Cold Hardiness Measurement to Time Fall Lifting[1]

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Abstract-Cold hardiness measurement has been found to be one of the most informative physiological tests developed over the last 15 years, and it is precise and quick enough to guide management decisions. The relationships among cold hardiness and other physiological attributes important to lifting and storage success are known for some species such as ponderosa pine and Douglas-fir, but this information is lacking for many other species important in reforestation. The objective of this study was to determine the relationship between cold hardiness at time of fall lifting and outplanting success. We sought a threshold of cold hardiness that would indicate that seedlings were ready to lift and store, as others have found (cf. Simpson 1990).

At cooperating nurseries, seedlings of several conifer species were lifted at weekly intervals during fall 1996 starting three weeks before the normal lifting season began. Cold hardiness was measured on a subsample and the remaining seedlings were overwintered in cold storage and then outplanted as a simulated forest plantation in spring 1997. In fall 1997 survival and growth were measured.

Preliminary results from the Saratoga Nursery (NY) showed that there was indeed a relationship between cold hardiness at time of lifting and field survival of white and red pine, but this relationship for two Norway spruce seedlots was more complex. White pine could have been lifted one week earlier than when operational lifting began, red pine two weeks earlier, and perhaps one of the two spruce seedlots could have been lifted three weeks earlier. Preliminary results at General Andrews Nursery (MN) showed that white pine was ready to lift when operational lifting began but not earlier. Red pine and an Ontario source of white spruce could have been lifted one week earlier, and a central MN source of white spruce would have had no loss in survival if lifted three weeks earlier.

For northern nurseries that must discontinue fall lifting prematurely because of frozen soil, it will usually be valuable to be able to start lifting a week or two earlier, if it can be done with confidence.

INTRODUCTION

Over the last 20 years physiological tests have been developed as a means to evaluate the condition of tree seedlings (Burr et al. 1990, Glerum 1984). Such tests are especially useful when the trees are not growing, and changes in condition are not readily seen. We have been researching physiological testing for the last 15 years and have found measurement of cold hardiness to be one of the most informative tests, as well as being precise, amenable to rigorous statistical analysis, and quick enough to use for management decisions (Burr et al. 1990).

We know a great deal about the dynamics of cold hardiness in Rocky Mountain ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm.), Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco), and Engelmarm spruce (*Picea engelmannii* (Parry) Engelmann). We also know how cold hardiness is related to root growth potential, bud dormancy, and general resistance to the stresses associated with lifting, packing, and overwinter storage. For the western species we have studied the threshold for readiness to be lifted and stored is a

50% index of injury of -22^{o}C. In fact, we have built a cold hardiness model which is driven by photoperiod, temperature, and degree of hardiness, and we have used the model successfully to tell the Forest Service Southwest Region when they should ask their contractor to pull and pack their container stock into cold storage, and when the operation needs to be completed (Tinus 1996).

However, we do not have this kind of information for many of the other species important to reforestation, although there is good reason to believe they would respond similarly. Furthermore, many nurseries begin fall lifting based on calendar date and past experience, but without a means to evaluate how year to year variations in climate affect the readiness of seedlings to be lifted. In northern climates especially, it would be valuable to be able to begin fall lifting a week or two earlier, if it could be done with confidence.

Therefore, the objective of the studies we initiated during the fall 1996 lifting season was to determine the correspondence between cold hardiness at time of lifting and outplanting success. We sought a threshold of cold hardiness that could be used as a readily measured indicator of when the trees were ready to lift.

THE STUDY

The two cooperating nurseries in the Northeastern Area were the General Andrews Nursery at Willow River, MN and the Saratoga State Nursery, Saratoga Springs, NY. The species selected were red pine (*Pinus resinosa* Alt.), white spruce (*Picea glauca* (Moench) Voss), and Norway spruce (*Picea abies* (L.) Karst), which are grown in high volume, and white pine (*Pinus strobus* L.) because of the current attention it is getting as a neglected component of the ecosystem. We used one seed origin of the pines and two of the spruces.

