The Container Tree Nursery Manual

Volume Seven

Chapter 2

Assessing Plant Quality

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7.2.1 Introduction

In his prophetic work "Planting the Southern Pines," Wakeley (1954) foresaw what we now hold as axiomatic —restoration, including forestation, can never be entirely successful until nurseries are able to produce crops of "high-quality" plants consistently and reliably. But how to distinguish a high-quality plant from a low-quality one was not always obvious, so the concept of plant quality remained obscure for many years. Wakeley also recognized that "morphological grades" often fell short in their ability to predict performance, and he hypothesized that "physiological grades" may be a better criterion of viability (Wakeley 1949). What exactly constituted a physiological grade, however, and how to measure it, eluded Wakeley and his contemporaries.

During the past 30 years, worldwide, nursery researchers and managers convened numerous workshops and symposia and published many reports on the subject of plant quality and how to measure it (for example, Colombo 2005; Duryea 1985; Haase 2008). This work generated a variety of quality tests; although many are ingenious, most failed to meet expectations. A few, however, stood the test of time and remain in operational use. In this chapter, we discuss the most practical ways of measuring plant quality and how these methods can be used in container nurseries.

7.2.2 Classes of Plant Quality Attributes

Forestry researchers have labored to identify quantifiable traits that could be used as indicators of plant quality and, better yet, predictors of performance after outplanting. Although an impressive list of such attributes has been assembled (for example, Grossnickle 2000), relatively few are used operationally in nurseries or on the outplanting site. In our view, plant quality can be divided into three broad classes:

Morphological attributes—These traits can be readily seen and easily measured, such as stem height, stem (root collar) diameter, root volume, and root and shoot dry weight. During the harvesting-to-outplanting process, these traits do not change appreciably.

Physiological attributes—These traits are not readily visible and need to be measured with instruments or through laboratory procedures. In contrast to morphological characteristics, physiological attributes change often and sometimes dramatically during the harvesting-to-outplanting process. Therefore, any measurement of physiological quality is a "snapshot," relevant for only a brief point in time. Some common physiological attributes include cold hardiness and bud dormancy.

Performance attributes—These traits can be assessed only by subjecting plants to certain predefined testing protocols and observing how they perform. Performance tests have great value because they assess and integrate a wide spectrum of morphological and physiological traits at once. Unfortunately, performance tests are laborious, time consuming, and therefore expensive. Nevertheless, because of their intuitive appeal, performance tests have found wide use in plant quality assessment. One of the oldest and still most commonly used performance tests is the root growth potential test.

7.2.3 Morphological Attributes

7.2.3.1 Introduction

Most nursery stock produced in the United States, Canada, and Europe during the 1970s was bareroot, and so most seedling morphology literature focuses on bareroot stocktypes (Frampton and others 2002; Ritchie and others 1997). The effects of morphology on performance of bareroot stock have been summarized in the literature (Mexal and Landis 1990; Thompson 1985; Wilson and Jacobs 2006); height, stem diameter, root system "quality" (volume or mass), and the ratio of the mass of the shoot to that of the root system are typically the best predictors of outplanting performance. Survival is best forecast by stem diameter, while shoot growth tends to be more related to initial seedling height. With bareroot stock, when stem diameter increases above about 5 mm (0.2 in.), other morphological indicators become less important (Mexal and Landis 1990). In addition, bareroot seedlings with larger root volumes at the time of outplanting have greater subsequent growth and survival than those with smaller root volumes (Rose and others 1997).

7.2.3.2 Morphological characteristics of container seedlings

Let's discuss, in order of importance, the major morphological factors that describe container stock quality.

Container volume. The most important morphological factor affecting plant quality in container nurseries is container size or volume. Container volume controls the amount of roots that a plant can produce, which in turn, determines how large a shoot can be produced in a given amount of time. In addition, the size of the container "plug" limits the moisture and mineral nutrient reserves that will be taken to the outplanting site. Compared with bareroot stock that has extremely variable root systems, it is easy to characterize the volume and depth of container root plugs; most container nursery stock is described by container volume. For example, in the Northwestern United States, a "Styro 20" refers to a plant that has been produced in a Styrofoam™ block container with cells that are 340 cm^3 (20 in^3) in volume.

Container volume is the most important factor controlling root egress after outplanting (fig. 7.2.1A). As container volume increases, the amount of exterior surface area of the root plug also increases (fig. 7.2.1B), which means that plugs of larger containers have more surface contact with the surrounding soil.

Among different container sizes, volume and growing density have the most significant effect on plant morphology (table 7.2.1). In studies with interior spruce (*Picea glauca* x *engelmanii* complex) (Grossnickle 2000); Douglas-fir, western hemlock (*Tsuga heterophylla*), and Sitka spruce (*Picea sitchensis*) (Arnott and Beddows 1982); black spruce (*Picea mariana*) (Jobidon and others 1998); and cherrybark oak (*Quercus pagoda*) (Howell and Harrington 2004), every morphological trait measured increased in value as container volume increased. In every case, container stock with larger root plugs grew larger after outplanting.

Because block containers have fixed cell spacing, it is more difficult to study the effects of changing plant density at the same cell volume. In contrast, the Ray Leach Conetainer® system allows cell spacing to be changed, allowing a few good research trials to be done. Douglas-fir seedlings grown at densities ranging from 270 to 1,080 plants/m^2 (25 to 100/ft^2) showed that shoot height increased with increasing density because of the competition for light in response to crowding (fig. 7.2.2). Stem diameter decreased, however, which shows that quality can be lessened by growing plants too closely together (Timmis and Tanaka 1976).

Within containers of the same size, stem diameter and shoot height have proved to be the most important morphological traits affecting quality and, therefore, are the two factors most often used in grading specifications (fig. 7.2.3A). More discussion on measuring height and stem diameter is provided in Volume One, Section 1.5.4.2.

Stem diameter ("caliper"). Stem diameter is typically measured, using a small caliper, at the root collar where the stem meets the root system. Root-collar diameter, or stem diameter, is always reported in millimeters. Numerous studies show that stem diameter is the best predictor of outplanting performance and, therefore, plant quality. When Engelmann spruce container seedlings with a range of stem diameters were outplanted on a high elevation site in Utah, survival after two growing seasons was strongly correlated with initial stem diameter (fig. 7.2.3B). This information

A

"Styro Super 4" or "160/90"	Plug characteristic	"Styro 20" or "45/340"
90 cm³ (5.5 in³)	Volume	336 cm³ (20 in³)
146 cm² (23 in²)	Surface area	292 cm² (45 in²)

B

Figure 7.2.1—*Root growth out of the plug and into the surrounding soil ("egress") is critical to plant survival and growth after outplanting (A). Container volume is important not only because it determines the amount of roots that a container plant has, but also because the surface area of the plug is in contact with the surrounding soil (B) (A, modified from Grossnickle 2000).*

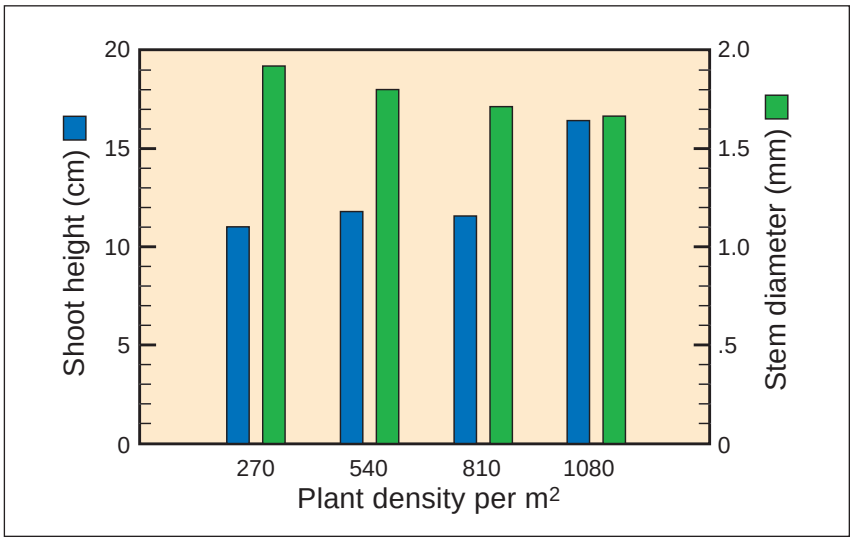


Figure 7.2.2—*When plants are grown in the same volume container but at different densities, shoot height increases with closer spacing whereas stem diameter decreases (modified from Timmis and Tanaka 1976).*

Table 7.2.1—*Effect of container volume on seedling morphology of 2-year-old interior spruce* (Picea glauca x Picea engelmannii)*

Seedling morphological attributes	Styroblock™ cell volumes		
	105 cm³ (6.6 in³)	**170 cm³ (10 in³)**	**340 cm³ (20 in³)**
Shoot height–cm (in)	24.2 (9.5)	29.7 (11.7)	33.3 (13.1)
Root-collar diameter–mm	4.4	5.0	6.8
Shoot dry weight–g (oz)	2.8 (0.10)	4.5 (0.16)	6.4 (0.23)
Root dry weight–g (oz)	1.1 (0.04)	1.4 (0.05)	2.1 (0.07)
Number of branches	18	24	33
Number of buds	50	67	86

* Source: Grossnickle (2000).

was used to develop grading standards; in this case, seedlings with stem diameters ≥ 2.5 mm were shippable, whereas smaller ones were not (Hines and Long 1986). Of course, this relationship varies with conditions on the outplanting site so standards must be developed for each species and for different outplanting conditions.

Shoot height. Height is the distance from the root collar to the tip of the terminal bud or shoot. It is usually reported in centimeters or millimeters, but in the United States it is often reported in inches. This results in the peculiar situation in which plants are characterized using both English and metric measuring systems; for example, a plant shoot that is 12 inches high with a 5 mm caliper. Height is correlated with the number of needles on the stem and, therefore, is a good estimate of photosynthetic capacity and transpirational area.

"Rootbound" plugs. The fact that excessive root growth becomes a quality issue in container plants has been known for decades but, until recently, no morphological index or rating system had been developed. Rootbound nursery stock can be defined as plants that have grown too large for their container, resulting in severe matting and tangling of the root system (fig. 7.2.4A). From a quality standpoint, this condition reduces plant survival or growth after outplanting (South and Mitchell 2006). Several studies have related rootbinding to the length of time that the plant has been in the container. Usually, the larger the container, the longer it takes for the plant to become rootbound. But time alone is not really useful, because root growth is also affected by cultural conditions at the nursery. A species growing rapidly in one nursery will become rootbound faster than the same species growing more slowly in another nursery. Similarly, a species in a large container given large amounts of fertilizer may become rootbound as fast as the same species in a smaller container given smaller amounts.

When plants are grown in the same volume container, outplanting survival has been shown to decrease after an optimum root-collar diameter is exceeded (fig. 7.2.4B). South and Mitchell (2006) propose a "root-bound index" based on root-collar diameter divided by container diameter or volume that must be calculated for each container type. From an operational standpoint, however, establishing a maximum stem diameter along with a visual assessment of root binding might be the most practical culling system.

Other morphological indices. Several other morphological criteria, such as biomass, shoot-to-root ratio, sturdiness, and appearance, have been used to describe plant quality. **Biomass** can be determined using volume or dry weight methods. Shoots and roots are usually measured separately. Dry weight is determined by cleaning, oven drying,

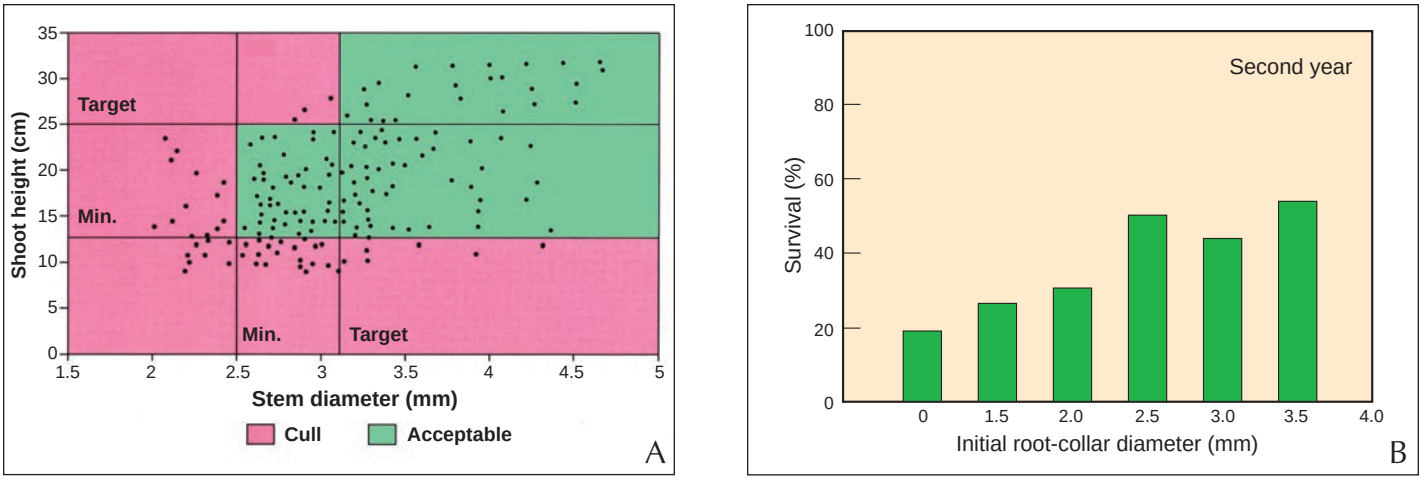


Figure 7.2.3—*Shoot height and stem diameter are the most common grading criteria in container nurseries (A), but stem diameter has proven to be the best single morphological indicator of seedling quality. When Engelmann spruce* (Picea engelmannii) *container stock were outplanted, plants with stem diameters larger than 2.5 mm outperformed smaller ones after the second year (B) (modified from Hines and Long 1986).*

and weighing plants. Volumes are determined using water displacement (Burdett 1979; Harrington and others 1994). **Shoot-to-root ratio** (shoot:root) is the ratio of the dry mass or volume of the shoot to the dry mass or volume of the root system and provides an indicator of the "balance" of the plant. When the shoot:root is "1" the size of the root mass equals the size of the shoot mass. More often, however, the ratio is greater than 1 because the shoot often outweighs the root system. Shoot-to-root ratios less than 2.5 are usually deemed most desirable. A **sturdiness ratio** is calculated by dividing shoot height (cm) by diameter (mm). It attempts to capture the idea of "sturdiness" (low value) in contrast to "spindliness" (high value). This ratio has found particular use in container stock, which can become tall and thin when grown at high densities and/or under lower lighting conditions. **Color, form, and damage** should also be accounted for when evaluating morphological quality. Foliar color is a general indicator of plant quality and can vary by species and time of season. Yellow, brown, or pale-green foliage indicates lower vigor and/or chlorophyll content than dark green foliage. The foliage of some species turns purple during winter dormancy, but this is not considered diagnostic (see Section 7.2.5.1). Multiple shoots, stem sweep, root deformity, physical damage, and any other noticeable characteristics that can affect plant performance are also important factors to note when assessing morphological quality. A single, but comprehensive study with Italian stone pine (*Pinus pinea*) container seedlings measured various morphological characteristics. The best single indicator of plant quality was the **ratio of container depth to stem diameter**, and target plants had a container depth-to-stem diameter ratio of 4 (Dominguez-Lerena and others 2006).

7.2.3.3 Effects of container size on outplanting performance

The main objective of measuring plant morphological traits is to predict performance after outplanting—specifically survival and growth.

So what traits, or combinations of traits, have the greatest positive effect on plant performance? The conventional wisdom is that bigger is generally better than smaller. All other factors being equal, big plants with proportionately larger stem diameters and root systems normally exhibit higher survival and greater growth than smaller plants or

Figure 7.2.4—*Container plants that have grown too long in the same container become "rootbound" which greatly reduces their quality (A). For a given species and container size, an optimum stem diameter exists that can be used for grading-out rootbound plants; this figure was developed for longleaf pine* (Pinus palustris) *(B) (B, modified from South and Mitchell 2006).*

those with poorly developed roots. In general, outplanting survival is more related to stem diameter, whereas shoot growth after outplanting depends more on initial plant height (Arnott and Beddows 1982).

As discussed in Chapter 7.1, survival and growth also depend strongly on environmental conditions of the outplanting site. After reviewing the literature on container size and performance, Grossnickle (2005) concluded that "large" seedlings performed better than "small" seedlings on moist sites where vegetative competition was severe. Conversely, smaller seedlings fared better on sites prone to water stress. On sites with heavy vegetative competition, the ability to access and process sunlight strongly determines survival and growth. Hence taller, branchier seedlings with a large photosynthetic area have an advantage over smaller seedlings that tend to become shaded by competing vegetation. For example, large white spruce (*Picea glauca*) seedlings outplanted in a boreal British Columbia forest were better equipped for competition than were smaller seedlings (McMinn 1982). Similarly, tall container seedlings of Douglas-fir, western hemlock, and Sitka spruce exhibited greater height growth after outplanting on a coastal British Columbia site than shorter seedlings (Arnott and Beddows 1982). In a study in Quebec, large spruce seedlings grew better than did smaller ones on mesic sites with heavy plant competition (fig. 7.2.5). Larger stock with thick stems also performs better on sites with animal predation and heavy snow, as shown with Engelmann spruce seedlings (Hines and Long 1986).

Contrast this with an outplanting site where hot and dry conditions cause high evapotranspirational demand. Here, the advantage lies with plants that have a relatively small transpirational surface area relative to a large, absorptive root system. Under these conditions, nursery plants with a large shoot and small root system (high shoot:root) are at a disadvantage because they transpire faster than they can absorb water from the soil. For these high-stress sites, specifying larger volume containers at lower growing densities (wider cell spacing) will produce plants that have a short shoot and thick stem diameter (Grossnickle 2005).

Miniplug transplants are a stocktype that results in large plants in a relatively short time (Landis 2007). Growers sow miniplugs (approximately 16 cm^3 [1 in^3] cavity volume) in a greenhouse during mid-winter and then transplant them a few months later to larger, wider spaced containers that are moved to outdoor growing areas or bareroot nursery beds. These "plug + plug" transplants have proved to be popular stocktypes for hot and dry outplanting sites (fig. 7.2.6).

