Auburn University
Southern Forest Nursery Management Cooperative

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WARM NIGHTTIME TEMPERATURES AFFECT THE ABILITY OF LOBLOLLY PINE (*PINUS TAEDA*) SEEDLINGS TO TOLERATE FREEZING

by
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INTRODUCTION

Nursery seedlings that undergo a hard freeze might result in poor outplanting performance if the roots have been injured by a freeze (Carlson 1985; Lantz 1985; Rowan 1985; Cameron RA and Lowerts GA 2007). In some cases, injury from a freeze will be increased if seedlings are grown at high seedbed densities (Dierauf and Olinger 1979). In contrast, top-pruning can increase freeze tolerance (South et al. 1993). Freeze injury is also affected by genotype (Kolb et al. 1985; Hodge and Weir 1993; Duncan et al. 1996; Kegley 1999). Therefore, when grown at the same location, Coastal Plain sources will be injured while Piedmont sources are relatively unaffected. There have been many reports where a hard freeze has injured pines (South 2006; 2007). It appears that freeze injury has become a frequent event over the past 2 decades. In some cases, deacclimation has occurred due to warm weather events and this increases the risk of freeze injury. Unfortunately, deacclimation of loblolly pine seedlings is often overlooked, in part because we have no guidelines that alert nursery managers to potential deacclimation events. Only a few research studies have addressed the deacclimation of pines with most of the research from more northern latitudes (Burr et al. 1990; Jokela et al. 1998; Ryyppö et al. 1998a; 1998b).

Laboratory tests that measure frost-induced electrolyte leakage (FIEL) have been developed to estimate the cold hardiness of conifers (Burr et al. 1990). Electrolytes leak from membranes when an environmental stress such as a freeze occurs. Needles, roots and stems have been used to measure electrolyte leakage and to estimate the resistance of conifers to freezing temperatures.

The FIEL test can also be used to evaluate the amount of deacclimation that may have occurred. The objective of this study was to determine if warm nighttime temperatures will deacclimate loblolly pine seedlings.

METHODOLOGY

Seed from loblolly pine (family 7-56) were sown at nurseries in Tennessee and Virginia. The seedlings were cultured using standard nursery practices. Multiple top-prunings were made at the VA nursery while seedlings were not top-pruned at the TN nursery. After natural chilling had occurred, two heated plastic tents were erected over the seedlings. Within each tent, heat was provided during eight nights using a propane gas heater. After 2, 4, 6 and 8 days of the study, seedling samples were collected from under each tent and samples were collected from beds that were outside of the tent. Seedlings were then sent to Purdue University for electrolytes leakage evaluation.

At Purdue, all seedling samples were subjected to 0°C, -2°C, -5°C, -8°C and -11°C treatments. A frost-induced electrolyte leakage (FIEL) test (Burr et al. 1990) was used to estimate cold hardiness. Needles and root system were rinsed with distilled water to remove ions from the surface. For each treatment (TN-outside, TN-tent, VA-outside and VA-tent) needles from 10 seedlings were cut into approximately 1-cm segments. This process was also followed for 1-cm root samples. From each experimental unit, 5 segments of roots and needles were placed into 5 separate vials (each vial for corresponding test temperatures; that is, 0°C, -2°C, -5°C, -8°C and -11°C. To avoid excessive super cooling during freezing, only 1 ml of deionized water was added to the vial prior to the freezing tests.

The vials containing needle and root samples were then placed in a programmable freezer (Ultra-Low Freezer; CH40-13, SO-LOW Environmental Equipment, Cincinnati, OH, USA) for 1 h at 0 °C, after which the control treatment vials were removed. The temperature was then decreased gradually at a rate of approximately -3 °C/h. Upon reaching -2 °C, the vials were briefly shaken by hand to help nucleate and freeze the water at as low of a temperature as possible. Upon reaching each successive test temperature, the temperature was held for 30 min before decreasing again. Once all the vials were removed and thawed, which was usually done on the next day, 9 ml of deionized water was added to each vial. The measurement of electrical conductivity was made using an EC/TDS meter (Seven Easy, Mettler Toledo, SevenEasy Co. Mettler Toledo GmbH, Switzerland). After the initial measurements were taken, the vials and their contents were autoclaved at 121 °C for 20 min. After cooling overnight at room temperature, the second measurement was taken which represented total electrolytes. Electrolyte leakage is expressed as percentage of the total electrolytes.

