | 14,856 | 34,431 | 548,196 | 78,552 | — | 626,748 | 661,179 |
| South Dakota | 234,822 | 1,070 | 235,892 | 273,975 | 12,780 | — | 286,755 | 52,2647 |
| Regional Totals | **656,942** | **44,926** | **701,868** | **1,182,171** | **991,332** | **0** | **2,173,503** | **2,875,371** |
| **Intermountain** | | | | | | | | |
| Arizona | — | 5,120 | 5,120 | - | 680 | — | 680 | 5,800 |
| Colorado | 60,225 | 39,124 | 99,349 | 12,925 | 119,114 | — | 132,039 | 231,388 |
| Idaho[1] | — | 63,739 | 63,739 | 1,994,408 | 5,118,614 | 3,942,980 | 11,056,002 | 11,119,741 |
| Montana[1] | 89,766 | 140,384 | 230,150 | — | 673,647 | 64,000 | 737,647 | 967,797 |
| Nevada | — | 2,600 | 2,600 | — | 170 | — | 170 | 2,770 |
| New Mexico | — | 4,500 | 4,500 | — | 54,000 | — | 54,000 | 58,500 |
| Utah | 20,000 | 1,500 | 21,500 | 5,000 | 2,000 | — | 7,000 | 28,500 |
| Wyoming | — | — | — | — | — | — | — | — |
| **Regional Totals** | **169,991** | **256,967** | **426,958** | **2,012,333** | **5,968,225** | **4,006,980** | **11,987,538** | **12,414,496** |
| **Alaska** | | | | | | | | |
| Alaska | — | — | — | — | — | — | — | — |
| **Pacific Northwest** | | | | | | | | |
| Oregon[1] | 1,806,650 | 2,309,540 | 4,116,190 | 33,924,235 | 37,864,909 | 786,850 | 72,575,994 | 76,692,184 |
| Washington[1] | 169,400 | 48,920 | 218,320 | 3,1457,050 | 18,315,297 | 2,008,550 | 51,780,897 | 51,999,217 |
| **Regional Totals** | **1,976,050** | **2,358,460** | **4,334,510** | **65,381,285** | **56,180,206** | **2,795,400** | **124,356,891** | **128,691,401** |
| **Pacific Southwest** | | | | | | | | |
| California | — | 72,900 | 72,900 | 1,155,489 | 23,334,217 | — | 24,489,706 | 24,562,606 |
| Hawaii | — | 10,500 | 10,500 | — | 500 | — | 500 | 11,000 |
| **Regional Totals** | **0** | **83,400** | **83,400** | **1,155,489** | **23,334,717** | **0** | **24,490,206** | **24,573,606** |
| **Totals** | **37,792,453** | **3,364,801** | **41,157,254** | **893,455,467** | **321,387,425** | **18,546,648** | **1,233,389,540** | **1,274,546,794** |

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

Table 2. Esitmated hardwood and conifer tree seedling acres planted for each State and each region during the 2020 planting year.

| State | Hardwood acres planted | Conifer acres planted | Total acres planted | FIA estimated acres planted[9] |
|---|---|---|---|---|
| **Southeast** | | | | |
| Florida[2] | 3,129 | 92,695 | 95,824 | 150,006 |
| Georgia[2] | 7,140 | 606,140 | 613,280 | 212,353 |
| North Carolina[2] | 420 | 120,935 | 121,355 | 108,401 |
| South Carolina[2] | — | 270,807 | 270,807 | 88,362 |
| Virginia[2] | 2,193 | 53,713 | 55,905 | 57,031 |
| **Regional Totals** | **12,882** | **1,144,289** | **1,157,171** | **616,153** |
| **South Central** | | | | |
| Alabama[2] | 9,202 | 211,455 | 220,656 | 218,748 |
| Arkansas[2] | 16,635 | 153,173 | 169,807 | 89,136 |
| Kentucky[3] | 1,256 | 17,662 | 18,918 | 1,142 |
| Louisiana[2] | — | 81,644 | 81,644 | 160,561 |
| Mississippi[2] | 2,098 | 154,033 | 156,131 | 140,495 |
| Oklahoma[2] | 825 | 4,647 | 5,473 | 31,659 |
| Tennessee[2] | 3,182 | 6,182 | 9,364 | 24,386 |
| Texas[2] | — | 139,602 | 139,602 | 126,044 |
| **Regional Totals** | **33,198** | **768,397** | **801,594** | **792,171** |
| **Northeast** | | | | |
| Connecticut[3] | 5 | 1 | 6 | - |
| Delaware[2] | - | - | - | 515 |
| Maine[5] | 3 | 6,005 | 6,009 | 4,069 |
| Maryland[2] | 1,922 | 1,164 | 3,086 | — |
| Massachusetts[3] | 126 | 9 | 136 | — |
| New Hampshire[3] | 39 | 425 | 464 | 402 |
| New Jersey[3] | 82 | 40 | 122 | — |
| New York[5] | 186 | 784 | 970 | 2,077 |
| Pennsylvania[3] | 184 | 25 | 209 | 1,847 |
| Rhode Island | — | — | — | — |
| Vermont[3] | 1 | 1 | 2 | — |
| West Virginia[3] | 191 | 105 | 295 | — |
| **Regional Totals** | **2,738** | **8,558** | **11,297** | **8,910** |
| **North Central** | | | | |
| Illinois[3] | 1,372 | 407 | 1,779 | 1,667 |
| Indiana[4] | 2,933 | 1,167 | 4,099 | 2,413 |
| Iowa[5] | 527 | 21 | 548 | — |
| Michigan[2] | 8,715 | 21,195 | 29,909 | 6,330 |
| Minnesota[2] | 47 | 1,970 | 2,017 | 8,403 |
| Missouri[3] | 2,381 | 1,147 | 3,528 | 223 |
| Ohio[3] | 14 | — | 14 | 2,173 |
| Wisconsin[6] | 360 | 1,589 | 1,949 | 8,256 |
| **Regional Totals** | **14,976** | **27,088** | **42,064** | **29,465** |

| State | Hardwood acres planted | Conifer acres planted | Total acres planted | FIA estimated acres planted[9] |
|---|---|---|---|---|
| **Great Plains** | | | | |
| Kansas[2] | 45 | 91 | 136 | 1,012 |
| Nebraska[2] | 739 | 2,200 | 2,939 | — |
| North Dakota[2] | 63 | 1,140 | 1,202 | — |
| South Dakota[2] | 429 | 521 | 950 | 164 |
| **Regional Totals** | **1,276** | **3,952** | **5,228** | **1,176** |
| **Intermountain** | | | | |
| Arizona[2] | 9 | 1 | 11 | - |
| Colorado[2] | 181 | 240 | 421 | 669 |
| Idaho[2] | 116 | 20,102 | 20,218 | 10,016 |
| Montana[2] | 418 | 1,341 | 1,760 | 4,506 |
| Nevada[2] | 5 | — | 5 | — |
| New Mexico[2] | 8 | 98 | 106 | — |
| Utah[2] | 39 | 13 | 52 | — |
| Wyoming | — | — | — | 846 |
| **Regional Totals** | **776** | **21,796** | **22,572** | **16,037** |
| **Alaska** | | | | |
| Alaska[2] | — | — | — | — |
| **Pacific Northwest** | | | | |
| Oregon[7] | 11,761 | 207,360 | 219,121 | 118,350 |
| Washington[7] | 624 | 147,945 | 148,569 | 96,376 |
| **Regional Totals** | **12,384** | **355,305** | **367,690** | **214,726** |
| **Pacific Southwest** | | | | |
| California[8] | 162 | 54,422 | 54,584 | 36,986 |
| Hawaii[8] | 23 | 1 | 24 | 568 |
| **Regional Totals** | **185** | **54,423** | **54,608** | **37,554** |
| **TOTALS** | **78,416** | **2,383,808** | **2,462,223** | **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] FIA = Forest Inventory and Analysis; average annual acreage planted estimated for all States 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.

