Seedling Quality Tests: Cold hardiness

by Gary Ritchie and Tom Landis

Introduction

In the Winter, 2003 issue of FNN, we initiated a series of articles on seeding quality tests with a discussion of the popular Root Growth Potential (RGP) test. In this issue we will consider a test that has been around much longer than RGP – the cold hardiness (CH) test.

Concepts Behind the Test

Cold injury to plants is one of the critical factors that determine where plants are able to survive in the Temperate Zone, and Hardiness Zones have been established based on tolerance to cold temperatures. Tree species exhibit a vast range of midwinter hardiness levels (Sakai and Weiser 1973), reflecting the climate of the regions in which the species occur. Boreal conifers, such as black and white spruce, jack pine and others attain hardiness levels below -112 ºF (-80ºC), while many Rocky Mountain conifers, such as lodgepole pine and Engelmann spruce, achieve this level or nearly this level. In contrast, Pacific coast conifers such as Douglas-fir, coast redwood and western redcedar, rarely harden to below -13ºF (-25ºC).

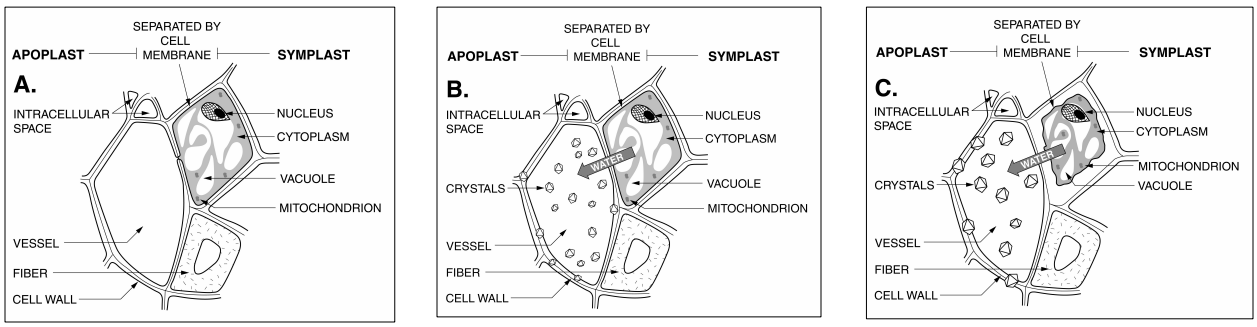
Although CH testing has been used since the early 1900's as a method of selecting cold hardy horticultural cultivars, its use as a seedling quality test has developed over only the past thirty or so years. As we will now discuss, CH tests have become of the most utilized tests of seedling quality with a variety of different applications in nursery management.

Annual Cold Hardiness Cycle. During the growing season, most temperate zone plants are killed when the air temperature drops to only few degrees below freezing. However, as winter approaches and growth slows, plants perceive the changing photoperiod (lengthening nights) and begin to develop tolerance to cold (Weiser 1970, Glerum 1976, 1985, Bigras and others 2001). When winter arrives, plants that would have been killed at slightly below freezing become conditioned to survive very cold temperatures. Then, as winter draws to a close and the growing season nears, this cold hardiness is rapidly lost and plants resume growth.

How Plant Cells Freeze. To understand how plants are able to progressively tolerate cold temperatures, it is necessary to discuss what happens inside plant tissue when it freezes. In a cross section of plant tissue (Figure 1A), there are various types of cells that have different functions. Some cells such as the fibers and vessels are empty while others are filled with living material called cytoplasm. The cells that contain cytoplasm are enclosed within a cell membrane made of a fatty material called lipid in which protein molecules are embedded. This membrane plays a key role in plant cold hardiness. All cells are surrounded by walls made primarily of cellulose, which is stiff and strong. The cell walls are packed tightly together, but occasionally spaces will occur between them – intracellular spaces that contain only air or water.

