

## A Simple Method for Evaluating Whole-Plant Cold Hardiness

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*Whole-plant assessment is direct, requires a minimum of equipment and expertise, and provides a good approximation of actual cold hardiness. Potted test plants are subjected to several target temperatures in a slightly modified domestic chest freezer. After gradual warmup, the plants are placed in a favorable growing environment for 7 to 14 days, and then the percent of cambial damage is determined. The temperature at which 50 percent damage occurs is determined graphically. Tree Planter's Notes 38(2):16-18; 1987.*

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To produce high-quality, properly hardened nursery stock in greenhouses and out-of-doors, it is essential to a) understand the cold hardening process, and b) test for cold hardiness as a guide for manipulating nursery and greenhouse environments. Basically, the objective of cold hardiness testing is to verify that the cultural treatments being applied are producing the desired results. Choosing an appropriate method to test for cold hardiness can be an overwhelming task because of the many testing methods used in research (1, 3, 4).

In this paper, we describe a simple whole-plant assessment method that is suitable for both

production nurseries and comparative research studies. Testing intact plants approximates actual cold hardiness and predicts plant survival better than does testing excised tissue samples. The method requires a minimum of equipment and skill, the procedure is simple, and the results can be correctly interpreted without calibration. However, a longer waiting period is required before the extent of damage can be measured.

Briefly, the whole-plant method consists of a) placing potted test plants in a chest freezer with roots insulated in a bed of vermiculite; b) lowering the temperature until target temperatures are reached, then removing seedlings from the freezer; c) after gradual warmup, placing the seedlings in a favorable growing environment for 7 to 14 days; d) determining the extent of cambial damage; and e) plotting the data and interpolating the temperature at which 50 percent damage occurs.

### Equipment

The test is conducted in a domestic chest freezer that can attain temperatures of -25 to -30 °C. A thermometer with remote readout is used so that temperatures in the chest can be monitored without opening the lid. A 50 cubic feet per minute fan is used to circulate the

air within the freezer. The fan should be less than 15 watts to avoid unnecessary heat load within the chest.

A cold room or refrigerator at +1 °C is ideal for slowly thawing the seedlings. If neither is available, a Styrofoam cooler will do. A greenhouse is best for allowing injury symptoms to develop, but a warm well-lighted room will suffice.

### Conducting the Freezing Test

The plants to be tested are potted with roots in moist growing medium. Styrofoam containers work well because they help insulate the roots. The number of seedlings per container is not important as long as their crowns are well spaced for adequate air circulation.

The objective is to choose target temperatures that bracket the killing temperature. A wrong estimate may leave the killing temperature off the scale of the test, in which case a second test must be run. We recommend at least three target temperatures, each represented by 10 plants. Before a test begins, randomly divide the plants among the target temperatures and give them color-coded labels to facilitate their removal.

Set the containerized plants in the bottom of the freezer and pour vermiculite around the containers so the pots are covered

with 5 centimeters of vermiculite. It is important that the root systems get no colder than  $-2\text{ }^{\circ}\text{C}$  during the tests ( $0\text{ }^{\circ}\text{C}$  if the roots are actively growing).

To run the test, turn the freezer and fan on and set the freezer control at its lowest setting. The rate of cooling down to  $0\text{ }^{\circ}\text{C}$  is not important, but below that level it should not exceed  $2$  to  $6\text{ }^{\circ}\text{C}$  per hour. At  $0$  to  $-10\text{ }^{\circ}\text{C}$ , cooling may be too fast, but can be reduced by inserting a small wedge (such as a pencil) under the chest lid to hold it open slightly.

When the first target temperature is reached, turn the fan off, then open the freezer and quickly remove the designated plants and place them in a cold room or in a Styrofoam cooler containing ice. Then repeat this procedure with the remaining target temperatures.

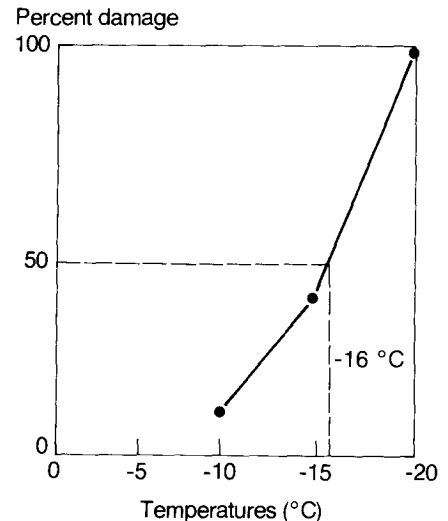
The rate of thawing can be much faster than the rate of freezing, up to  $20\text{ }^{\circ}\text{C}$  per hour. An overnight thawing period is sufficient. Then move the potted plants to a favorable growing environment and keep them well watered.