## 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 2020 planting season (i.e., seedlings that were planted from fall of 2019 through spring of 2020).*

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).*

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 seedlings are produced in Canada and imported for planting in those States. Estimates of the amount 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 1.27 billion forest tree seedlings were planted in the United States in fiscal year (FY) 2020. This production level is a decrease from FY 2019 (table 3).

**Table 3.** Annual forest nursery seedling production in each region for FY 2012 to FY 2020.

| Year | Total seedling production | West (17 States) | East (20 States) | South (13 States) |
|---|---|---|---|---|
| FY 2020 | 1,274,546,794 | 168,554,874 | 30,846,680 | 1,075,145,240 |
| 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, 2020), Harper et al. (2013, 2014), Hernández et al. (2015, 2016, 2017, 2018)

In spring 2020, coronavirus pandemic restrictions coincided with lifting season and resulted in an inability of some nurseries to finish lifting seedlings. This decline in seedling production is especially notable in the Eastern States (Pennsylvania, Michigan, and Minnesota). In addition, year-to-year variation is attributed to inconsistent participation from nurseries during data collection and shifting planting needs 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, more than 2.4 million ac (977,542 ha) of tree seedlings were planted during the fall 2019 through spring 2020 planting season.

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# Arbuscular Mycorrhizal Fungi Associated With Rhizosphere of *Casuarina* in Morocco

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## Abstract

The presence and diversity of arbuscular mycorrhizal fungi (AMF) in the rhizosphere of *Casuarina* trees was studied in four regions of Morocco. The results showed that all the sampled roots were mycorrhizal and various AMF structures were evident (arbuscules, vesicles, hyphae, spores, and non-specialized hyphae) in all regions. AMF colonization and diversity varied among regions, but all regions had a relatively high level. A total of 83 morphotypes belonging to 14 genera and 10 families were isolated and documented. *Glomus* was the most common and widespread genus found. Understanding the association of AMF with this important restoration species has implications for nursery production and outplanting strategies.

## Introduction

The *Casuarinaceae* family comprises 86 species of trees and shrubs distributed in 4 genera (*Allocasuarina*, *Casuarina*, *Ceuthostoma*, and *Gymnostoma*) (Steane et al. 2003). These species are actinorhizal plants forming nitrogen-fixing nodules with the actinomycete *Frankia* (Dommergues et al. 1999). The *Casuarina* genus belongs to tropical and subtropical trees from Australia, Southeast Asia, and the Pacific Islands (Sougoufara et al. 1992). *Casuarina* are characterized by a conifer-like appearance with articulated and needle-shaped foliage that gradually reduce to tiny green-twined teeth (Zhong et al. 2010). The vegetative and floral parts develop with considerable scleromorphism (Midgley et al. 1983, Pinyopusarerk et al. 1996, Subba-Rao and Rodriguez-Barrueco 1995).

*Casuarina* sp. trees are widely used as shelterbelts (Castle 2017, Poynton 1995) and are planted along coasts, mobile dunes, and eroded slopes for controlling erosion. *Casuarina* sp. are also used for improving soil fertility due to their nitrogen-fixing ability and production of organic litter (Parrotta 1993, Zhong et al. 2010). Additionally, these species have been used as ornamental trees and for timber (Beadle 1981, Castle 2017, El-Lakany 1983, Kondas 1983, Midgley et al. 1983, Turnbull 1990).

In Morocco, *Casuarina* trees are planted in all regions, especially *Casuarina cunninghamiana* Miq, for windbreaks and shelterbelts (Ducousso et al. 2003). Several studies have reported the symbiotic association between roots of *Casuarina* sp. and *Frankia* as well as mycorrhizal fungi (Diagne et al. 2013). Arbuscular mycorrhizal fungi (AMF) is a type of endomycorrhizae which form a symbiotic association with plants (Redecker et al. 2000, Schübler et al. 2001). AMF are the most common mycorrhizal fungi (Wipf 2014) and are associated with 80 percent of green plants (Béreau et al. 2003). AMF are characterized by the formation of several structures (arbuscules, vesicles, spores, and non-specialized hyphae) (Béreau et al. 2003, Tommerup 1984, Wipf 2014).

The symbiotic associations between AMF and the host plant contribute to nitrogen fixation at similar rates to those of nodulated legumes (Zhong 1993). *Casuarina* trees with AMF have significantly improved mineral nutrition and increased tolerance to drought, flooding, and salt stress. Thus, this association enhances the host plant's ability to thrive in challenging environments (Elumalai and Raaman 2009, Evelin et al. 2009, Osundina 1997, Zhong et al. 2010) which can be vital for replanting forest species in their natural environment, especially during the first few months after outplanting (Nouaim and Chaussod 1994). AMF can also improve seedling quality in the nursery by improving rooting and initial growth and thus make it possible to compensate for stress after outplanting (Bousselmame et al. 2002).

Research on microorganisms of *Casuarina* sp. in Morocco is limited. Ducousso et al. (2003) noted that the frequency and intensity of AMF are generally low in *Casuarina* sp. but can be high in *C. cunninghamiana* growing in nurseries. Tellal (2008) reported AMF spore morphotypes of *C. cunninghamiana* and *C. glauca* Siebold ex Spreng. belonging to *Acaulospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. Touati et al. (2016) found proteoid roots in *Casuarina* sp. with or without an endomycorrhizal inoculum.

The objective of our study was to evaluate the diversity of AMF and their development in the rhizosphere of *Casuarina* sp. in four regions of western Morocco.

## Materials and Methods

### Sites and Sampling

Surveys were carried out in four regions (Allal Tazi, Had Kourt, Kenitra, and Sidi Slimane) in western Morocco (figure 1). These regions have a flat geography with average elevations reaching 60 m, the height of the border dunes to the west (El Jihad et al. 2014). The Mediterranean climate is characterized by alternating wet seasons (October to April) and dry, hot seasons (May to September) (Anonymous 2013). In each region, three sites were selected for soil collection (figure 2). At each site, fine roots and soil samples were collected from three *Casuarina* trees (1 kg soil/tree) from 0 to 20 cm depth.

### Root Staining for the Evaluation of AMF Root Colonization

Roots were evaluated for AMF colonization using the technique described by Phillips and Hayman (1970). The roots were washed with tap water and then cut into fragments approximately 1-cm long. These fragments were bleached with a solution of 10-percent potassium hydroxide for 45 min at 90 °C and then whitened for 5 min by adding four drops of 33-percent hydrogen peroxide. Next, the root fragments were rinsed with distilled water and stained with a solution of brilliant cresyl blue for 15 min at 90 °C in a water bath. Following staining, roots were rinsed with distilled water and observed using a microscope to determine the proportion of mycorrhizal roots in each sample.

