COLD TOLERANCE VARIATION IN LOBLOLLY PINE NEEDLES FROM DIFFERENT BRANCH TYPES, FAMILIES, AND ENVIRONMENTS

T. E. Kolb and K. C. Steiner

Abstract.--The cold tolerances of loblolly pine needles from different open-pollinated families, branch types, field blocks, and test locations were measured by the electrical conductivity method. Significant differences in tolerance were found between families, "upper" and "lower" growth internodes, blocks, and locations. Family differences in tolerance were more pronounced among 1-year-old seedlings in a nursery environment than among 12- and 13-year-old trees in plantation environments. Results indicate that non-genetic sources of variation and genotype x environment interaction may bias assessments of cold tolerance genetic variation in loblolly pine.

Keywords: Pinus taeda, cold tolerance, genetic variation.

INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is the preferred pulpwood species for many areas located immediately north of its natural range (Allen 1953, Aughanbaugh 1957, Lambeth et al. 1984). However, winter injury to loblolly pine in these areas may reduce growth rates or cause mortality, thus reducing productivity below the potential for the site (Boggess and McMillan 1954, Minckler 1952, Thor 1967, Wells and Rink 1984). Consequently, loblolly pine genetic improvement programs should stress the development of varieties possessing both cold tolerance and rapid growth rates.

Reliable techniques of screening loblolly pines for genetic differences in cold tolerance are needed to expedite the production of hardy varieties. Assessments of cold tolerance differences among progenies in field tests are desirable since the trees acclimate under natural environmental conditions. However, field assessments depend on the fortuitous occurrence of adequate test winters and may require observations over many years to detect anything but large differences in tolerance. A modification of the electrical conductivity method (Dexter et al. 1930, 1932) was used by Kolb et al. (1985) to accurately measure differences in cold tolerance among open-pollinated families of loblolly pine growing in a western Kentucky field test. This paper reports the results of two studies designed to identify non-genetic sources of variability which may bias assessments of cold tolerance genetic variation in loblolly pine. Study One emphasized within-tree and field block sources of variability, while Study Two emphasized ontogenetic and plantation sources of variability.

[1/] The authors are, respectively, Graduate Research Assistant, and Associate Professor of Forest Genetics, Forest Resources Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802. Journal Article No. of the Pennsylvania Agricultural Experiment Station. The authors wish to acknowledge the financial assistance provided by Westvaco Corp. in performing the research and the valuable contributions of Henry F. Barbour in executing the study.

STUDY ONE

Methods and Materials

On December 15, 1982, needle samples from three open-pollinated families (1-31, 6-20, 18-94) were collected from each of four field blocks in the Westvaco Corporation's 1976 progeny test in Calloway County, Kentucky. These families represent a wide range of hardiness based on assessments of winter injuries that occurred in the progeny test in 1977 (Kolb et al. 1985). Family 1-31 was the most hardy of these families, 6-20 was intermediate, and family 18-94 was the **least** hardy.

In each field block, collections consisted of two lower and two upper growth internodes from branches which flushed two or three times in 1982. The "upper" growth internodes on three-flush branches were defined as the middle internode, and "lower" internodes were consistently those in the lowermost position on both two-and three-flush branches. Two- and three-flush branches were collected from one tree each in a ten-tree row plot for each family in a block. The collection of samples was limited to branches formed from the main stem in 1981 on the north side of the **tree.** This sampling scheme produced 48 treatment combinations in factorial arrangement: 3 families, 4 field blocks, 2 branch types (two- and three-flush), and 2 internodal positions, each combination represented by samples from two branches on the same tree. Samples were promptly packed into coolers and mailed to University Park, Pennsylvania, where they arrived the morning following collection.

Samples were prepared for laboratory exposure to low temperatures by bulking an approximately equal number of needles from the two branches representing each treatment combination, cutting these into 5 cm segments from the fasicle end, and randomly choosing approximately 25 segments for each desired temperature exposure. Needle samples of each treatment combination were commonly exposed in a freezing chamber to the following temperatures: 5°C (unfrozen control), -10°C, -15°C, -20°C, -25°C, -30°C, -35°C, -40°C, -45°C. The temperature in the chamber was lowered at a rate of 4°C per hour, and each desired temperature exposure was maintained for 30 minutes. Needle samples were removed from the chamber following each desired exposure, and slowly thawed to an ambient temperature of 5°C. Electrolytes from each sample diffused into 10 ml of deionized water for 24 hours after thawing. The electrical conductivity of each diffusate solution was measured both before and after an autoclaving treatment at 245°C for 30 minutes.

