Auburn University Southern Forest Nursery Management Cooperative

RESEARCH REPORT 00-4

FREEZE DAMAGE TO LOBLOLLY PINE SEEDLINGS AS INDICATED BY CONDUCTIVITY MEASUREMENTS AND OUTPLANTING SURVIVAL

by

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INTRODUCTION

Plant cell walls are damaged during freezing and typically results in the leakage of cell contents into surrounding tissue. Electrolytes that conduct electricity are a small portion of this material. If damaged tissues are placed in water and allowed to "leak" into solution, the conductivity of the solution can then be measured with a conductivity meter. The greater the cell damage, the greater the leakage, and the higher the conductivity. This measure of conductivity then becomes an indicator of freeze damage, with high conductivity readings indicating increased damage.

Previous research tested various combinations of tissue type, sample size, and water volumes. Standardized procedures were developed and presented in Research Report 99-4. The next step was to further explore the relationship between conductivity estimates and seedling survival.

METHODOLOGY

Two separate tests were conducted for this work. In both cases, loblolly pine seedlings were lifted by hand from a Coastal Plain nursery in late October, 1999, transported to Auburn and placed in cold storage ($\pm 2^{0}$C). The seedlings were selected for a generally uniform size regarding diameter and top length. A specified number of seedlings were placed in a top-loading freezer that had been previously shut off and allowed to come to room temperature. The seedlings were laid on a cardboard box and their roots covered with dry paper towels. The freezer was then turned on and seedlings sampled at pre-determined times. Conductivity measurements were conducted on needles, stem, and taproot tissue using 15 mm sample lengths, 20 ml of water volume, 24 hour shaking times, and the

conductivity meter set at 2000 ohms (Research Report 99-4). Percent damage is determined by dividing this reading by a second conductivity reading after all sample tissue had been completely disrupted through autoclaving at over 100°C for 8-10 minutes.

Test 1

A group of 40 seedlings was placed in the freezer. Eight seedlings were pulled from this group at 0, 1, 2, 3, and 4 hours after restarting the freezer. Five seedlings of each sample time were planted in the trophotron at Auburn (deep sand, no irrigation). The remaining three seedlings were used for conductivity tests, with individual seedling measurements of needle, stem, and taproot samples. This procedure was repeated with four bundles of seedlings over 4 days.

Test 2

A group of 40 seedlings was placed in the freezer. Four seedlings were pulled from this group at 0, 60, 75, 90, 105, 120, 135, 150, 165, and 180 minutes. Three seedlings from each sample time were planted in the trophotron at Auburn. A needle sample of each of these three seedlings was taken for conductivity measurements. The remaining seedling of the four seedlings sample was used for conductivity measurements of needle, stem, and taproot tissue. This procedure was repeated 5 times over just as many days.

RESULTS

The freezer temperature during cool down and subsequent seedling survival is presented in Figure 1. Air in the freezer reached zero degrees centigrade about 45 minutes after starting the test. Normal

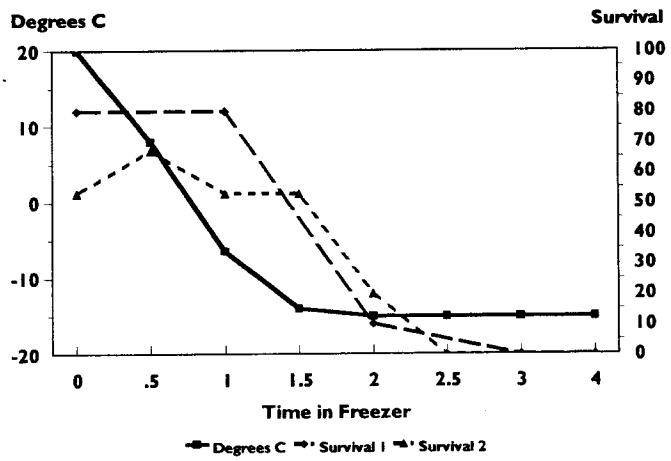

Figure 1. Time in freezer, temperature change, and survival for Tests 1 and 2.

operating temperature of -15°C was obtained after 1.5 to 2 hours. In both Test 1 and Test 2, survival began to decline between 1 and 2 hours in the freezer. Survival fell to zero after 2.5 to 3 hours in the freezer. Test 1 seedlings averaged higher survival than Test 2 seedlings. Although such a comparison is confounded by planting date, it is possible the additional cold storage time of Test 2 seedlings

contributed to lower survival. The last replication of Test 2 was done on November 30. October lifted seedlings had been in cold storage for five weeks by that date.

Conductivity measurements for needle, stem, and root tissues in Test 1 (Figure 2) indicated increasing tissue damage between one to three hours in the freezer. The sigmoid nature of the damage curve is apparent and matches the survival curve. At first, survival and damage remain steady, followed by a period of rapid change, followed by a leveling off. The high R^2 values indicate a strong relationship between tissue damage and time in the freezer for all three tissue types. The results of Test 1 indicated the biggest influence on survival occurred from 1 to 2 hours. Test 2 was therefore initiated to obtain more data for this critical period of time. Seedlings in Test 2 also showed increasing needle, stem, and root tissue damage as measured by conductivity (Figure 3) with survival gradually decreasing as damage increased. There was a significant relationship between damage and time in the freezer.

