Auburn University Southern Forest Nursery Management Cooperative

RESEARCH REPORT 99-5

TESTING ELECTROLYTE LEAKAGE AFTER FREEZE DAMAGE TO LOBLOLLY PINE

by
Ken Mc Nabb and Ernesto Takahashi

INTRODUCTION

All plant tissues have ions in their cells that conduct electricity. The K+ ion is a good example, which is abundant in plant cells. Under normal conditions, these electrolytes are contained within the cell walls by membranes. When these membranes are damaged, electrolyte leakage occurs. Plant physiologists have related electrolyte leakage to plant damage from biotic factors such as disease and abiotic factors such as severe heat and cold. There are a few studies relating conifer seedling quality to electrolyte leakage, particularly in the case of freeze damage. Most of this work has been done on boreal species. The U.S. Forest Service has done work with longleaf pine and has used it to estimate freeze damage on nursery seedlings. Union Camp has done some work with top-pruned seedlings (South et al. 1993).

Assessing freeze damage to loblolly pine seedlings has practical applications. Severe freezes often damage seedlings in the nursery. A technique to assess the severity of this damage would be useful. Also, refrigeration trucks periodically malfunction, freezing an entire load of seedlings. Regeneration foresters would benefit from an accurate and fast test of seedling damage to assess determine if the seedlings should be destroyed. To this end, a series of tests were initiated at Auburn to test the possibility of electrolyte leakage as a measure of loblolly pine freeze damage,

METHODS

Conductivity measurements were done following some basic procedures.

1. a tissue sample (root, stem or needle) was cut from a seedling
2. the sample was placed in deionized water in a 50 ml vial and shaken for a specific length of time so electrolytes could "leak out"

3. conductivity of the water was measured using a Fisher digital conductivity meter
4. the tissue sample was autoclaved to release all possible electrolytes.
5. conductivity was again measured
6. The number of seedlings used per treatment/replication was 3 or 4. A total of 48 samples could be run at a single time.

Using this procedure, tests were conducted to quantify the influence of several variables. The following is a list of tests and objectives.

Test 1 To determine the effect of water volume and organ sampled on the amount of electrolyte leakage. Samples were taken at 1 - 2.5 cm below the root collar (taproot sample), 1 - 2.5 cm above the root collar (stem sample), and a 1.5 cm length of a needle from the upper third of the seedling.

Test 2 To determine how leakage is affected by the size of the sample. Taproot, stem, and needle samples of 5, 10, 15, and 20 mm were tested.

Test 3 To determine if the diameter of the seedling affects conductivity levels. Stem and taproot samples from seedlings of 3, 5, and 7 mm DRC were used.

Test 4 To determine the effect of the location of a root sample on the variability and level of leakage. Taproot sections with and without first order lateral roots (lateral roots pruned off), FOL roots adjacent to the taproot, and FOL roots 15 cm removed from the taproot were used for the test.

Test 5 To test the effect of freezing seedlings on electrolyte leakage. Eight seedlings were placed in a deep freezer for 0, 4, 8, and 24 hours. Taproot, stem, and needle samples were used for leakage determination on three seedlings, the other 5 were outplanted to a stress pit containing washed sand. There were two replications spaced 2 days apart.

Test 6 This was a repeat of test 5 but using exposure times of 0, 1, 2, and 4 hours. There were 4 replications.

All seedlings used in these tests were typical field run loblolly pine lifted in February and kept in cold storage.

RESULTS

Test 1

The 20 ml water volume was determined to be best. The 10 ml volume sometimes posed a problem with getting the conductivity probe sufficiently deep. The 30 ml volume tended to dilute the solution, particularly for needles. The values for different plant organs varied considerably (Figure 1), with needles averaging 6 mmhos/cm, stem samples averaging 27 mmhos/cm, and taproot samples averaging 45 mmhos/cm at 24 hours of soaking and prior to autoclaving.

Test 2

The length of tissue samples did affect conductivity measurements of stems and taproots. There was no detectable effect on needles. Figure 2 shows the results of the stem sections.

Test 3
The diameter of the stem or root sample also influenced leakage results. Larger diameter samples leaked more than smaller diameter samples (Figure 3).

Test 4
Having first order lateral roots (FOLR) removed from taproot samples did not influence the total leakage of samples (Figure 4). FOLR samples probably had lower conductivity values because they were simply smaller than other samples. An important aspect of this test was to determine the variability in conductivity measures using these 4 types of root tissue. The coefficient of variation for the taproot/no FOLR, taproot with FOLR, FOLR near taproot, and FOLR 15 cm from the taproot were 21.5%, 20.0%, 37.7% and 81.7%, respectively. If smaller FOLR are to be used in conductivity tests, larger sample sizes are required.