As initially described by Dexter et al. (1930, 1932), the electrical conductivity of diffusate from plant tissues injured by low temperatures is higher than that of diffusate from uninjured tissues. To obtain a measure of injury due to low temperature exposure, a "relative electrical conductivity" was calculated for the diffusate solution from each sample by dividing the electrical conductivity before autoclaving by the electrical conductivity after autoclaving. This index eliminates spurious effects caused by the tendency of some samples to have a higher electrical conductivity due to differences in needle sample size or nutrient status (Wilner 1959).

Analysis of variance on observations of relative electrical conductivity were used to determine if cold tolerance varied between needles from different sources. In these analyses, the temperature x needle source interaction was of primary interest since the significance of this term indicated whether needles from different growth internodes, branch types, families, or blocks varied in their injury response to temperature and consequently cold tolerance.

Results

The first hypothesis of interest was that the cold tolerance of loblolly pine needles does not differ between lower and upper growth internodes. This was tested by an analysis of variance on observations of relative electrical conductivity for upper and lower growth internodes averaged over two- and three-flush branches and families. Needles from lower growth internodes were significantly ($p < 0.01$) less cold tolerant then those from upper growth internodes. This difference is illustrated in Figure 1-- needles from lower internodes were injured more rapidly in response to decreasing temperature than needles from upper growth internodes.

The second hypothesis of interest was that tolerance does not differ between needles from two- and three-flush branches. This was tested by analysis of variance which compared the tolerances of needles from the two branch types averaged over families for lower and upper internodes, respectively. Needles from comparable internodes (lower or upper) did not differ significantly in tolerance when obtained from either two- or three-flush branches. As shown in Figure 2, needles from lower internodes on two- and three-flush branches were injured approximately the same. However, Figure 3 shows a possible difference in response between needles from upper internodes of two- and three-flush branches. Thus, needles from upper internodes may differ slightly in cold tolerance between branch types (although not significant in this experiment), while needles from lower internodes appear to be relatively stable in tolerance with respect to branch type.

The final hypotheses of interest were that the cold tolerance of needles does not differ among the three families and four field blocks sampled in this experiment. To test these hypotheses, data for the three families were averaged over two- and three-flush branches using only lower internodes, as suggested by the results of the previous analysis. Families did not differ significantly in tolerance. However, the pattern of response of families to temperature shown in Figure 4 is identical to that suggested by winter injury to families under field conditions in 1977: family 18-94 was injured the most rapidly, 6-20 was intermediate, and 1-31 was injured the least rapidly. This comparison suggests that cold tolerance varied among families, but that these differences were not statistically detectable at the level of precision present in this experiment.

It was also apparent in this analysis that needles from the four field blocks differed significantly ($p < 0.005$) in tolerance. As shown in Figure 5, needles from block one were injured much more rapidly than needles from other blocks. These block influences on cold tolerance are presumably related to variations in microsite within the plantation and perhaps related to the fact that block one is located in a somewhat moister area than the others.

STUDY TWO

Methods and Materials

On February 8, 1984, needle samples were collected from each of four open-pollinated families of loblolly pine (3-4, 3-41, 6-8, 6-22) at Westvaco's 1972 test in Livingston County, Kentucky (13-year-old trees), Westvaco's 1973 test in Hickman County, Kentucky (12-year-old trees), and in Westvaco's progeny test seedlings at the J. P. Rhody (Kentucky State) Nursery, Marshall County, Kentucky (1-year-old trees). For each family, progenies at all three test locations originated from the same seed orchard, but not necessarily the same seedlot. Progenies 3-4 and 3-41 are from the Champion International seed orchard in Newberry, South Carolina, and progenies 6-8 and 6-22 are from the Champion International seed orchard in Tillary, North Carolina.