Everything within the plant that is enclosed by the membrane system is called, collectively, the symplast and is living tissue. Everything outside the membrane (cell walls, intercellular spaces, empty cells, etc.) is

Figure 1. Diagrammatic cross section through plant tissue illustrating the events that occur when tissue freezes: A - Living cell contents (symplast) are separated from non-living cell contents (apoplast) by the cell membrane. B - When temperatures fall below freezing, ice crystals begin to form in the apoplast. As these crystals grow, they draw water across the cell membrane causing dehydration of the cell contents. C - As temperature continues to fall, more water is drawn from the cells, the cytoplasm becomes severely dehydrated, and the membrane can rupture, and/or lose its semi-permeable properties. When this occurs, cell contents can leak into the apoplast resulting in severe injury or death.



referred to as the apoplast, and is non-living (Figure 1A). Both the symplast and apoplast are bathed in water. The apoplast water is nearly pure, so its freezing point is close to 32 °F (0 ºC) . The water in the symplast, however, contains dissolved sugars and salts, suspended starch granules and protein molecules. These materials cause an osmotic effect and depress the freezing point of the water in the symplast to considerably below freezing. When this tissue is exposed to increasingly colder temperatures, the relatively pure apoplastic water begins to freeze and small ice crystals form within the cell walls, intracellular spaces and other voids within the apoplast (Figure 1B). The water in the symplast, with its lower freezing point, resists freezing. Thus, the ice that forms within the plant tissue is contained in the apoplast and does little or no damage to living plant tissue.

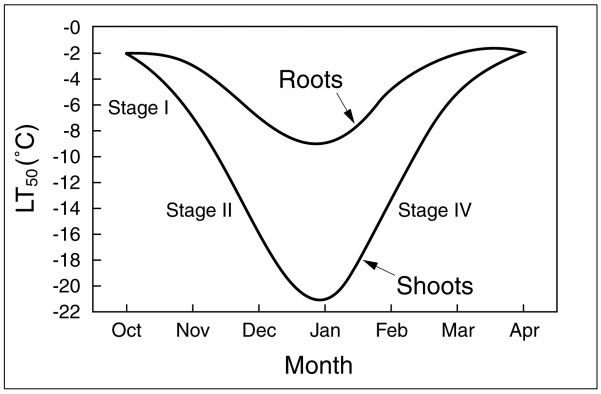
Ice has a very strong affinity for water – so strong that the ice crystals in the apoplast pull water tenaciously across the membrane and out of the symplast (Figure 1B). Since the membrane is permeable to water only, the dissolved sugars and other materials remain in the symplast even as water is being drawn out. This raises the concentration of the dissolved solutes, further lowering the freezing point of the symplast water. So, the more water that is pulled out of the symplast, the more stubbornly it resists freezing. When the temperature increases, the ice crystals gradually melt and the water trapped in the ice crystals is pulled back into the symplast by osmosis. The symplast regains its lost water, the living cells re-hydrate, and no tissue damage occurs.

Throughout winter, this process occurs over and over - even on a daily basis when nights are cold and days are warm. Ice routinely forms and melts in the apoplast, and water moves into and out of the symplast across the membrane. However, when plants are not cold hardy or when the temperature falls below their seasonal level of hardiness, the size of the ice crystals become larger causing severe dehydration of symplastic cells. When this happens, proteins denature and cell membranes are killed or damaged which allows cell contents to leak into the apoplast. Eventually, cells plasmolyze and their cytoplasmic volume decreases sharply, leading to cell death (Figure 1C). It is not clear whether low temperature itself, or desiccation, or both actually incite the damage (Adams and others 1991).

Mechanisms of cold hardiness. Cold hardy plants avoid cold injury by several mechanisms (Sutinen and others 2001, Öquist and others 2001). Solutes accumulate either actively or passively in the symplast lowering their freezing point. In addition, the properties of cell membranes change, making them physically more resistant to desiccation and rupture. Another important avoidance mechanism is deep supercooling of water (Quamme 1985). Pure water can cool to nearly -40 °F (-40 ºC) without forming ice crystals if no ice nuclei are present. Some plants are able to exploit this property of water to prevent ice crystal formation down to nearly this temperature. However, when this "supercooled water" freezes it is nearly always lethal. The observation that many plant species do not occur north of the -40ºF mid-winter isotherm, suggests that they avoid cold damage by this mechanism (George and others 1974). Midwinter temperatures of about -40 °C also occur commonly at timberline, causing Becwar and others (1981) to speculate that supercooling may also limit survival of certain species to below timberline. Many conifers (pines excepted) employ supercooling as a method of avoiding cold damage. However, many tree species can survive temperature far below -40 °C, so they are able to resist cytoplasmic desiccation by other, less well understood, mechanisms.