Test 5
All seedlings from the freezing treatments died after planting indicating that freeze damage was extensive. Two out of ten check seedlings died. When looking at electrolyte leakage as a percent of autoclaved values (the maximum possible leakage), the values for needles, stems, taproots, and secondary roots were 97%, 85%, 81%, and 61%, respectively.

Test 6
All the seedlings from the freezing treatments died after planting. Seven out of 20 check seedlings died. There was good treatment separation for both the needle and stem samples (Figure 5). However, it is evident that the roots were damaged by the treatments with the percent conductivity of taproots at 71 % after only 1 hour of exposure to freezing.

In summary, these tests indicated: (1) water volume, seedling diameter, and sample length all influence the absolute amount of electrolytes leaked. We decided to standardize to 20 ml water volumes, 15 mm sample length, and shaking for 24 hours. (2) Different seedling organs vary in absolute amounts of electrolytes leaked, a function primarily of their size. Smaller samples such as needles and lateral roots will have more variability from sample to sample. (3) Seedling organs vary in response to freezing, with roots more sensitive than tops. (4) The point at which electrolyte leakage of either above or belowground parts actually indicates mortality is yet to be determined.

MANAGEMENT IMPLICATIONS

Electrolyte leakage tests are a relatively fast and inexpensive way to measure loblolly pine seedling damage resulting from freezing. If nursery managers wish to employ this method, standardized techniques are important. Moreover, the exact relationship between electrolyte leakage and seedling mortality and the consistency of that relationship still needs to be worked out.

LITERATURE CITED

South, D.B., D.G.M. Donald, and J.L. Rakestraw, Effect of nursery culture and bud status on freeze injury to *Pinus taeda* and *P. elliottii* seedlings. Suid-Afrikaanse Bosboutydskrif-nr. 166, Sept. 1993:37-46

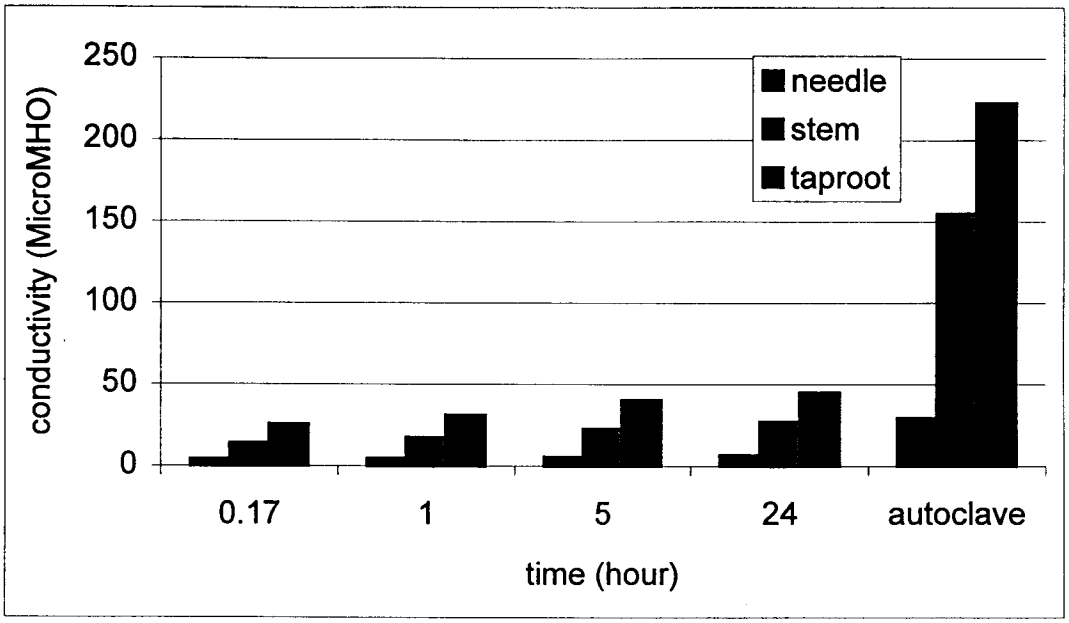

Figure 1: Conductivity by tree organ and shaking time (20 ml water volume).