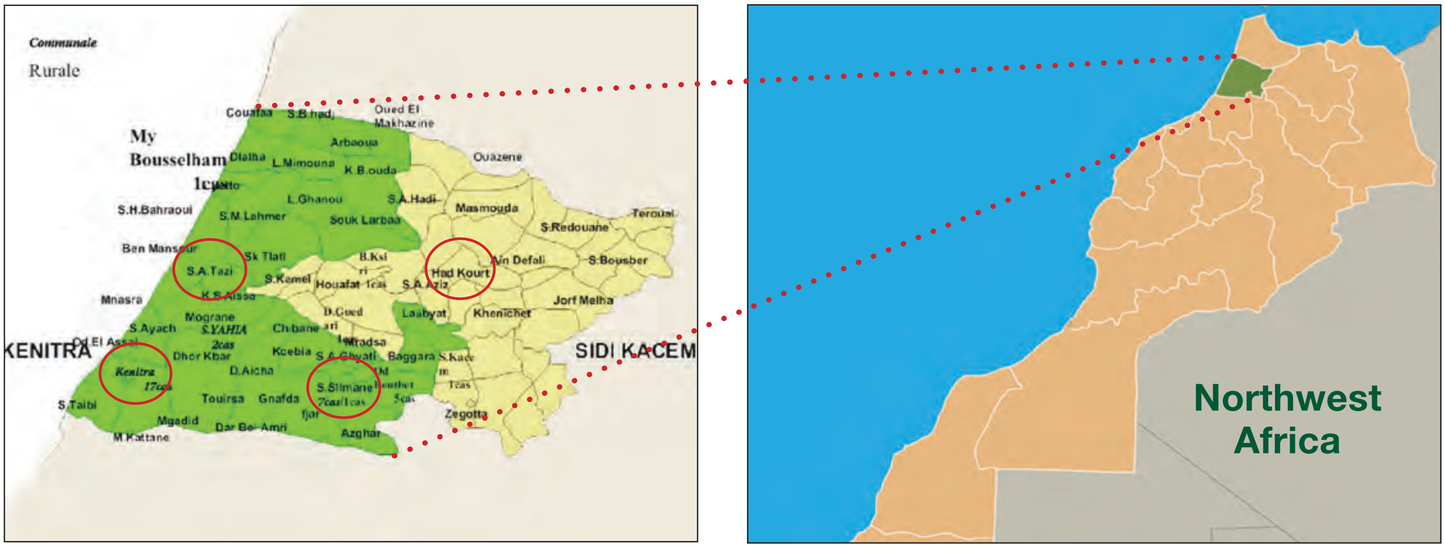


**Figure 1.** Samples were collected in four regions (Allal Tazi, Had Kourt, Kenitra, and Sidi Slimane) (Belomaria et al. 2007) in Morocco (Source: https://fr.wikinews.org/wiki/Fichier:Gharb-Chrarda-B%C3%A9ni_Hssen.svg).

**Figure 2.** Typical sampling site for the study. (Photo by N. Hibilik, 2015)

## Evaluation of the Mycorrhization Rate

Mycorrhization parameters were evaluated by assessing 30 fragments from each region as described by Trouvelot et al. (1986) and Amir and Renard (2003). Root fragments were observed at 100 and 400 magnifications. Mycorrhizal intensity (MI), arbuscule content (A), and vesicle content (V) were measured by assigning an index of mycorrhization from 0 to 5 (Derkowska et al. 2008) as follows: 0=none, 1=trace, 2=less than 10 percent, 3=11 to 50 percent, 4=51 to 90 percent, and 5=more than 91 percent.

Mycorrhizal frequency (MF) reflects the colonization percentage of the root system:

$$MF = 100 \times (N - n0)/N$$

Where:

N = total number of root fragments

n0 = number of nonmycorrhizal root fragments

MI estimates the proportion of colonization in the entire root system:

$$MI = (95n5 + 70n4 + 30n3 + 5n2 + n1)/N$$

Where:

n = number of fragments with the index 0, 1 2, 3, 4, or 5 of colonization (according to the scale developed by Derkowska et al. 2008)

N = total number of root fragments

A estimates the proportion of the root cortex containing arbuscules:

$$A = (100\ mA3 + 50\ mA2 + 10\ mA1)/100.$$
$$mA = (95\ n5A + 70\ n4A + 30\ n3A + 5\ n2A + n1A)/N$$

Where:

n and N are determined as above for MI

A1: 1 to 10 percent, A2: 11 to 50 percent, A3: 51 to 100 percent

nA denotes the number of root fragments for a given n and A (e.g., n4A3 is the number of fragments denoted 4 with A3)

V estimates the proportion of the root cortex containing vesicles and is calculated in the same way as for A:

$$V = (100\ mV3 + 50\ mV2 + 10\ mV1)/100.$$
$$mV = (95\ n5V + 70\ n4V + 30\ n3V + 5\ n2V + n1V)/N$$

## Spore Collection

AMF spores were extracted from *Casuarina* rhizosphere soil samples from each region using the wet sieving method described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100 g of each composite soil sample was immersed in 0.5 L of tap water and stirred for 1 min with a spatula. After 10 to 30 seconds of decantation, the supernatant was passed through four superposed sieves with a decreasing mesh size (500, 200, 80, and 50 microns). This operation was repeated twice. The contents recovered after passing through the different sieves were divided into two tubes and centrifuged for 4 min at 9,000 rpm. The supernatant was discarded, and a viscosity gradient was created by adding 20 ml of a 40-percent sucrose solution to each centrifuge tube (Walker et al. 1982). The mixture was rapidly stirred, and the tube was returned to the centrifuge for 1 min at 9,000 rpm. In contrast to the first centrifugation step, the supernatant was poured into the sieve with a mesh size of 50 microns. The resulting substrate was rinsed with distilled water to remove sucrose and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with a little distilled water in a flask.

The number of spores in soil was estimated by counting the spores in 1 ml of supernatant which was proportionate to the total spore number in 100 ml. If no spores were detected, the supernatant was concentrated to 1 ml and observed again. The characteristics (color, shape, size, and number of separation membranes) of spores were observed using an optical microscope.

Spore identification was based on the criteria developed by Berch (1986), Dalpé (1994, 1995), Ferrer and Herrora (1981), Hall (1984), Morton and Benny (1990), Mukerji (1996), Schenck and Perez (1987), Schenck and Smith (1982), Walker (1992), and available information in different databases (INVAM 2017).

Species richness was determined based on the total number of observed species per collection site. The frequency of occurrence corresponds to the percentage of sites where a species was detected.

### Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) for a completely randomized design. Significant differences among the four regions were determined using the least significant difference test at the 5 percent threshold. Data were analyzed using Statistica software (Stat Soft Inc.).

## Results

In all four regions, *Casuarina* trees were associated with AMF, and characteristic AMF structures were observed (figure 3). The Had Kourt site tended to have the highest mycorrhizal colonization (MF, MI, A, and V) compared with the other three regions, and the Sidi Slimane site tended to have higher colonization compared with the Kenitra and Allal Tazi sites, though all four regions had relatively high AMF levels (figure 4). Average AMF spore densities and species richness followed the same pattern among regions (figure 5).

Spore identification revealed a total of 83 morphotypes present in the rhizosphere of *Casuarina* trees (table 1, figure 6). Dominant arbuscular mycorrhizal fungi varied among regions (table 1). Based on Oehl et al. (2011), morphotypes were divided into 14 genera (*Acaulospora, Ambispora, Cetraspora, Claroideoglomus, Dentiscutata, Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Pacispora, Paraglomus, Rhizoglomus, Scutellospora, Septoglomus*) occurring within 10 families (Acaulosporaceae, Ambisporaceae, Dentiscutataceae, Diversisporaceae, Entrophosporaceae, Gigasporaceae, Glomaceae, Pacisporaceae, Racocetraceae, Scutellosporaceae) and 5 within orders (Archeosporales, Diversisporales, Gigasporales, Glomerales, Paraglomerales).

## Discussion

Our analyses show that *Casuarina* trees in four regions of western Morocco were associated with AMF. Tellal (2008) also found AMF associated with this species. We found characteristic structures including arbuscules, vesicles, internal hyphae, and external hyphae. The presence of arbuscules reveals it is a mycotrophic plant. Arbuscules are sites of nutrient exchange between symbionts (Smith and Read 1997). Differences in colonization and spore density among regions may be attributable to influences of seasons, edaphic factors (pH level and soil moisture), dormancy period, and the distribution of AMF in soil (Lugo et al. 2008).

