EVALUATING THE COLD HARDINESS OF CONTAINER-GROWN LONGLEAF PINE SEEDLINGS[1]

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ABSTRACT-Root systems of container-grown longleaf pine (*Pinus palustris* Mill.) seedlings stored outside in fall and winter can be severely damaged by low temperatures in the South. The freeze-induced electrolyte leakage (FIEL) test was used to evaluate the cold hardiness of container-grown longleaf pine. Results indicated that longleaf pine seedling roots should not be exposed to temperatures below 26.5 °F. Moreover after mid-January, the minimum temperature associated with permanent seedling damage may increase. One alternative to risking damage from low temperature in winter is planting seedlings in fall. If seedlings must be retained for winter and spring planting, the placement of black polyethlyene over seedlings can avoid damage from overnight freezing.

INTRODUCTION

Longleaf pine (*Pinus palustris* Mill.) forests of the Southern United States have been reduced from approximately 92 million acres to less than 5 million acres within the last century (Landers and others 1995). During the first 90 years of this period, efforts to reestablish longleaf pine were unsuccessful (Landers and others 1995, Outcalt 1997), and alternative land management options led to natural and artificial regeneration of loblolly (*P. taeda* L.) and slash pine (*P. elliottii* Engelm. var. *elliottii*) on land that originally supported longleaf pine (Barnett and Dennington 1992, Landers and others 1995).

Recent research has defined desirable longleaf pine seedling characteristics and developed optimum cultural programs (Barnett and McGilvray 1997, Barnett and others 1990). This information has enabled the consistent establishment of container-grown longleaf pine throughout the South (Landers and others 1995, McRae and Starkey 1996). As a result, production and planting of container-grown longleaf pine has increased twelvefold within the last decade (McRae and Starkey 1997).

Although cultural improvements have made the large-scale planting of container-grown longleaf pine a reality, not all efforts have been met with success. Current cultural and planting practices often dictate that container-grown seedlings must be stored in a shade house environment during December and January. Without insulation, the root systems of container-grown seedlings are exposed to near ambient air temperature. Exposure to one or more days of cold weather has been associated with failed establishment of container-grown longleaf pine seedlings that appeared healthy at the time of planting. Knowledge of the minimum temperature tolerated by container-grown longleaf pine and methods to prevent damage from freezing are needed.

The whole-plant freeze test (WPFT) and freeze-induced electrolyte leakage (FIEL) test are commonly used in northern climates to determine the pattern of cold acclimation and deacclimation of nursery seedlings (Burr and others 1990, Rietveld and Tinus 1987). This information allows accurate prediction of optimum lifting windows (Burr and others 1990). Unlike the WPFT, which requires a 7- to 14-day incubation period prior to obtaining accurate results, the FIEL test can be completed within a 3-day period (Burr and others 1990). The FIEL test, therefore, provides the opportunity to quickly adapt cultural activities to the 'current seedling crop rather than rely on delayed information or predictive tools based on data from previous seedling crops.

Low temperatures that result in the mortality of bare-root nursery stock have not been encountered in the South. However, without insulation of the growth medium, the root systems of container-grown seedlings have been damaged by exposure to winter temperatures. The objectives of this study were to: (1) apply the FIEL test to determine the pattern of cold acclimation and deacclimation associated with the root system of container-grown longleaf pine, (2) evaluate the level of cold hardiness of longleaf pine during December through February, and (3) develop recommendations to reduce the risk of seedling damage due to cold temperature.

METHODS

Longleaf pine seedlings were sampled from the 1996-97 crop of container-grown seedlings produced at the U.S. Forest Service W.W. Ashe Nursery in Brooklyn, MS. The seed source was a 1992 bulk Mississippi orchard collection. In late March 1996, seeds were sown in Multipot 4/96 containers in peat-vermiculite (1:1) growth medium, Standard operational cultural practices were applied to

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seedling production (Barnett and McGilvray 1997). The crop was grown and hardened in full sunlight on platforms elevated 10 cm above the ground, and remained outside in containers until packaging and immediate transport to planting locations.

Before packaging, 20 trays of seedlings were randomly identified and permanently marked. On 7 dates in December 1996 through February 1997 (December 1, 17, and 31, 1996; January 15 and 29, 1997; February 12 and 26, 1997), 30 seedlings were randomly extracted from the permanently marked trays, packaged, and shipped to the Southern Research Station laboratory in Pineville, LA, for cold hardiness testing.