For the 1972 and 1973 tests, a lower internodal segment of 1983 twig growth from a lateral branch formed on the main stem in 1982 was collected from seven trees per family in each of three field blocks. For each family in the nursery, twenty whole seedlings were collected from each of two blocks. All collections were packed into coolers and mailed to University Park, Pennsylvania, where they arrived the next morning.

The preparation of samples, the freezing process, and the measurement of injury was identical to that described for Study One with the following exceptions: 1) needles collected from blocks in the field were divided into four replications for laboratory analysis, and 2) temperature treatments of -10°C, -40°C, and -45°C were excluded. Relative conductivity data from each of the test locations were subjected to analysis of variance to determine if genetic differences in tolerance among families were detectable in each environment. A combined analysis of variance was used to determine whether test locations influenced overall levels of tolerance, as well as relative differences among families.

To make specific comparisons of cold tolerance, injury response curves were formulated by regressing mean relative conductivity on temperature treatment using the following model:

$$Y_{ij} = b_{oij} + b_{1ij}X + b_{2ij}X^2 + E_{ij}, \text{ where}$$

Y_{ij} = mean relative conductivity for family "i" at test location "j" for each temperature treatment

X = temperature treatment

E_{ij} = residual for family "i" at test location "j"

Similar regressions were performed on overall means at each test location to compare injury responses among environments. The model fit the data adequately, as indicated by R^2 values which ranged from 0.91 to 0.99.

Results

Overall levels of tolerance differed significantly ($p < 0.005$) among the three test locations as illustrated by injury response curves shown in Figure 6. One-year-old seedlings from the nursery were generally the least tolerant, trees from the 1973 test were intermediate, and trees from the 1972 test were the most tolerant. Test location also influenced differences in tolerance among families (family x temperature x location interaction significant, $p < 0.005$). Injury response curves shown in Figures 7 and 8 indicate no significant differences in tolerance among families in the 1973 and 1972 test. In contrast, family 3-41 was significantly ($p < 0.005$) **less** tolerant than the other families in the nursery (Figure 9).

DISCUSSION

These studies indicate that differences in the cold tolerance of loblolly pine needles may arise from both genetic and non-genetic sources of variation. Consequently, confounding the sampling of tissues from families or clones with branch positions, field blocks, or test locations may seriously bias genetic assessments of cold tolerance. Consistent sampling is especially important when measuring cold tolerances by the electrical conductivity method because of its sensitivity in detecting differences in injury. Exploratory studies to identify potential sources of variation are a necessary precaution in using indirect measures such as the electrical conductivity method for genetic assessments of cold tolerance in any species.

The differential injury response of families from the three test locations suggests that genetic variation in tolerance may be more pronounced among seedlings in the nursery than among older trees in the field. The reasons causing this interaction cannot be determined in this study since environmental and ontogenetic effects were confounded. It is conceivable that expression of genetic variation was greatest in the nursery environment because of a correlation between tolerance and some aspect of seedling physiology such as response to fertilizers or other cultural treatments used in the nursery. Useful assessments of cold tolerance in loblolly pine breeding programs will be complicated if such genotype x environment or genotype x age interactions are common.

LITERATURE CITED

Allen, J. C. 1953. A half century of reforestation in the Tennessee Valley. J. For. 51:106-113.

Aughanbaugh, J. E. 1957. Loblolly pine winning Ohio pine race. Ohio Farm and Home Res. 42:95-96.

Boggess, W. R., and F. W. McMillan. 1954. Cold weather and glaze damage to forest plantations in southern Illinois. Bull. Ill. Agr. Exp. Sta. No. 574.

Dexter, S. T., W. E. Tottingham, and L. F. Garber. 1930. Preliminary results in measuring the hardiness of plants. Plant Physiol. 5:215-223.

Dexter, S. T., W. E. Tottingham, and L. F. Garber. 1932. Investigations of the hardiness of plants by measurements of electrical conductivity. Plant Physiol. 7:63-78.

Kolb, T. E., K. C. Steiner, and H. F. Barbour. 1985. Seasonal and genetic variations in loblolly pine cold tolerance. For. Sci. in press.