Starting three weeks before the nurseries normally would begin lifting and continuing each week until lifting was complete (or until the soil froze), 112 seedlings of each selected species and seed origin was lifted. One hundred of these were packaged and placed in cold storage using the standard packing and storage technique at that nursery. The other 12 were sent by overnight mail to the Forest Service Coeur d'Alene Nursery's Quality Assurance Laboratory, Coeur d'Alene ID, for measurement of cold hardiness. In addition, the study nurseries reported their daily maximum and minimum temperatures, which were used in an attempt to model cold hardiness as a function of local photoperiod and temperature.

COLD HARDINESS MEASUREMENT

There are a number of ways to measure cold hardiness, but the one selected was the freeze-induced electrolyte leakage test (FIEL), because it is precise, amenable to rigorous statistical analysis, and gives results within three days (Burr et al. 1990).

The procedure is to remove a sample of needles, cut them into 1 cm segments, and put 10 segments in each of 28 culture tubes with 0.5 ml of distilled water. Four of the tubes are put in a refrigerator at about 2^{o}C and not frozen. These are the controls which measure the amount of electrolytes that leak out when there is no damage except for the cut ends of the needle segments. The remaining tubes are placed in a low temperature alcohol bath at -2^{o}C, where the deionized water in the tubes is nucleated to form ice with #8 lead shot chilled to -

80°C. The temperature of the bath is then lowered at a rate of 5°C per hour, so that the rate of cooling does not confound the effects of the actual test temperature. At each of six preselected temperatures four tubes are removed and placed in a refrigerator to thaw. The temperatures are selected to span the expected range from no damage to complete kill, so that the relationship between temperature and injury can be determined. After thawing, distilled water is added to bring the volume to 6 ml to aid measurement, and all of the tubes are placed on a shaker to incubate 20 hours overnight.

The next day, conductivity of the water in each tube is measured. The reading measures the electrolytes that have leaked from the tissue in response to damage from cold. Next, the tubes are boiled to kill the tissue completely, and they are returned to the shaker overnight. On the third day the conductivity is measured again. This time the reading measures the total electrolytes present.

From this body of data an index of injury is calculated which represents the percent of electrolytes that leaked out in response to low temperature damage. From this we calculate the temperature that causes a 50% index of injury and its 95% confidence level. In terms of avoiding damage in the nursery, this is not the figure to use, because, of course, 50% injury is much greater than is acceptable[4] (I_{50}), but we use it for comparisons because we can estimate it most precisely with the greatest level of confidence.

The seedlings that had been lifted at weekly intervals and stored overwinter were outplanted in May 1997 at each nursery in a simulated forest planting. The seed sources and lift dates were divided and planted as four randomized blocks for statistical purposes.