The cold hardiness of seedling root systems was evaluated using a modification of the foliar FIEL test (Burr and others 1990). On the morning after seedlings were received in Pineville, the growth medium was washed from root systems, primary lateral roots were removed from the taproot, and the upper one-third of the taproot was excised. Excised taproot segments were kept submersed in distilled water until all seedlings were processed. A 1.2-cm section was excised from the upper portion of each taproot and placed in a capped test tube (12 X 125 mm) containing 2 g washed sand and 1 ml distilled water. Five test tubes were refrigerated (1 .O °C), and five subsets of five test tubes were grouped into glass beakers. Copper-constantan thermocouples were submersed in two test tubes per subset and wired to a data acquisition unit. The beakers of test tubes, enclosed in a Styrofoam ice chest, were placed in the bottom of an upright freezer. Temperatures were recorded at 1 O-minute intervals. The speed of freezing was regulated by adjusting the lid of the Styrofoam ice chest and freezer door so that solution temperatures did not decrease by more that 1 .O °C per 12 min. When temperatures reached -2 to -3 °C, the test tubes were shaken to induce freezing. One subset of test tubes was transferred from the freezer to the refrigerator when temperatures decreased to approximately -3.0, -4.5, -6.0, -7.5, and -9.0 °C (+/- 0.1-0.2 °C). Test tubes were thawed in the refrigerator (3 °C). Five ml of deionized water was added to each test tube after thawing and the tubes were agitated on a horizontal shaker for 24 h. The average solution temperature of the two test tubes with thermocouples at the time of removal from the freezer was calculated and applied to the three test tubes per subset that did not contain thermocouples.

After 24 h, electrical conductivities (EC) of solutions were recorded. Test tubes were boiled for 20 min, placed back on the shaker, and EC measurements were repeated 24 h later. The percentage of EC before, relative to that after boiling, was calculated for each solution, and data were expressed as indices of injury by the method of Flint and others (1967). Indices of injury and temperatures at which solutions were removed from the freezer were fit to both linear and Weibull sigmoid models. Lethal temperatures with 95-percent confidence intervals, were estimated by indices of injury equal to 10, 30, and 50 percent (LT10, LT30, and LT50) from the modeled data. Lethal temperatures predicted by the model, either linear or sigmoid, that yielded the smallest confidence interval were chosen for cold hardiness evaluations. Past research has shown a high degree of

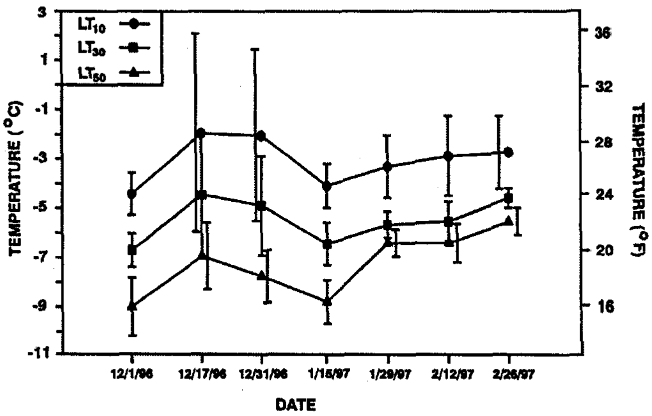

Figure I-Root cold hardiness of container-grown longleaf pine seedlings grown at the U.S. Forest Service W.W. Ashe Nursery in Brooklyn, MS, during 1996. Lethal temperatures associated with an index of injury equal to 10 percent (LT10) are the threshold of minimum temperature that seedlings can be exposed to without significant damage. Lethal temperatures associated with indices of injury equal to 30 percent (LT30) and 50 percent (LT50) represent temperatures at which seedlings will be damaged beyond use.

correlation between the electrolyte leakage of conifer seedling tissues and tissue viability, percent live root mass and seedling survival (Bigras 1997, Burr and others 1990). Based on this information, temperatures at which the seedling crop would not be significantly damaged were defined as LT10 values, and temperatures at which the seedling crop would be damaged beyond use and completely dead were defined as LT30 and LT50 values, respectively. Significant differences among LT1 0, LT30, or LT50 values associated with the seven sampling dates were determined by the test of nonoverlapping 95-percent confidence intervals (Jones 1984).

RESULTS

Between December 1, 1996, and February 26, 1997, LT1 0 values averaged 26.5 °F. (-3.0 °C), and were not significantly different among sampling dates since the 95-percent confidence intervals overlapped (fig. 1). Between December 1, 1996, and January 15, 1997, LT30 values averaged 21.9 °F. (-5.6 °C) and were not significantly different. However, the LT30 on February 26, 1997, was significantly higher than that on December 1, 1996; January 15, 1997; and January 29, 1997. Similarly, LT50 values in December 1996 and January 15, 1997, averaged 17.4 °F. (-8.1 °C) and were not significantly different. As winter progressed, however, LT50 values on January 29, February 12, and February 26, 1997, were significantly higher than those on December 1, 1996, and January 15, 1997.