Although much less research has been done on broadleaved (hardwood) species, the review by Wilson and Jacobs (2006) notes that, as with conifers, height and stem diameter are the most frequently used grading criteria for hardwoods, with stem diameter usually providing the most consistent prediction of field performance.

7.2.3.4 Morphological attributes: Summary

Shoot height and stem diameter are the most frequently measured morphological traits and the most common grading criteria. Morphological attributes are easily assessed and do not change appreciably during the harvesting-to-outplanting process. Nearly all morphological traits reflect container volume and/or growing density; large container volumes and low growing density promote development of large stock.

Effects of morphology on performance of container stock mirror those for bareroot stock:

- Initial stem diameter tends to be correlated with survival.
- Initial height tends to be correlated with shoot growth.
- Morphological traits can interact. For example, stem diameter may influence survival in plants with poor root systems but not in those with good root systems.
- Larger stock generally performs better than smaller-stock, but this depends on conditions on the outplanting site. Tall stock with thick, stiff stems and a large photosynthetic surface is best for sites with plant competition, animal predation, or heavy snow loads. Short stock with thick, stiff stems and extensive root systems is best for droughty sites.

As discussed earlier, physiological traits of nursery plants differ significantly from morphological characteristics in that they are not readily seen, they change often and sometimes dramatically throughout the harvest-to-outplanting process, and they must be measured with laboratory equipment.

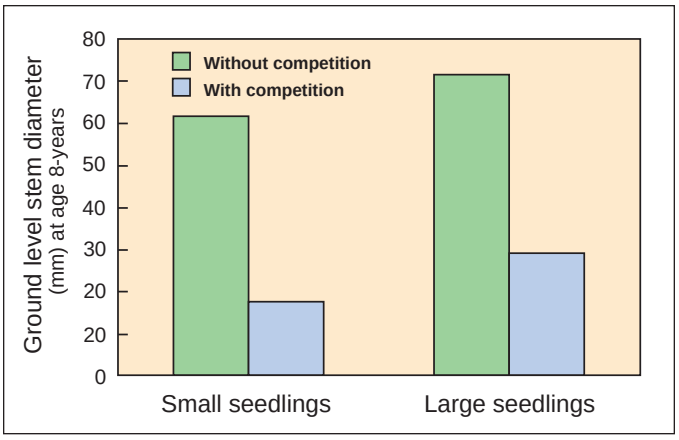


Figure 7.2.5—*Large black spruce and white spruce container stock outperformed smaller plants when measured 8 years after outplanting in southeastern Quebec (modified from Thiffault 2004).*

Most physiologically based quality tests measure only one plant function, such as cold tolerance, water status, or photosynthetic efficiency. It is helpful to think of plant quality in layers: morphological characteristics are the base layer, whereas physiological traits are the second layer. A batch of plants may have ideal shoot height and stem diameter, but these morphological traits alone are insufficient to guarantee high quality. Physiological tests are needed to provide a more comprehensive picture.

In the next section, we discuss four tests of physiological quality: plant moisture stress, cold hardiness, root electrolyte leakage, and chlorophyll fluorescence.

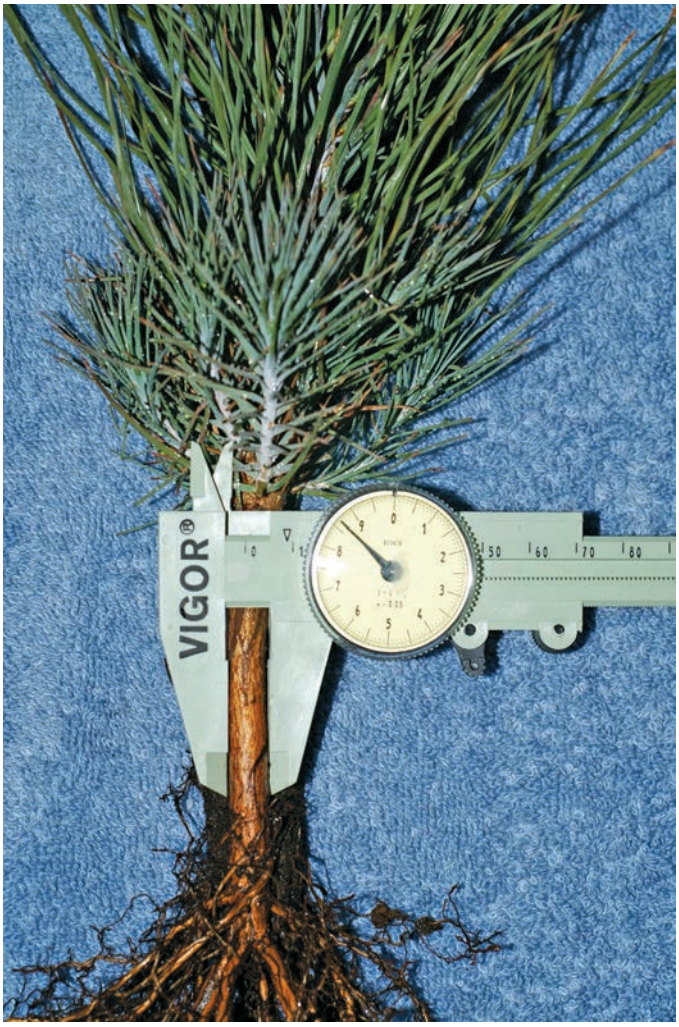


Figure 7.2.6—*For hot and dry outplanting sites, these "Q-plug + one" Jeffrey pine (*Pinus jeffreyi*) have the ideal morphology — short stocky shoots (A) with large stem diameters and root mass (B).*

7.2.4 Physiological Attributes

7.2.4.1 Plant moisture stress (PMS)

Plant moisture stress, or PMS, is one of the oldest and most commonly used tests to measure quality. Its popularity rests on its simplicity and robustness, and the fact that PMS equipment is relatively inexpensive, intuitive, and portable. Although PMS measurements are easily made, their interpretation can be more difficult.

What is PMS? Without a steady supply of good quality water, plants cease growing and ultimately die. The amount of water needed to meet the basic metabolic needs of a plant is quite low. During photosynthesis, atmospheric carbon dioxide (CO_2) diffuses into leaves through stomata and, once inside the leaf, this CO_2 is converted to sugars. Photosynthesis is, however, a very "leaky" process because, while CO_2 is diffusing into the leaves, water is diffusing out—this loss of water is called transpiration. Plants can reduce transpiration by closing stomata, but this impedes photosynthesis. So, in order to grow, plants must transpire vast amounts of water.

Transpiration generates a tension (or "stress") that due to water's high cohesion, is transmitted through vascular tissue from the leaf down through the stem and into the roots. During daylight, when stomata are open, transpiration typically exceeds the plant's ability to extract water from the soil. Therefore, during the day, plants are always under some degree of water stress. This stress is perfectly normal and not injurious unless it reaches high levels for a prolonged period of time.

In very simple terms, plant moisture stress can be modeled as:

$$PMS = A - T + S$$

where A is the absorption of water from the soil, T is transpirational loss, and S is storage of water in the plant's stem and roots, which is negligible in seedlings but important in large trees. During daylight, T almost always exceeds A.

Water potential. A more precise way to model the state of water in plants is the thermodynamic approach, which is based on water potential and represented by the Greek letter psi (ψ). The total water potential (ψ_W) is a measure of the free energy or chemical potential of water. In plants, ψ_W is the sum of two component potentials: the pressure potential (ψ_P), which can be either positive or negative, and the osmotic potential (ψ_O), which is always negative:

$$\psi_W = \psi_P + \psi_O$$

Potentials are expressed in units of pressure and, although MegaPascals (MPa) are the official SI units, bars are most commonly used by nursery and reforestation personnel. By definition, the ψ_W of pure water at standard temperature and pressure is 0 bars, or 0 MPa. ψ_P and ψ_O are continually changing as transpiration and osmosis cause water to move across membranes, in and out of cells, and up the transpiration stream.

The components of water potential have different properties depending on where the water is located within the plant tissues. Water is contained within cell membranes as part of the symplast and outside cell membranes as part of the apoplast. In the apoplast, water is nearly always under hydrostatic tension from transpirational pull, so pressure potential (ψ_P) is always negative (table 7.2.2). In the symplast, however, ψ_P is normally positive owing to the inward turgor pressure that cell membranes and walls exert on cell contents. The exception would be for a cell that has lost all turgor (wilted), in which case $\psi_P = 0$. This is often called the "zero turgor point," which is discussed below. The osmotic component (ψ_O) is normally near 0 in the apoplast whereas, in the symplast, ψ_O is always negative owing to effects of dissolved solutes (ions) in the cells (table 7.2.2). These component potentials are continually changing as water moves across cell membranes due to osmosis or up through the plant due to transpiration. Because ψ_W is the sum of these two components, it is almost always negative and the plant is almost always under some level of water deficit, or stress.

The interplay of these component potentials in the symplast can be visualized with a Höfler diagram (fig. 7.2.7). The X-axis is the water content of the cell expressed as a percentage of full turgor. The Y-axis gives the component potentials. At full hydration (A in figure 7.2.7), plants are turgid and the positive turgor pressure of cell walls (ψ_P) balances the negative osmotic potential (ψ_O) of cell contents. At this point, $\psi_W = 0$ MPa. As cells lose water, ψ_P falls and the concentration of solutes in cells increases.

This drives ψ_O down, so ψ_W also falls. When ψ_P reaches 0 MPa (B in figure 7.2.7), cells collapse and plants wilt. The value of ψ_W at which this occurs is known as the "zero turgor point" or, as it is more commonly known, the "permanent wilting point" (C in figure 7.2.7).

Units of water potential. Thermodynamic water potential terminology (Slatyer 1967) has sometimes been troublesome for growers because negative values are hard to visualize and tricky to manipulate algebraically. For this reason, water potential is often expressed as a positive value and is called "Plant Moisture Stress" (PMS). These values can be easily converted because –1.0 MPa equals 10 bars. This relationship and some examples are shown in table 7.2.3. For example, a PMS value of 10 bars indicates a "moderate" level of stress and is equivalent to ψ_W of –1.0 MPa. From a theoretical standpoint, however, thermodynamic terminology is useful because it is consistent through the soil-plant-atmosphere continuum (fig. 7.2.8).

Diurnal patterns of plant water potential. As already mentioned, ψ_W is dynamic and this affects its usefulness as an index of plant quality. Consider, for example, a container plant whose growing medium is at field capacity with water. During daylight, while stomata are open, low humidity (high vapor pressure deficit) draws moisture from the leaves. This creates an imbalance between transpiration and water absorption, resulting in the development of PMS at midday (ψ_W decreases). During nighttime, stomata tend to close, relative humidity rises to nearly 100 percent, and transpiration ceases. The negative ψ_W in the plant pulls water from the soil or growing medium, thereby relieving the stress. By early the next morning, pre-dawn ψ_W reaches a dynamic equilibrium with soil moisture potential ($\psi_W = \psi_{soil}$).

If no water is added to the container, the growing medium dries out, and predawn and midday plant moisture stress increase daily as ψ_{soil} decreases. After a few days, the plant will close its stomata during midday to retard transpiration. This can be seen occurring in days 4 and 5 in figure 7.2.9, and results in a moderating of the midday PMS. ψ_{soil} will eventually become so negative that the plant will be unable to equilibrate during the night. Throughout this time, the midday stress will continue to increase. When irrigated, the system will return to the ini-

Table 7.2.2—*Properties of component water potentials in the symplast and apoplast*

Component potential	Apoplast (outside cells)	Symplast (inside cells)
Pressure potential (ψ_P)	Always negative	Generally positive Zero when wilted
Osmotic potential (ψ_O)	Generally slightly negative	Always negative
Water potential (ψ_W)	Always negative	Variable

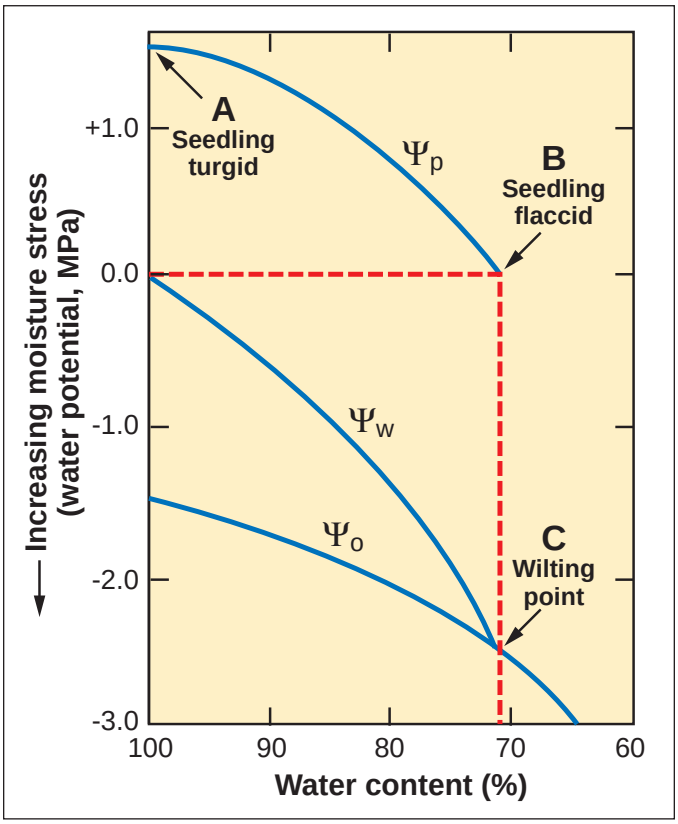


Figure 7.2.7—*The interrelationships between plant water potential (ψ_W) and its components, osmotic potential (ψ_O) and pressure potential (ψ_P), change over the range of plant water contents from turgidity (A) to the permanent wilting point (PWP) (C) (modified from Ritchie 1984b).*

tial state shown in day 1 unless the plant has experienced irreversible damage from the high PMS.

Note that the ability to track moisture stress levels of both soil and plant in figure 7.2.9 shows the advantage of using water potential units rather than PMS, which reflects only plant stress.

Measurement of plant moisture stress. Over the years, as plant physiologists labored to understand the dynamics of plant water relations, many attempts were made to develop methods of measuring ψ_W (Lopushinsky 1990). As far as nursery work goes, the most significant development was invention of the "Scholander Pressure Chamber" (Scholander and others 1965), based on an earlier glass pressure chamber devised by Dixon (1914). Waring and Cleary (1967) modified the chamber for trees and seedlings and outlined basic measurement procedures.

The modern pressure chamber consists of a metal pressure vessel connected to a nitrogen gas source through a pressure regulator. To measure plant moisture stress, the stem is cut and inserted through a rubber or compression gasket. A new model pressure chamber from the PMS Instrument Company comes equipped with a "rubber gland" instead of a gasket, which greatly improves the speed and accuracy of measurements. This is then sealed into a hole in the chamber lid with the foliage inside the chamber and the cut stem protruding (fig. 7.2.10). Nitrogen gas is slowly bled into the chamber while the cut stem is closely observed. When a droplet of water appears at the end of the stem, the chamber pressure is noted. The gas pressure required to force water to the surface is equal to the moisture stress of the plant. For a detailed theoretical description and procedural guide see Ritchie and Hinckley (1975).

The pressure chamber is the standard technique used for measuring PMS in forest nurseries, on outplanting sites, and in plant research facilities. For example, the Forest Service J.H. Stone Nursery in Central Point, Oregon, uses pressure chambers to measure PMS for scheduling bareroot seedling irrigation and to detect dangerous PMS levels during lifting and packing operations (J.H. Stone Nursery 1996).

Table 7.2.3—*Comparison of units and terms for plant water potential and plant moisture stress (modified from Landis and others 1989)*

Plant water potential (MPa)	Plant moisture stress (bars)	Relative moisture stress rating	Relative moisture condition
0.0	0.0	Very low	Wet
-0.5	5.0	Low	
-1.0	10.0	Moderate	
-1.5	15.0	High	
-2.0	20.0	High	
-2.5	25.0	Very high	Dry

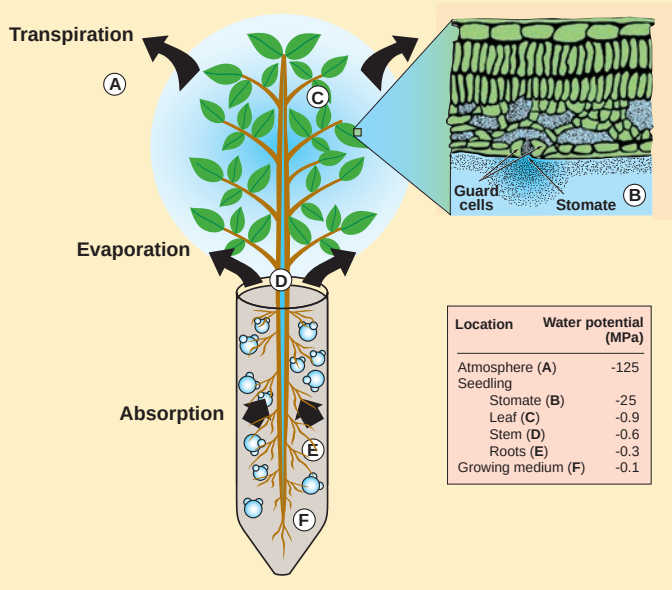


Figure 7.2.8—*Water is pulled along a gradient of water potential that is driven by evapotranspiration, from higher (less negative) levels in the growing medium through the plant to lower (more negative) levels in the surrounding air (modified from McDonald and Running 1979).*

Pressure chambers and supplies are available from the following companies:

PMS Instrument Company
1725 Geary Street SE
Albany, OR 97322 USA
Tel: 541-704-2299
Fax: 541-704-2388
E-mail: info@pmsinstrument.com
Web site: http://pmsinstrument.com/

or

Soil Moisture Equipment Corporation
Santa Barbara, CA
Tel: 805-964-3525 ext. 248
E-mail: alle@soilmoisture.com
Web site: http://www.soilmoisture.com/

Interpretation of PMS values. PMS measurements are used extensively in plant physiology and ecological research because they are robust, easy to obtain, and their relationship to plant physiology is easy to demonstrate. For example, when container white spruce was subjected to extended moisture stress, stomata closed and photosynthesis ceased abruptly at –2MPa (20 bars) (fig. 7.2.11). Unless this stress is relieved, plant growth will most certainly be restricted and death may occur.