AMF has also been found in the rhizosphere of other plant species in Morocco. In the western region of Morocco, AMF spores have been found associated with sugarcane (*Saccharum officinarum* L.) (Selmaoui et al. 2017), citrus (*Citrus aurantium* L.) (Artib et al. 2016), and olive (*Olea europaea* L.) (Chliyeh et al. 2014, Msairi et al. 2020). In south Morocco, AMF has been found in association with argan (*Argania spinosa* L.) (Nouaim and Chaussod 1994, Ouallal et al. 2018, Maazouzi et al. 2021), date palm (*Phoenix dactylifera* L.) (Bouamri and Dalpé 2006, Sghir et al. 2014), and carob (*Ceratonia siliqua* L.)(El Asri et al. 2014).

*Glomus* was the most widespread genus in our soil samples and is typically the most encountered genus in Moroccan soils. This genus has been reported in several studies in tropical and rainforest areas such as Latin America (Cruz 1989, Lopes et al. 1983), China (Zhao et al. 2001), and Mexico (Guadarrama and Alvarez-Sanchez 1999). The genus has also been found in arid and semi-arid areas such as Ethiopia (Jefwa et

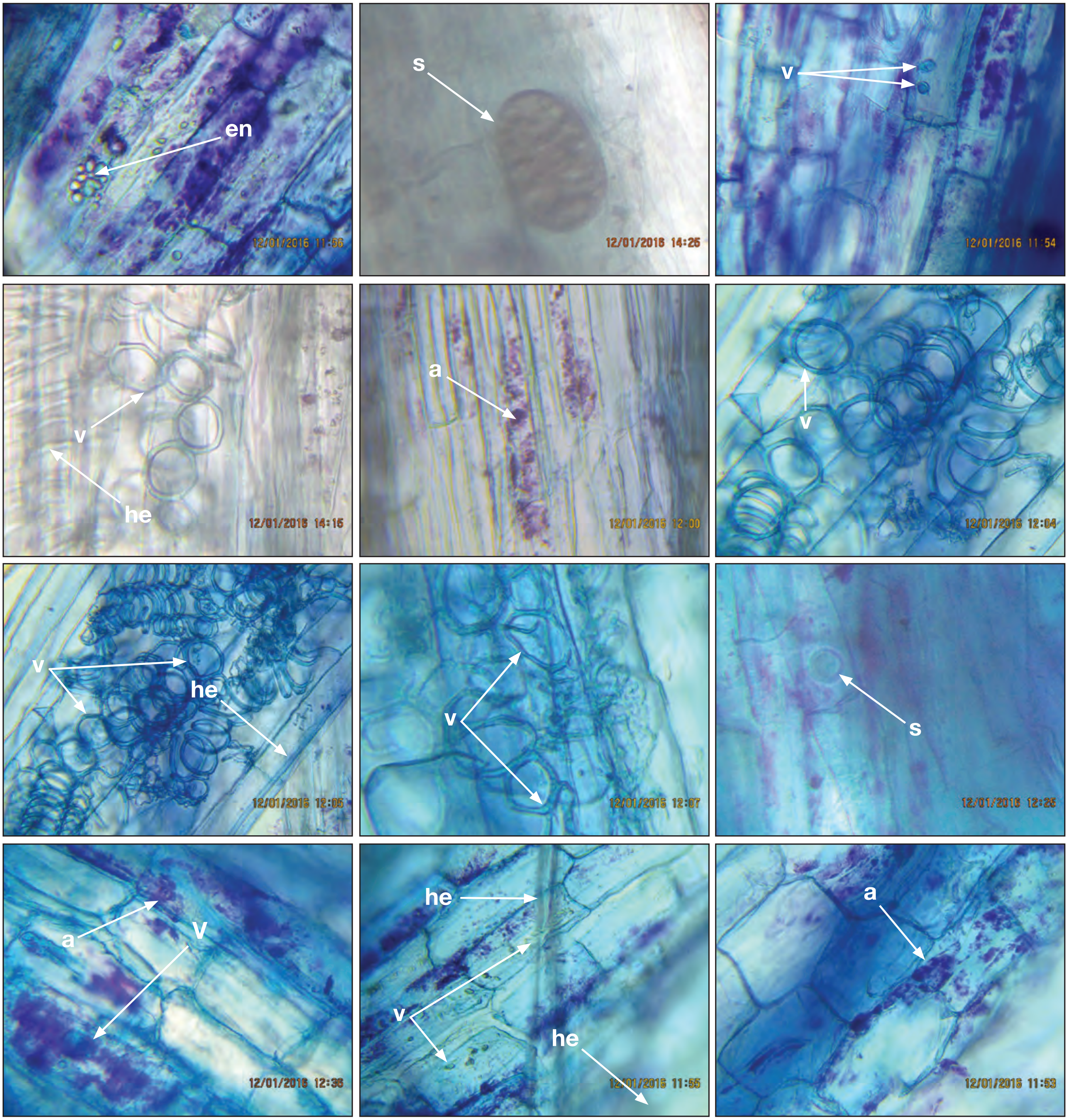


**Figure 3.** Different structures of arbuscular mycorrhizal fungi observed in the roots of *Casuarina* trees included (a) arbuscules, (h) extracellular hyphae, (s) spores, (v) vesicles, and (e) non-specialized hyphae (G× 400). (Photos by N. Hibilik, 2015)

**Table 1.** Morphological characteristics and regional distribution of endomycorrhizal fungi isolated from the *Casuarina* rhizosphere in four Morroco regions (see also figure 6).

