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SHORT COMMUNICATION

Nursery Culture Impacts Cold Hardiness in Longleaf Pine (*Pinus palustris*) Seedlings

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Abstract

Success in restoring longleaf pine ecosystems depends on outplanting high-quality longleaf pine seedlings. One important and relatively understudied attribute of seedling quality is cold hardiness. A suite of trials was conducted to investigate the influence of common nursery cultural practices on longleaf pine cold hardiness. Cold hardiness was increased with higher rates of nitrogen, unaffected by copper coating containers, greater for foliage than for root-collar tissue, and tended to increase with increases in container size.

Key words: freeze-induced electrolyte leakage, nursery production, seedling quality.

Introduction

Populations of longleaf pine (*Pinus palustris* Mill.), endemic to the southeastern United States, have been greatly reduced from historic levels because of extensive harvesting and conversion of land to agricultural uses. It is a critical component of current restoration programs because of a relatively high value and the habitat it provides for many threatened and endangered species (Outcalt 2000). Recent research in longleaf pine seedling production has focused on nursery techniques relating to morphology (Sword Sayer et al. 2009; Jackson et al. 2010), and Dumroese and Barnett (2004) identified a need to better understand seedling physiology. As using appropriate nursery stock is necessary to meet restoration objectives (Pinto et al. 2011; Tepe & Meretsky 2011) such information is in high demand.

Cold hardiness is the ability of a seedling to withstand exposure to freezing conditions. This is an important physiological component of seedling quality that has yet to be fully investigated (Tinus et al. 2002). Using the freeze-induced electrolyte leakage (FIEL; Burr et al. 1990) method to quantify cold-induced damage, Tinus et al. (2002) identified that the temperature at which 30% of maximum leakage occurred (LT_{30}) was the critical damage threshold for longleaf pine seedlings.

With the objective of determining the impact of three common nursery cultural practices (fertilization, copper-coated containers, and container size) on seedling LT_{30}, three studies were initiated. Tissue source (foliar and root-collar) also compared as the principal tissue in earlier work (Tinus et al. 2002), and foliage, which would be a faster and non-destructive sample.

Methods

Longleaf pine seedlings were grown in a fully controlled greenhouse in Moscow, Idaho. Containers (Table 1) were filled in mid-May with a 1:1 (v:v) sphagnum peat moss:vermiculite medium. Seeds from a Louisiana source (Louisiana Forest Seed Company, Woodworth, LA, U.S.A.) were sown and thinned to a single seedling per cavity after 3 weeks.

To test nitrogen rate, seedlings were grown in Superblock 412A containers. Four weeks after sowing and then once per week for 19 weeks (20 applications total), seedlings received one of the three N rates: 0.5, 2.0, or 4.0 mg N $seedling^{-1}$ $week^{-1}$ (hereafter mg N). A blended fertilizer was used to achieve these nutrient ratios: 100N ($50NO_3^-$:$50NH_4^+$):55P:41K:27Ca:16Mg:34S plus micronutrients (Peters Professional S.T.E.M., The Scotts Company, Marysville, OH, U.S.A.). Irrigation or fertigation (irrigation with soluble fertilizer added; applied by hand) frequency was determined gravimetrically and applied when container mass reached 75% of field capacity mass. Weekly calculations were made to supply the appropriate mg N and return the containers to field capacity.

To test effects of copper root pruning, seedlings were grown in either Superblock 412A or Copperblock 412A containers, identical except for a proprietary application of copper oxychloride to the surface of each cavity in the Copperblock. Seedlings were grown as described above at the 2 mg N rate. To test container size, seedlings were grown in Copperblock containers of all four sizes (Table 1) as described above at the 2 mg N rate.

In December, FIEL was measured on five seedlings randomly selected across replicates from each treatment in each

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Table 1. Characteristics of Superblock and Copperblock containers.