The relationship between PMS readings and plant quality, unfortunately, is not always as straightforward as one might hope. This is partly because PMS, as an estimate of ψ_W, integrates several variables into one reading and, therefore, much information is lost. In addition, because the components of water potential change seasonally, a given value of PMS might have a different interpretation if taken in spring as opposed to winter. For example, figure 7.2.12 shows how the "zero turgor point" changes seasonally in roots and stems of Douglas-fir seedlings (Ritchie and Shula 1984). Looking at stem values, a PMS reading of –2.5 MPa (25 bars) would be a potentially lethal value if taken in April, because it would be near the zero turgor point. But the same value, if measured in January, would be of little concern. On the other hand, root systems with PMS near –2.0 MPa (20 bars) would be suspect most of the year.

As illustrated in figure 7.2.9, PMS can vary sharply throughout the day and from day to day. Daytime PMS

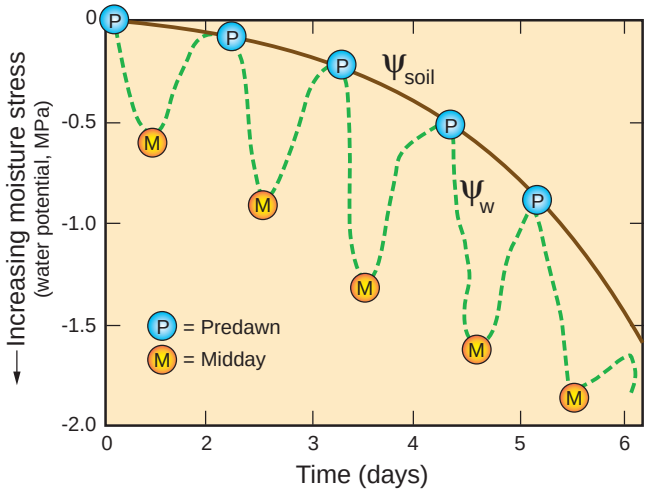


Figure 7.2.9—*For a plant growing in a nonirrigated container, the plant water potential (ψ_W) gradually decreases as the growing medium (ψ_{soil}) dries (modified from Slatyer 1967).*

values can fluctuate widely on days with intermittent sunshine and wind, providing only brief "snapshots" of PMS that have little diagnostic value. Probably the most useful PMS value is what is known as "predawn PMS." This is the PMS that occurs just before sunrise when ψ_W is in dynamic equilibrium with ψ_{soil} (fig. 7.2.9) and provides an estimate of the minimum stress the plant might experience that day. If this minimum value is high, it may be cause for concern. With the above caveats in mind, we present some suggested guidelines for interpretation of predawn PMS measurements as they relate to plant growth and cultural implications (table 7.2.4).

Is PMS an indicator of plant quality? As pointed out by Lopushinsky (1990), the commonly used plant quality indicators (root growth potential, cold hardiness, stress resistance, and dormancy intensity) are not correlated with PMS. Therefore, PMS cannot be used as a proxy indicator of any of these. So, can PMS alone be a useful indicator of quality?

In our opinion, PMS reflects quality only when stress is moderately high and sustained for several days. For example, nursery stock with *predawn* PMS values in the range of –1.5 to –2.5 MPa (15 to 25 bar) range is under severe stress (table 7.2.4), especially if these readings persist after irrigation. We should also point out that dead plants can exhibit very low PMS values because dead roots retain

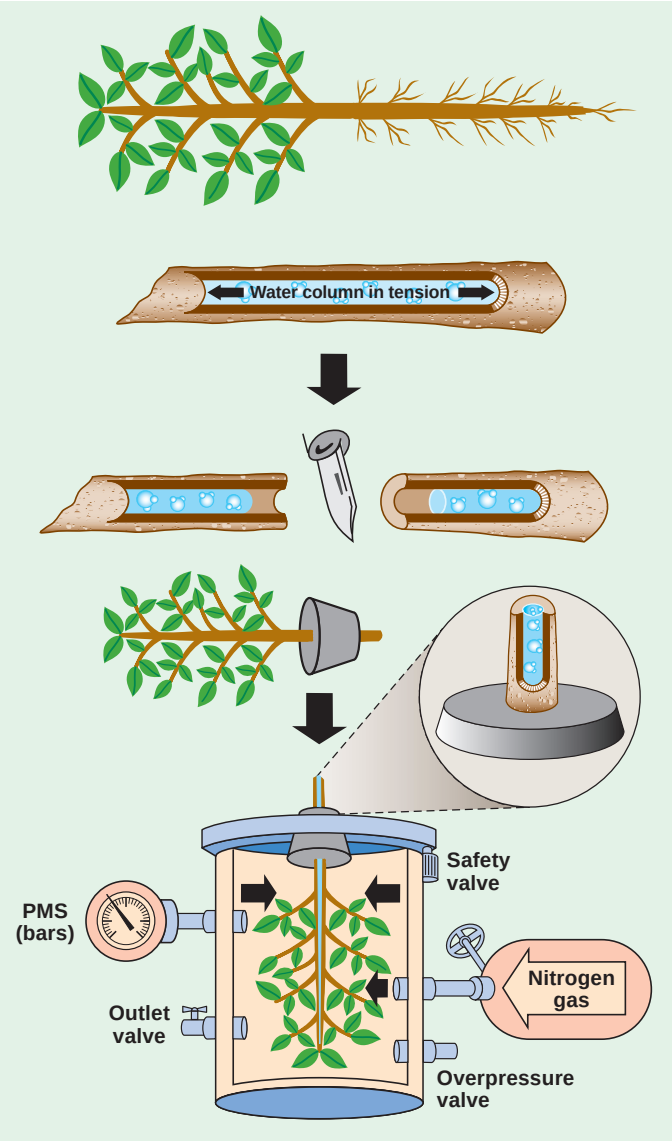


Figure 7.2.10—*How to measure plant moisture stress (PMS) with a pressure chamber. A plant stem is severed and the cut end forced through a hole in the center of a rubber gland, which is then inserted into the lid of the chamber. Nitrogen gas is slowly introduced into the chamber until a drop of water is forced to the surface of the cut stem. The gauge pressure at which this occurs is equal and opposite to the forces holding the water in the stem and is known as PMS.*

the ability to absorb water. So, low PMS values are not necessarily indicators of healthy stock.

PMS is also used operationally to monitor plant condition during the harvest-to-outplanting process. For example, stock that has a plant water potential (PMS) value of, say, –1.0 MPa (10 bars) coming out of refrigerated storage would certainly be cause for concern. Likewise, nursery stock should have low PMS values immediately before outplanting, when high values indicate overheating or exposure to sun or wind.

You may have noted that all research has been done with conifers. Use of PMS as a performance predictor for deciduous hardwoods also shows some promise, although Wilson and Jacobs (2006) point out that much work is needed to define critical PMS values for a given species.

PMS as a snapshot of plant water status. The fact that PMS is not always a good predictor of plant quality should not be interpreted to mean that monitoring PMS is a waste of time. Pressure chambers should be used to check plant moisture status at several times during nursery tenure. Using predawn PMS readings to fine-tune nursery irrigation practices is a good idea, because pressure chamber measurements show the actual water status of a plant at a given time.

PMS measurements during harvesting can alert nursery managers to dangerously dry conditions or excessive plant exposure (MacDonald and Running 1979). PMS can also be used to check the moisture status of stock immediately before outplanting. For example, a very strong relationship was found between PMS readings taken immediately before outplanting of radiata pine (*Pinus radiata*) seedlings and root growth potential (Mena-Petite and others 2001) (fig. 7.2.13).

Plant moisture stress: Summary. Plants normally lose water more rapidly through transpiration than they absorb from the soil, so they are almost always under some level of water stress, commonly known as plant moisture stress (PMS). PMS is linearly correlated to, but differs in sign from, plant water potential (ψ_W). PMS shows strong diurnal variations as transpiration rates adjust in response to changes in temperature, vapor pressure deficit, and stomatal aperture. The most useful value of PMS is that which

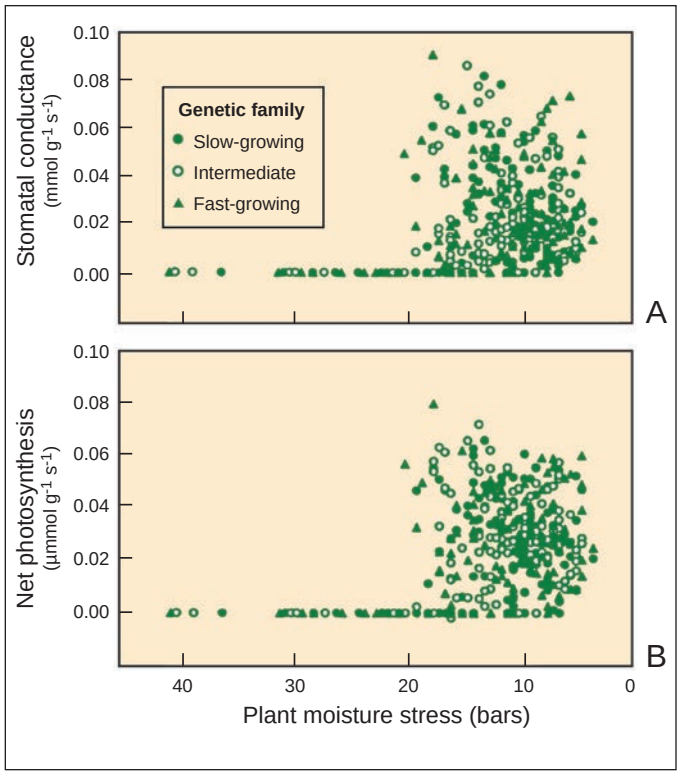


Figure 7.2.11—*Plant moisture stress can give an instantaneous indication of nursery stock water status. When different families of white spruce seedlings were placed under increasing water stress, the stomata closed (A) and all photosynthesis ceased at –2 MPa (20 bars) (B, modified from Bigras 2005).*

occurs just before dawn (pre-dawn PMS), when ψ_W is in near equilibrium with ψ_{soil}. The Scholander pressure chamber, introduced in the mid 1960s, remains the most robust and useful method for measuring PMS. In this test, a stem is severed from a plant, sealed in a pressure chamber, and gas under pressure is introduced into the chamber until a water drop forms at the cut surface. The pressure at which this occurs is equal and opposite to the forces holding the water in the stem and provides an estimate of PMS. Although there are strong seasonal variations in critical PMS (plant water potential) values, readings in the range of –0.5 to –1.5 MPa (5 to 15 bars) are normal whereas those below –1.5 MPa (above 15 bars) can be cause for concern.

PMS is not directly correlated with any of the classical plant quality indicators, but predawn PMS measurements

Table 7.2.4—*Growth response and cultural implications of inducing moisture stress in conifer seedlings in Northwestern United States nurseries (modified from Landis and others 1989)*

Predawn PMS value (bars)	Moisture stress rating	Seedling response and cultural implications
0 to 5	Slight	Rapid growth
5 to 10	Moderate	Reduced growth Best for hardening
10 to 15	High	Restricted growth Variable hardening may result
15 to 25	Severe	Potential for injury
> 25	Extreme	Injury or mortality

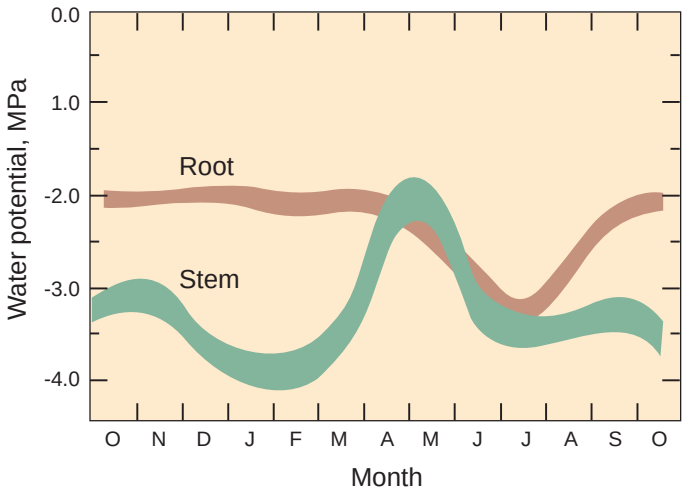


Figure 7.2.12—*The value of water potential at zero turgor varies differently through the year for roots and stems of Douglas-fir seedlings (modified from Ritchie and Shula 1984).*

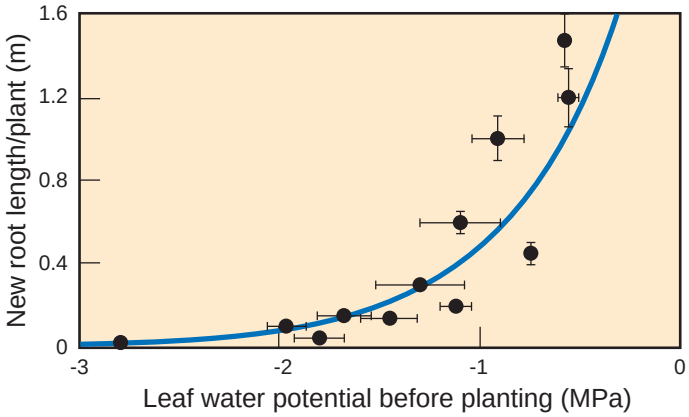


Figure 7.2.13—*In some studies, plant moisture stress was found to be a good predictor of the ability to grow new roots after outplanting (modified from Mena-Petite and others 2001).*

can be used in nurseries to determine irrigation amount and timing, and are the best measurements for monitoring stress levels during hardening. PMS reading during harvesting can alert nursery managers to stressful conditions, and plant users can use PMS to check moisture status of their stock immediately before outplanting.

7.2.4.2 Cold hardiness

Cold hardiness (CH) testing has been used in horticulture since the early 1900s as a method of selecting cold hardy cultivars. Its use as a plant quality test in forest and conservation nurseries has developed over the past 30 or so years, but it stands now as perhaps the second most-often used test of forest planting stock quality.

Concepts behind the test. During the growing season, most temperate zone plants are killed when the air temperature drops below freezing. As winter approaches and growth slows, however, plants respond to the changing photoperiod (lengthening nights) and develop tolerance to cold (Bigras and others 2001; Glerum 1976, 1985; Weiser 1970). In general nursery terminology, this is known as "hardening" and this cold tolerance is indicative of general stress resistance. When winter arrives, plants that would have been killed at slightly below 0 °C (32 °F) during the growing season are able to survive temperatures far below that. As winter draws to a close and the growing season nears, this resistance to low temperatures is rapidly lost and plants resume growth.

What happens when plant tissues freeze? To understand how plants withstand subfreezing temperatures, it is first necessary to understand what happens inside a plant when it freezes. Consider a generalized cross section of plant tissue showing the cellular structure (fig. 7.2.14A). Cells are enclosed by flexible walls made primarily of cellulose, which is stiff and strong. Cells are typically packed tightly together, but occasionally spaces that contain only air and/or water occur between them (intercellular).

Plant tissue is composed of many types of cells that have different functions. Some cells, such as vessels and tracheids, are hollow and transport water from roots to the leaves, or photosynthate back down from leaves. Living cells that function in photosynthesis and other physiological processes are filled with cytoplasm, which is surrounded by a semipermeable membrane composed of a fatty material called lipid in which protein molecules are embedded. This membrane plays a key role in plant cold hardiness; everything within it is referred to as symplast and is living tissue. Everything outside this membrane (cell walls, vessels, intercellular spaces, empty cells, etc.) is referred to as apoplast and is not living (fig. 7.2.14A).

Both the symplast and apoplast normally contain some water. Apoplast water is nearly pure, so its freezing point is close to 0 °C (32 °F). In contrast, the symplast contains dissolved sugars and salts, suspended starch granules, and protein molecules. These solutes act as "antifreeze," depressing the freezing point of the symplast to considerably below 0 °C. So, when cells are exposed to sub-freezing temperatures, the apoplastic water begins to freeze. As it does, small ice crystals form within the cell walls, intercellular spaces, and other voids within the apoplast (fig. 7.2.14B). The symplast water, with its lower freezing point, resists freezing. Therefore, the ice that forms within the plant tissue is contained in the apoplast and does little or no damage.

Ice, however, has a very strong affinity for water—so strong that ice crystals pull water tenaciously across the membrane and out of the symplast. Because the membrane is permeable only to water, the dissolved sugars and other materials remain in the symplast even as water is drawn out. This raises the concentration of the dissolved solutes, further lowering the freezing point of the symplast water. When plant tissues are not cold hardy, or

when the temperature falls below the plant tissues' seasonal level of hardiness, the cytoplasm can become severely dehydrated to the point at which: (1) proteins denature; (2) membranes are killed or damaged allowing cell contents to leak into the apoplast; (3) cells plasmolyze; and (4) cytoplasmic cell volume decreases sharply, signaling cell death. It is not clear whether low temperature itself, or desiccation, or both actually incite the damage (Adams and others 1991; Sutinen and others 2001).

Cold injury must be distinguished from winter desiccation that results when cell water is pulled across the cell membrane to feed ice crystals growing outside the cells. This can severely dehydrate cytoplasm and injure membranes causing them to leak cell contents. Even cold hardy plants can be damaged by winter desiccation.

Cold hardiness mechanism. For plants to resist freezing, several changes must occur in the physical and chemical properties of the membranes and the cytoplasm during the hardening process (Öquist and others 2001; Sutinen and others 2001). First, membranes change physically, becoming more permeable to water. This enables water molecules to move out of the cells rapidly, permitting intracellular solute concentrations to increase quickly. In addition, the membranes become physically more rigid. This helps protect them from being pierced by ice crystals that are rapidly growing in the apoplast, while enabling them to resist being torn and pulled away from the cytoplasm and/or cell walls as the cytoplasm dehydrates and shrinks. The cytoplasm itself undergoes profound physical-chemical changes that enable it to survive severe dehydration. These adaptations take place in response to changes in photoperiod and lowering temperatures and are orchestrated by suites of genes that are turned "on" or "off" by these environmental signals.

