

USING ELECTROLYTE LEAKAGE TESTS TO DETERMINE LIFTING WINDOWS AND DETECT TISSUE DAMAGE

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Seedling quality, conductivity, FIEL

Physiological testing is rapidly coming into use as a means to determine the condition of nursery stock and predict how it will respond to treatment or use. One such test, the electrolyte leakage test, can be used to measure cold hardiness and detect tissue damage. The principle of this test is that when cell membranes are damaged, electrolytes leak out into the water in which the tissue is immersed and can be measured by the conductivity of the solution. The test for damage is nonspecific in that anything that damages the membranes, such as cold, heat, disease, or mechanical injury, will cause electrolyte leakage. In the case of cold hardiness measurement, we know the damaging agent because we freeze the tissue.

Why measure cold hardiness? This is an important attribute in its own right, but it is also well correlated with a number of other important attributes such as bud dormancy, root growth potential, and ability to be lifted and successfully stored over winter.

We have used the measurement of cold hardiness to determine when conifer seedlings are ready to be fall lifted, so that they will store well over winter and survive well when out planted; these results have been reported at previous nursery meetings. Now, starting with 2 species of juniper, we have created a computer program in which you enter the current date, results of a cold hardiness measurement made in the early fall, and the threshold of cold hardiness needed for high out planting survival. The program will compute the number of days before you can begin lifting.

RUNNING THE TEST

Sample preparation

The sample can be any tissue. In measuring cold hardiness to determine the lifting windows of conifers we use foliage because it is available year-round, and it is nondestructive of the trees. However, stems or roots can also be used, if either is the critical tissue.

Day 1: Cut the sample into 1-cm segments and place 10 of the segments, cut at both ends, into each of 28 12- by 100-mm culture tubes. Add 0.5 ml distilled water and a few grains of washed quartz sand. Place 4 of the tubes in a refrigerator at about 2 °C. These are the control samples that measure the amount of electrolytes that leak out when there is no damage except for the cut ends. The rest of the samples are arranged in 6 groups of 4, and at least one tube in each group is fitted with a thermocouple immersed in the liquid and connected to a readout device. Cap each tube with a vented cap. Place the rack of tubes in a styrofoam box, and set it inside a chest freezer. Monitor the decrease in temperature, and when the liquid reaches -2 to -3 °C, shake the tubes. This will cause the sand to nucleate the water and freeze it. You will know this has happened because the temperature of the liquid plus ice will rise to 0 °C. It is important that ice be in contact with the tissue and that it is frozen at as warm a temperature as possible to avoid artifacts. Thereafter, adjust the lid of the box and/or temperature of the freezer so that the decrease in temperature is no more than 5 °C per hour.

In order to get a precise measurement you need to have some idea of how cold hardy the tissue is. Select 6 target temperatures that you expect will span the range from no damage to a complete kill. As a group of tubes reaches each of the successively lower temperatures, remove them and place them in a refrigerator to thaw.

After all of the tubes have been frozen to their target temperatures and thawed, add 5.5 ml distilled water and put them on an orbital shaker at about 120 rpm. The surface of the water should be rotating and agitating the foliage, but there should be no danger that the tubes would spill or be thrown off the shaker.

Day 2: Leave the tubes on the shaker for about 20 hours. Measure the conductivity, being careful not lose any foliage or transfer it from one tube to the next. Start with the controls and proceed to groups frozen to successively lower temperatures. This way you can measure one tube after another without having to clean the probe between tubes. The readings can be recorded on a form or entered directly into a computer spreadsheet. If you have selected the target temperatures correctly, you will notice that the conductivity rises with lower freezing temperature.

Next, recap the tubes, place them in beakers of water, and boil for 20 minutes. This is to kill the tissue completely and release all of the electrolytes that can leak out. Put the tubes back on the shaker overnight for about 20 hours.