Lambeth, C. C., P. M. Dougherty, W. T. Gladstone, R. B. McCullough, and O. O. Wells. 1984. Large-scale plantings of North Carolina loblolly pine in Arkansas and Oklahoma: a case of gain versus risk. J. For. 82:736-741.

Minckler, L. S. 1952. Loblolly pine seed source and hybrid tests in southern Illinois. U.S. For. Ser. Central States For. Exp. Sta. Tech. Pap. 128.

Thor, E. 1967. A ten-year-old loblolly pine seed source test in Tennessee. J. For. 65:326-327.

Wells, O. O., and G. Rink. 1984. Planting loblolly pine north and west of its natural range. Proc. Southern Silvicultural Research Conf. p. 261-265.

Wilner, J. 1959. Note on an electrolytic procedure for differentiating between frost injury to roots and shoots in woody plants. Can. J. Plant Sci. 39:512-513.

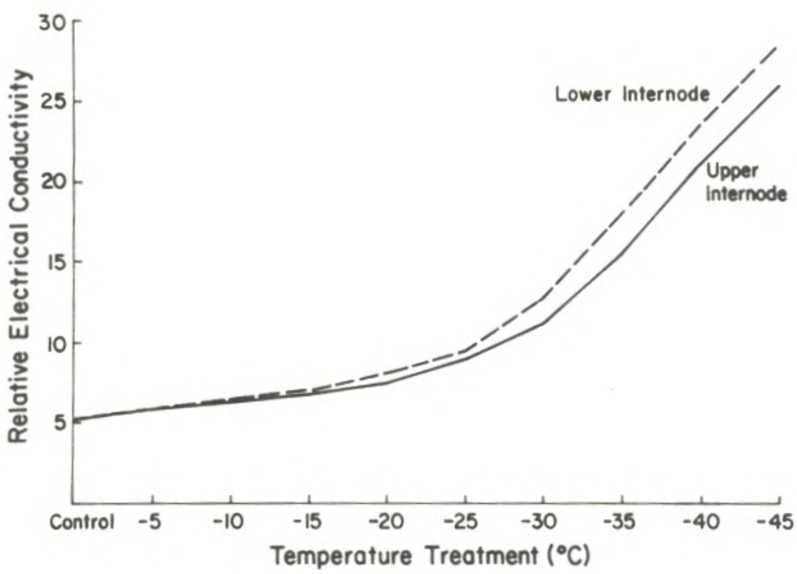

Figure 1.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for "lower" and "upper" growth internodes. Graphs adjusted to a common y-intercept.

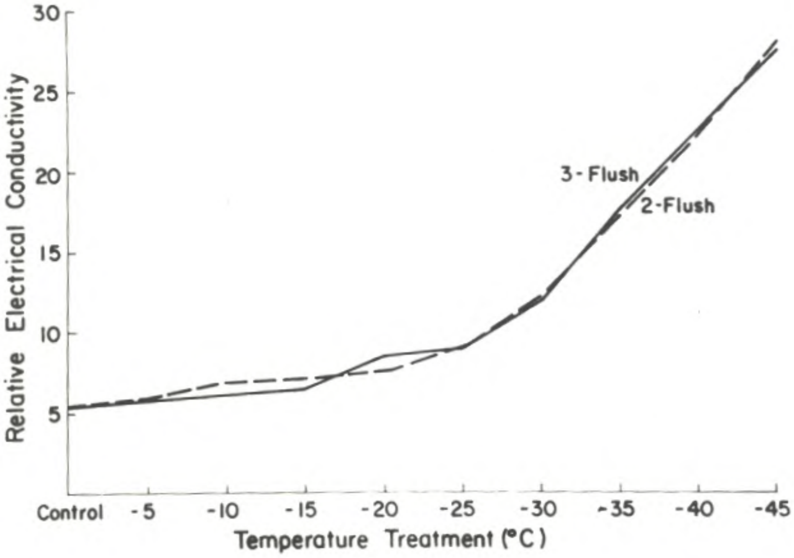

Figure 2.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for lower internodes of "two-flush" and "three-flush" branches. Graphs adjusted to a common y-intercept.