An important hardiness avoidance mechanism is deep supercooling of water (Burr and others 2001; Quamme 1985). Pure water can cool to nearly –40 °C (–40 °F) without forming ice crystals when no ice nuclei are present, and some plants exploit this property. When supercooled water does freeze, however, it is nearly always lethal. The observation that many plant species do not occur north of the –40 °C midwinter isotherm suggests they avoid cold damage primarily by this mechanism (George and others 1974). This same midwinter isotherm

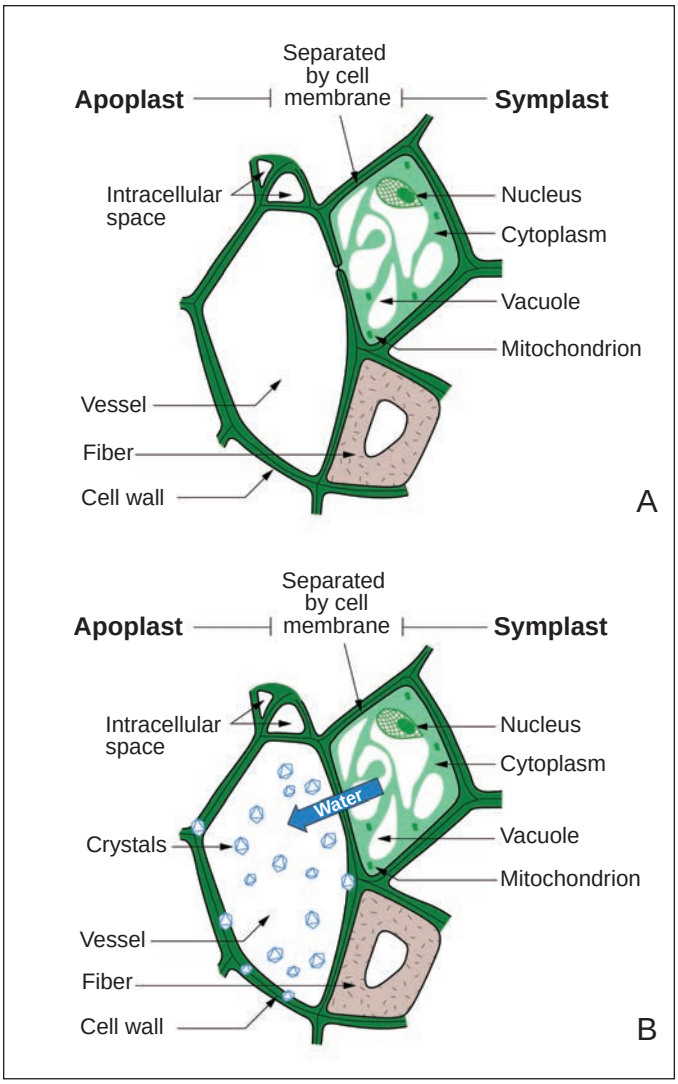


Figure 7.2.14—*Living cell contents (symplast) are separated from nonliving cell contents (apoplast) by the cell membrane (A). When temperatures fall below freezing, ice crystals begin to form in the apoplast. As these crystals grow, they draw water across the cell membrane causing dehydration of the cell contents (B). If the cytoplasm becomes severely dehydrated, the membrane can rupture, and cell contents leak into the apoplast, causing cell injury.*

Table 7.2.5—*Stages of cold hardening and dehardening for coastal Douglas-fir seedlings (compare with figure 7.2.15)*

Hardening stage	Season	Environmental cues	Temperature tolerance as LT_{50}
Hardening begins slowly	Early fall	Shortening photoperiod	–2 to –5 °C (28 to 23 °F)
Hardening increases rapidly	Late fall	Increasing lower temperatures, especially at night	–10 to –20 °C (14 to –4 °F)
Maximum hardiness	Midwinter	Very cold temperatures	–15 to –40 °C (5 to –40 °F)
Dehardening happens quickly	Late winter	Rising temperatures and longer days	Rapidly rising to –2 °C (28 °F)

commonly coincides with timberline, causing Becwar and others (1981) to speculate that supercooling may also limit survival of certain species to below timberline. Many conifers (pines excluded) employ supercooling as a method of avoiding cold damage. However, many tree species can survive temperatures far below –40 °C so they are able to resist cytoplasmic desiccation by other, less well understood, mechanisms.

Stages in cold hardening. Cold hardening (also known as cold acclimation) occurs in a series of stages depending on plant species (Cannell and Sheppard 1982; Timmis 1976; Timmis and Worrall 1975). Table 7.2.5 gives a generalized cold hardening pattern for coastal Douglas-fir shoots and root systems, which is illustrated in figure 7.2.15. The Y-axis represents the LT_{50} value—the temperature that is lethal to 50 percent of a sample population—which is the most common index of cold hardiness.

Further information on the environmental cues that trigger and sustain the various stages of hardening and dehardening are discussed in Greer and others (2001).

Hardiness variation in plant tissues, species, and ecotypes. Different plant tissues harden and deharden at different rates (Bigras and others 2001; Rose and Haase 2002). In particular, the fact that roots do not harden as deeply as shoots (fig. 7.2.15) has very important implications for container growers (Colombo and others 1995). Burr and others (1990) tested cold hardiness of Engelmann spruce seedlings throughout winter and separately examined buds, needles, and lateral cambium (fig. 7.2.16). Stems and needles hardened more rapidly and achieved greater midwinter hardiness than buds. All three tissues dehardened very rapidly in late winter.

Tree species and ecotypes exhibit a vast range of midwinter hardiness levels depending on the regional climate where they naturally occur (Sakai and Weiser 1973). Boreal conifers, such as black and white spruce, jack pine (*Pinus banksiana*), and others, attain hardiness levels below –80 °C (–112 °F). Many Rocky Mountain conifers, such as lodgepole pine (*Pinus contorta*) and Engelmann spruce, also reach this cold hardiness level. In contrast, Pacific coast conifers, such as Douglas-fir, coast redwood (*Sequoia sempervirens*), and western redcedar (*Thuja plicata*), rarely acclimate to below –20 °C (–22 °F). Note that the cold tolerance of wide-ranging species, such as Douglas-fir, varies by ecotype (–20 °C [–4 °F] for Washington State, but interior sources from the Rocky Mountains can tolerate –20 to –30 °C [–4 to –22 °F]).

Cold hardiness testing methods. Although plants can be tested for cold hardiness by several methods (Burr and others 2001), two tests are widely used: the whole plant freezing test (WPFT) (Tanaka and others 1997) and the freeze-induced electrolyte leakage (FIEL) test (Burr and others 1990; Dexter and others 1932; McKay 1992). Both tests employ two steps (Burr and others 2001; Ritchie 1991). First, plants or plant parts are exposed to a freezing stress and, second, the amount of cold injury is rated. These tests are compared in table 7.2.6.

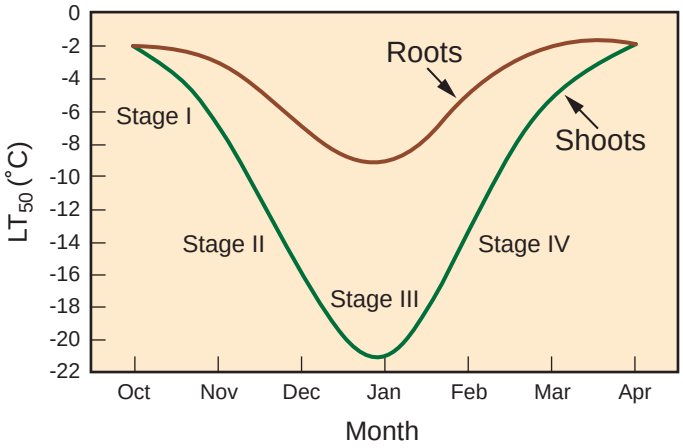


Figure 7.2.15—*These typical cold hardening trends for conifer seedlings show that shoots and roots follow the same general pattern, reaching peak hardiness in January. It is important to note that some species and ecotypes do not reach Stage III Hardening, and roots do not attain the same level of hardiness as shoots.*

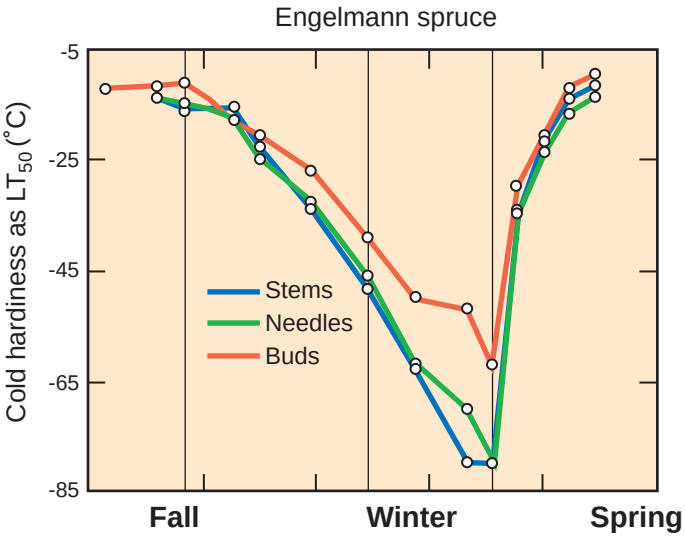


Figure 7.2.16—*Plant tissues harden at different rates in the fall, but all deharden very rapidly in the spring (modified from Burr and others 1990).*

Whole plant freezing test. To begin, a representative sample of plants is subjected to a series of subfreezing temperatures in a programmable chest freezer (fig. 7.2.17A&B) or a Thermotron for a predetermined time period, often a few hours. Next, plants are incubated in a warm environment, such as a greenhouse, for several days to allow symptoms to develop. Finally, the stem, buds, and foliage of test plants are assessed for cold damage by evaluating visible damage or "browning" in the bud, cambial, and foliar tissues (fig. 7.2.17C–E). Mortality is determined based on the severity and position of tissue damage (Tanaka and others 1997).

Freeze-induced electrolyte leakage test. This test is based on the fact that freeze-damaged cell membranes leak electrolytes that can be measured with an electrical conductivity (EC) meter. To begin, sample tissues (foliage, buds, or roots) are cut from the test plants (fig. 7.2.18A), and subjected to freezing temperatures (fig. 7.2.18B). They are then placed into deionized water, which has zero electrical conductivity (fig. 7.2.18C). The electrolytes that leak from damaged cells increase the EC of the water, and this relative increase in EC (described below) is a measure of the amount of cold injury. Although this test can be done on any plant tissue, samples of foliage or roots are most commonly used.

A relative conductivity (RC) index of freeze damage, described by Ritchie (1991) and Burr and others (2001), is determined as follows: (1) place tissue into vials containing deionized water; (2) expose the tissue to sub-freezing temperatures; and (3) incubate the vials until the EC reading stabilizes. This point is known as the initial solution conductivity (EC_1). Finally, the sample is completely killed by heating or freezing and the final conductivity (EC_2) is measured. Relative conductivity is calculated as:

$$RC\ (\%) = (EC_1 - B_1) \times 100 / (EC_2 - B_2)$$

where B_1 and B_2 are optional blanks included to account for possible ion leakage from vials.

So, as you can see, the FIEL test provides a quick and easy way to measure cold hardiness of plant tissues.

Differential thermal analysis. Differential thermal analysis (DTA) is based on the theory that when supercooled water

Table 7.2.6—*Comparison of two main cold hardiness tests*

Factor	Whole plant freezing test (WPFT)	Freeze-induced electrolyte leakage (FIEL)
Plant tissue tested	Intact plant (foliage, buds, stem, and roots)	Detailed tissue (foliage, buds, stems, or roots)
Time	Several days to a week	1 to 2 days
Required testing equipment	Programmable freezer and growth chamber or greenhouse	Programmable freezer, electrical conductivity meter, autoclave, oven, or microwave
Evaluation criteria	Degree of tissue damage (browning) or chlorophyll fluorescence (see Section 7.2.4.4)	Numerical reading

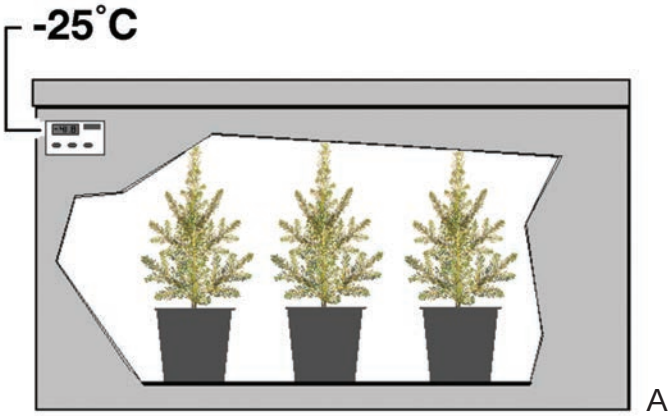
freezes it almost always indicates significant tissue injury. Two plant tissue samples (stem or bud) are collected and one is killed by heat or cold and then dried. Two tiny thermocouples, wired in series, are placed in the sample material—one in the dead tissue and one in the living tissue. The samples are placed into a freezing cabinet capable of freezing down to about –40 °C (–40 °F).

As the temperature is slowly lowered, the temperature difference between the samples remains at zero until a freezing event happens. At this point a "spike" is registered. The first spike often occurs when the temperature reaches –5 °C to –10 °C (23° to 14 °F) and represents the freezing of intercellular (apoplastic) water. In tissues that supercool, a second spike will occur at a lower temperature (down to –40 °C [–40 °F]). Evidence suggests that the temperature of this second spike indicates the lethal temperature for that sample (Ritchie 1991).

While this method seems to offer promise for determining hardiness levels of species that supercool, various technical problems have hindered its operational use (Burr and others (2001).

Cold hardiness testing through gene expression. We indicated earlier that changes in environmental signals, specifically photoperiod and temperature, trigger changes in gene expression that ultimately result in cold hardiness development. A novel approach to measuring hardiness, described by Balk and others (2007), involves identifying genes known to be implicated in this process. These genes are responsible for production of enzymes, proteins that trigger all physiological processes in organisms. To create an enzyme, the cell must first transcribe the genetic information stored in the DNA into messenger RNA (mRNA). The strand of mRNA then moves over to a ribosome, a site of protein synthesis, where amino acids are stitched together using the mRNA blueprint. The subsequent chain of amino acids is another enzyme that folds into its characteristic shape, floats free, and begins performing a specific reaction (fig. 7.2.19A). Changes in levels of enzymes triggered by these genes signal acquisition or loss of cold hardiness. An advantage is that these signals can be detected much earlier (indicating that nursery treatments used to trigger cold hardiness development were effective, or that plants are losing cold hardiness in spring) than waiting for measurable changes in cold hardiness values using tests like whole plant freezing and freeze-induced electrolyte leakage.

Research with Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) identified three indicator genes and their subsequent enzymes that together provide enough information to give an accurate estimate of the cold hardiness stage of nursery plants (Balk and others 2007). Subsequent work with Douglas-fir showed similar results (Balk and others 2008). Chemical assays were developed to detect the enzymes created by the indicator genes, and a private company, N-Sure, now offers this test. A composite sample of bud tissue is collected by the nursery manager, stabilized using chemicals provided in a sampling kit, and



A

B

C

Figure 7.2.17—*In the whole plant freezing test, plants are exposed to cold temperatures in a chest freezer (A) with programmable capabilities (B). After a specified exposure period, plant tissues are rated for "browning" of buds (C), foliage (D), and lateral cambium (E).*

D

E

Figure 7.2.18—*In the freeze-induced electrolyte leakage test, plant tissue samples (A) are exposed to freezing temperatures (B) and then immersed in deionized water. The relative increase in electrical conductivity is an indication of cold injury (C) (C, courtesy of Sonia Gellert).*

mailed to the test laboratory (fig. 7.2.19B). Results are available in a few days.

Applications of cold hardiness testing. Container nurseries use CH testing for several purposes.

1. CH tests can be used to track the hardiness of crops as they go through natural hardening in the fall or through cultural hardening procedures, such as blackout. In outdoor compounds, CH tests at regular intervals can be used to determine when frost protection measures are needed (Perry 1998).

2. CH test are commonly used to determine the "lifting window" for container crops. For example, the ability to tolerate -18 °C (0 °F) is being used as an indication of when conifer crops in British Columbia can be lifted for freezer storage (Burdett and Simpson 1984). Different reference temperatures should be developed for other species and ecotypes.

3. CH tests provide a good estimate of overall plant stress resistance (Ritchie 2000), which is a key quality attribute (see Section 7.2.5.2).

Cold hardiness: Summary. Plants that are easily killed by freezing temperatures during the growing season can survive much lower temperatures in winter when they are cold hardy. Cold injury must be distinguished from winter desiccation that results when cell water is pulled across the cell membrane to feed ice crystals growing outside the cells. This can severely dehydrate cytoplasm and injure membranes, causing them to leak cell contents. Even cold hardy plants can be damaged by winter desiccation.

Cold hardening is triggered in late summer by photoperiod and increases during early winter as plants are exposed to increasingly lower temperatures. The level of hardiness can vary greatly among species and ecotypes and is highly influenced by the climate of origin. Peak hardiness occurs in January in temperate zone plants. Following this peak, hardiness can be rapidly lost as plants respond to lengthening photoperiod and warming temperatures.

The most commonly used CH tests are the whole plant freeze test, in which entire plants are exposed to freezing temperatures then evaluated for their response, and the

freeze-induced electrolyte leakage test, which is used to test foliar and root samples. Tests based on genetic indicators are now becoming available.

Cold hardiness tests can be used to establish lifting windows, for indicating when frost protection may be needed in the nursery, and as a surrogate for stress resistance testing.

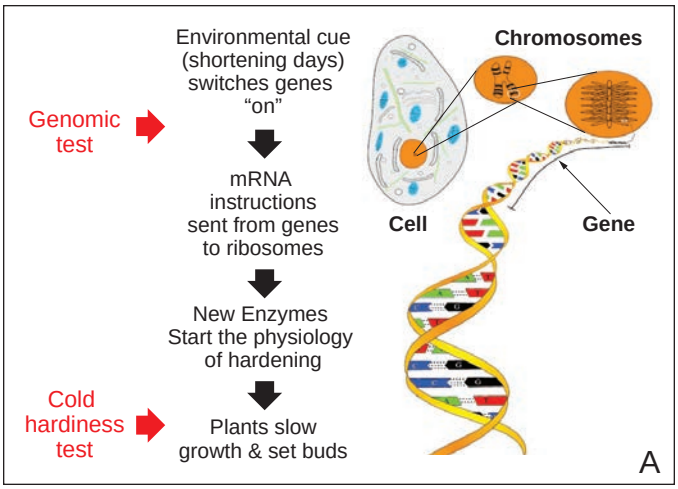
7.2.4.3 Root electrolyte leakage

Roots are among the most fragile parts of plants and, hence, are sensitive to many environmental and operational stresses. This is particularly true of container stock whose root systems are not insulated by surrounding soil. Stresses include high and low temperatures (Lindström and Mattsson 1989; Stattin and others 2000), desiccation (McKay and Milner 2000), rough handling (McKay and White 1997), improper storage (Harper and O'Reilly 2000; McKay 1992; McKay and Mason 1991), and even water logging and disease. It is sometimes possible to detect root damage using the time-honored thumbnail scraping and browning examination, but often the damage is invisible or difficult to quantify. A more rigorous test is called root electrolyte leakage (REL). Because it measures the health and function of root cell membranes, REL can be used as an indication of root injury and, therefore, quality.