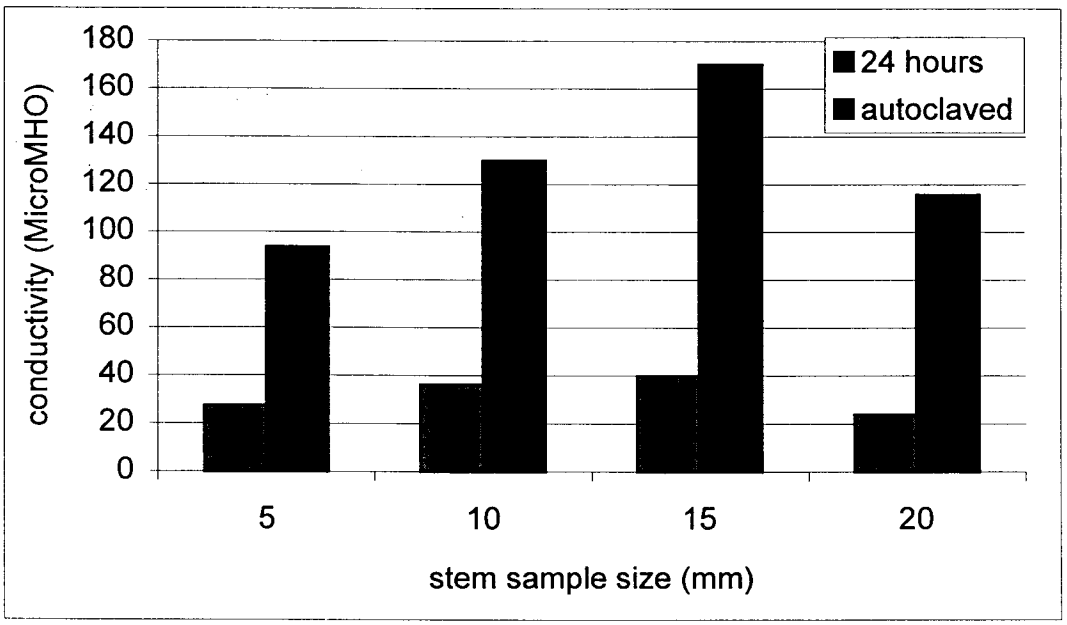

Figure 2: Conductivity by length of stem tissue sample.

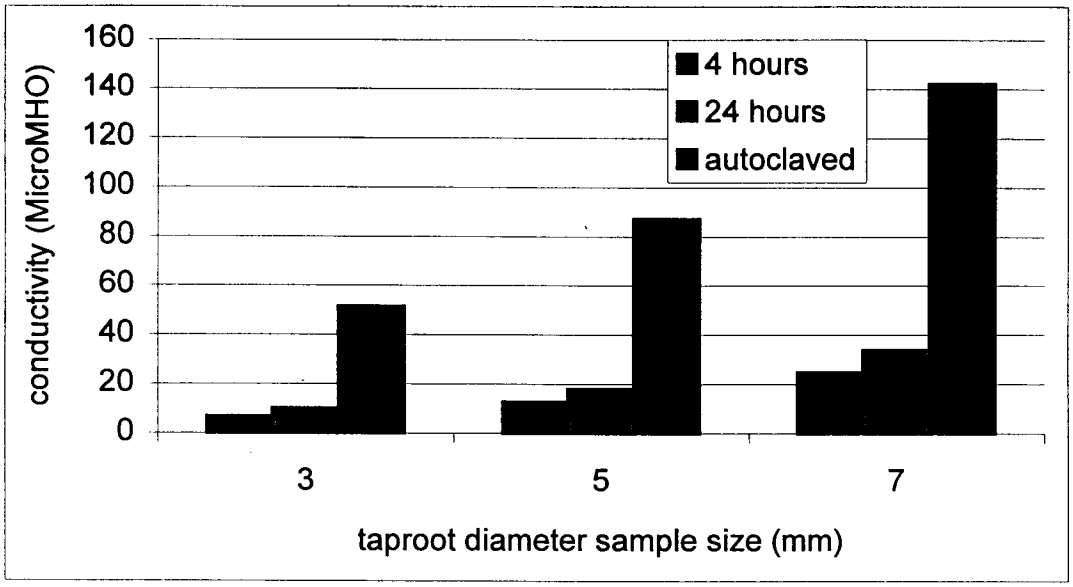

Figure 3: Conductivity of taproot on samples of different diameters.