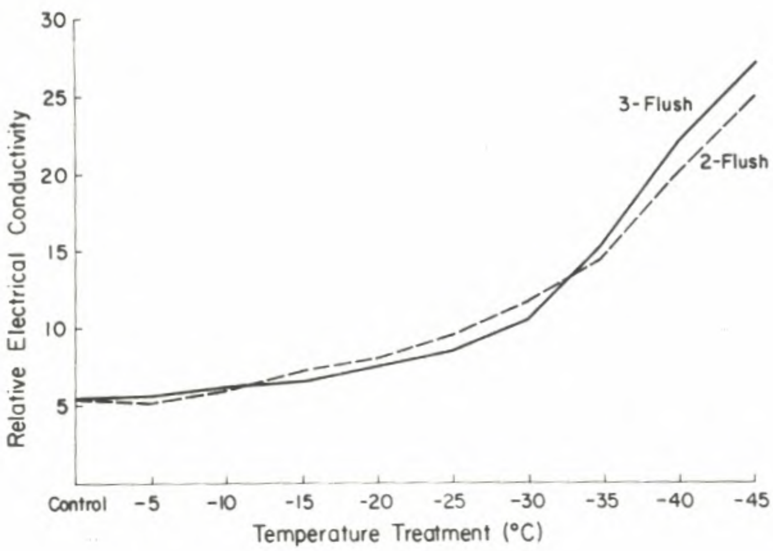

Figure 3.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for upper internodes of "two-flush" and "three-flush" branches. Graphs adjusted to a common y-intercept.

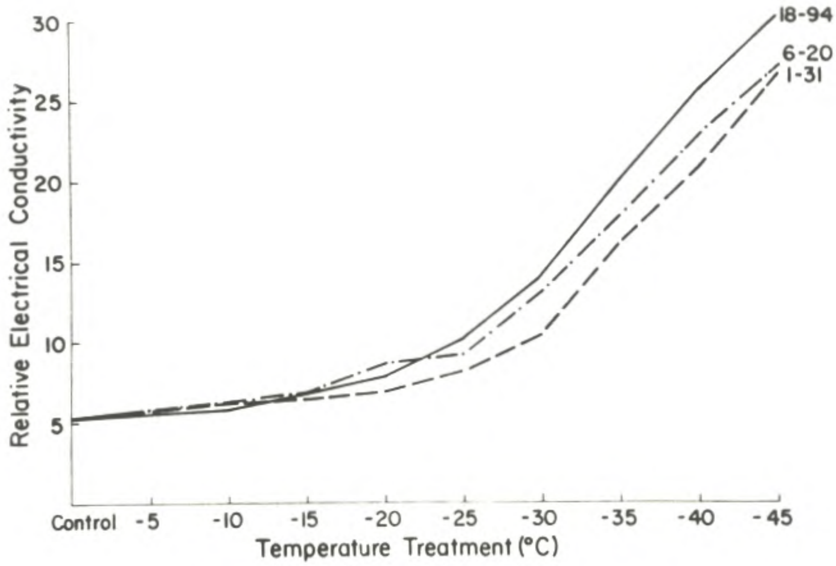

Figure 4.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for lower internodes of three open-pollinated families. Graphs adjusted to a common y-intercept.

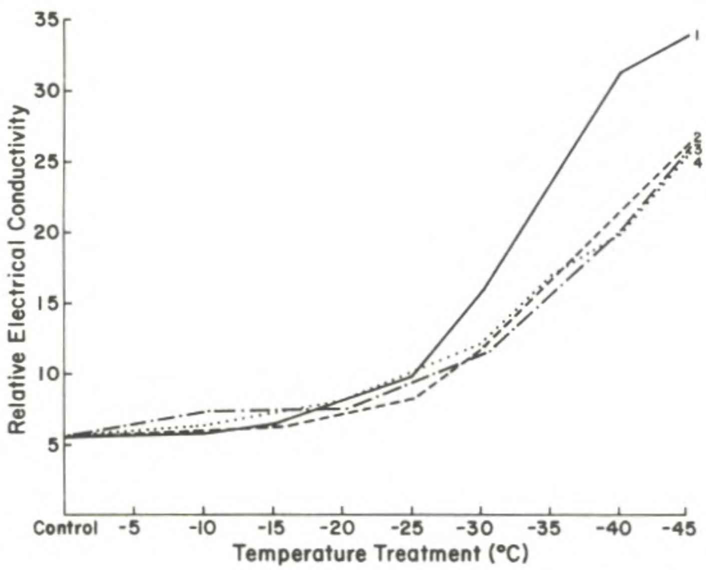

Figure 5.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for four field blocks. Graphs adjusted to a common y-intercept.

