

RESEARCH REPORT 07-05

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

by

David B. South, Tom Starkey and M. Anisul Islam

INTRODUCTION

Seedlings that undergo a hard freeze while in the nursery will sometimes result in poor performance after outplanting if the roots have been injured by a freeze (Carlson 1985; Lantz 1985; Rowan 1985; Cameron and Lowerts 2007). In some cases, freeze injury will be affected by seedbed density (Dierauf and Olinger 1979) and top-pruning (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, Coastal Plain sources will be injured in the nursery while Piedmont sources are relatively unaffected. There have been cases of freeze injury to pines throughout the 20th century (South 2006). It seems that seedling injury to freeze has become a frequent event over the past two 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. Most of this type of research has been done for more northern species (Burr et al. 1990; Jokela et al. 1998; Ryyppö et al. 1998a; 1998b).

Laboratory tests that measure electrolyte leakage (EL) 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 by researchers to measure electrolyte leakage and to estimate the resistance of conifers to freezing

temperatures. The EL test can be used to evaluate the amount of deacclimation. The objective of this study was to determine if warm nighttime temperatures will deacclimate loblolly pine seedlings.

METHODOLOGY

Seed from family 7-56 were sown at two 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. Heat was provided at night using a propane gas heater. After 2, 4, 6 and 8 days, seedling samples were collected from under each tent and samples were collected from beds that were outside of the tent. Seedlings were sent to Purdue University for electrolyte leakage evaluation. At Purdue, seedlings were subjected to 1°C, -2°C, -5°C, -8°C and -11°C and the electrolyte leakage () was recorded. Following the fourth sampling period, the seedlings were transported to Auburn University and were subjected to two freezing temperatures (-5° and -10°C). Seedlings were kept at these temperatures for two hours and then they were removed from the freezer. Seedlings were allowed to thaw and then were outplanted at the Coop stress facility.

RESULTS

At both nurseries, the pattern of chilling accumulation was similar (Figure 1). By New Year's Day, there were about 254 hours of chilling at Chattanooga, TN and 183 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.

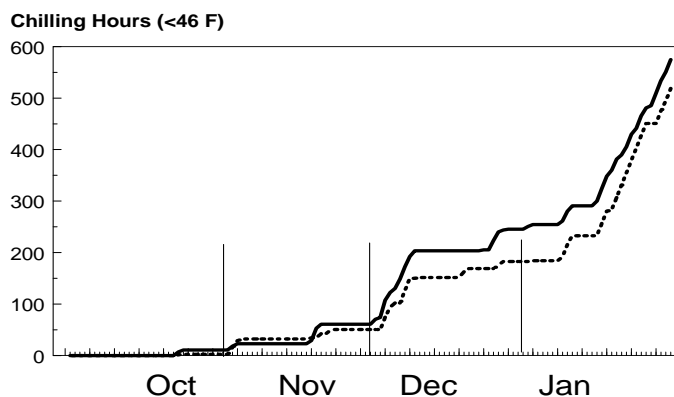


Figure 1. Estimated chilling hours (<8°C) for Chattanooga, TN (solid line) and Franklin, VA (dashed line) for 2006-07.

Temperatures in tents

The minimum and maximum temperature under the tent and the outside minimum temperature are shown in Figure 2 for the TN nursery. The four sampling dates were on Jan. 30, Feb. 1, 4, and 6. We had some initial problems with keeping the heaters running on Jan. 30 and 31 so the minimum temperatures were about the same as for the outside. During this cold period, the temperatures outside were below freezing during most mornings (except for February 2 and 7). Temperatures inside the tent fell below freezing on four days (Jan. 31, Feb. 3, 5, and 6).