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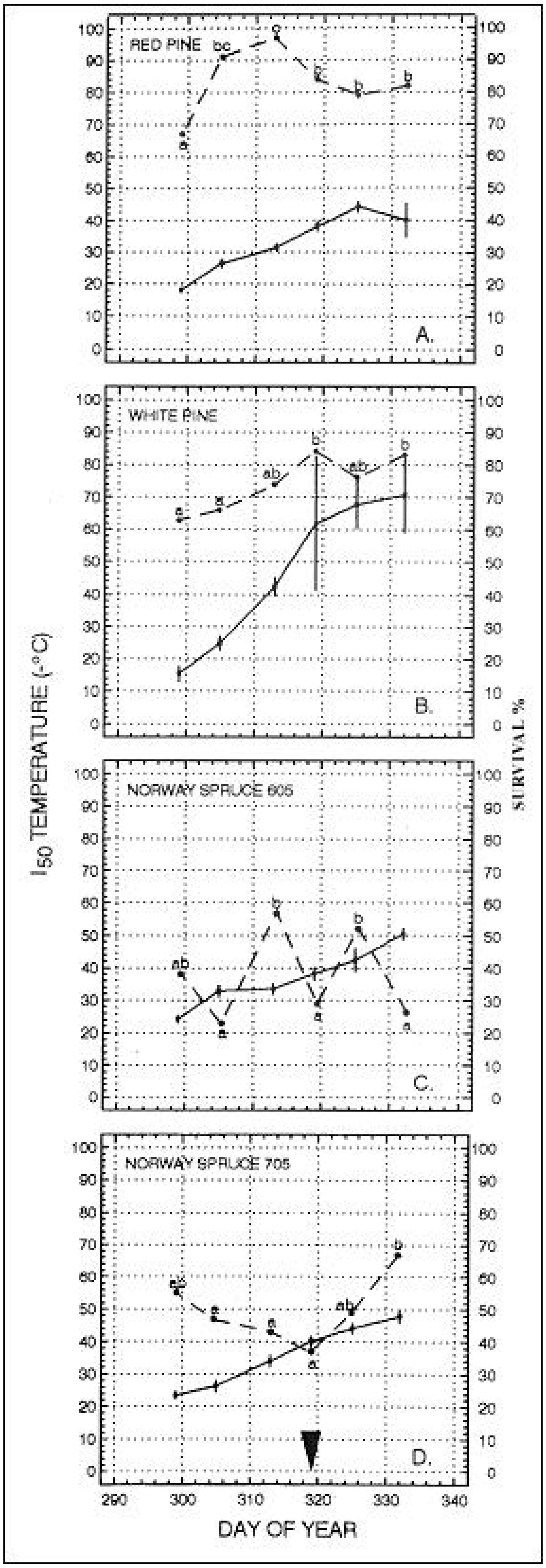
[4]A 30% index of injury is approximately equivalent to an LT_{50}, in a whole plant freeze test, and a 10% index of injury or less represents damage the seedlings can usually recover from.

COLD HARDINESS RESULTS

With regard to the spruces, the behavior of the two sources of Norway spruce in New York was indistinguishable, and they were hardier than the pines from the beginning (Fig. 1C & D). However, in Minnesota the two sources of white spruce hardened at the same rate, but the Ontario source hardened 15 days behind the central MN source (Fig. 2C & D). In both MN and NY (not shown) throughout the measurement period, the rate of hardening of the spruces was rapid and linear (Fig. 3).