REL has been used in Canada (for example, Folk and others 1999) and is currently one of a battery of plant quality tests developed by the Ontario Ministry of Natural Resources (Colombo and others 2001). In the United States, electrolyte leakage has primarily been used to test the cold hardiness of foliage, but application of this technique to roots is uncommon.

The REL method is relatively simple, uses readily available equipment, produces results quickly, and can be useful with deciduous trees, which are leafless in winter (Wilson and Jacobs 2006). Interpretation of REL results, however, can be problematic due to species, seedlot, and seasonal effects.

Theory. REL tests are based on the same principle as the FIEL test described in the previous section. The main difference, however, is that the REL test measures all types of root injury, not just cold damage. The basic idea is that measuring the quantity of ions that leak across damaged



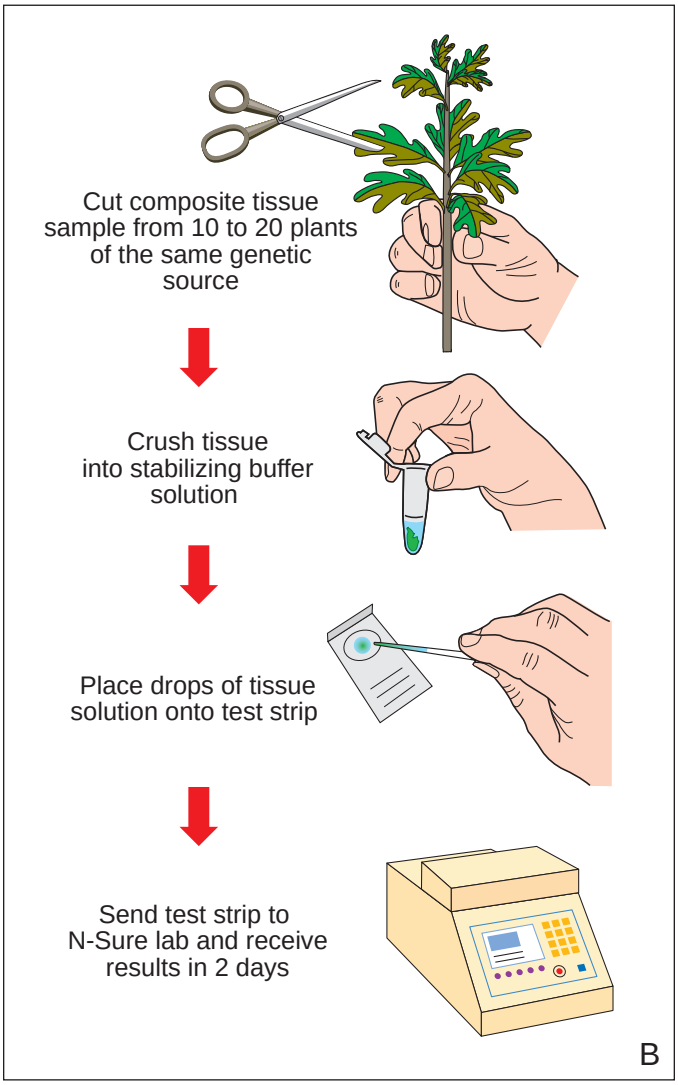


Figure 7.2.19—*Genomic cold hardiness tests allow early detection of the chemical signals that trigger cold hardiness and can serve as an early indicator (A). The N-Sure test provides a quick and accurate way to monitor cold hardiness of nursery stock (B).*

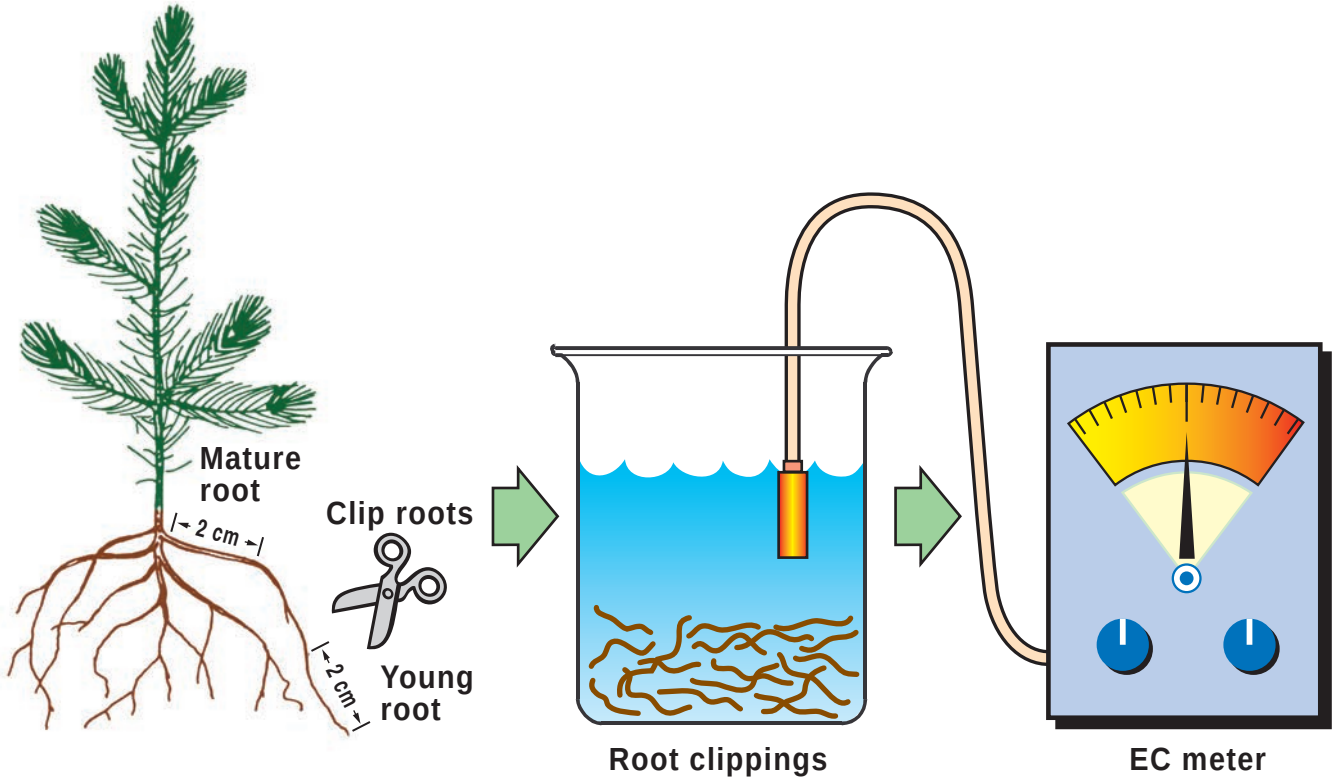


Figure 7.2.20—*In the root electrolyte leakage test, measuring the change in electrical conductivity of root tissue gives an indication of the amount of membrane damage. Because this test reflects all types of root injury, it can be used to indicate how well roots will grow out after outplanting.*

root membranes provides an estimate of the relative "viability" of the root system (Palta and others 1977). When damaged roots are placed in distilled water, the amount of membrane leakage can be easily and quickly measured with an electrical conductivity (EC) meter.

The biological significance of REL. McKay (1998) offers the following explanation for why the REL test has application as a plant quality test. After outplanting, the main cause of plant mortality is transplant shock induced by water stress. Plants with existing, viable root systems are more efficient in extracting water from soil, and REL measures that root system viability. A low REL reading indicates high root viability, allowing water uptake to mitigate transplant shock.

Measurement procedure. The technique most often used (McKay 1992, 1998) has changed little from the initial protocol described by Wilner (1955, 1960). The steps are as follows (fig. 7.2.20):

1. Roots are first washed in water to remove soil, then in deionized water to remove any surface ions that may be present.

2. A central mass of roots is removed from the plant—with nursery plants this is often a band about 2 cm wide running across the midsection of the root system.

3. Roots with diameter > 2 mm are removed from the sample, leaving only "fine" roots.

4. Fine roots are placed into a vessel containing deionized water.

5. The vessel is then capped, shaken, and left at room temperature for about 24 hours.

6. Conductivity of the solution (C_{live}) is measured with a temperature-compensated EC meter.

7. Root samples are removed and killed by autoclaving for 10 minutes at 100 °C (212 °F) or heating in an oven at 90 °C (194 °F) for 6 hours.

8. Conductivity of the solution surrounding the dead root samples (C_{dead}) is measured.

9. REL is calculated as the ratio of the EC of live roots divided by the EC of dead roots:

$$REL = (C_{live} / C_{dead}) \times 100$$

Applications of REL in nurseries. The REL test is most often used to assess effects of cold damage, poor storage conditions, root exposure causing desiccation, or rough handling of nursery stock. Nearly all the published work has been with bareroot conifer seedlings, primarily Douglas-fir, spruces, pines, and larch. Use of REL to detect freezing damage to roots is applied in one of two contexts: evaluation of cold hardiness test results, and detection of root injury following unseasonably cold weather.

Measuring root cold hardiness. REL cold hardiness testing is the same process as FIEL as explained in Section 7.2.4.2. For example, root samples from bareroot Norway spruce seedlings were exposed to either –5 °C (23 °F) or –10 °C (14 °F) biweekly from September through December in Sweden (Stattin and others 2000). As winter progressed, the difference in REL between cold-treated and nontreated seedlings became smaller, indicating that seedlings were becoming increasingly more cold hardy (fig. 7.2.21).

Detecting cold or heat injury to roots. Because roots of container plants are not protected by the thermal mass of soil, they can be easily injured by extreme temperatures. This is especially true when nursery stock is overwintered outdoors under snow, as is done in eastern Canada and Scandinavia (Lindström and Mattsson 1989). If snow fails to accumulate, or a sudden warm period occurs, container crops are often exposed long enough for their roots to be severely damaged. The REL test is ideally suited for making rapid assessment of potentially damaged nursery stock (for example, Coursolle and others 2000).

Determining lifting windows. REL has been used to indicate when it is safe to harvest bareroot nursery stock (McKay and Mason 1991). For example, Douglas-fir seedlings harvested during midwinter showed much lower REL readings and, therefore, less root injury than stock harvested earlier (fig. 7.2.22).

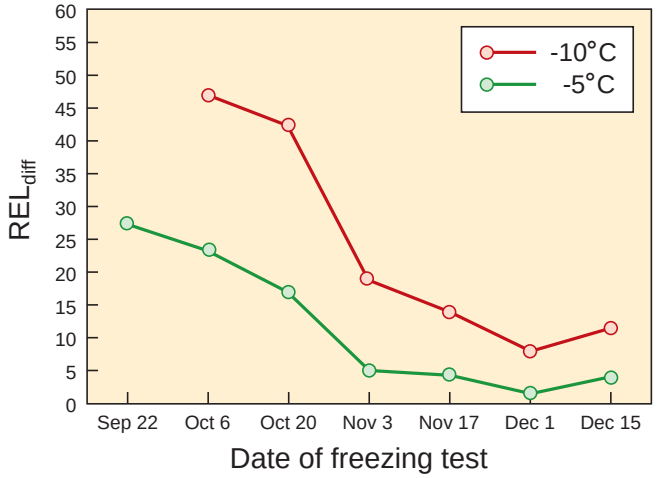


Figure 7.2.21—*Root electrolyte leakage measurements of Norway spruce seedlings show the development of root hardiness during fall. REL_{diff} is the increased electrolyte leakage from roots following exposure to –5 or –10 °C (23 or 14 °F) compared with leakage from nonfrozen seedlings (modified from Stattin and others 2000).*

Monitoring quality of stored seedlings. REL can be used to monitor quality during overwinter storage (McKay 1992, 1998; McKay and Morgan 2001). In one test (McKay 1998), spruce and larch seedlings were harvested throughout winter, beginning October 1, and then placed in storage at 1 °C (33 °F). All seedlings were removed from storage, tested for REL, and then outplanted in April. With both species, REL decreased and survival increased as harvesting was delayed. In another experiment (Harper and O'Reilly 2000), Douglas-fir seedlings were harvested in October, November, December, and January; "warm stored" at 15 °C (59 °F) for 7 and 21 days; and then tested for REL. The REL readings taken at the time of harvest decreased with later harvesting dates, indicating that seedlings were becoming hardier. For each harvest date, however, the readings increased sharply with storage duration suggesting that warm storage contributed to fine root degradation (fig. 7.2.22).

Desiccation and rough-handling effects. Bareroot Sitka spruce and Douglas-fir seedlings were held in controlled environment chambers with their roots exposed to drying conditions for up to 3 hours (McKay and White 1997). The REL readings increased with the intensity of the desiccation treatment, indicating root injury. Injury was confirmed when the desiccation treatments had poor outplanting performance on sites with low spring rainfall in Great Britain.

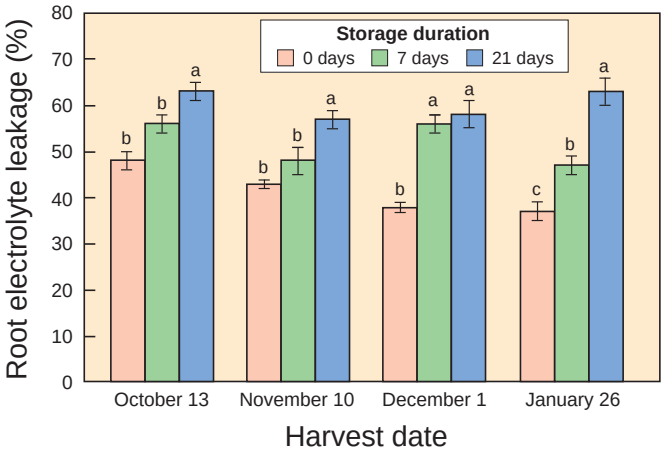


Figure 7.2.22—*REL can be used to determine harvesting (lifting) windows and monitor stock quality during storage. Douglas-fir seedlings harvested during midwinter showed lower REL levels than stock harvested earlier in fall. The same stock was warm-stored after harvesting, and REL measurements at each date showed that less warm storage yielded lower REL levels (modified from Harper and O'Reilly 2000).*

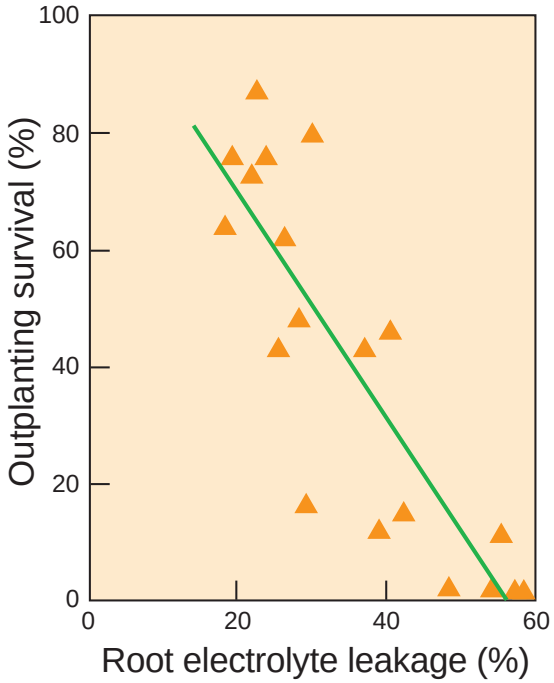


Figure 7.2.23—*Root electrolyte leakage has shown good correlation with outplanting performance in this study with Japanese larch, but not so in many other studies (modified from McKay and Mason 1991).*

Rough handling in combination with root desiccation was assessed in bareroot Douglas-fir, Sitka spruce, Japanese larch (*Larix kaempferi*), and Scots pine using REL (McKay and Milner 2000). Rough-handling treatments were simulated by dropping bags of seedlings from a height of 3 m (9.8 ft). Desiccation was achieved by exposing roots to warm, dry air for 5 hours. Although effects varied with harvesting date and species, REL was significantly higher in stressed seedlings across species and treatments.

REL as a predictor of outplanting performance. The ultimate objective of any plant quality test is to predict how well nursery stock will survive and grow after outplanting, and many studies have used REL for this purpose with mixed results. REL was closely correlated with relative water content of radiata pine (*Pinus radiata*) seedlings 20 days after planting (Mena-Petite and others 2004). With Sitka spruce and Japanese larch seedlings, REL was closely related to both survival and height growth (fig. 7.2.23). In Sitka spruce and Douglas-fir seedlings, REL was correlated with survival on some sites but not others (McKay and White 1997). REL predicted establishment of Japanese larch seedlings to some extent, but root growth potential was a better predictor (McKay and Morgan 2001). Similar results were found with black pine *(Pinus nigra)* (Chiatante and others 2002), while Harper and O'Reilly (2000) reported that REL was a poor predictor of survival potential in warm-stored Douglas-fir seedlings.

Limitations of REL. Why does REL predict survival in some cases but not all? As with many things, "the devil is in the details."

Genetics. REL has been shown to vary with species and even seed sources within species. For example, jack pine and black spruce exposed to a range of damaging root temperatures had REL values in the range of 27 to 31 percent, while white spruce exposed to the same temperatures had REL between 36 and 38 percent (Coursolle and others 2000). Sitka spruce seedlings from Alaska, the Queen Charlotte Islands (QCI), and Oregon provenances were evaluated for their ability to withstand root drying and rough handling (McKay and Milner 2000). Oregon and QCI seedlings exposed to root drying had lower REL values than Alaska seedlings, while Alaska and QCI seedlings, when exposed to rough handling had lower values

than Oregon seedlings did. In another study, Douglas-fir had higher REL values than did Sitka spruce, Scots pine, and Japanese larch, regardless of the type of stress encountered (McKay and Milner 2000). Two coastal seedlots of Douglas-fir (British Columbia) gave different relationships between REL and survival (Folk and others 1999).