Following the fourth sampling period (on Day 8), additional seedling samples were transported to Auburn University and subjected to two -5° and -10°C using a freezer. Seedlings were maintained at these temperatures for two hours and then removed from the freezer. Seedlings were allowed to thaw and then were outplanted into sand at the Nursery Cooperative seedling testing facility.

RESULTS

At both nurseries, the pattern of chilling accumulation was similar (Figure 1). By New Year's Day, there were about 254 h of chilling at Chattanooga, TN and 183 h at Franklin, VA. By the beginning of the test (VA January 23; TN January 29), there were about 533 and 379 chilling hours, respectively. By the end of the test (VA February 1; TN February 7), there were about 673 and 599 chilling hours, respectively.

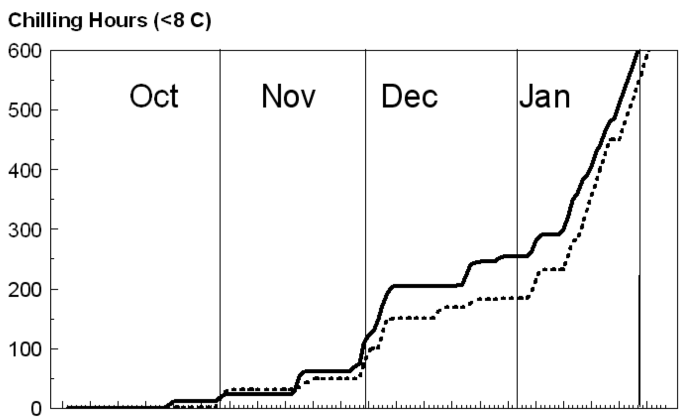

Figure 1. Estimated chilling hours (<8°C) for Chattanooga, TN (solid line) and Franklin, VA (dashed line) for 2006-07.

Temperatures in tents

The minimum and maximum temperature under the heated tent and the outside minimum temperature for the TN nursery are shown in Figure 2. The four sampling dates were Jan. 30, Feb. 1, 4, and 6, 2007. There were initial problems with the heaters running on Jan. 30 and 31, so the minimium temperatures for these dates were about the same as the outside. During this cold period, the temperatures outside were below freezing during most mornings (execpt for February 2 and 7). Temperatures inside the tent fell below freezing on four days (Jan 31, Feb 3, 5, and 6). When considering only the "nightime" (from 7PM to 7AM), the heat treatments resulted in 26 to 29 h of temperatures above 10°C (Table 1).

Table 1. Hours of cold and warm temperatures from 7PM to 7AM at the at Tennessee and Virginia nurseries during the test period.

Nursery	**Location**	**< 0°C**	**0-8°C**	**> 8°C**	**> 10°C**
Tennessee	outside	68	33	7	0
	tent	17	45	46	29
Virginia	outside	76	12	8	0
	tent	21	42	33	26

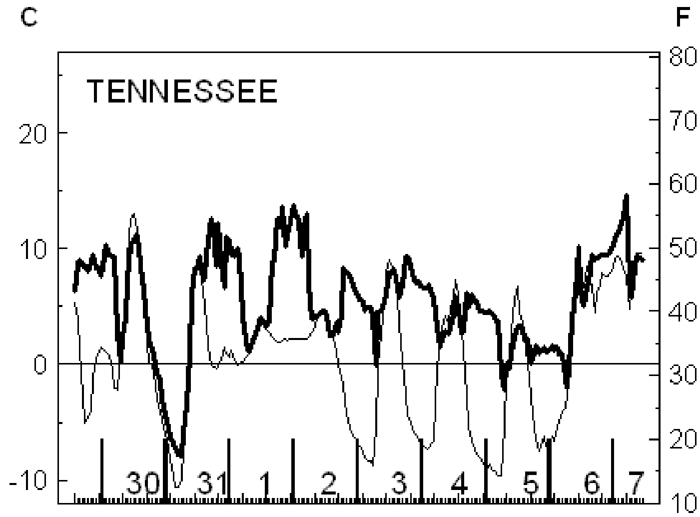

Figure 2. Temperatures at Tennessee nursery during the test period. The dark, thick line represents temperature under the tents while the thin line represents temperature outside the tent.

The maximum temperatures under the tent were slightly higher at the VA nursery (Figure 3). However, the minimum temperatures outside the tent fell below freezing on all nine days. Minimum temperatures inside the tent fell below freezing on six days.