| | | | | | | | | Number of spores per 100 g of soil in each region | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Number** | **Name** | **Form** | **Color** | **Spore size (µm)** | **Wall size (µm)** | **Hypha length** | **Spore surface** | **Had Kourt** | **Sidi Slimane** | **Kenitra** | **Allal Tazi** |
| 1 | *Acaulospora alpina* | Globular | Yellow | 119.88 | | | Grainy | 5 | 1 | - | - |
| 2 | *Acaulospora capsicula* | Oval | Orange | 173.16 | 3 | | Grainy | 4 | - | - | - |
| 3 | *Acaulospora cavernata* | Subglobose | Orange | 139.86 | | | Grainy | - | 3 | - | - |
| 4 | *Acaulospora colossica* | Globular | Yellow green | 99.90 | 2.1 | | Grainy | 4 | - | 10 | - |
| 5 | *Acaulospora delicata* | Globular | Yellow | 103.23 | 1 | | Grainy | 5 | - | - | - |
| 6 | *Acaulospora denticulata* | Oval | Dark yellow | 126.54 | 1.3 | | Grainy | 4 | 11 | 2 | 2 |
| 7 | *Acaulospora elegans* | Globular | Brown | 73.26 | 1 | 99.90 | Grainy | - | - | 2 | - |
| 8 | *Acaulospora excavata* | Globular | Yellow | 129.87 | 1 | | Grainy | 14 | - | - | - |
| 9 | *Acaulospora gedanensis* | Globular | Yellow | 116.55 | 1 | 73.26 | Smooth | - | 4 | - | - |
| 10 | *Acaulospora gerdemanii* | Globular | Brown | 106.56 | 3 | | Grainy | - | - | 2 | - |
| 11 | *Acaulospora koskei* | Subglobose | Dark yellow | 213.12 | 1 | | Grainy | - | 4 | - | - |
| 12 | *Acaulospora lacunose* | Subglobose | Yellow | 116.55 | 1 | 33.3 | Grainy | 5 | - | - | - |
| 13 | *Acaulospora laevis* | Globular | Orange | 99.90 | 2 | 49.95 | Smooth | 1 | - | 4 | 4 |
| 14 | *Acaulospora longula* | Globular | Brown | 133.20 | 1 | | Smooth | - | - | 1 | - |
| 15 | *Acaulospora mellea* | Globular | Yellow | 106.56 | 1 | 33.30 | Smooth | - | - | 2 | - |
| 16 | *Acaulospora morrowiae* | Oval | Brown | 103.23 | 2 | | Grainy | 2 | 8 | 2 | - |
| 17 | *Acaulospora nicolosonii* | Globular | Orange | 166.50 | 1.3 | | Grainy | 4 | 7 | - | - |
| 18 | *Acaulospora polonica* | Globular | Yellow | 133.20 | 1.8 | | Grainy | 4 | 4 | 3 | - |
| 19 | *Acaulospora reducta* | Globular | Yellow | 109.89 | 1 | 49.95 | Grainy | 1 | - | - | - |
| 20 | *Acaulospora rehmii* | Subglobose | Light yellow | 186.48 | 0.1 | | Grainy | - | - | 2 | - |
| 21 | *Acaulospora scrobiculata* | Globular | Yellow | 113.22 | | | Grainy | 10 | 5 | 5 | - |
| 22 | *Acaulospora* sp1 | Globular | White | 116.55 | 2 | | Grainy | 1 | - | - | - |
| 23 | *Acaulospora* sp2 | Subglobose | Yellow green | 209.79 | 1.2 | 66.60 | Grainy | 2 | - | - | - |
| 24 | *Ambispora callosa* | Subglobose | Yellow green | 119.88 | 1 | 73.26 | Grainy | 3 | 4 | - | - |
| 25 | *Cetraspora helvetica* | Globular | Yellow | 66.60 | 1.5 | 66.60 | Grainy | 1 | - | - | - |
| 26 | *Claroideoglomus etunicatum* | Globular | Beige | 119.88 | 1.2 | 99.90 | Smooth | 4 | 3 | 1 | |
| 27 | *Dentiscutata reticulata* | Globular | Beige | 103.23 | | | Grainy | - | - | 1 | - |
| 28 | *Diversispora epigea* | Globular | Beige | 103.23 | | | Grainy | 4 | - | - | - |
| 29 | *Diversispora omani* | Globular | Brown | 133.20 | | | Smooth | - | 1 | - | - |
| 30 | *Entrophospora infrequens* | Subglobose | Yellow | 103.23 | | | Grainy | 7 | 5 | 3 | - |
| 31 | *Entrophospora kentinensis* | Globular | Yellow | 93.24 | | 66.60 | Grainy | 6 | 5 | 1 | 1 |
| 32 | *Funneliformis caledonius* | Subglobose | Dark orange | 76.59 | 3 | | Smooth | - | 13 | - | - |
| 33 | *Funneliformis mossae* | Globular | Yellow | 99.90 | 1 | 3.33 | Grainy | - | 8 | - | - |
| 34 | *Gigaspora albida* | Globular | Orange | 153.18 | 1.2 | | Smooth | 4 | 3 | - | - |
| 35 | *Gigaspora margarita* | Globular | Dark yellow | 99.90 | 2.1 | 43.29 | Smooth | 5 | 4 | - | - |
| 36 | *Gigaspora* sp1 | Subglobose | Yellow Green | 113.22 | 1 | | Grainy | - | 3 | - | - |
| 37 | *Gigaspora* sp2 | Globular | Green | 166.50 | | | Grainy | 5 | - | - | - |
| 38 | *Glomus aggregatum* | Globular | Dark yellow | 99.90 | 2 | | Smooth | 5 | 4 | 3 | - |
| 39 | *Glomus albidum* | Globular | Dark yellow | 119.88 | 1.5 | | Grainy | - | 6 | - | - |
| 40 | *Glomus arenarium* | Subglobose | Brown | 193.14 | 1 | 33.30 | Smooth | - | - | - | 2 |
| 41 | *Glomus aureum* | Globular | Dark yellow | 86.58 | 1.2 | 39.96 | Smooth | - | 5 | - | - |
| 42 | *Glomus boreale* | Subglobose | Dark yellow | 159.84 | 2.3 | | Grainy | - | 7 | 4 | - |

| Number | Name | Form | Color | Spore size (µm) | Wall size (µm) | Hypha length | Spore surface | Number of spores per 100 g of soil in each region | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Had Kourt | Sidi Slimane | Kenitra | Allal Tazi |
| 43 | *Glomus botryoides* | Globular | Orange | 106.56 | 2.8 | 99.90 | Grainy | - | 1 | - | - |
| 44 | *Glomus caesaris* | Globular | Yellow | 109.89 | 1 | 3.33 | Smooth | 8 | - | - | - |
| 45 | *Glomus callosum* | Globular | Yellow | 106.56 | 1 | | Smooth | - | - | - | 1 |
| 46 | *Glomus clarum* | Globular | Yellow | 109.89 | | 99.90 | Grainy | 8 | 4 | 3 | 2 |
| 47 | *Glomus constrictum* | Globular | Orange | 139.86 | 3 | | Smooth | 4 | 3 | - | - |
| 48 | *Glomus coronatum* | Globular | Yellow | 113.22 | 1 | | Grainy | - | - | - | 3 |
| 49 | *Glomus deserticola* | Globular | Dark orange | 136.53 | 2.2 | | Grainy | 4 | 5 | 2 | - |
| 50 | *Glomus etunicatum* | Globular | Beige | 109.89 | 2 | | Grainy | 8 | 6 | - | 3 |
| 51 | *Glomus fasiculatum* | Globular | Yellow | 66.60 | 2 | | Grainy | 5 | - | - | - |
| 52 | *Glomus fecundisporum* | Globular | Beige | 99.90 | 0.1 | 43.29 | Grainy | 4 | - | - | - |
| 53 | *Glomus formasum* | Globular | Brown | 126.54 | 1 | | Grainy | 2 | - | - | - |
| 54 | *Glomus geosporum* | Globular | Dark orange | 119.88 | 2 | 116.55 | Smooth | 4 | - | - | 2 |
| 55 | *Glomus globiferum* | Globular | Dark orange | 103.23 | 1 | | Grainy | - | 5 | - | - |
| 56 | *Glomus heterosporum* | Globular | Yellow | 133.20 | 1 | | Smooth | - | 6 | - | 1 |
| 57 | *Glomus intraradices* | Oval | Yellow | 99.90 | 1 | 43.29 | Smooth | 7 | 5 | 2 | 8 |
| 58 | *Glomus lamellosum* | Globular | Green | 119.88 | 2.5 | | Grainy | - | - | 1 | - |
| 59 | *Glomus macrocarpum* | Globular | Brown | 106.56 | 1.5 | 33.30 | Smooth | - | 4 | - | 2 |
| 60 | *Glomus manihoti* | Globular | Orange | 119.88 | | | Grainy | 16 | - | - | - |
| 61 | *Glomus monosporum* | Globular | Dark yellow | 96.57 | 2 | | Grainy | 4 | - | 7 | - |
| 62 | *Glomus mossae* | Globular | Yellow | 99.90 | 1 | 33.3 | Grainy | 6 | 7 | - | 3 |
| 63 | *Glomus radiatus* | Globular | Orange | 133.20 | | | Grainy | 4 | - | 1 | - |
| 64 | *Glomus rubiformis* | Globular | Yellow | 163.17 | 2.3 | 119.88 | Smooth | - | - | 3 | -- |
| 65 | *Glomus* sp1 | Subglobose | Dark yellow | 206.48 | 1.2 | | Grainy | - | 5 | - | - |
| 66 | *Glomus* sp2 | Globular | Gray | 69. 93 | 0.1 | | Smooth | - | - | - | 2 |
| 67 | *Glomus tetrastratosum* | Globular | Dark yellow | 103.23 | 2 | | Grainy | - | 5 | - | - |
| 68 | *Glomus verruculosum* | Globular | Dark yellow | 99.90 | 2.1 | 43.29 | Smooth | 1 | - | - | - |
| 69 | *Glomus versiforme* | Oval | Yellow | 113.22 | 1 | | Grainy | 3 | 5 | - | 6 |
| 70 | Multicolored *Glomus* | Globular | Orange | 139.86 | 2.3 | | Smooth | 8 | - | - | - |
| 71 | *Pacispora boliviana* | Globular | Yellow | 119.88 | 1.5 | 3.33 | Grainy | _ | - | 3 | - |
| 72 | *Pacispora scintillans* | Globular | Yellow | 103.23 | 0.1 | 186.48 | Smooth | 4 | 4 | - | 2 |
| 73 | *Paraglomus pernambucanum* | Globular | Yellow | 156.51 | 1 | 33.30 | Grainy | - | - | 1 | 3 |
| 74 | *Rhizoglomus fasiculatum* | Subglobose | Orange | 113.22 | 1.2 | 76.59 | Smooth | - | 8 | - | - |
| 75 | *Scutellospora armeniaca* | Globular | Dark orange | 99.90 | 1.2 | 76.59 | Smooth | - | 3 | - | - |
| 76 | *Scutellospora biornata* | Globular | Beige | 99.90 | 1 | 200 | Grainy | 3 | 2 | - | - |
| 77 | *Scutellospora calospora* | Globular | Beige | 46.62 | 166.50 | | Smooth | 2 | - | - | - |
| 78 | *Scutellospora dipapillosa* | Subglobose | Yellow | 106.56 | 1 | 66.6 | Grainy | 3 | 2 | - | - |
| 79 | *Scutellospora nigra* | Globular | Black | 99.90 | | | Smooth | 6 | 4 | 1 | - |
| 80 | *Scutellospora pellucida* | Oval | Light yellow | 149.85 | 1 | | Grainy | 4 | - | 2 | 1 |
| 81 | *Scutellospora scutata* | Globular | Yellow green | 133.20 | 0.1 | 49.95 | Smooth | - | - | - | 2 |
| 82 | *Septoglomus constrictum* | Globular | Dark yellow | 99.90 | 1 | 76.59 | Smooth | 4 | - | - | - |
| 83 | *Septoglomus deserticola* | Globular | Dark yellow | 106.56 | 66.60 | | Smooth | 6 | - | - | - |