Dormancy status. McKay and Milner (2000) found that the resistance to stresses mentioned above varied seasonally and was correlated with the intensity of bud dormancy. A similar result was reported by Folk and others (1999) for Douglas-fir seed lots who concluded that REL must first be calibrated to bud dormancy status before it can be effectively used to assess root damage in Douglas-fir.

Seedling age. REL gave good correlations with survival in 2-year-old black pine seedlings, but correlations were weak for 1-year-old seedlings (Chiatante and others 2002). The authors speculate that the efficiency of REL as a quality assessment tool could be closely related to the developmental state of the root system.

Root electrolyte leakage: Summary. Electrolyte leakage from fine roots (REL) is a measure of the ability of membranes within the root system to contain ions. Damaged membranes tend to leak ions so, if ion leakage is quantified, it can provide an indicator of root viability. The REL test is a fast and easy way to evaluate the effects of cold damage, rough handling, desiccation, cold and warm storage, and other stresses on root viability and plant vigor. REL is sometimes closely correlated with plant survival, but in other cases these correlations are weak. This is because factors other than root damage can affect REL, including species, seedlot, plant age, season, and bud dormancy intensity. Fortunately, REL can be calibrated for these effects.

7.2.4.4 Chlorophyll fluorescence

Although technology for measuring chlorophyll fluorescence (CF) has been in place for more than 50 years, it has been applied to tree seedling physiology only since the late 1980s (Mohammed and others 1995). In early trials, forestry researchers considered CF to be an important research tool for potential applications such as assessing effectiveness of irrigation and fertilization, determining harvest windows, and evaluating plant vigor after storage. CF was predicted to be a "simple, rapid, reliable and non-destructive method of evaluating seedling physiological status during the nursery production cycle" (Vidaver and others 1988).

In the intervening years, CF has not lived up to those early expectations. Because CF has such great potential, however, both plant producers and users should have a basic understanding of CF and what it can and cannot do.

What is chlorophyll fluorescence? When solar radiation strikes a leaf, some light energy is reflected, some is transmitted through the leaf tissue, and some is absorbed. Plants absorb much more light energy than is required for photosynthesis. In fact, < 20 percent of the photosynthetically active radiation absorbed by a leaf is actually used in photosynthesis (fig. 7.2.24). Red and blue wavelengths are absorbed by chlorophyll and other pigments, but green wavelengths are reflected, giving living plants their green color. To dissipate all that excess energy that would otherwise be damaging, plants have developed ingenious processes known collectively as "energy quenching." Three types of energy quenching are recognized. Photochemical quenching (qP) is energy used in photosynthesis. Nonphotochemical quenching (qN) is energy dissipated mainly as sensible heat. Fluorescence quenching (qF) is energy emitted as fluorescence and is the basis for the chlorophyll fluorescence test. The largest amount of the absorbed energy is dissipated as sensible heat (qN), while a much smaller amount is given off as fluorescent light (qF) (fig. 7.2.24). These three quenching mechanisms operate simultaneously and in competition with one another.

If these quenching mechanisms are overloaded by high light, the surplus energy drives a biochemical process called the "Moehler reaction." This generates free radicals, mainly oxides and peroxides toxic to the plant. To protect themselves, leaves synthesize scavenging molecules that mop up free radicals and render them harmless. The yellow carotenoid pigments, for example, serve this function. When light intensity is so high, however, as to overwhelm these scavenging systems, then photodamage occurs (Demig-Adams and Adams 1992). This often appears as leaf "scorching" and is common in nursery plants that have been moved too quickly from shade to full sun.

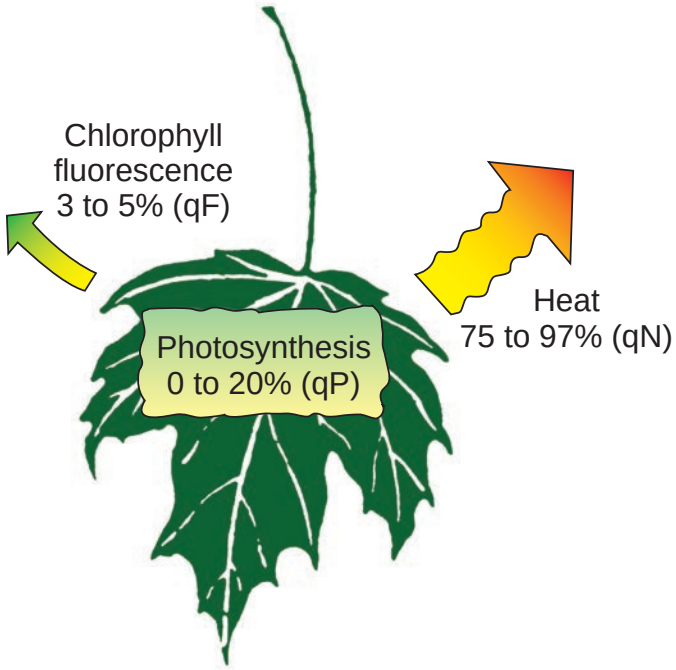


Figure 7.2.24—*Only a small amount of photosynthetically active radiation is absorbed by leaves and actually used (quenched) by photosynthesis. The rest of the surplus energy is quenched as heat loss or as fluorescence.*

The manner in which a plant is able to manage the light energy it absorbs is a sensitive indicator of stress (Krause and Weis 1991). The CF technique, which quantifies energy quenching, is useful for studying plant responses to stress and therefore plant quality.

Photosynthesis and chlorophyll fluorescence. Photosynthesis embodies three sequential processes (Vidaver and others 1991):

1. Light harvesting—light energy is absorbed by light-sensitive pigments (including chlorophyll) in the leaves.
2. Photochemistry—the absorbed light energy is converted into chemical energy.
3. Biochemistry—chemical energy is used to drive Calvin cycle reactions that convert atmospheric carbon into simple sugars.

CF provides a view into the photochemistry process. Because all three processes are intimately interconnected, a perturbation to one part of one process affects the entire set of reactions. These changes in the photosynthetic process are reflected in variations in the amount and rate of CF emissions.

Light energy enters the leaf of a plant and is "captured" by light harvesting pigments (fig. 7.2.25). Depending on the wavelength of the captured light, it enters one of two reaction centers: Photosystem I (PSI) and Photosystem II (PSII), which are located on membranes in the chloroplasts. When a chlorophyll$_a$ (Chl_a) molecule in PSII absorbs a photon of energy, one of its electrons is raised to a higher energy state. While in this excited state, it is captured by an electron acceptor pool from which it funnels down through an electron transport chain into PSI, where a similar process occurs (PSI and PSII are named in the order in which they were discovered, not the order of the reaction). This energy transfer leads to the generation of ATP and ultimately the reduction of NADP to NADPH. The energy contained in ATP and the reducing power of NADPH contribute to the fixation of CO_2 molecules and their ultimate conversion to simple sugars in the Calvin Cycle.

"Water splitting" is another key part of the light reaction. In order to replenish the electrons that are lost from Chl_a in PSII, the plant splits water molecules, releasing oxygen atoms into the atmosphere and providing electrons that feed into PSII (fig. 7.2.25).

For any of a number of reasons, many of the excited electrons from Chl_a in PSII are not captured by the acceptor pool and they decay back to their ground state. The energy lost in this decay process is given off as fluorescent light (qF), which emanates entirely from Chl_a in PSII (Krause and Weis 1991) as it decays to its ground state. This is shown in figure 7.2.25 as a wavy line and occurs when the acceptor pool is fully reduced or when the electron transport pathway is backed up. In other words, when more excited electrons are produced than can be processed, they fall back to their ground state, releasing their excitation energy as fluorescence.

This fluorescence emission is too weak to be visible to the naked eye but can easily be detected by an instrument called a chlorophyll fluorometer. The fluorometer measures and quantifies the nature of this fluorescence emission and forms the basis of the CF test.

Measuring chlorophyll fluorescence. The German plant biochemist Hans Kautsky first observed chlorophyll fluorescence in the late 1920s (Govindjee 1995). Kautsky darkened a leaf, then illuminated it with a brief flash of intense

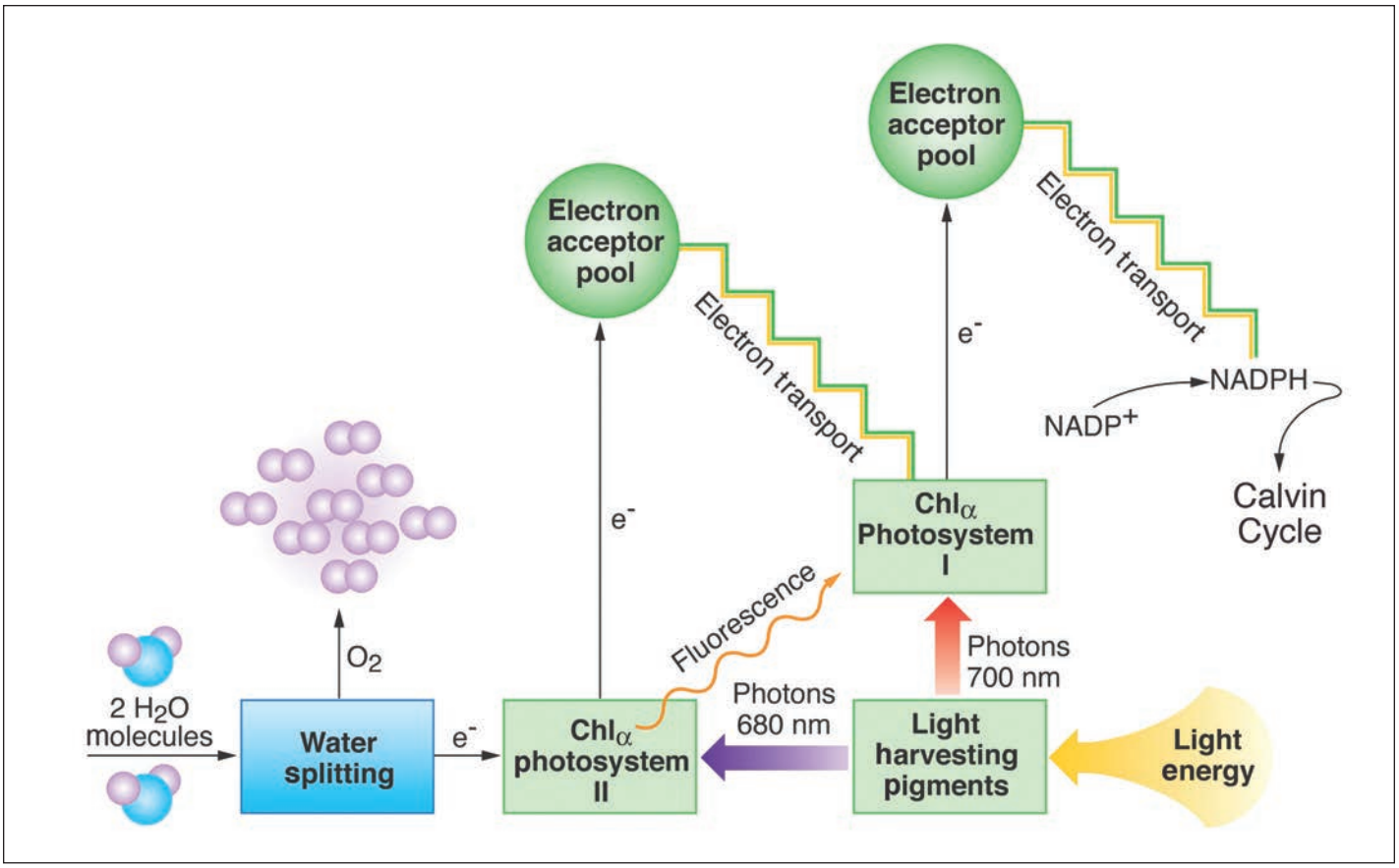


Figure 7.2.25—*Simplified diagram of the light reaction of photosynthesis. Chlorophyll fluorescence emanates from chlorophyll$_\alpha$ in Photosystem II. This fluorescence can be measured with a fluorometer and can be used to diagnose stresses.*

light, and noted an emission of fluorescent light followed the light pulse. Surprisingly, he found that in healthy tissue the emission disappeared within a few minutes, but when the tissue was killed with cyanide or by freezing, the fluorescence emission persisted much longer. It has since been determined that poisoning or freezing leaf tissue disables the electron flow pathway, causing excited electrons to fall back to their ground state and give off measurable fluorescence. In healthy tissue, by contrast, more electrons are quenched in the electron transport pathway, thereby reducing fluorescence emissions.

Kautsky fluorometers. Kautsky's observation led to the development of instruments called "Kautsky" fluorometers. Originally large and cumbersome and the staple of laboratory research on photosynthesis, Kautsky fluorometers have evolved into small, affordable, portable, and user-friendly devices. They contain a light source, two sets of filters, a microprocessor, and a photosensor, and they typically interface with a laptop computer (fig. 7.2.26A). The light source sends a pulse of photosynthetically active light through a fiberoptic cable to the leaf surface where it activates Chl_a in PSII. The Chl_a emission returns back through the cable and passes through a second filter that transmits fluorescent light to the photosensor, which records the emission. The process is controlled by the microprocessor, which is programmed using the laptop computer.

The CF measurement process begins with "dark adapting" the leaf for about 20 minutes. This ensures that: (1) all chlorophyll is in an unexcited, or ground, state; (2) the acceptor pools are empty; and (3) the electron transport pathway is clear before the light pulse is received. Following the light pulse, the fluorometer generates a curve in which the intensity of the resulting fluorescence emission is plotted over time (fig. 7.2.26B). In the Kautsky curve, Fo is fluorescence emanating from the light harvesting pigments in the leaf, not from PSII. Fm is the maximum fluorescence, and Fv is the variable fluorescence coming from PSII.

This curve has many diagnostic features, but the most useful is the ratio of variable fluorescence to maximum fluorescence, or Fv/Fm. This ratio provides a direct estimate of the efficiency of the light reaction (Genty and others 1989) and is the most often used CF output.

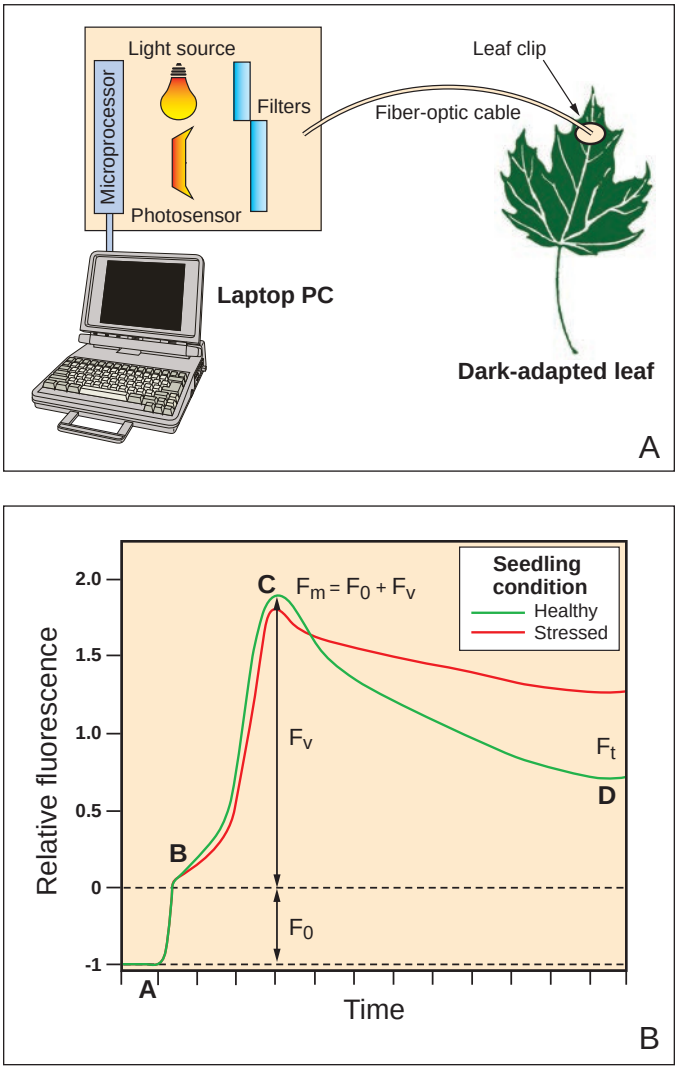


Figure 7.2.26—*A Kautsky fluorometer consists of a light source, two filters, a photosensor, microprocessor, and a fiberoptic cable that attaches to a leaf. Instructions are sent to the fluorometer from a laptop computer (A). A quenching curve is generated after a light pulse is delivered to a dark-adapted leaf. These curves are diagnostic because healthy and stressed plants differ in the amount and duration of their fluorescence emission (B). For example, the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) is a good indicator of photosynthetic efficiency. See table 7.2.7 for explanation of symbols (B, modified from Rose and Haase 2002).*

Pulse amplitude modulated fluorometers. A more recent development in fluorometry is an instrument called the pulse amplitude modulated (PAM) fluorometer (Schreiber and others 1995). After delivering an initial excitation light pulse, the PAM generates a rapid stream of high-intensity, saturating light pulses that overwhelm acceptor pools, thus canceling out photochemical quenching. The fluorescence emission differentiates between these peaks and the fluorescence decay curve is, therefore, nonphotochemical quenching.

This powerful procedure enables simultaneous measurement of the three energy-quenching components, along with determination of overall process efficiency at several levels. One of these instruments, the PAM-2000, is manufactured by Heinz Walz in Germany (http://www.walz.com). PAMs have become an essential tool for seedling physiology research. A PAM-2000 run produces estimates of quantum yield (Fv/Fm), effective quantum yield (Y), photochemical quenching (qP), nonphotochemical quenching (qN), electron transport rate (ETR), and many other variables.

Normal values of CF parameters in plants. The biochemistry of photosynthesis is essentially uniform across all species of C_3 plants. Therefore, CF parameters in "normal" healthy plants would not be expected to vary across a broad range of species. Discussions with other scientists, as well as perusal of the CF literature, led to the development of table 7.2.7. This gives what are often considered to be "normal" values for the CF parameters and can be used as a guide to interpreting literature values.