Freezing damage to leaves (or needles), buds, and cambium can be assessed separately and combined into a damage scale, but we prefer to measure only cambial damage. Damage symptoms to cambial tissue require 7 to 14 days to develop, depending on species and environment.

To assess damage, use a knife or razor blade and scrape the entire length of the stem down to the cambium. The color of healthy tissue is fresh green, but damaged tissue changes over time from fresh green to drab olive green to brown.

The symptoms of injury will vary from one species to another, and the rate of symptom development will depend on the hardiness of the plants and conditions in the greenhouse or room where the plants are incubated. With plants in early stages of cold acclimation, symptoms will be readily visible after 7 days. Hardy plants take longer. It is usually desirable to estimate damage as soon as possible, but the longer the incubation period, the less the chance of error.

Measure the length (+ 0.5 centimeters) of the damaged stem down from the shoot tip, then measure the total stem length, excluding the 5 centimeters covered with vermiculite. Calculate the percent damage for each plant as: (damaged length per total length)  $\times$  100. Determine the mean percent damage for the test plants representing each target temperature. Next, plot percent damage versus temperature (fig. 1). From the graph, determine the temperature at which 50 percent damage would occur; this is the lethal temperature for 50 percent damage, or  $LT_{50}$ . Or, you may wish to determine the temperature at which no



**Figure 1**—Percent cambial damage plotted for three freezing test temperatures ( $-10$ ,  $-15$ , and  $-20\text{ }^{\circ}\text{C}$ ), as determined with the whole plant assessment method. The temperature for 50 percent damage ( $LT_{50}$ ), in this case about  $-16\text{ }^{\circ}\text{C}$ , is determined from the graph.

damage occurs, or 20, 50, or 100 percent damage—depending on your preferences and the purpose of the nursery stock.

### Discussion

A domestic chest freezer has enough cooling capacity for testing during the hardening period to determine if plants have attained sufficient cold hardiness to withstand the natural environment. If you desire additional cooling capacity, replace the refrigerant to extend the lower limit to about  $-30\text{ }^{\circ}\text{C}$ . Or place a pan of liquid nitrogen in the

freezer. Determining the maximum cold tolerance of plant materials requires more sophisticated (and expensive) equipment capable of reaching -70 °C.

Our method assumes that the youngest parts of the seedling are the most vulnerable to freezing injury, so that the severity of injury increases as more of the stem is damaged, from the top down. If the species you are working with shows a different pattern of freezing injury, devise an alternative damage rating scheme.

The amount of damage that occurs in a freezing cycle depends on the low temperature attained, on the rates of freezing and thawing, and on the duration of the low-temperature period. Some authors recommend that each target temperature be maintained for a specified time period (1 to 3 hours) before removing the plants (1). We prefer to remove plants immediately once the target temperature is reached.

A test that requires holding the plants at specified minimum temperatures requires more time and equipment than one in which plants are removed immediately upon reaching a benchmark temperature. Although the results of the two methods may

be somewhat different, they are both usable for predicting field reaction to freezing temperatures. The important issue here is to choose a technique, then apply it uniformly and consistently.

If maintaining each target temperature is desirable, you can install a microswitch temperature controller (or for more money, a cam-operated or electronic temperature controller) on the outside of the freezer control at its lowest setting. Then the temperature controller and light bulb, working against the freezer's cooling system, will maintain any desired temperature.

In some freezing tests, the plants are inoculated with ice to minimize the damaging effects of supercooling. Plant tissue supercools if the temperature of the cell solution falls below the freezing point without the formation of ice. When ice does form in supercooled tissues, crystals form very rapidly and are more damaging to tissue than ice that forms slowly in association with little or no supercooling.

Supercooling is of more concern when target temperatures fall in a range of 0 to -5 °C. Wrapping the seedling stems with a 1- to 2-centimeter-wide

strip of damp cheesecloth inoculates plants with ice and circumvents supercooling during freezing tests (2). Because our method does not include seeding with ice crystals, supercooling and increased damage may occur. But results of the test are consistent. The cooling rate of 2 to 6 °C per hour should be maintained as uniformly as possible within and between tests.

#### Literature Cited

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