DISCUSSION

Temperatures that cause an index of injury equal to 10 percent represent the threshold of minimum temperature that seedlings can be exposed to without significant damage. Our results indicate that container-grown longleaf pine at the Ashe Nursery cannot withstand temperatures below 26.5 °F. Minimum temperatures reached 28.5 °F. or less on 11 nights at the Ashe Nursery in winter 1996-l 997 (fig. 2). During periods of potentially damaging temperature, seedlings were covered with black polyethylene which

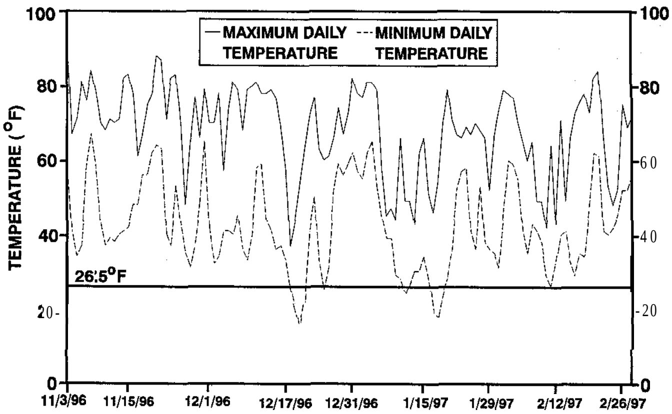

Figure P-Daily maximum and minimum temperatures at the U.S. Forest Service W.W. Ashe Nursery in Brooklyn, MS, during November 1996 through February 1997. Note that on two occasions the exposed longleaf pine seedlings needed protection to prevent root damage by cold temperatures.

prevented damage. Air and growth medium temperatures were monitored during one period when seedlings were protected by polyethylene, which conserved the heat in the growth medium so that the temperature of the root system was 10 to 13 °F. warmer than the outside air temperature. This suggests that at the Ashe Nursery, container-grown longleaf pine seedlings covered with black polyethylene can withstand short periods of air temperature as low as 16.5 °F. before root damage occurs.

The degree of cold acclimation and deacclimation exhibited by tree species varies by the climate of their natural range. For example, Tinus (1996) used the FIEL test to compare the cold hardiness of Aleppo pine (*P. halepensis* Mill.), radiata pine (*P. radiata* D. Don), and Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) roots. The roots of radiata pine, native to a cool Mediterranean climate, hardened 2 °C, and those of Aleppo pine, native to a warm Mediterranean climate, exhibited no cold hardiness; whereas, the roots of Douglas-fir, a species that naturally occurs in cold temperate climates, hardened 10 °C.

The natural range of longleaf pine is limited to the lower and middle Coastal Plain portion of the Southeastern United States (Loveless and others 1989), and is characterized by a warm temperate climate. Therefore, it is likely that LT10 values of container-grown longleaf pine from other origins in the South are similar to those observed at the Ashe Nursery. Although FIEL tests are required for validation, longleaf pine native to the northern range in southeastern Virginia, and montane longleaf pine in northern Alabama and Georgia, may exhibit more cold hardiness than longleaf pine seedlings from other portions of the species' natural range.

Significant seasonal differences among lethal temperatures were observed at indices of injury of 30 and 50 percent. For example, significantly different LT50 values indicate that longleaf pine seedlings lost approximately 4.6 °F. of cold hardiness between mid-January and late February. This information suggests that seedlings reached maximum cold hardiness in December and early January and lost cold hardiness in late January and February.

Our results indicate that longleaf pine seedling roots should not be exposed to temperatures less than 26.5 °F. Furthermore after mid-January, the minimum temperature associated with permanent seedling damage may increase. Longleaf pine is successfully established by fall planting (McRae and Starkey 1997). Therefore, one alternative to risking damage from suboptimum temperature in winter is planting seedlings in fall. If container-grown longleaf pine seedlings must be retained for winter and spring plantin methods to protect the seedings from low temperatures should be developed at nurseries where air temperatur reach 26.5 °F. or below. In the South, the placement of b polyethylene over container-grown longleaf pine seedli is one method to avoid short periods of damaging low temperatures. However, this method of seedling proteci should be evaluated at each location before it is relied operationally.

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