FROST HARDINESS TESTING FO} NURSERY STOCK

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Frost damage has long been a problem in forest nurseries. The damage often goes unnoticed until spring when needles turn red, buds fail to flush and often a large percentage of the crop is lost. This is particularly frustrating when the frost event occurs after the last seedling inventory because the manager has no means to determine the amount of loss. A method to assess frost hardiness of seedlings during susceptible periods in the spring or fall would allow the nursery manager to protect crops not frost hardy enough to withstand the expected low temperatures. If frost damage occurs, then losses can be estimated prior to harvest before more money is spent on lifting and planting damaged or dead stock.

Two methods commonly used to assess frost hardiness are the electrolytic method and the whole seedling assessment method. In both of these methods the seedling or a section of seedling tissue is exposed to the desired freezing temperature in a programmable freezer; the following procedure is common to both methods.

1. Randomly select a sample of seedlings from the population of interest (15-40 seedlings).

2. Place the seedlings or tissue sample in the freezer.

3. Lower the temperature at a set rate (5°C/hr is recommended by Timmis 1980).

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4. Hold the test temperature for a set time period (2 hours - Timmis 1980).
5. Return to the starting temperature at a given rate (not to exceed 20°C/hr - Timmis 1980).

The rate at which seedling tissue is frozen is critical as more rapid rates of freezing and thawing may compound the damage. Increasing the exposure period to the low temperature may also cause more damage. Whatever rates or exposure periods of freezing are chosen, all tests should be carried out in precisely the same manner for results to be comparable (Levitt 1980). Repeated freezing also tends to increase damage (Green and Warrington 1978). Various freezing chambers and their relative advantages and disadvantages are discussed in a comprehensive review by Warrington and Rook (1980).

The electrolytic method of measuring frost damage is based upon the fact that when a cell freezes, the cell membrane is ruptured and cellular contents begin to leak out. If the plant tissue is surrounded by a water solution, these cellular contents raise the salt content and hence the electrical conductivity of the solution. The amount of frost injury, therefore, can be assessed by measuring the relative increase in electrical conductivity with a conductivity meter (Columbo et al., 1984).

The sample of seedling stem segments or shoot tips is divided in half. The first half are used as controls and are rinsed in deionized water, placed in jars, covered with additional water, and allowed to soak overnight at room temperature. The samples are then shaken and the EC of the solution is measured and recorded as "EC control". The same samples are then killed to determine the total electrolytic content of the tissue - this is accomplished

by heating in a 90°C oven for about 2 hours and then allowing the sample to cool overnight. The EC of the solution is then measured and recorded as "EC killed". The proportion of electrolyte leakage is calculated as % RC Control in Equation A, Table 1.

The second half of the seedling samples are exposed to the desired cold temperature to determine the amount of electrolyte leakage after freezing. The samples are treated as before: placed in a jar, rinsed with deionized water, and then covered with water and capped. The sample jars are placed in a sheet of foam insulation to moderate the freezing process, placed into the freezer and allowed to remain overnight. Upon removal, they are shaken and the EC of the solution recorded as "EC frozen". The samples are then killed completely in the oven and the "EC killed" is recorded. The proportion of electrolyte leakage is calculated as % RC frozen using Equation B (Table 1).

The final calculation of the Index of Injury (I_t) is calculated by comparing the relative amount of electrolyte leakage in the frozen and control samples, Equation C (Table 1).

This method is being used on an operational basis by the Ontario Ministry of Forests to track frost hardiness development in container nurseries. This test has the advantages of being relatively quick (results in 4 days) and large numbers of samples can be run concurrently.

The whole seedling assessment for determining frost hardiness involves, as the name implies, freezing whole seedlings. After completion of the freezing

procedure, seedlings are placed in a greenhouse for 7-10 days and then the needles, buds and cambium are visually assessed for damage. Needles that are frost-killed will turn olive-drab green and then red whereas bud mortality is indicated by brown meristematic tissue. The cambium will turn olive-drab green when damaged and brown when killed outright. Frost killed needles and buds are obvious within three days after being frozen and placed in a warm, lighted environment, but the cambium tissue takes a full 7-1U days to develop damage symptoms. Table 2 (Johnson 1983) shows the assessment scale for nursery stock. This scale is used primarily for Douglas-fir but may be adapted for other species. The emphasis is on economic viability of nursery stock as opposed to whether the seedlings are simply alive or dead--seedlings with over half of the top dead may be alive but are not economically viable for the nurseryman who will likely cull them during packing.

The Whole Seedling Assessment method is being used by the Industrial Forestry Association in Washington and Oregon to track frost hardiness of 1+0 seedlings in order to designate periods when frost protection is necessary. It is also being used by two seedling vigor testing services in Washington and Oregon as an indicator of seedling physiological status both in nurseries and after packing for outplanting status. This method has the advantages of being relatively quick (results in 7-10 days) and looks at the cold hardiness of the whole seedling rather than at a small section of tissue. Bud mortality is essential in assessing growth potential and this technique permits evaluation of both terminal and lateral buds. Container seedlings are particularly susceptible to frost kill at the ground line or just below the root collar (Alden and Hermann 1971) and the whole seedling assessment would detect this type of injury.

Frost hardiness is an extremely important seedling physiological attribute and using either the electrolytic or the whole seedling assessment method will take a lot of the guess work and often painful surprises out of the nurseryman's life.

Table 1 - Calculations for Determining Index of Injury

A. $$\text{RC control} = \frac{\text{EC control}}{\text{EC control/killed}} \times 100$$

B. $$\text{RC frozen} = \frac{\text{EC frozen}}{\text{EC frozen/killed}} \times 100$$

C. $$I_t\ (\%) = \frac{\text{RC frozen} - \text{RC control}}{1 - \frac{\text{RC control}}{100}}$$

where EC = electrical conductivity of control (not frozen), frozen, and killed shoot tips.

RC = relative conductivity

and I_t = Index of Injury

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Table 2 - Frost Damage Assessment Procedure

Seedlings are individually rated by examining the needles, buds and the cambium layer of the stems and assigning an overall frost damage score. Combining the scores for a 20 seedling sample yields a rating for the population.

Needles: Rate from 1 to 10
% needles dead, e.g., 10% = 1, 20% = 2, etc.

Buds: Rate from 1 to 10
Check up to 10 buds if available and record % dead, e.g., 1 bud in 10 dead = 10% = 1, 1 bud in 3 dead = 33.3% = 3.

Stem: Rate from 1 to 4
Divide the seedlings into 1/4's and rate according to the following damage scale:

1 = Top 1/4 dead or girdled
2 = Top 1/2 dead or girdled
3 = Stem girdled in lower 1/4
4 = Entire stem dead

No economic damage (Score = 0.0)

Needles	Buds	Stem
0 - 6	0 - 8	0 - 1

Half Kill (score = 0.5)

Needles	Buds	Stem
0 - 6	0 - 5	0 - 2
0 - 6	9	0 - 1

Total Kill (score = 1.0)

Needles	Buds	Stem
0 - 10	9 - 10	2 - 4
0 - 10	0 - 8	3 - 4
0 - 10	10	0 - 2
7 - 10	0 - 9	0 - 2