Cold Hardiness Patterns and stages. Cold hardening and dehardening (also referred to as cold acclimation and deacclimation) occur in a series of two (Cannell and Sheppard 1982) or three (Timmis 1976, Timmis and Worrall 1975) stages depending on species. A typical cold hardiness pattern for coastal Douglas-fir shoots and roots for the Pacific Northwest is illustrated in Figure 2. The X-axis shows time from fall to spring and the Y-axis represents the LT_{50} value - the cold temperature that is lethal to 50% of a sample population. When discussing the relative cold hardiness of plants, the LT_{50} is traditionally used as a basis for comparison.

Figure 2. Temperate zone plants go through a seasonal cycle of hardening and dehardening. This generalized curve for coastal Douglas-fir seedlings shows that peak hardiness for both shoots and roots occurs in January. However, note that roots do not attain the same level of hardiness as shoots.



Stage 1 - By October, in response to shortening photoperiod and growth cessation, the LT_{50} begins to drop to around 28º to 23ºF (-2º to -5ºC).

Stage 2 - This stage begins in November and can take the plants down to -4°F (-20°C) or lower. This stage is apparently promoted by exposure to increasingly lower temperatures – normally at night. During this stage intercellular sugar concentration, soluble proteins, membrane permeability and cytoplasmic permeability increase.

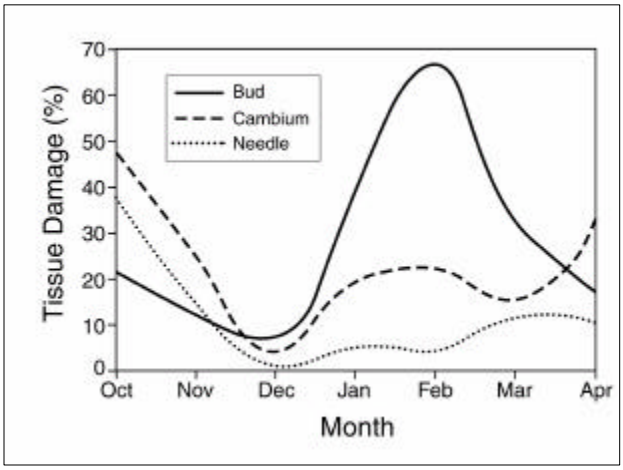
Stage 3 - Peak hardiness is normally achieved by mid-January. By then, hardening can take plants down to -148 ºF (-100ºC) or lower for very hardy species.

Stage 4 - By late winter and early spring, dehardening is triggered by longer days and especially warmer temperatures. This stage continues until active growth resumes in spring, at which time cold hardiness is completely lost.

The environmental cues that trigger and sustain the various stages of hardening and dehardening are discussed and evaluated in the interesting review of Greer and others (2001).

Differential tissue hardiness. Different plant tissues harden and deharden at different rates (Bigras and others 2001). For example, the roots of Douglas-fir seedlings do not harden nearly as much as the shoot although they exhibit the same seasonal hardiness pattern. This has important implications for outdoor container growers (Colombo and others 1995). The Oregon State University Nursery Technology Cooperative tested Douglas-fir seedlings through winter looking at hardiness of the buds, needles and cambium separately (Figure 3). In fall, buds were the most hardy tissues, with cambium the least hardy. By December, however, all tissues had similar hardiness. During late winter, buds dehardened most rapidly, followed by the cambium and finally needles, which retained hardiness into late winter. One would expect, then, to see more cambial damage resulting from fall frosts and more bud damage from spring frosts.

Figure 3. Douglas-fir seedling tissues exhibit differential sensitivity to cold during a winter season. In fall, buds show the greatest hardiness. In spring, this trend reverses, with foliage being hardiest and buds least hardy (used with permission: D. Haase and R. Rose, Oregon State University Nursery Technology Cooperative).



Cold Hardiness Testing

Practical Applications. Nurseries can use CH testing for a wide variety of purposes:

1. Monitoring Development of Hardiness - In fall, when the likelihood of cold fronts increases, it is useful to keep track of the hardiness level of outdoor nursery crops (Perry 1998). If a cold event is forecast to drop below the crop hardiness level, this signals the need for frost protection.