RED PINE
A.
WHITE PINE
B.
NORWAY SPRUCE 605
C.
NORWAY SPRUCE 705
D.
I50 TEMPERATURE (-°C)
SURVIVAL %
DAY OF YEAR

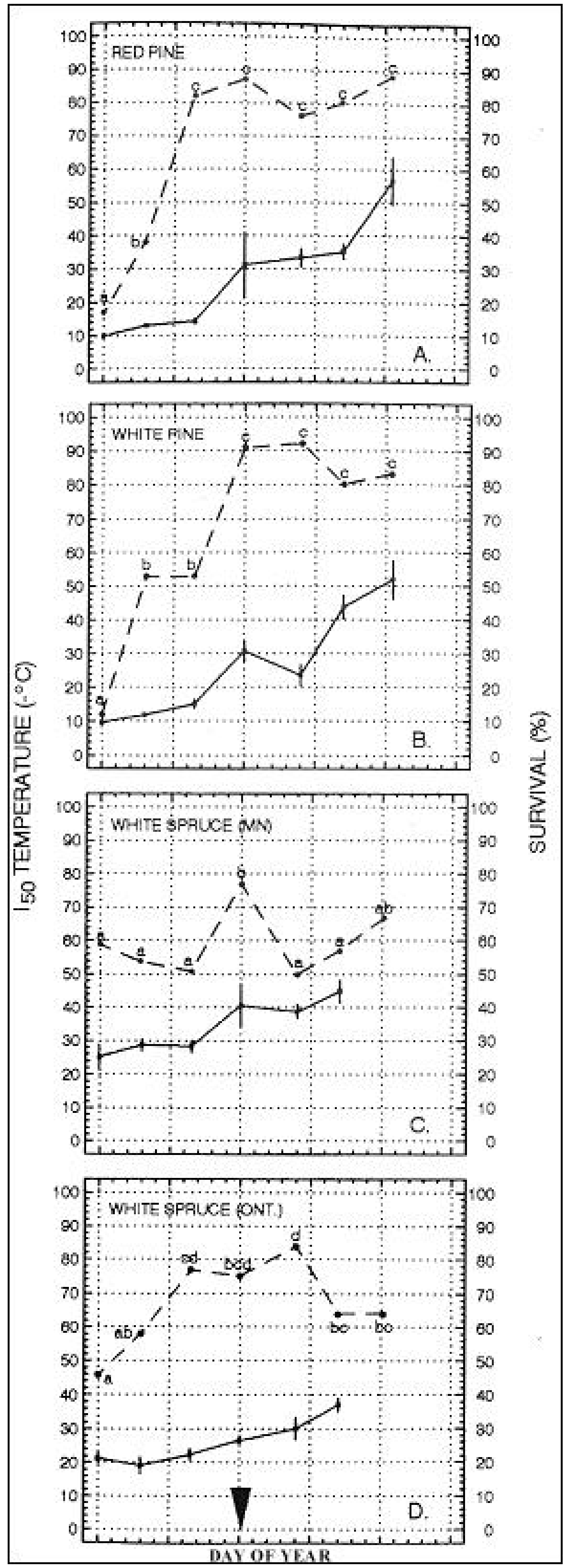

RED PINE
A.
WHITE PINE
B.
WHITE SPRUCE (MN)
C.
WHITE SPRUCE (ONT.)
D.
I50 TEMPERATURE (-°C)
SURVIVAL (%)
DAY OF YEAR

Figure 1. Cold hardiness at time of lifting (solid line) and field survival (dashed line) at Saratoga State Nursery (NY) of (A) red pine, (B) white pine, and Norway spruce (C) lot 605, and (D) lot 705. Vertical lines (cold hardiness) are 95% confidence intervals; if they do not overlap, data points are deemed significantly different. Letters indicate differences at p =.05 by the Chi squared test; data points that do not have the same letter are significantly different. Arrow indicates date when operational lifting began.

Figure 2. Cold hardiness (solid line) at time of lifting and field survival (dashed line) at General Andrews Nursery (MN) of (A) red pine, (B) white pine, and white spruce (C) from central MN, and (D) from Ontario. Vertical lines (cold hardiness) are 95% confidence intervals; if they do not overlap, data points are deemed significantly different. Letters indicate differences at p =.05 by the Chi squared test; data points that do not have the same letter are significantly different. Arrow indicates date when operational lifting began.

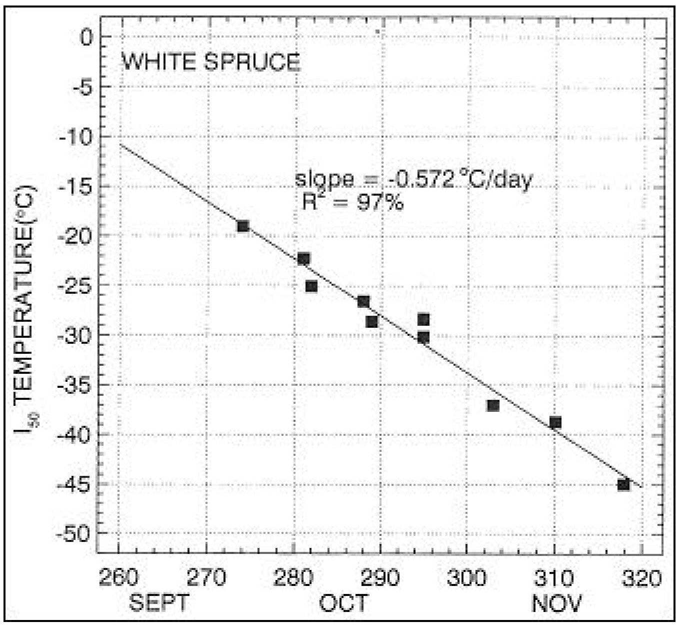


Figure 3. Regression of cold hardiness of MN white spruce versus day of year 1996 showing a high degree of linearity. Both seedlots are represented, but the Ontario source has been moved earlier by 15 days to overlap the MN source with maximum R^2.