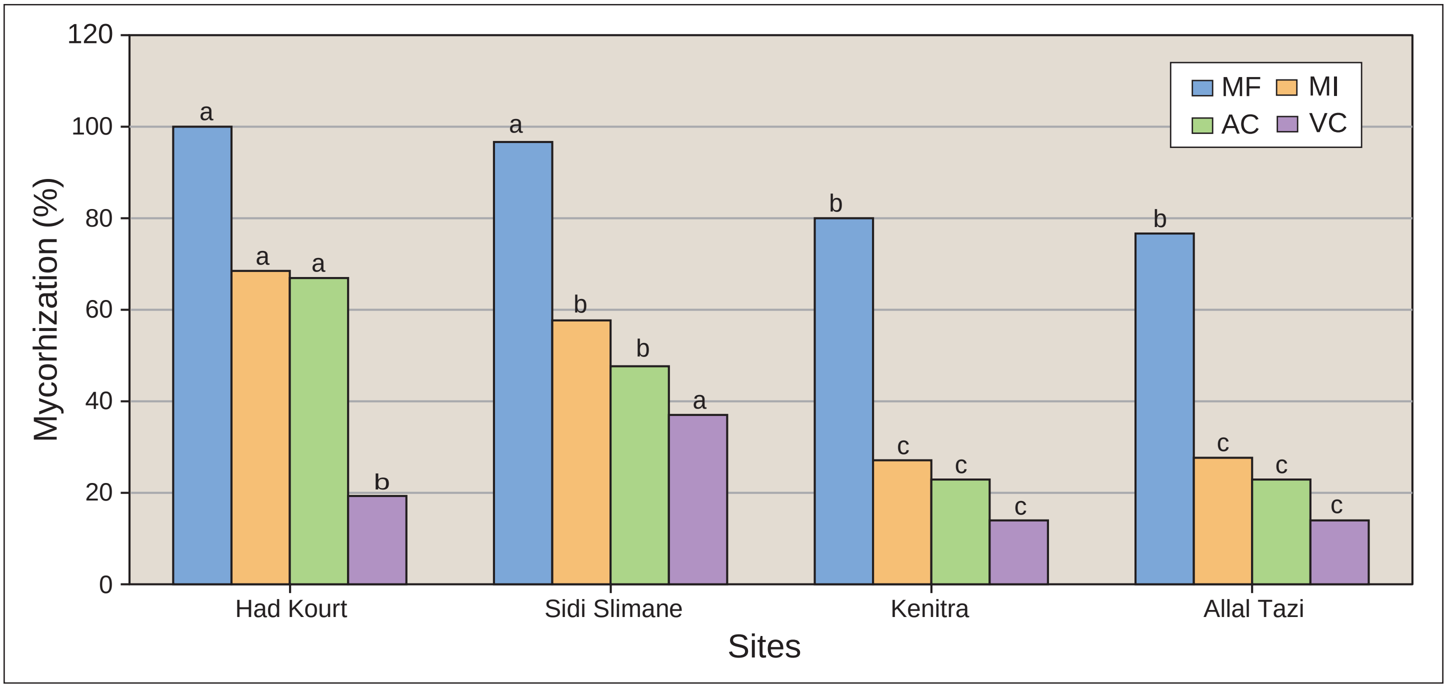


**Figure 4.** Mycorrhizal intensity (MI), mycorrhizal frequency (MF), arbuscular content (A), and vesicle content (V) varied among regions but were relatively high overall. For each variable, bars with the same letter are not significantly different at the 5 percent level.

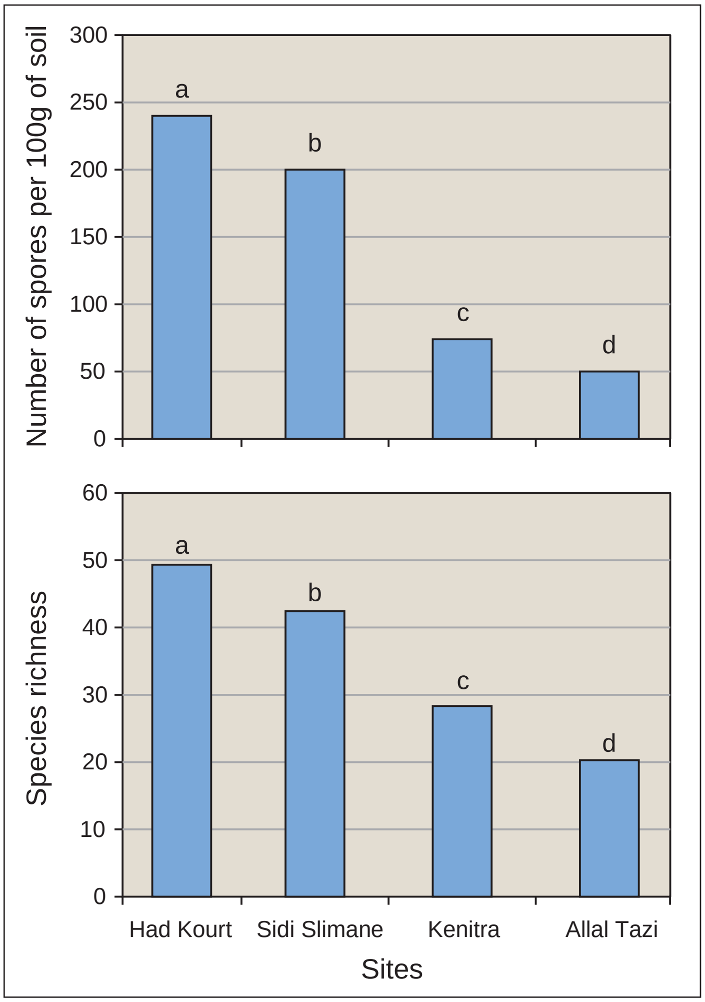
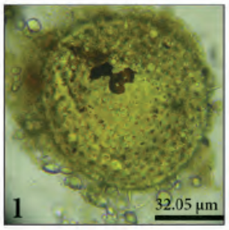


**Figure 5.** The (a) density of AMF spores and (b) species richness in the rhizosphere of *Casuarina* sp. differed significantly among all four sites.

al. 2009, Muleta et al. 2008, Tesfaye et al. 2004), Jordan (Mohammad et al. 2003), and several coastal dune areas (Bergen and Koske 1984, Hatimi and Tahrouch 2007, Giovannetti et al. 1983, Nicolson and Johnston 1979).