Use of CF in plant-quality assessment. At the present time, CF is primarily a research tool but is beginning to be used operationally in some nurseries.

Dormancy. Although attempts to use CF as an indicator of plant phenological condition or dormancy status have been done, we are not yet convinced that these studies are verifiable or repeatable.

Cold hardiness. Currently, the most common use of CF is in detecting and assessing cold injury (Binder and others 1997). For example, when 17 species of *Abies* were tested for cold hardiness, the damage to buds, foliage, and lateral cambium were all well correlated with CF ratings (Jones and Cregg 2006). When compared with other cold

hardiness tests, CF was shown to be a quick, nondestructive indication of cold injury of the foliage and stems of Scots pine container stock (Peguero-Pina and others 2008). Rather than rating cold injury with visual, electrolytic, or other methods (see Section 7.2.4.2), the CF approach uses the response of the photosynthetic process as an index of damage. "Normal" plants will typically have Fv/Fm values from 0.700 to 0.830, or slightly lower in winter. When this value falls to < 0.600 following freezing, it indicates significant damage to the photosynthetic process (table 7.2.7).

Outplanting performance. Some studies have attempted to correlate CF variables with outplanting performance. For example, measures of effective quantum yield predicted variations in survival and plant health of stored and nonstored Douglas-fir seedlings in an Irish nursery (Perks and others 2001).

Storage effects. Short-term (2-week) cooler storage of radiata pine seedlings caused depressions of Fv/Fm, Fv/Fo, and other CF parameters as leaf water potential, stomatal conductance, and net photosynthesis also dropped (Mena-Petite and others 2003). These reflected storage-related damage to photosynthetic apparatus and portended reduced post-planting performance. CF is being used as a plant-quality test after storage in some Ontario nurseries (Colombo 2009).

Drought stress. Long-term drought affects photosynthesis directly by depressing leaf water potential, which closes stomata. Recent evidence suggests that prolonged drought also disrupts photosynthesis at the photochemical level. When white spruce seedlings were exposed to 21 successive days without water in a controlled environment chamber (Bigras 2005), Fo and qN were unaffected, but Fm, Fv, Fv/Fm, and qP were depressed when water potential fell below –1.0 MPa (10 bars PMS). Fv/Fm measured in dormant Norway spruce seedlings was unaffected by 4 weeks of post-planting drought in the field, but the same drought exposure depressed Fv/Fm from 0.83 to about 0.28 in seedlings lacking bud dormancy (Helenius and others 2005).

Chlorophyll fluorescence: Summary. Plants have evolved intricate mechanisms for dissipating, or quenching, the light energy they absorb. Some of this energy is used in photosynthesis (photochemical quenching, qP), while the remainder is dissipated by nonphotochemical (qN) or fluorescence (qF) quenching.

Stress caused by high and low temperature, disease, drought, inadequate nutrition, and so on impairs a plant's ability to manage energy quenching. Thus, by measuring and interpreting the three components of quenching with CF, it is possible to detect damage resulting from subtle, transient stress as well as long-term, severe stress. Three important CF parameters that are often reported in the nursery literature are qP, qN, and Fv/Fm.

Damaged or stressed plants have the ability to recover quickly, so it is important to measure CF parameters over a course of several days following stressful events before conclusions about plant damage can be reached. If Fv/Fm remains low and qN high for several days, this indicates that significant damage to the photosynthetic system has probably occurred. Still, much more research is needed before CF will be an operational quality test.

7.2.4.5 Mineral nutrient content

Intuitively, the amount of mineral nutrients that is stored in a plant should be related to its quality. Mineral nutrients such as nitrogen and phosphorus supply the building materials for new growth, and newly outplanted seedlings must rely on a supply of stored nutrients until they are established in the field. Because they reflect actual mineral nutrient uptake, plant tissue tests are the best way to monitor a fertilization program. Analytical laboratories are able to accurately and precisely measure the levels of all 13 mineral nutrients in a small sample of plant tissue, and nursery managers can obtain results in as little as a week. By also measuring tissue biomass, nutrient content can be calculated from the laboratory results for nutrient concentration. That data can then be examined using vector diagrams for relative differences among fertilizer regimes for nutrient dilution, toxicity, sufficiency, or deficiency (Haase and Rose 1995). Although tentative guidelines for analyzing mineral nutrient levels exist, they are for general classes such as "conifer seedlings" (table 7.2.8) and are of limited usefulness for precision monitoring of fertilizer programs. Most published test results are for commercial tree species and almost nothing is known about other native plant species (Landis and others 2005).

Another problem is that correlation between foliar nutrient levels and outplanting survival is not good. One problem is that a plant could be severely stressed or even dead and

Table 7.2.7—*Normal ranges of chlorophyll fluorescense emissions parameters in C4 plants (extracted from the literature)*

CF parameter	Definition	Description	Normal range	Stress range
Fo	Ground state fluorescence	Fluorescence which emanates from the light-harvesting pigments of the leaf; generally considered a "background level" fluorescence that is zeroed out when measuring PSII chlorophyll fluorescence.	0.2 to 0.4	> 0.7
Fs	Steady-state fluorescence	Fluorescence level (sometimes referred to as Ft)		Low Ft indicates stress
Fv	Variable fluorescence	Height of the fluorescence peak above Fo following exposure to the actinic light pulse (Fv = Fm – Fo)		
Fm	Maximal fluorescence	Fv + Fo	1.2 to 1.5	
Fv/Fm	Maximum quantum yield	An estimate of the ratio of moles of carbon fixed per mole of light energy absorbed (Genty and others 1989); theoretical maximum value for C_3 photosynthesis is approximately 0.830.	0.70 to 0.83	< 0.60
Y	Effective quantum yield	(Fm-Fs)/Fs	0.40 to 0.60	0.10 to 0.20
qN	Nonphotochemical quenching	Dissipation of absorbed light energy by means other than photosynthesis (mainly as sensible heat)	0.4 to 0.6	Prolonged values > 0.6
qP	Photochemical quenching	Use of absorbed light energy via photosynthesis	0.7 to 0.8	Prolonged values < 0.6
ETR (in full sun)	Electron transport rate	Speed at which electrons are transported through the photosystem	< 300 µmol electrons $m^{-2}s^{-1}$	

still contain ideal mineral nutrient levels. Even though mineral nutrient levels are not a guarantee of vitality, foliar nitrogen levels appear to be a good predictor of growth after outplanting (Landis 1985). For example, van den Driessche (1984) found a strong correlation between foliar nitrogen and the shoot growth of Sitka spruce seedlings when measured 3 years after outplanting (fig. 7.2.27A). This makes sense only because, after a plant is established, it needs good reserves of nitrogen to repair any injuries and build new cells. Some nurseries have established foliar nitrogen targets at the time of harvest as one indication of plant quality; for instance, provincial nurseries in Quebec specify a minimum foliar nitrogen level for their nursery stock depending on container size (Government of

Quebec 2007). Therefore, the best recommendation is for nurseries to develop their own foliar nutrient standards for the plant species that they grow.

The latest research into the relationship between seedling nutrient levels and outplanting performance involves a concept called "nutrient loading" with nitrogen. The idea is that "supercharging" a seedling with nitrogen will help it survive and grow better on the outplanting site where mineral nutrients are usually limiting. Nutrient loading involves fertilizing seedlings during the hardening phase until their nitrogen content is in the luxury consumption area of the growth curves (fig. 7.2.27B). This process has been successful with black spruce (*Picea mariana*) on sites with heavy plant competition as chronicled by Timmer and his associates (for example, Timmer 1997). The concept of nutrient loading with nitrogen is certainly attractive and it is hoped that this technique will be tested with more species on a wide variety of outplanting sites (Landis and others 2005). Possible problems with increased animal predation and lower frost hardiness also need to be investigated.

7.2.4.6 Carbohydrate reserves

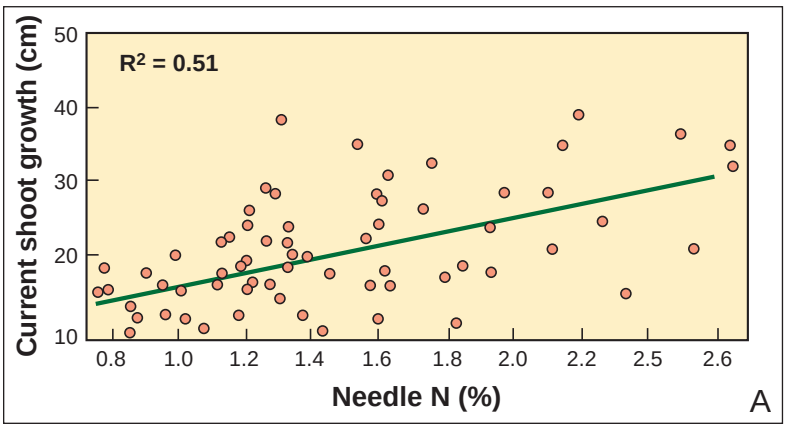
It seems logical that the amount of food stored as carbohydrates in nursery stock should be a good indication of plant quality. After outplanting, nursery plants must rely on this stored "food" to fuel new growth until the plants can start photosynthesizing. Marshall (1983) gives an excellent review of carbohydrates in plants and presents a good comparison of how stored carbohydrates would be used in two different seedlings. Seedling 1 contains adequate levels when harvested, but carbohydrates are gradually consumed during storage; after outplanting, even more are used until the plant becomes established and generates new carbohydrates through photosynthesis (fig. 7.2.28A). Plants that suffered stress or injury would use even more carbohydrates to repair tissues and fuel metabolic recovery. In fact, carbohydrate reserves were found to influence the growth of nursery stock for up to 2 years after outplanting (Ronco 1973).

Unfortunately, research trials have not shown carbohydrate reserves to be a good predictor of plant quality and little has been done with container nursery stock. For example, the carbohydrate reserves of bareroot Scots pine seedlings were evaluated as an indicator of stock quality, and the results followed the general trend in figure 7.2.28A. When reserves dropped below 2 percent total glucose during storage, significant mortality occurred (fig. 7.2.28B). The author concluded that difficulties in measuring carbohydrate concentrations and the dynamics of carbohydrate metabolism make tests of carbohydrate reserves impractical for operational use as a plant-quality index (Puttonen 1986).

Measuring performance attributes can be thought of as a "bioassay" that integrates the functioning of all plant systems into one performance variable. Although they are often robust indicators of plant performance potential, performance attributes do not identify what, specifically, is wrong when performance potential is low. In addition, they also suffer from being very time consuming to measure directly, which can limit their usefulness to plant producers and users.

Table 7.2.8—*Target concentrations for the essential mineral nutrients in the foliage of conifer nursery stock (modified from Landis 1985)*

Nutrient	Symbol	Acceptable range
Macronutrients (%)		
Nitrogen	N	1.30 to 3.50
Phosphorus	P	0.20 to 0.60
Potassium	K	0.70 to 2.50
Calcium	Ca	0.30 to 1.00
Magnesium	Mg	0.10 to 0.30
Sulfur	S	0.10 to 0.20
Micronutrients (ppm)		
Iron	Fe	40 to 200
Manganese	Mn	100 to 250
Zinc	Zn	30 to 150
Copper	Cu	4 to 20
Boron	B	20 to 100
Molybdenum	Mo	0.25 to 5.00
Chloride	Cl	10 to 3,000



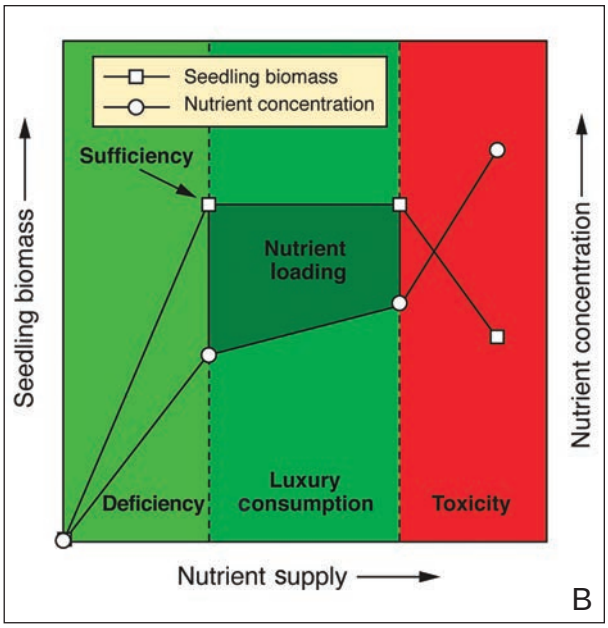
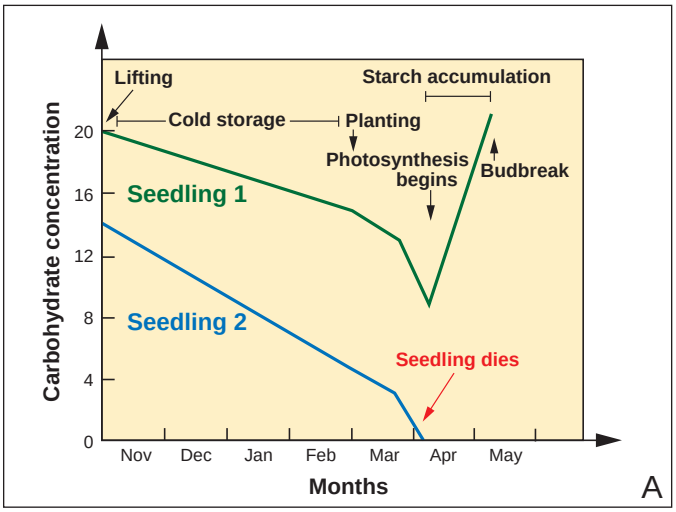


Figure 7.2.27—*Foliar nitrogen (N) concentration was shown to be a good predictor of the shoot growth of Sitka spruce seedlings when measure 3 years after outplanting (A). "Nutrient loading" conifer seedlings with high levels of nitrogen (B) has been shown to be beneficial on wet outplanting sites with heavy plant competition (A, modified from van den Driessche 1984; B, modified from Timmer 1997).*



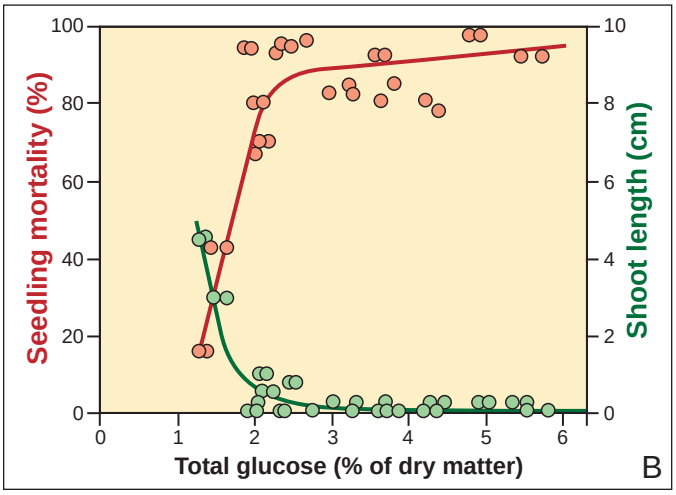


Figure 7.2.28—*Nursery plants consume significant amounts of stored carbohydrates from harvest through storage and outplanting. Seedling 1 contained adequate reserves and survived until it became established on the outplanting site and replenished carbohydrates through photosynthesis. Seedling 2 started out with inadequate carbohydrate storage and died soon after outplanting (A). With* Pinus sylvestris *seedlings, mortality increased and shoot growth decreased after outplanting when total glucose levels dropped below 2 percent (B) (A, modified from Marshall 1983; B, modified from Puttonen 1986).*

7.2.5 Performance Attributes

7.2.5.1 Bud dormancy

The notion that nursery stock quality is related to its dormancy status is strongly ingrained in the minds of plant producers and users, especially foresters. When pressed to explain this relationship and why it is important, however, few are able to articulate a clear view of what dormancy is, how it works, or how it affects quality. So, our intent is to discuss this important concept with the caveat that dormancy intensity can vary between species and ecotypes. In particular, plants from higher latitudes and elevations will show stronger dormancy than those from lower latitudes and elevations.

The concept of dormancy. Dormancy is one of the oldest concepts in plant science. Nursery workers learned by trial and error that plants could be transplanted and outplanted most successfully when they were not actively growing. In the temperate zone, this occurs in winter, so nurseries have traditionally harvested stock then. The concept of the "lifting window" was developed by harvesting and outplanting seedlings from late fall through early spring and measuring survival and growth (Jenkinson and others 1993). These trials supported the traditional practice of harvesting during midwinter, and people interpreted these results to mean that plants were most "dormant" during this period. As we will show, however, this concept of a midwinter dormancy peak is not correct.

Defining dormancy. Dormancy can be broadly defined as a state of minimal metabolic activity, or any time that a plant tissue is predisposed to grow, but does not (Lavender 1984). In other words, dormancy is that condition in which plant growth—cell division and enlargement—is not occurring. In horticulture, dormancy can refer either to seed dormancy or plant dormancy. In the published literature, plant dormancy has been studied much less than seed dormancy but plant dormancy is what we are concerned with here.

Two kinds of plant dormancy are recognized:

> External dormancy, also known as "quiescence," occurs when environmental conditions (for example, severe water stress) will not support growth (Lavender 1984). Plants exhibiting imposed dormancy will resume growth when these unfavorable conditions improve (when it rains).