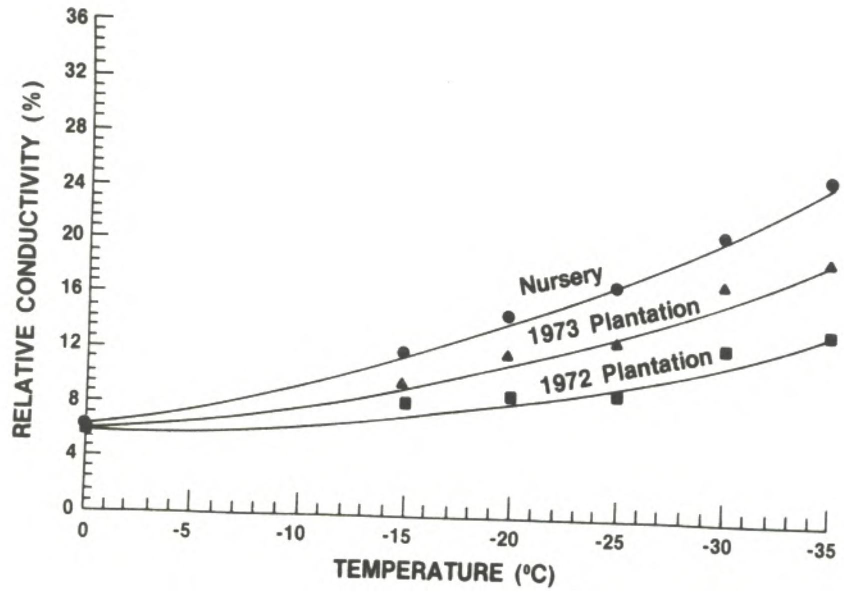

Figure 6.--February 1984 injury (Relative conductivity) to needle tissues versus temperature treatment for four families in each of three environments. Graphs adjusted to a common y-intercept.

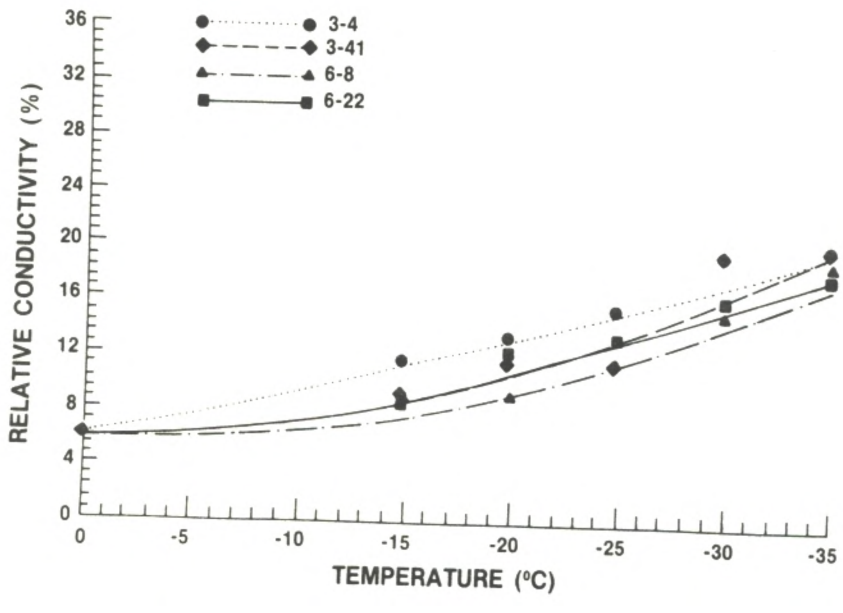

Figure 7.-- February 1984 injury (Relative conductivity) to needle tissues versus temperature treatment for each of four families growing in the 1973 plantation environment. Graphs adjusted to a common y-intercept.