Day 3: Measure the conductivity again. This time the conductivity will be high in all of the tubes. Next, package the data in an ASCII file named FIELCOND.DAT and run FIEL.EXE (Appendix 1). The program subtracts the conductivity of the unfrozen controls from that of the frozen samples and then calculates an index of injury for each frozen sample. This represents the proportion of the total electrolytes that leaked out in response to the freezing. Sometimes the results are obvious by inspection, but it is better if you have a graphics program that will accept a data set and plot index of injury (Y axis) against freezing temperature (X axis) (Figure 1). We use the 50% index of injury as the benchmark for cold hardiness because it is usually near the midpoint on the curve and has the smallest confidence interval.

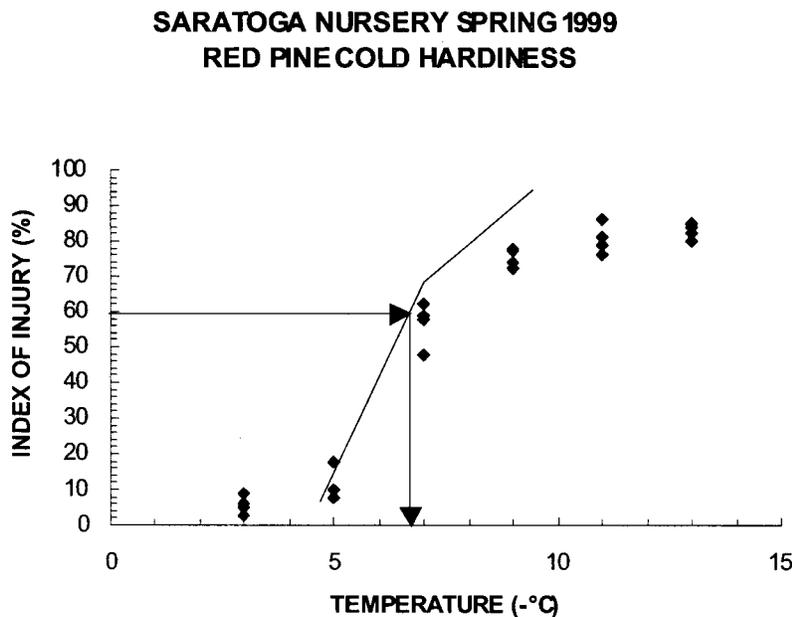


Figure 1. To determine the temperature of the 50% index of injury graphically, plot the points as shown. Draw lines that best fit the data through the part of the curve that includes the 50% index of injury. Where the drawn line crosses the 50% level, project down to the horizontal axis and read the temperature.

Detection of damage: Another use of the electrolyte leakage test is to determine whether trees have been damaged. In this case the tissue selected should be the one on which the damage is suspected. For instance, if container stock has recently been moved out of the greenhouse and is subjected to an unexpectedly cold freeze, the partially lignified new shoot tips would be the most vulnerable. Take samples of the tissue that may be damaged. For the control, take samples of comparable tissue that you are sure is not damaged. Ten is a good number of replications for each. Place the samples in culture tubes in sufficient distilled water to cover them (the same volume in each), and place them on the shaker for 20 hours. From here on, the procedure is the same as for the measurement of cold hardiness. To run FIEL.EXE code the control temperature as +1 and the test samples as -1. Our experience has been that if the index of injury is 10% or less, the trees will recover. At a 30% index of injury, the trees are badly damaged and not shippable. At 50%, they are mostly dead.

APPENDIX ONE

Program File
 Calculation of Index of Injury for grouped data based on:

- 1) averaged control data within the group, or
- 2) control datum for each individual within the group

User is interactively prompted to specify calculation method

Program input is from file FIELCOND.DAT

- 1) Data for each group should reside in contiguous records
- 2) Control data should be identified with TEMPERATURE=1
- 3) For individual calculation approach, control data must be grouped preceding treatment data, and be in the same order

Format of data input is:

Col		
Group ID	1-7	up to 7 numbers and/or characters
Temperature	8-12	1=CONTROL, otherwise negative
Replicate No.	13-14	up to 2-digit number
CONBB	15-19	conductivity before boiling
CONAB	20-24	conductivity after boiling

Program output is to file INJURY.DAT

Format of data output is:

Col	
Group ID	1-7
Temperature	9-13
Rep ID	15-16
Relative Conductivity (percent)	18-22
Index of Injury (percent)	24-28