*Casuarina* mycorrhizae greatly improve plant growth and survival in difficult environments (Potgieter et al. 2014). Mycorrhizae have also been found to improve nutrient uptake (Abbott and Robson 1982) and to promote the symbiosis of *Frankia* in *Casuarina,* thereby increasing nitrogen fixation (He and Critchley 2008). This symbiosis also increases tolerance to drought (Abdelmoneim et al. 2013), flooding (Osundina 1997), acid soils (Diem et al. 2000), salt stress (Evelin et al. 2009), and disease (Akhtar and Siddiqui 2008, Liu et al. 2007). In a study on *Casuarina equisetifolia* L., a triple inoculation with endomycorrhizae, ectomycorrhizae, and *Frankia* significantly increased root and shoot AMF colonization (Elumalai and Raaman 2009).

Tacon et al. (1997) concluded that trees cannot survive without mycorrhizae in forest ecosystems. The interaction of the AMF and host plant must be both structurally and physiologically compatible. This compatibility depends on the host plant, mycorrhizal species, and environmental factors (Koïde and Scheiner 1992, Plenchette et al. 1983). AMF associations can also contribute to the maintenance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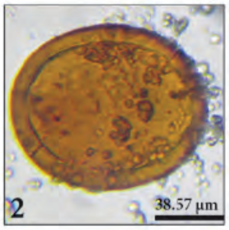
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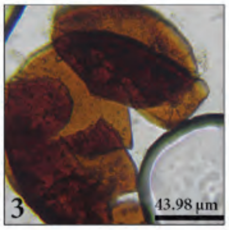
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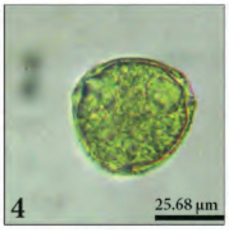
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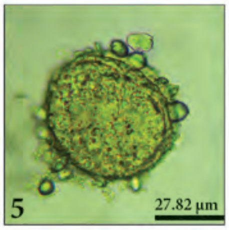
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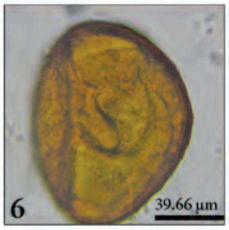
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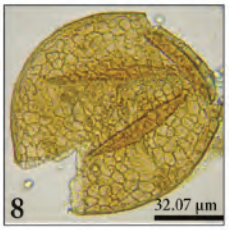
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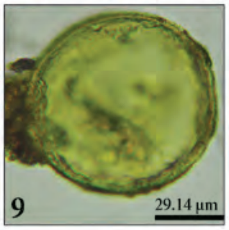
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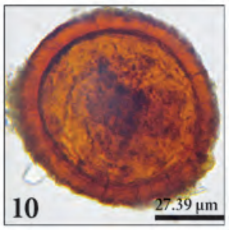
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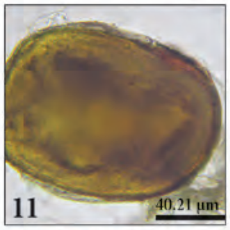
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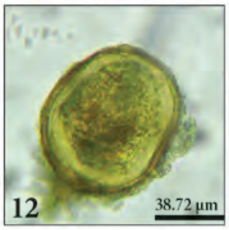
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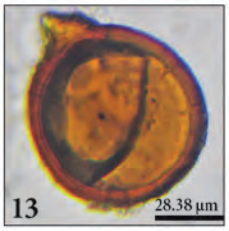
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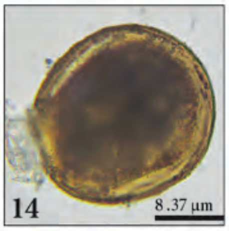
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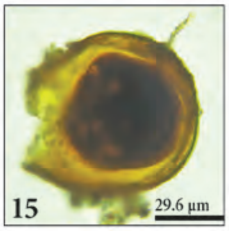
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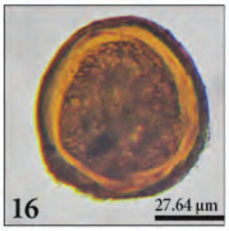
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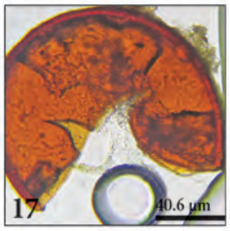
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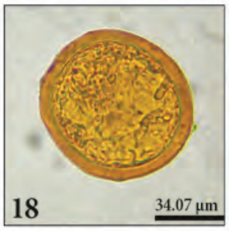
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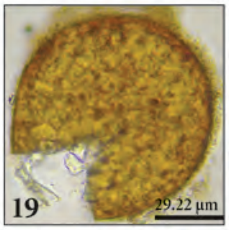
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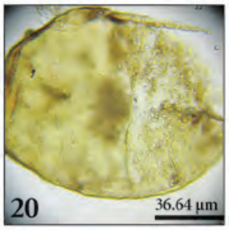
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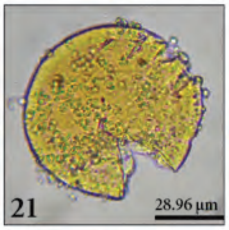
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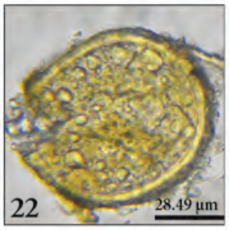