Container	*Density (Seedlings m^{-2})*	*Volume (cm^3)*	*Depth (cm)*	*Top Diameter (cm)*
615A	213	336	15.1	5.9
412A	364	125	11.6	4.2
412B	530	95	11.6	3.6
313A	936	60	13.2	2.8

Containers measure 35.2 cm wide by 60.0 cm long and are manufactured by Beaver Plastics, Ltd., Acheson, Alberta, Canada. Copperblock and Superblock containers are identical except Copperblocks have the interior surface of each cavity coated with a proprietary application of copper oxychloride.

experiment. From each seedling, five 1-cm sections were excised from secondary needles and randomly assigned to one of the five temperatures: 2 (control), −5, −10, −15, and −20°C. The copper experiment also included a 1-cm section of stem tissue excised at the root-collar. Tissues were placed in 20-mL copolymer polypropylene vials (RPI Corp., Mt. Prospect, IL, U.S.A.) containing 15 mL deionized water. Control vials were placed in a refrigerator. Remaining vials went into a programmable freezer within which air temperature was reduced by 0.25°C $minute^{-1}$ and held for 20 minutes at each test temperature before respective vials were removed for thawing inside the refrigerator. FIEL (total dissolved solids; ppm) was measured with a SevenEasy conductivity meter (Mettler Toledo, Columbus, OH, U.S.A.) and samples were then autoclaved for 20 minutes at 120°C (Market Forge Sterilmatic, Vernon Hills, IL, U.S.A.) before measuring maximum conductivity. For each of the five seedling replicates per treatment per experiment, FIEL at each temperature was regressed against test temperatures and the point of 30% damage calculated (LT_{30}).

Data conformed to analysis of variance (ANOVA) assumptions and were analyzed using PROC GLM in SAS version 9.2 software (SAS, Inc., Cary, NC, U.S.A.). Means among treatment levels were compared with pairwise t-tests.

Results

Nitrogen rate significantly affected cold hardiness ($p = 0.0048$, Fig. 1). Seedlings grown with 2 and 4 mg N were more hardy than those grown with 0.5 mg N. Cold hardiness of seedlings grown with ($LT_{30} = -12.58 \pm 1.39$°C; M ± SE) or without ($LT_{30} = -13.18 \pm 1.37$°C) copper was similar. Root-collar tissue ($LT_{30} = -10.40 \pm 1.50$°C) was significantly ($p = 0.0081$) more susceptible to cold than foliage ($LT_{30} = -15.36 \pm 0.45$°C). Container size significantly affected cold hardiness ($p = 0.0328$, Fig. 2). Seedlings in 412A containers were more cold hardy than those in the 313A. Cold hardiness of seedlings grown in the 615A and 412B containers did not differ from those grown in 412A and 313A containers.

Discussion

Longleaf pine seedling size increases as N rates increase from 0.5 to 4 mg N (Jackson et al. 2010). In this study, seedlings grown at the higher N rates were more cold hardy than those receiving the least N. Similarly, Bigras et al. (1996) found that *Picea mariana* [Mill.] B.S.P. seedlings with lower foliar N (as influenced by fertilizer regimes) were less cold hardy. Islam et al. (2009) demonstrated that fall fertilization of *Pinus resinosa* Ait. seedlings increased cold hardiness, indicating manipulation of N, particularly late in the growing season, may be a useful tool for adjusting cold hardiness.

Copper coating can yield superior root form (Sword Sayer et al. 2009) but did not affect cold hardiness, unsurprising given the more dominating effects of temperature, moisture, and photoperiod on cold hardiness (Jacobs et al. 2008). Thus, copper can be used to modify root systems without concern about ancillary negative impacts on cold hardiness.

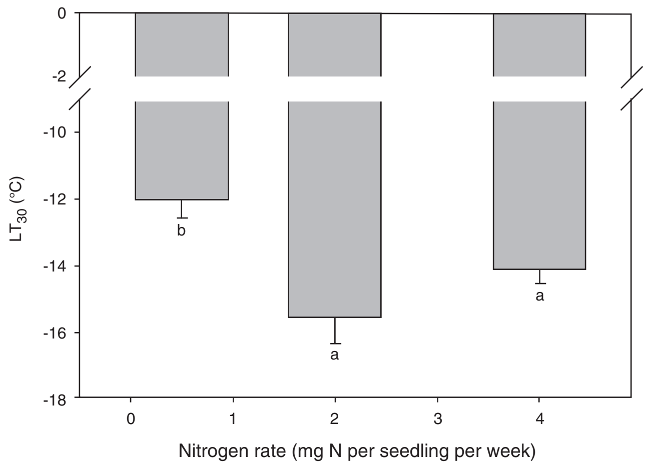

Figure 1. Cold hardiness (M ± SE) of needles from longleaf pine seedlings grown with different nitrogen application rates after 20 weeks of fertigation in a greenhouse. Different letters show significant differences at $\alpha = 0.05$.