The red and white pine in Minnesota behaved almost identically (Fig. 2 A& B). Both had little hardiness when measurements began, but gained it slowly over the next two weeks and thereafter more rapidly. Using the -22°C criterion for southwestern conifers, the pines would not have been ready until October 14, which is when the nursery normally begins lifting. However, the pines in New York hardened at different rates. The white pine hardened much faster than the red pine, but by the end of lifting both appeared to be reaching maximum hardiness,-40°C for red pine and -70°C for the white pine (Fig.2 A & B).

OUTPLANTING SURVIVAL

Survival reported here is preliminary, because the tally was taken in NY the last week of July and in MN on August 11. Additional mortality and growth is expected by the end of the growing season.

Survival of red pine in New York lifted when operational lifting began (day #319) was high. Although it might have been even higher if lifted one or two weeks earlier, corresponding to an 1.0 of -26^{o}C, this may not be biologically significant. However, three weeks earlier (Fig. 1A, first lift date) is clearly too soon.

Plantation survival of white pine in New York lifted when operational lifting began (day #319) was high and remained so for the duration of lifting (Fig. 1B). It could have been lifted a week earlier without significant loss of survival, corresponding to an 1.0 of about -40^{o}C, but not two or three weeks earlier.

Survival of Norway spruce source 605 also showed an unusual pattern (Fig. 1C). Significant week to week changes in cold hardiness and root growth potential have been observed a number of times before, but this is the first time we have seen it in field survival. There have been many lifting window studies in the past, but seedling samples have usually been lifted at two week or monthly intervals, so these studies would not have picked up the weekly variation seen here (cf. Heidmann and Haase 1991). Overall, there was no progressive change in survival, so disregarding the week to week variation, lifting of the 605 source could have started three weeks earlier without loss of field survival, corresponding to an I^{50} of -23^{o}C.

Survival of Norway spruce was very poor, probably because of the very sandy site, and rainfall from the time of planting in May through the last week in July was about half of normal. Survival of source 705 was at its lowest when operational lifting began and increased significantly by the end of lifting (Fig. 1D). Survival of seedlings lifted up to three weeks earlier was at least as good as when operational lifting began.

At General Andrews Nursery white pine was ready to lift when operational lifting began (day #290), but not earlier, corresponding to an I^{50} of -30^{o}C (Fig. 213). Red pine could have been lifted a week earlier when it had an I^{50} of -15^{o}C (Fig. 2A). The Ontario source of white spruce could have been lifted one week earlier when it had I^{50} of -23^{o}C, but not sooner (Fig. 2D). The central MN source of white spruce could have been lifted up to three weeks earlier with no loss in survival, corresponding to an I^{50} of -25^{o}C (Fig. 2C). However, with one exception survival throughout the lifting period was not very good, probably due to the site being very sandy, although summer rainfall was average.

DISCUSSION AND CONCLUSIONS

Results reported here are preliminary for two reasons. First, in order to present the material at the nursery meeting, survival tallies were made well before the end of the growing season. First season growth has yet to be measured, and some additional mortality can be expected, although this is likely to increase, rather than blur, the differences in survival between early and later lifted stock. Second, the first year of a study like this one must be considered a case history until it can be repeated.

The rate of hardening of most of the species and seed lots was very linear and did not appear to respond to short term fluctuations in the local temperature (Fig. 3 for example). If this is repeatable year to year, it will simplify modeling of weather data to predict cold hardiness. It may become possible to take one or a few cold hardiness measurements early in the season and project hardening in time to a benchmark I^{50} temperature when the seedlings will be ready to lift. Results from the first year of this study are very promising.

ACKNOWLEDGEMENTS

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