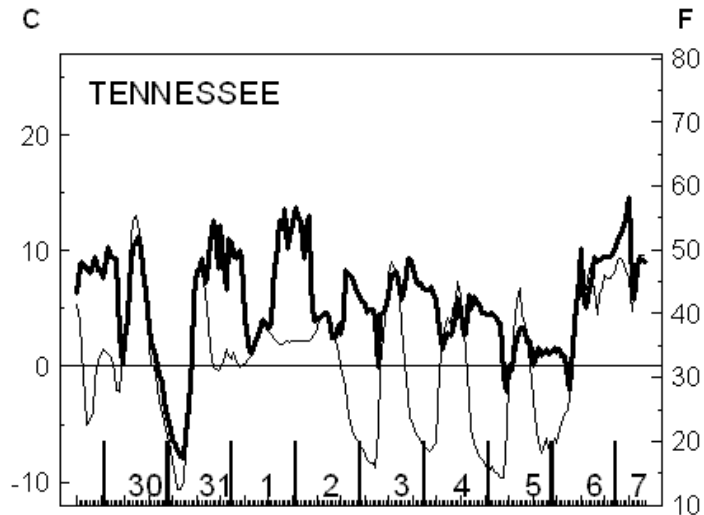


Figure 2. Temperatures at Tennessee nursery during the test period. The dark, thick line represents temperature under the tents while the thin line represents temperature outside.

The 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 in the tent fell below freezing on six days.

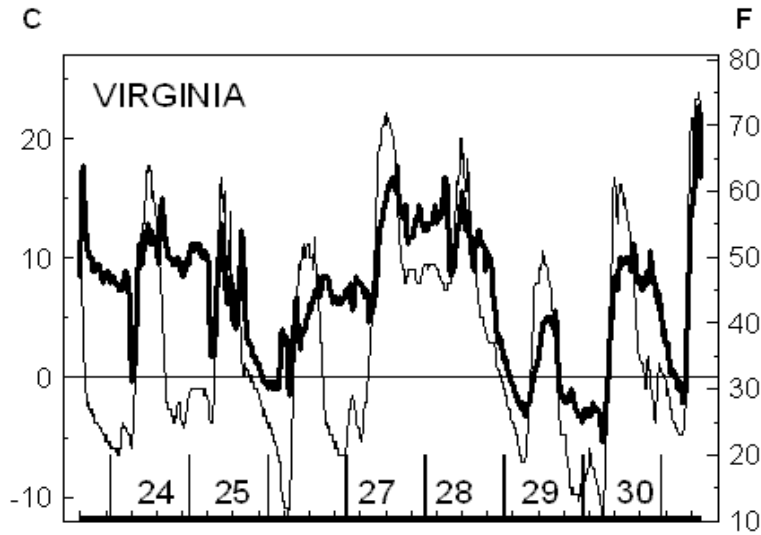


Figure 3. Temperatures at the Virginia nursery during test period. The dark, thick line represents temperature under the tents while the thin line represents temperature outside.

Electrolyte leakage

In general, shoots are more tolerant of freezing temperatures than roots. As a result, the 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 (South et al. 1993). This indicates that cold weather acclimation that occurs in the shoots is not translocated to the roots.

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 rate of leakage. Therefore, the -8° and -11°C temperature 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 ($p > F = 0.759$).

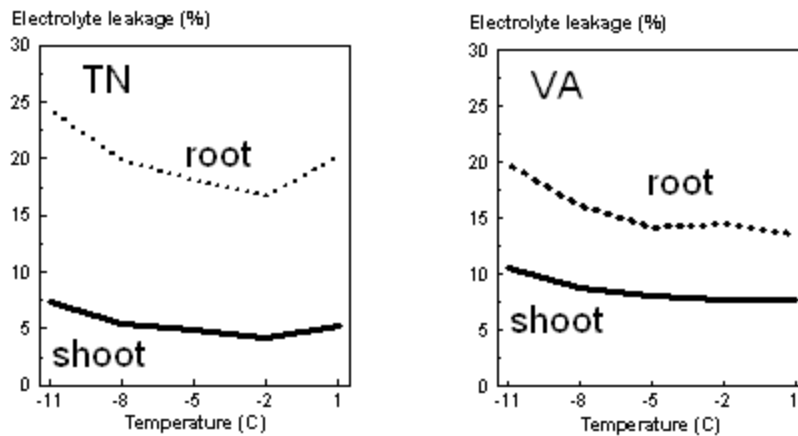


Figure 4. Percentage electrolyte leakage as affected by tissue type and test temperature. At the VA Nursery, the cold weather during the test appeared to reduce the amount of electrolyte leakage, both inside and outside of the tents (Figure 5). Seedlings tested on day 8 had less electrolyte leakage than seedlings tested on day 2. In contrast, seedlings inside of the tents in TN had more electrolyte leakage as the test progressed.