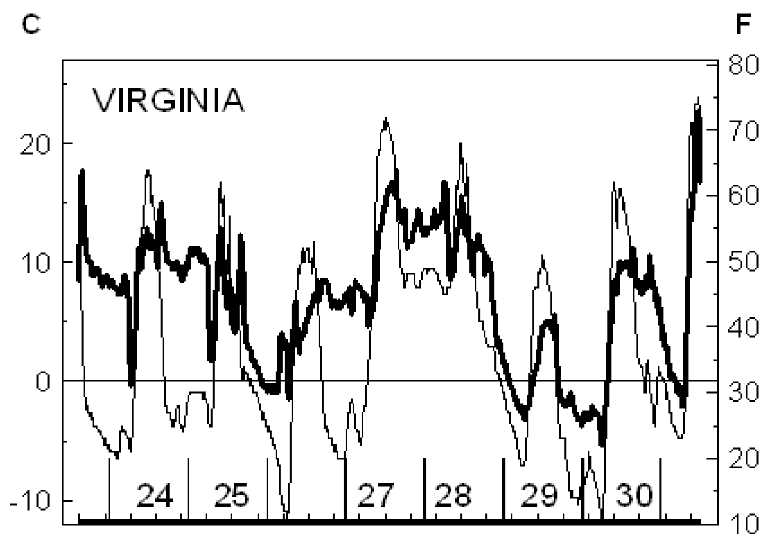

Figure 3. Temperatures at the Virginia nursery during test period. The dark thick line represents temperature under the tents while the thin line represents temperature outside.

Electrolyte leakage

In general, seedling shoots are more tolerant of freezing temperatures than roots. As a result, pine roots at both nurseries had more electrolyte leakage than the shoots (Figure 4). This is consistent with other reports that indicate that electrolyte leakage is higher at the root-collar than at a height of 15 cm above the root-collar (South et al. 1993).

In general, electrolyte leakage did not increase unless temperatures were lower than -5°C. Temperatures of either -8° or -11°C were low enough to increase the amount of leakage. Therefore, the -8° and -11°C temperatures were used to examine the effect of treatment (i.e. tent+heat) on electrolyte leakage of roots (Figure 5). The heat treatment clearly had an effect at the TN nursery (P=0.009) but had no effect at the VA nursery (p> F = 0.759).

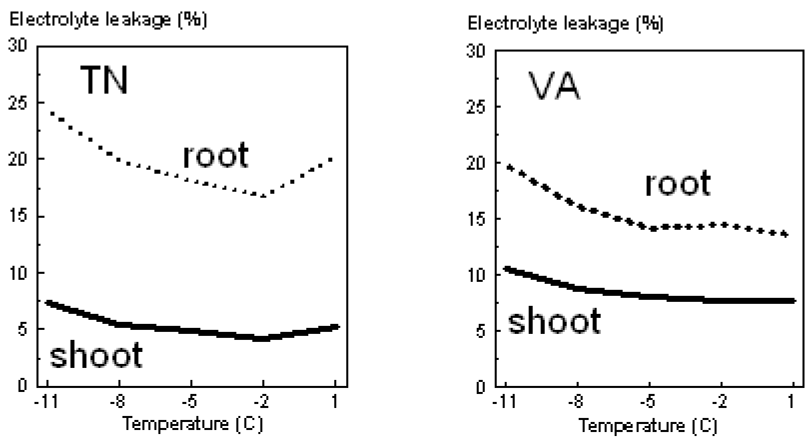

Figure 4. Percent electrolyte leakage as affected by tissue type and test temperature.

At the VA Nursery, seedlings tested on Day 8 had less electrolyte leakage than seedlings tested on Day 2 (P=0.002). In contrast, seedlings inside tents in TN had more electrolyte leakage over time as the test progressed.

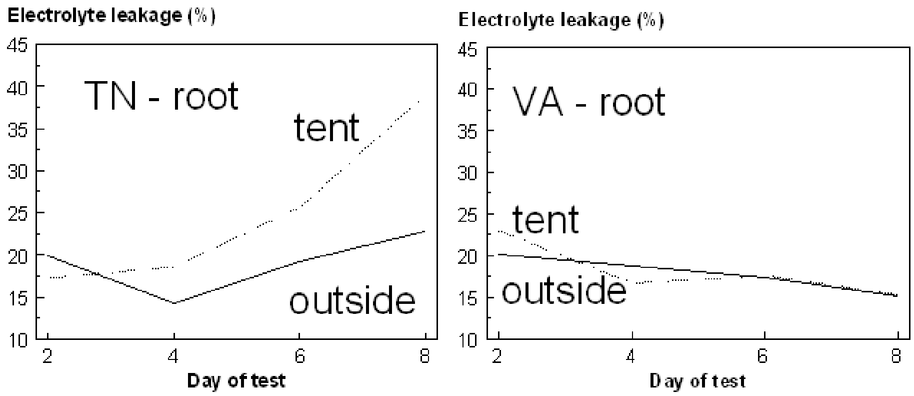

Figure 5. Percent electrolyte leakage of roots (using -8° and -11°C) as affected by treatment and test temperature. The tent+heat treatment made a difference at the TN nursery.