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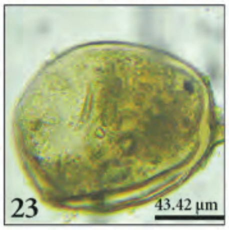
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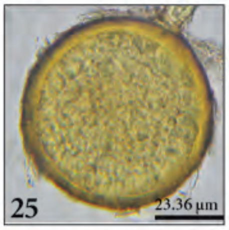
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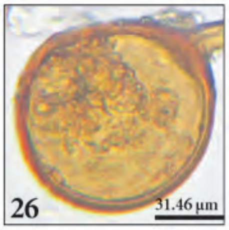
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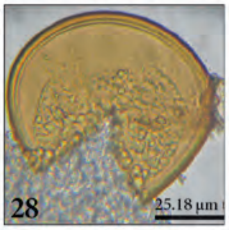
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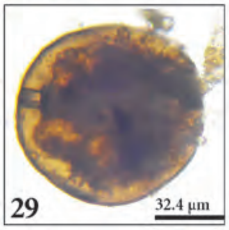
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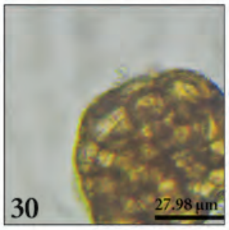
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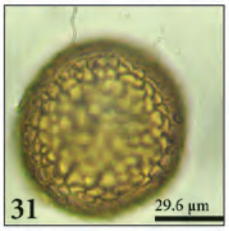
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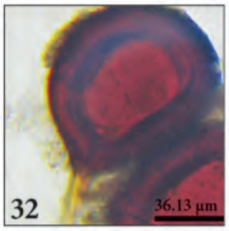
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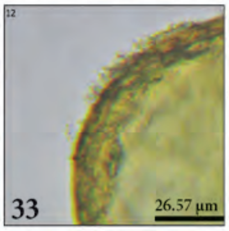
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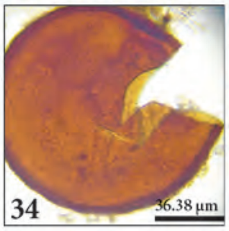
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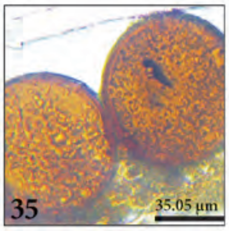
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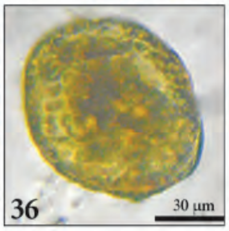
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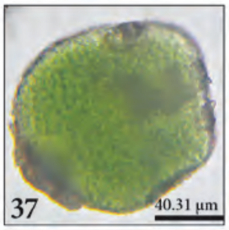
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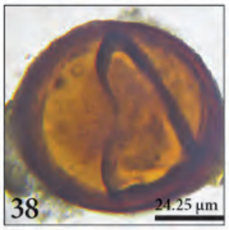
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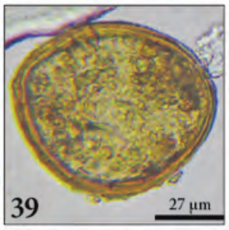
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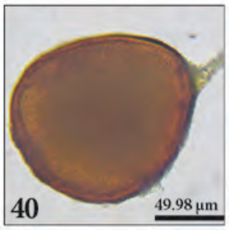
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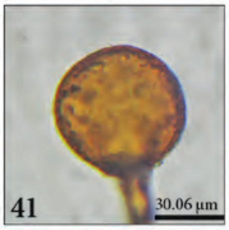
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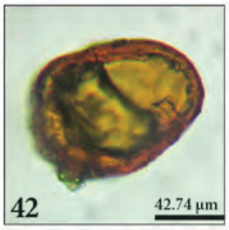
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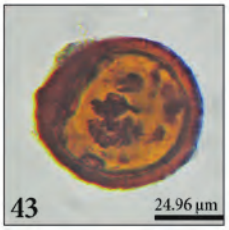
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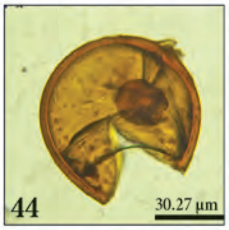
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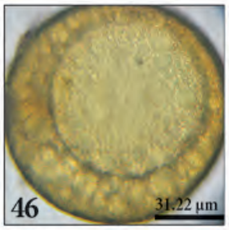
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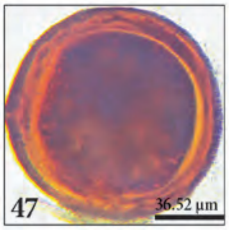
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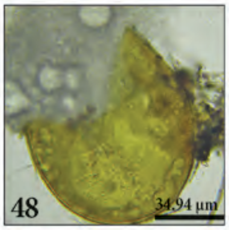
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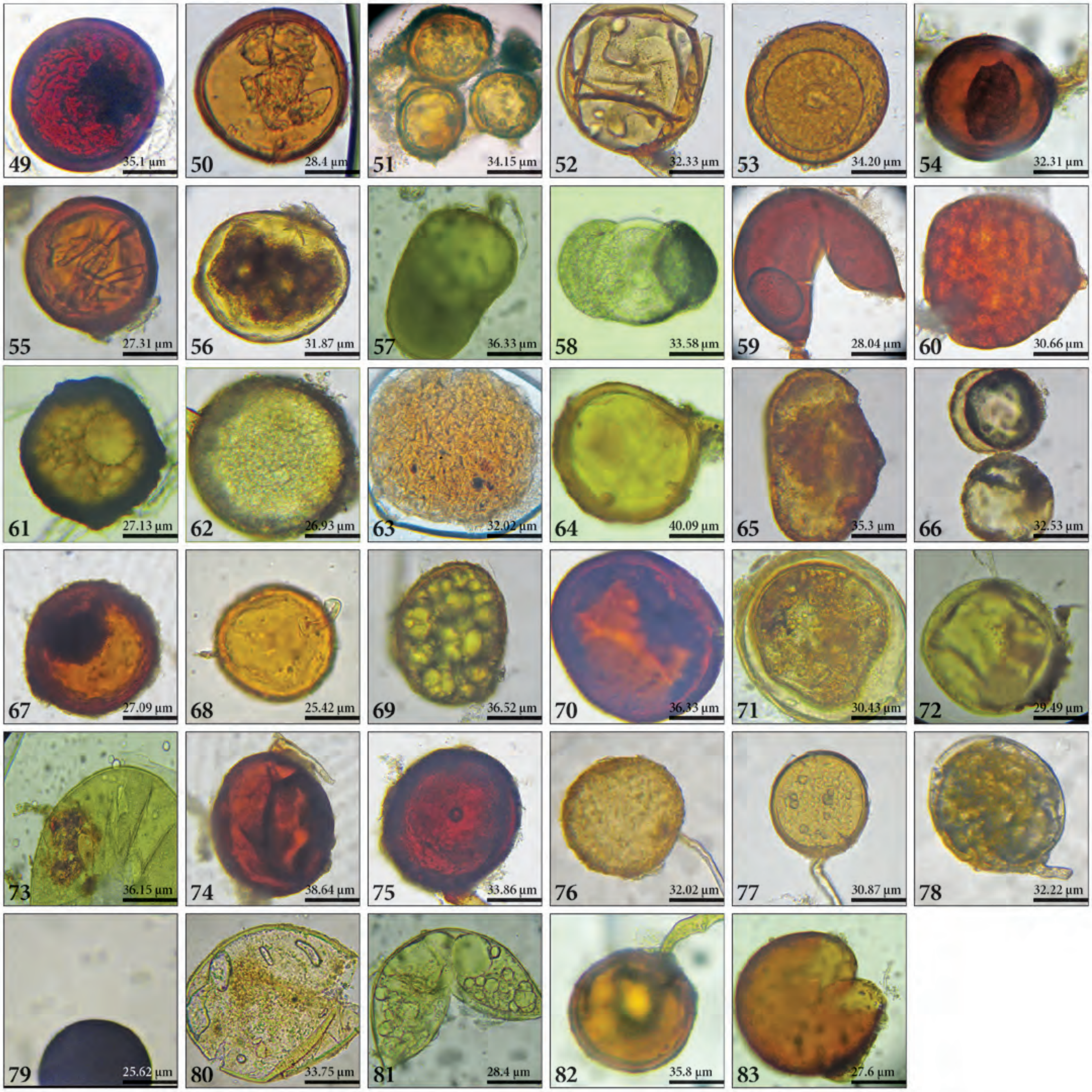

**Figure 6.** A total of 83 morphotypes of endomycorrhizal fungi were isolated from the rhizosphere of *Casuarina*. See table 1 for additional details. (Photos by N. Hibilik, 2015)

of plant biodiversity and thus have a positive impact on terrestrial ecosystems (Duponnois et al. 2013).

## Conclusion

This study demonstrates that *Casuarina* sp. is highly mycotrophic with a high diversity of AMF. This diversity enhances the capacity of trees to thrive in difficult environments by improving mineral nutrition, increasing tolerance to drought, floods, salt stress, and diseases. Thus, AMF inoculation has great potential for use in reforestation and restoration programs including growing *Casuarina* and other plants in the nursery and outplanting them to degraded ecosystems.

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