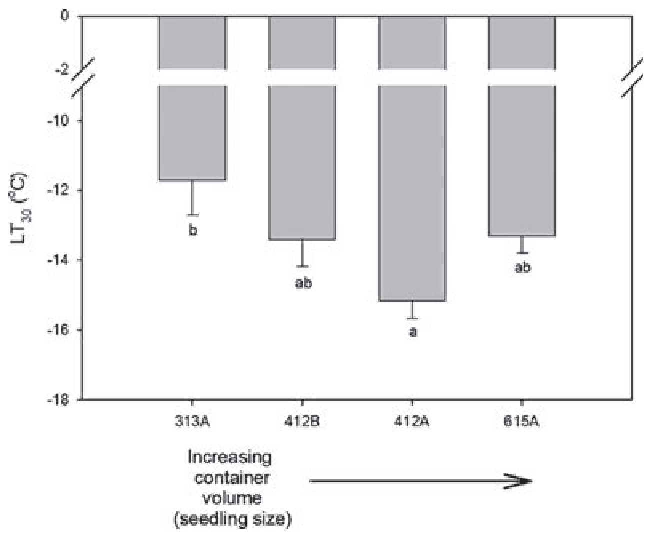

Figure 2. Cold hardiness (M ± SE) of needles from longleaf pine seedlings grown in four different containers. Different letters show significant differences at $\alpha = 0.05$.

Sword Sayer et al. (2009) showed that longleaf pine seedling size increases with cavity volume, a result echoed in this study (A.S. Davis 2007, University of Idaho, Moscow, ID, personal observation). Differences in initial seedling size can influence subsequent field performance (South et al. 2005). Timmis and Tanaka (1976) found that smaller Douglas-fir (*Pseudotsuga menziessii* Mirb.) seedlings were less cold hardy than larger ones. This study provided evidence in support of these findings, with seedlings in the 125 cm^3 cavities being more cold hardy than those grown in 60 cm^3 cavities. A larger sample size may have further clarified the relationship between seedling size and cold hardiness. Furthermore, observations indicate that seedlings in the largest container may have benefited from a longer growing period or more aggressive fertilization regime, both of which could have yielded larger seedlings and affected cold hardiness.

Longleaf pine root-collar tissue was more susceptible to cold than needle tissue. Root-collar tissue in this study was hardier than that reported by Tinus et al. (2002); they reported LT_{30} values between -5 and -7°C. Colombo et al. (1995) found that *P. mariana* root tissue was less cold hardy than shoot tissue, but the difference between the two tissue types followed a gradient rather than occurring at a discrete point (i.e. the root-collar). Additional studies should be implemented to identify seasonal variability in this relationship. Because cold hardiness is transient (Tinus et al. 2002; Jacobs et al. 2008), quantifying these temporal and spatial differences could allow rapid, non-destructive measurement of foliage which can provide nursery managers information on overall plant susceptibility to cold damage.

A broad array of non-tree seedlings is being successfully established as a means to initiate successful restoration of longleaf pine-wiregrass ecosystems (Aschenbach et al. 2009). Critical to continued success is outplanting high-quality longleaf pine seedlings that will survive and grow well (South et al. 2005). Nursery cultural practices can be optimized to yield seedlings designed to meet cold hardiness targets. Further research is needed to quantify the effect of such cultural practices on other physiological parameters.

Implications for Practice

- Seedling users can manipulate cold hardiness through nitrogen fertilization.
- Smaller seedlings may not be as cold hardy as larger ones.
- Cold hardiness can be tested non-destructively using foliage but values differ from root-collar tissue.

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