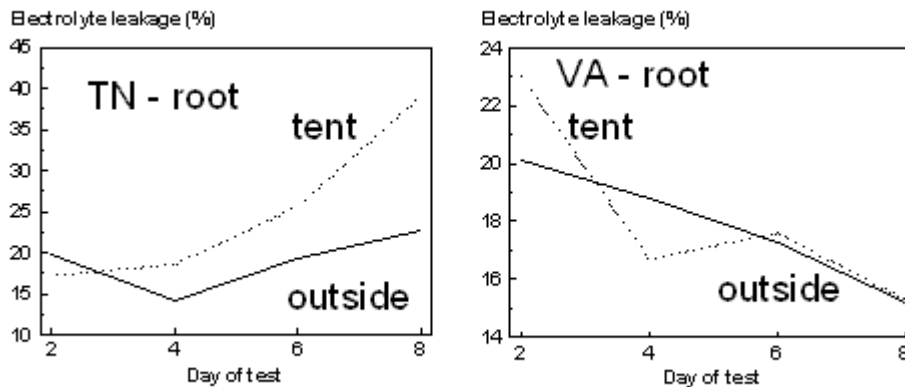


Figure 5. Percentage electrolyte leakage of roots (using -8° and -11°C) as affected by treatment and test temperature. The tent-heat treatment made a difference at the TN nursery.

Seedling survival

Seedlings that were not placed in the freezer exhibited higher mortality in the stress pits than seedlings frozen at either -5°C or -10°C . The reason for the greater mortality 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 (tent = 31% mortality vs. 14% mortality for outside seedlings). 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).

DISCUSSION

In regards to survival after artificial freezing, there was no difference between the two nurseries ($P > F = 0.879$). Mortality of seedlings grown outside was 11% for seedlings from VA and 17% for seedling from TN (Table 1). In both cases, the LT_{20} (i.e. temperature required to kill 20% of the population) was colder than -10°C . This indicates 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 USDA hardiness zone 7 can survive a short-term artificial freeze event. Prior to the final lift date, seedlings grown outside of the tents in VA had 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 7-56. From the whole-plant freezing test, 8 warm nights (or less) resulted in enough deacclimation to make seedlings more susceptible to an artificial freeze test. Results from the electrolyte leakage test using TN seedlings suggest it may only take 3 nights of warm weather to begin to deacclimate seedlings.

We examined the relationship between electrolyte leakage and survival. For the TN seedlings, mortality was greater for the tent/heat treatment and leakage of roots was greater than that for shoots (Table 1). However, for VA seedlings, the electrolyte leakage was not affected by the tent/heat treatment. The lack of correlation between seedling mortality 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 freezing test is poor precision associated with small samples.

Table 1. Effect of nighttime heat treatment (8 days) on seedling mortality due to artificial freezing at -5° and -10°C and electrolyte leakage at -8° and -11°C.

Location	Seedling mortality	Electrolyte leakage roots	Electrolyte leakage 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%

The study was installed in VA and TN in hopes that seedlings grown in cooler nurseries would be acclimated to cold temperatures by the end of January. However, it was somewhat unfortunate that during the test period, the weather was among the coldest of the season. Preliminary results at Auburn indicated the heaters could elevate nighttime temperatures 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 the tent-heat treatment did cause deacclimation, the nighttime temperature was not as warm as we had hoped. This might help explain why electrolyte leakage of roots from VA appeared to decline over time (Figure 5) instead of increase.

MANAGEMENT IMPLICATIONS

To reduce the risk of freeze injury early in the lifting season, nursery managers will sometimes irrigate during the freeze event or 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. Still, 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 suggest that seedlings of family 7-56 can deacclimate to some degree with as little as 3-to-7 warm nights.