2. Lifting and Outplanting Windows - CH testing can be used as a quick and easy way to determine when bareroot and container stock is hardy enough for lifting, processing and storage. This test is being used operationally in British Columbia where conifer seedlings are considered ready to lift and cold store when they tolerate freezing to –18 °C (0 °F) with no more than 25% visible cold injury to the foliage (Burdett and Simpson 1984).

3. Overall Stress Resistance - Cold hardiness is a good surrogate measure for resistance to the many different stresses that occur during lifting, handling, storage, shipping, and outplanting. As such, CH tests have great value as a indication of overall stress resistance, which is otherwise difficult to measure (Ritchie 2000).

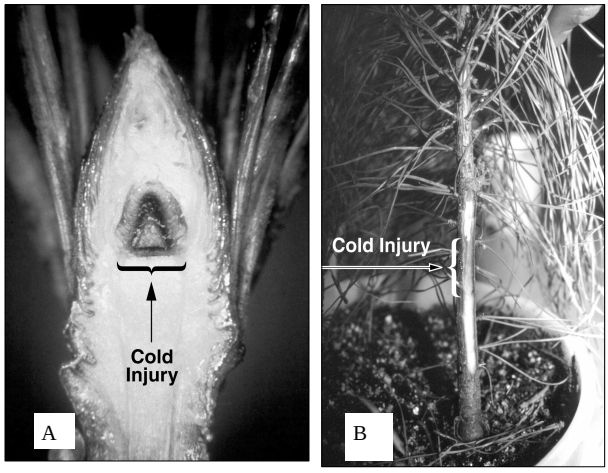
Cold Hardiness Testing methods

There are many ways to test seedlings for cold hardiness (Burr and others 2001), but only two types of tests are being widely used in forestry today: the whole plant freezing test (WPFT) (Tanaka and others 1997) and the freeze induced electrolyte leakage test (FIEL) (Dexter and others 1932, Burr and others 1990, McKay 1992). Both tests entail two steps (Ritchie 1991, Burr and others 2001). In the first step, plants or plant parts are exposed to a freezing stress. In the second step the stress damage sustained by the sample is evaluated.

Whole Plant Freezing Test. First, note that this is a

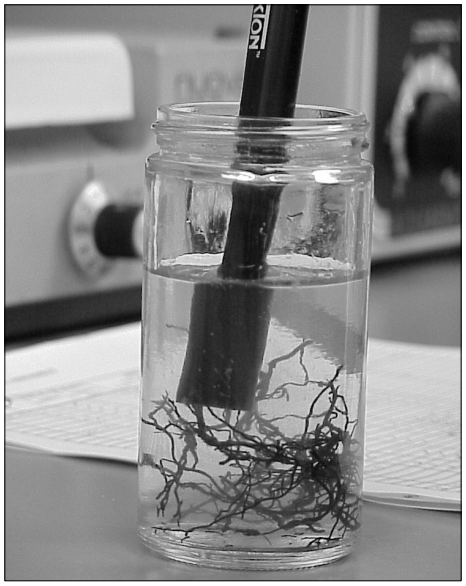
"whole plant test" rather than a "tissue test". This means that the hardiness of several different tissues can be tested at once which will give a good indication of overall cold hardiness. WPFT is a bit of a misnomer, since root systems are normally protected during the low temperature exposure step. In the WPFT a representative sample of seedlings is subjected to a sub-freezing temperature, or a series of bracketing sub-freezing temperatures, for a pre-determined time period – often a few hours. This can be accomplished in a programmable chest freezer or a Thermotron. Next, the seedlings are incubated in a warm growth promoting environment such as a greenhouse for several days. Finally, the test plants are evaluated for cold injury. A wide range of techniques have been used for assessing damage to stem, buds and foliage including visible injury, freeze induced electrolyte leakage, pressure chamber analysis (Ritchie 1990), and chlorophyll fluorescence (Mohammed and others 1995). Each of these methods has its advantages and disadvantages but visible injury is the most widely-used because it is quick, easy and does not require any sophisticated equipment. When plant tissue is injured by cold temperature, the cell membranes begin to leak and the contents become oxidized. The injured tissue turns brown in a few days (Figure 4), and this can be used to rate cold hardiness (Tanaka and others 1997).

Figure 4 . In the whole plant freezing test, seedling tissue turns brown (arrow) after being exposed to the test temperature. The degree and extent of the browning give a good indication of the total damage.