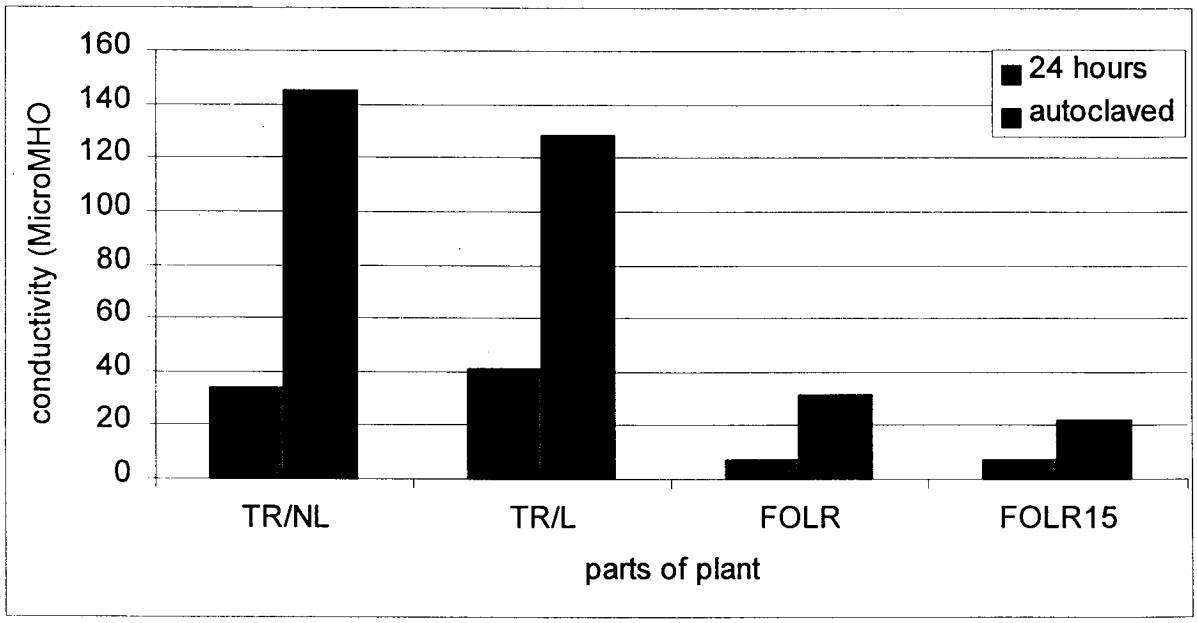

Figure 4: Conductivity of root segments after 24 hrs of shaking and autoclaving. TR/NL-Taproot segment with no first order lateral roots, TR/L-Taproot segment with first order lateral roots pruned, FOLR-first order lateral root segment adjacent to taproot, FOLR15-first order lateral root segment 15 cm from the taproot.

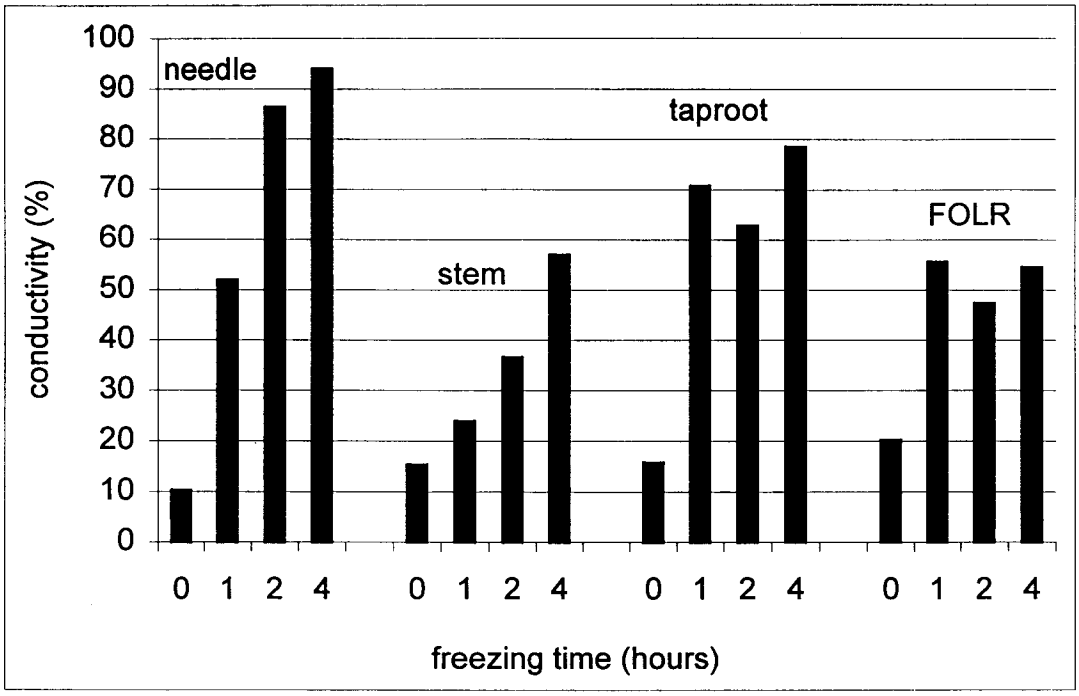

Figure 5: Conductivity of tissue samples by time of exposure to freezing temperatures. Expressed as a percent of the autoclaved value (FOLR-first order lateral root segment adjacent to taproot).