ACKNOWLEDGEMENT

This research was funded by the USDA Forest Service State and Private Forestry under terms of the Forest and Rangeland Renewable Resources Research Act of 1978. Many thanks go to Dwight Stallard of the Garland Gray Forestry Center and John Conn of the East Tennessee Nursery for the collecting and shipping of seedlings.

LITERATURE CITED

Burr KE, Tinus RW, Wallner SJ and King RM (1990) Comparison of three cold hardiness tests for conifer seedlings. *Tree Physiology* 6(4):351-69

Carlson WC (1985) Cold damage to *P. taeda* seedlings. In: CW Lantz (Tech. coord.) Proceedings of the 1984 Southern Nursery Conferences. *United States Department of Agriculture Forest Service*, pp. 1-21

Cameron RA and Lowerts GA (2007) A new visual technique for diagnosing cold damage in stems of bareroot loblolly pine seedlings. *Auburn University Southern Forest Nursery Management Cooperative Technical Note* 07-01, 7 pp

Dierauf T and Olinger HL (1977) January 1977 cold damage to loblolly seedlings at New Kent Nursery. *Virginia Division of Forestry Occasional Report* 51, 4 pp

Duncan PD, White TL and Hodge GR (1996) First-year freeze hardiness of pure species and hybrid taxa of *Pinus elliottii* (Engelmann) and *Pinus caribaea* (Morelet). *New Forests* 12:223-241

Hodge GR and Weir RJ (1993) Freezing stress tolerance of hardy and tender families of loblolly pine. *Canadian Journal of Forest Research* 23:1892-1899

Jokela A, Sarjala T and Huttunen S (1998) The structure and hardening status of Scots pine needles at different potassium availability levels. *Trees structure and Function* 12:490-498.

Kegley AJ (1999) Evaluation of Atlantic Coastal and Piedmont sources of loblolly pine (*Pinus taeda* L.) seedlings and their hybrids for growth and cold hardiness. MSc thesis, North Carolina State University, Raleigh, North Carolina, 74 pp

Kolb TE, Steiner KC and Barbour HF (1985) Seasonal and genetic variations in loblolly pine cold tolerance. *Forest Science* 31:926-932

Lantz CW (1985) Freeze damage to southern pine seedlings in the nursery. In: CW Lantz (Tech. coord.) Proceedings of the 1984 Southern Nursery Conferences. *United States Department of Agriculture Forest Service*, pp. 20-29

Mexal JG, Timmis R and Morris WG (1979) Cold-hardiness of containerized loblolly pine seedlings. Its effect on field survival and growth. *Southern Journal of Applied Forestry* 3:15-19

Rowan SJ (1985) Impact of Christmas 1983 freeze on growth and survival of slash, loblolly and longleaf pine seedlings from Alabama and Georgia nurseries. In: CW Lantz (Tech. coord.) Proceedings of the 1984 Southern Nursery Conferences. *United States Department of Agriculture Forest Service*, pp. 30-38

Ryöppö A, Repo T and Vapaavuori E (1998a) Development of freezing tolerance in roots and shoots of Scots pine seedlings at nonfreezing temperatures. *Canadian Journal of Forest Research*, 28(4): 557–565

Ryöppö A, Iivonen S, Rikala, R, Sutinen, ML and Vapaavuori E (1998b) Responses of Scots pine seedlings to low root zone temperatures in spring. *Physiologia Plantarum*, 102(4): 503-512.

South DB (2006) Freeze injury to southern pine seedlings. In: KF Connor (Ed.) Proceedings of the Thirteenth Biennial Southern Silvicultural Research Conference. *General Technical Report, Southern Research Station, USDA Forest Service* SRS-GTR-92: 441-447

South DB, Donald DGM and Rakestraw JL (1993) Effect of nursery culture and bud status on freeze injury to *Pinus taeda* and *P. elliottii* seedlings. *South African Forestry Journal* 166:37-45