Seedling survival

Seedlings collected from both nurseries and planted in the seedling stress facility (without freezing) exhibited higher mortality in the sand pits than seedlings artificially frozen at either -5°C or -10°C. The reason for the greater mortality from the control seedlings is not known. Therefore, survival data were analyzed using only results from seedlings that were placed in the freezer. The analysis showed no difference between nurseries (P>F = 0.880) and no difference between the two freezing temperatures (P>F = 0.605). However, the tent+heat treatment resulted in an increase in seedling mortality (Table 2). There was no treatment by nursery interaction (P >F = 0.357).

Note the color difference between 7-56 (foreground) and TN sources (background).

Note the color difference between 7-56 (left bed) and VA sources (right bed).

Table 2. Effect of nighttime heat treatment (8 days) on seedling mortality due to artificial freezing (mean of -5° and -10°C) and electrolyte leakage (mean of -8° and -11°C).

		Electrolyte leakage	
Location	Seedling mortality	Roots	shoots
TN-outside	17%	22.7%	9.0%
TN-tent	29%	39.0%	6.0%
VA-outside	11%	15.2%	8.3%
VA-tent	34 %	15.3%	11.3%

DISCUSSION

There was no difference between the two nurseries with respect to the ability of seedlings to survive an artificial freeze. Mortality of seedlings grown outside ranged from 11% (VA) to 17% (TN). In both cases, the temperature required to kill 20% of the population was below -10°C.

The data above indicate that when acclimated seedlings are lifted during the first week of February (at about 600 chilling hours), more than 80% of the seedlings grown in either TN or VA (in USDA hardiness zone 7) can survive a short-term artificial freeze event. In fact, prior to the final lift date, seedlings grown outside of the tents in VA had also been exposed to a short-term natural freeze event of -10°C.

The objective of this study was to determine how many warm nights are required to deacclimate loblolly pine family 7-56. Results from the whole-plant freezing test (Table 2) indicate that 8 warm nights (perhaps only 5 warm nights) resulted in enough deacclimation to make seedlings more susceptible a hard freeze. It might take only 50 h of warm nighttime temperatures (> 8°C) to result in deacclimation (Table 1). Results from the electrolyte leakage test using TN seedlings suggest it may only take 3 nights of warm weather to begin to deacclimate seedlings.

When the relationship between electrolyte leakage and outplanting survival of TN seedlings was examined, mortality was greater for the tent+heat treatment and electrolyte leakage of roots was greater (Table 2). However, for VA seedlings, electrolyte leakage was not affected by the tent+heat treatment. The lack of correlation between seedling mortality (after freezing the whole seedling) and electrolyte leakage might be due to the variability associated with the whole-plant freezing test. According to Burr et al. (1990), a disadvantage of the whole plant freeze test is poor precision associated with small samples.

The study was installed in VA and TN to ensure that seedlings would be acclimated to cold temperature by the end of January. However, it was unfortunate that during the test period, the weather was among the coldest of the season. Preliminary test results at Auburn indicate the heaters could elevate nighttime temperature in the tent about 22°C above ambient. However, when the outside temperatures fell to -10°C, the temperatures in the tent were barely above freezing. Therefore, although deacclimation occurred inside the heated tent, the nighttime temperature was not as warm as we had hoped. At both nurseries, there was less than 30 h of nighttime temperatures above 10°C (Table 1). This might help explain why electrolyte leakage of roots from VA did not increase over time (Figure 5).

MANAGEMENT IMPLICATIONS

To reduce the risk to freeze injury early in the lifting season, nursery managers will sometimes irrigate during the freeze event or will place covers over the seedlings. At some container-nurseries, heat is sometimes applied to keep seedling roots from freezing. However, once seedlings have received a sufficient amount of chilling, many managers assume seedlings will be tolerant of hard freeze events in January or February. However, freeze injury of Coastal Plain sources can result during these months if seedlings have deacclimated due to several nights of unusually warm temperatures. The data from these studies are not definitive, but they indicate that seedlings of loblolly pine family 7-56 can deacclimate to some degree with as little as 3-to-7 warm nights.

ACKNOWLEDGEMENT

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