Freeze-Induced Electrolyte Leakage. The FIEL test is a tissue test that is based on the fact that freeze-damaged cell membranes tend to leak electrolytes into the apoplast. When freeze-damaged tissue samples are placed into de-ionized water, this leakage of electrolytes will increase the electrical conductivity of the water which can be measured with a conductivity meter. The technique can be used on foliage, stem segments or root sections.

Figure 5. In the freeze induced electrolyte leakage test, samples of foliage, roots, or stem tissue are exposed to the test temperature and then the relative amount of cellular leakage is measured with an electrical conductivity meter.



The first step is to expose the tissue to sub-freezing temperatures in a programmable freezer or Thermotron. One advantage of the FIEL test is that the samples take much less space than the entire seedlings in the WPFT. After exposure to the desired temperature, the sample is sectioned and placed into vials containing deionized water where they are incubated until leakage stabilizes (Figure 5). Next, the initial conductivity of the solution (EC_1) is measured. The sample is then completely killed by heating or freezing and the final conductivity (EC_2) is measured. A relative conductivity index is calculated as:

$$RC\ (\%) = (EC_1 - B_1) \times 100 / (EC_2 - B_2) \quad [1]$$

Where B_1 and B_2 are optional blanks included to account for possible ion leakage from the vials. See Burr and others (2001) for a detailed discussion of this method.

The FIEL test has been widely used because it is relatively simple and produces a numerical result, compared to the subjective assessment in the WPFT. Some researchers prefer to test foliage whereas others use root tissue as the definitive indication of seedling cold hardiness.

Sources of Seedling Quality Testing

In the introductory article we presented a table listing all

the seedling quality testing facilities in North America. However, several readers pointed out that we missed one - the Laboratory for Forest Soils and Environmental Quality in Eastern Canada. Hopefully, the following table is complete but, if not, let us know and we'll make any additions or corrections.

Conclusions and Recommendations

Seedlings that are easily killed by temperatures near freezing during the growing season can survive much lower temperatures in winter when they are cold hardy. Winter injury is generally caused by the loss of cell water as it is pulled across the cell membrane to feed ice crystals growing outside the cells. This can severely dehydrate cytoplasm and injure membranes callusing them to leak cell contents.

Hardiness develops in fall triggered by photoperiod, and increases during early winter as seedlings are exposed to increasingly low temperatures. Peak hardiness occurs in January in plants from the northern temperate zone. Following peak hardiness, as photoperiod begins to lengthen and temperatures begin to rise, hardiness is rapidly lost.

Cold hardiness testing is often used along with Root Growth Potential testing to provide quantitative information on the physiological status of forest planting stock. The most commonly used CH tests are the whole plant freezing test, in which entire seedlings are exposed to low temperature stress then evaluated for their response, and the freeze induced electrolyte leakage test, which can be applied to foliage, stems, or root segments.

Cold hardiness tests can be used to indicate when frost protection may be needed in the nursery, to determine lifting and outplanting windows for different species and stock types, and as a surrogate index for overall stress resistance.

References

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Table 1—Seedling Quality Testing Facilities and Their Procedures

Company	Address	Types of Tests Offered			
		Morphology	Root Growth Capacity	Cold Hardiness	Others
Roseburg Forest Products	34937 Tennessee Rd. Lebanon, OR 97355 TEL: 541.259.2651 FAX: 541.259.3661 E-mail: mjalbrecht@msn.com	X	X	X	X
Nursery Technology Cooperative	Seedling Quality Evaluation Services OSU Dept. of Forest Science 321 Richardson Hall 3015 SW Western Ave. Corvallis, OR 97331 TEL: 541.737.6576 FAX: 541.737.1393 E-mail:SQES@orst.edu	X		X	
KBM Forestry Consultants	SQA Coordinator 349 Mooney Avenue Thunder Bay, ON CANADA P7B 5L5 TEL: 807.345.5445 ex. 34 E-mail: sgelleert@kbm.on.ca	X	X	X	X
Laboratory for Forest Soils and Environmental Quality	Tweeddale Centre for Industrial Research 1350 Regent Street Fredericton, NB E3C 2G6 TEL: 506.453.4507 FAX: 506.453.3574 E-MAIL: lfsez@unb.ca		X	X	X

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