At the time seedlings in Test 2 were outplanted, a needle fascicle (with 3 needles) was sampled from each of the 3 trees planted. This sample resulted in a very strong relationship between damage and time in the freezer with an R^2 of 0.77, indicating that over three fourths of the change in needle damage as measured by conductivity could be explained by time in the freezer (Figure 4). Using this data, percent survival was regressed against percent needle damage. The relationship is linear (Figure 5) with a moderately strong R^2 of 0.49. For this data set, every increase of 10% in damage resulted in a survival decrease of 8%.

MANAGEMENT IMPLICATIONS

The use of conductivity to quantify seedling damage from freezing appears to be feasible. There is a demonstrable relationship between damage measurements and seedling quality. We are confident that damage levels of 70 to 80% indicate seedling mortality. Further work is needed, however, to further define the survival effect when damage is in the 30 to 50% range.

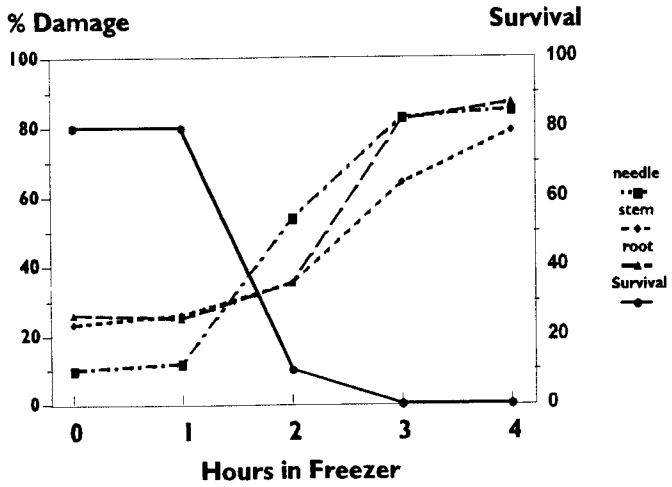

Figure 2. Percent damage by tissue type and survival for Test 1. R^2 for Needles = .75, stem = .88, and root = .83.

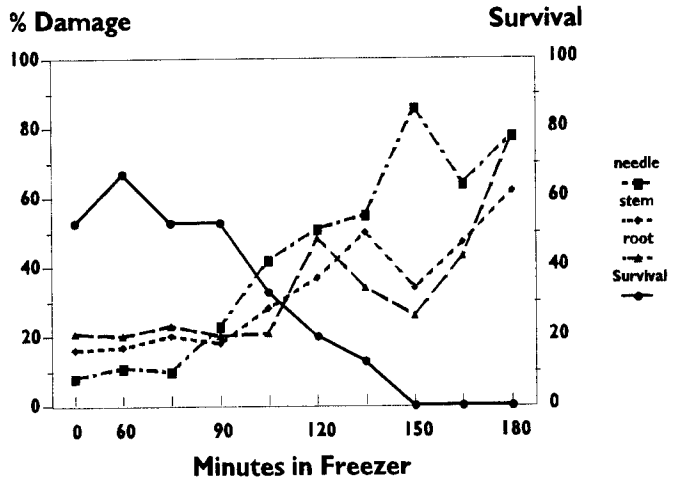

Figure 3. Percent damage by tissue type and survival for Test 2. R^2 for needles = .63, stem = .49, and root = .51.

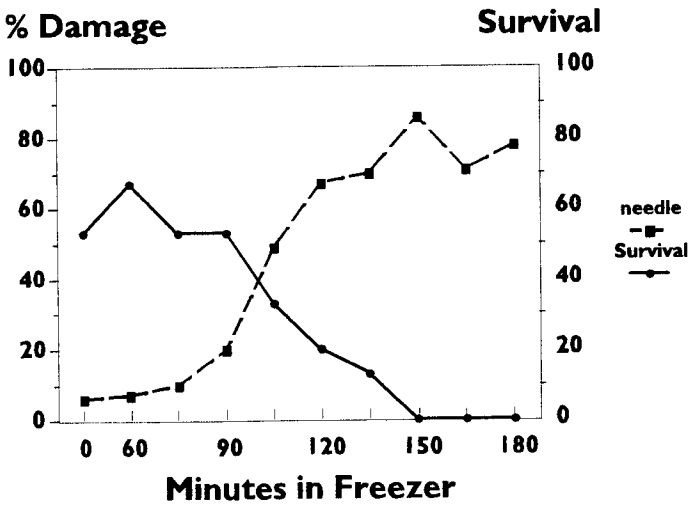

Figure 4. Percent needle damage and survival of trees planted in Test 2. R^2 needles =.77.

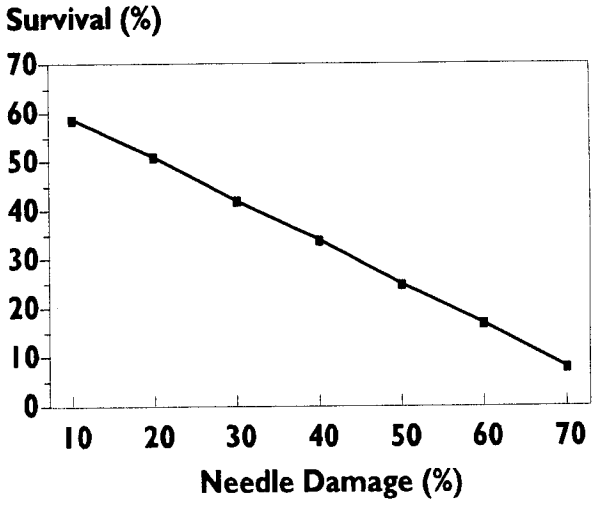

Figure 5. The relationship of survival to needle damage in Test 2.
Survival = 67.7 + (-.85)damage R^2=.49