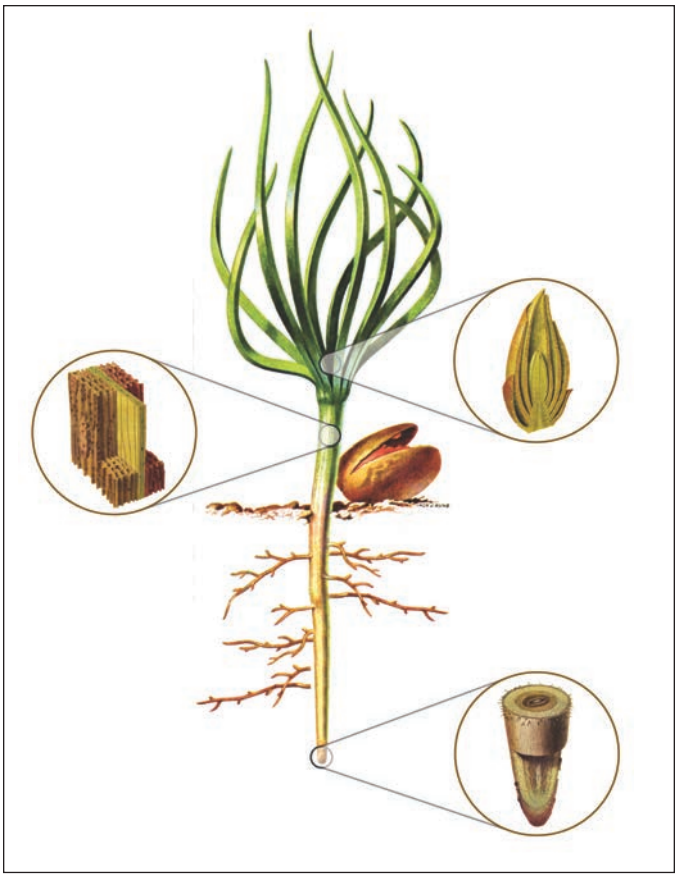
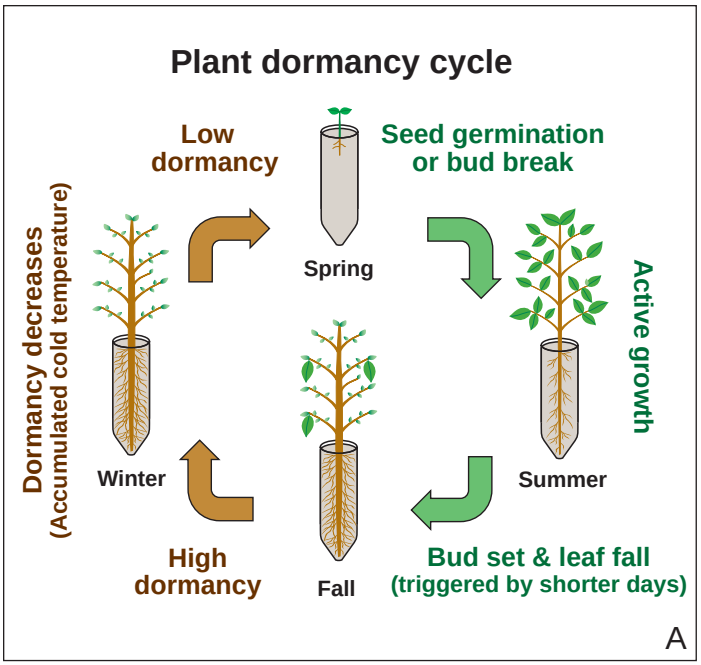


Figure 7.2.29—*Dormancy refers to the activity of the meristematic tissues: buds, lateral meristems in the stem, and root tips. In the normal context of plant quality, bud dormancy is the primary concern.*

> Internal dormancy, or "deep dormancy," is a condition in which plants will not resume growth until they have experienced a long period of exposure to low temperatures (Perry 1971). This condition is also called "winter rest." In this chapter, we are concerned with deep dormancy and how this physiological condition affects nursery culture and outplanting success.

Dormancy refers to tissues, not entire plants. In everyday nursery jargon we talk about plants, or even entire crops, being dormant. While this is common terminology, it is important to understand that plant dormancy refers to a specific meristematic tissue, usually buds (fig. 7.2.29). In the same plant, buds may be dormant while the lateral meristem may not. Root meristems never truly go dormant and will grow anytime that environmental conditions, especially temperature, are favorable. Because we are




B


C

Figure 7.2.30—*The buds of perennial plants in the temperate zone, including forest and conservation nursery stock, undergo a seasonal cycle of shoot growth and dormancy. Note that peak dormancy occurs in late fall instead of midwinter, as is often believed, and that dormancy is released by cumulative exposure to cold ("chilling requirement"). Some dormant plants exhibit morphological changes: firm "winter buds" and bluish needles due to waxy deposits (B), and purplish foliage in others species. Due to extreme variation among individuals (C), these color changes cannot be used to predict dormancy.*

concerned with quality testing, we will be discussing bud dormancy, which is most clearly observed in the behavior of terminal buds.

The dormancy cycle. Perennial plants that grow in temperate regions exhibit a pronounced seasonal "cycle of dormancy" (fig. 7.2.30A). In spring, as day length and temperature increase, plant buds begin to exhibit dimensional increases reflecting both cell division and expansion—in other words, they begin to grow. Shoot growth persists through spring and into summer. In summer, as day length (photoperiod) begins to shorten, the increasing length of the dark period is perceived by the phyto-chrome system in leaves as a signal to begin preparing for winter. At this point shoot growth slows and winter bud development proceeds (Burr 1990). By early fall, some plants form a dormant bud and exhibit other morphological changes, such as leaf color change and abscission in hardwood stock (fig. 7.2.30A), increased needle waxes on conifer needles (fig. 7.2.30B), and purplish needle color in other plants. These visual changes should not be considered proof of dormancy, however, as considerable variation occurs among individuals in the same seedlot (fig. 7.2.30C). In a study with Scots pine seedlings, no predictive relationship could be developed between purplish foliage and cold hardiness test results (Toivonen and others 1991).

The chilling requirement. In late summer, plant buds enter the condition of imposed dormancy. As summer surrenders to autumn, imposed dormancy gradually gives way to deep dormancy and buds reach maximum dormancy in late fall (fig. 7.2.30A). As we just mentioned, dormancy is then released by exposure of the plants to an extended period of low temperatures; this is known as a "chilling requirement" and is sensed by the buds. This evolutionary adaptation ensures that plants will not resume shoot growth (break bud) during a midwinter warm spell only to be killed by a return of cold weather. Once this chilling requirement is satisfied, warm spring temperatures and, to a lesser extent, lengthening photoperiod, will trigger and sustain a resumption of shoot growth (Campbell 1978). Although temperatures in the range of about 3 to 5 °C (37 to 41 °F) are most efficient at

releasing bud dormancy (Anderson and Seeley 1993), temperatures above and below this range also are effective to a lesser degree (fig. 7.2.31).

Orchardists and other horticulturalists have developed elaborate models to predict the date of flower bud opening in cold-sensitive crops such as peaches (see, for example, Richardson and others 1974). These models take into account the efficiency of chilling and the fact that warm interruptions during late fall can negate some chilling that has occurred up to that time. In forest and conservation nurseries, however, a simpler process for calculating chill sums or chilling hours is often used. The details are given in the following section.

Measuring dormancy. Because of the tremendous importance of measuring dormancy to nursery management, many attempts have been made to develop a simple way to measure it. As we will now discuss, this objective has been elusive.

Dormancy meters. In the 1970s, researchers observed that changes in electrical resistance of plant tissue provided a useful way to determine whether tissues were injured or dead. Building on these observations, they constructed a "dormancy meter" (fig. 7.2.32) with the objectives of measuring dormancy in fall and telling nursery managers when it was safe to harvest their stock. Unfortunately, subsequent tests showed that these meters were unreliable (Timmis and others 1981). The idea of a simple "black box" quality test is still attractive, but it is doubtful that any equipment or technique will be able to instantaneously measure bud dormancy.

Chilling sums. This is the easiest and most practical method for estimating the intensity of bud dormancy and is based on the chilling requirement just discussed. Chilling sums have immediate application, because they can be used to establish harvesting windows or monitor bud dormancy as it weakens during winter. The concept is logical enough: the cumulative exposure of plants to cold temperatures controls the release of dormancy. So, by measuring the duration of this exposure, it is possible to estimate the intensity of dormancy indirectly.

In actual practice, chilling hours, or degree-hardening-days (DHD), have been used. The process involves meas-

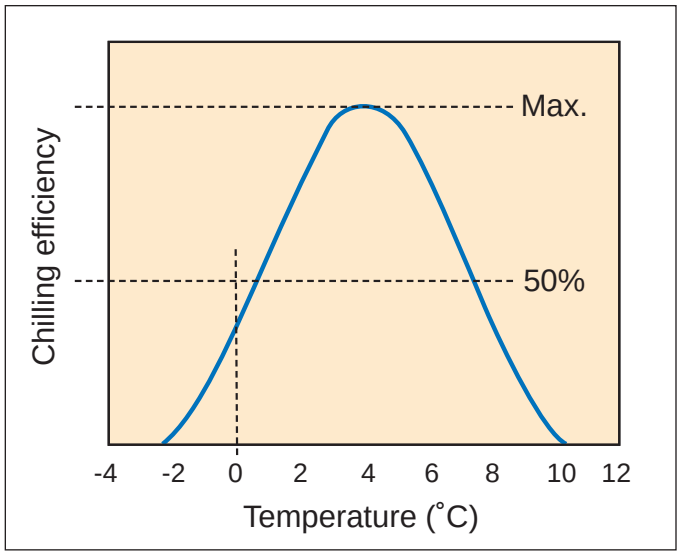


Figure 7.2.31—*Chilling temperatures and their efficiency at breaking bud dormancy (modified from Anderson and Seeley 1993). Note that temperatures in the range of refrigerated storage (–1 to +1 °C [30 to 33 °F]) release dormancy very slowly.*

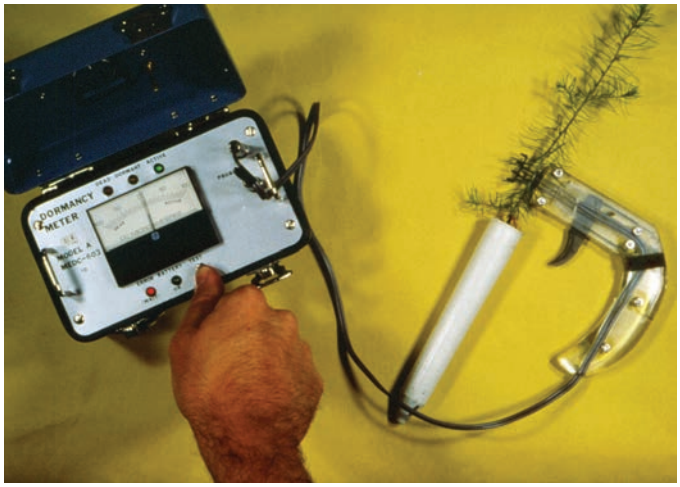


Figure 7.2.32—*The "dormancy meter" was an attempt to find a simple and easy way to measure dormancy and to determine when plants were ready for harvest. Operational testing showed that such devices were unreliable.*

Table 7.2.9—*An example of how to calculate chilling sums using degree days, calculated from an average of daily maximum and minimum temperatures and a 40 °F (4.5 °C) base temperature*

Day	Base temperature (°F)	Daily temperatures (°F)			Degree days	Chilling sum
		Maximum	Minimum	Average		
One	40	40	20	30	10	10
Two	40	45	35	40	0	10
Three	40	50	40	45	0	10
Four	40	40	30	35	5	15

uring the temperature each day and calculating the amount of time below a specific reference temperature. A method sometimes used in forest and conservation nurseries is to simply count the number of hours during which the air temperature is at or below a threshold value, such as 5 °C (41 °F) (Ritchie and others 1985). Reference temperatures will vary with nursery location and species; for example, 8 °C (46 °F) has been used for southern pines (Grossnicle 2008). One shortcut method is to record the daily maximum and minimum temperatures, average them, and subtract this average from the base temperature. Note that, when calculating chilling sums, only negative values are recorded (table 7.2.9).

Bud break test. The more dormant a plant is, the more slowly the terminal buds will resume growth (break) under ideal growing conditions. This phenomenon forms the basis of the only direct way of measuring dormancy intensity—the bud break test. With access to a greenhouse or other growth-promoting structure that can maintain ideal growing conditions through the winter, the intensity of dormancy in nursery stock can be measured by observing days to bud break (DBB) in this "forcing" environment.

The procedure is relatively simple. Grow plants to shippable size and, in the late summer, harden them to the fully dormant condition by exposing them to ambient conditions. By early fall, plants typically have formed a dormant bud and exhibit the other morphological changes, such as leaf color change and abscission in hardwood stock (fig. 7.2.30A) and increased needle waxes on conifer needles (fig. 7.2.30B). Place a temperature recording device at plant height and check temperatures at least weekly to compute chilling sums (table 7.2.9).

Set the environmental controls in the testing greenhouse to maintain spring forcing conditions with warm days, cool nights, and long photoperiods created with photoperiod lights. Then, beginning around Halloween, harvest a sample of plants, pot and label them, and bring them into the forcing greenhouse. Keep the sample plants watered and count the number of days required for the terminal buds to resume growth—this is DBB. Repeat this process at every major holiday: Thanksgiving (late November), Christmas (late December), New Year's Day (early January), Valentine's Day (mid-February), and St. Patrick's Day (mid-March). Starting at the first sample date in September, keep track of the sum of chill hours, all hours when the temperature was, say, 5 °C (41 °F) or lower throughout this test period.

When finished, plot the DBB values over the chilling sums. The number of days required for the terminal buds to break is a direct measure of dormancy intensity. (Note: the Halloween plants may never break bud.) It is likely results will be similar to those shown in figure 7.2.33, which came from coastal Douglas-fir in western Washington and Oregon (Ritchie 1984a) and are in agreement with the general curve proposed by Lavender (1984). As the chilling sum accumulates during winter, the DBB will shorten dramatically. Similar experiments with many tree species, including several hardwoods (birch, dogwood, hawthorn, and oak) have yielded similar results

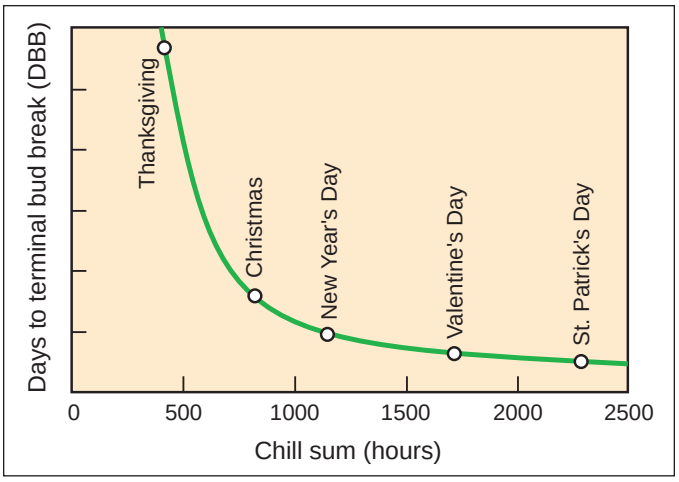


Figure 7.2.33—*The only reliable test for bud dormancy intensity is a bud break test that can be performed by harvesting plants at regular intervals during late fall and winter and bringing them into a greenhouse. As they break bud, the number of days to bud break (DBB) is plotted against the chilling sum for each lift date. The data shown are typical of Douglas-fir nursery seedlings (modified from Ritchie 1984a).*

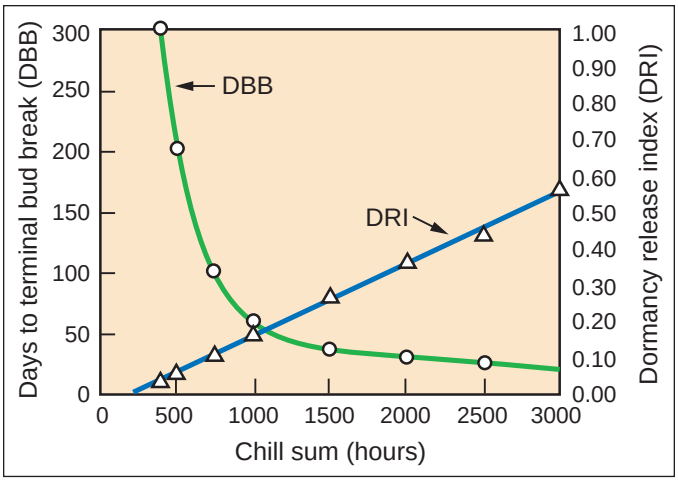


Figure 7.2.34—*Because days to bud break (DBB) over chill sum is a curvilinear relationship, it is useful to convert it to a linear dormancy release index (DRI). In this example, DRI = 10/DBB because Douglas-fir seedlings resumed growth (broke bud) in 10 days when their full chilling requirement was satisfied (modified from Ritchie 1984a).*

(Sorensen 1983, Lindqvist 2000). After this curve has been developed for a nursery, it can be used subsequently to estimate dormancy intensity for a given species and seed zone directly from chilling sums.

From this experiment, it is clear that bud dormancy intensity is very high in fall and drops sharply in early winter, in contrast to the common misconception that deepest dormancy occurs in midwinter when plants are most stress resistent. In addition, this test illustrates that there is no simple "chilling requirement" for any species. Rather, there is a curvilinear relationship between chilling and dormancy in which more chilling will result in more rapid budbreak under forcing conditions. For example, Douglas-fir seedlings with only 800 hours of chilling exposure will eventually break bud, but not nearly as rapidly as those exposed to 2,000 hours of chilling (fig. 7.2.33).

Calculating the dormancy release index. Now that DBB for a given crop can be estimated from chill sums, how is this information used? If DBB were measured on a group of Douglas-fir seedlings that were fully released from dormancy (that is, the chilling requirement was completely fulfilled), the buds would break in about 10 days. Taking this number as the denominator, an index can be calculated that expresses the dormancy intensity on a linear scale:

Dormancy release index (DRI) = 10/DBB

DBB is the days to bud break of a test group of plants as described in the experiment above.

Buds at peak dormancy have a DRI value near zero (for example, DRI = 10/300 = 0.03). As dormancy weakens, DRI approaches 1 (for example, DRI = 10/15 = 0.67). This relationship is shown in figure 7.2.34. DRI is useful because it transforms the curvilinear relationship between dormancy intensity and chilling sum to a more useable linear form. This linear regression can then be used to provide a benchmark and common scale for comparing stock lots in a given plant species.

McKay and Milner (2000) developed a variation on this approach; they estimated DRI by counting the days required for 50 percent of the terminal buds to break in Sitka spruce, Douglas-fir, Japanese larch, and Scots pine. Their results also closely resemble those of figure 7.2.34. The DRI has been particularly useful as an indicator of plant stress resistance—a key performance attribute. We will discuss this relationship and how it is used in Section 7.2.5.2.

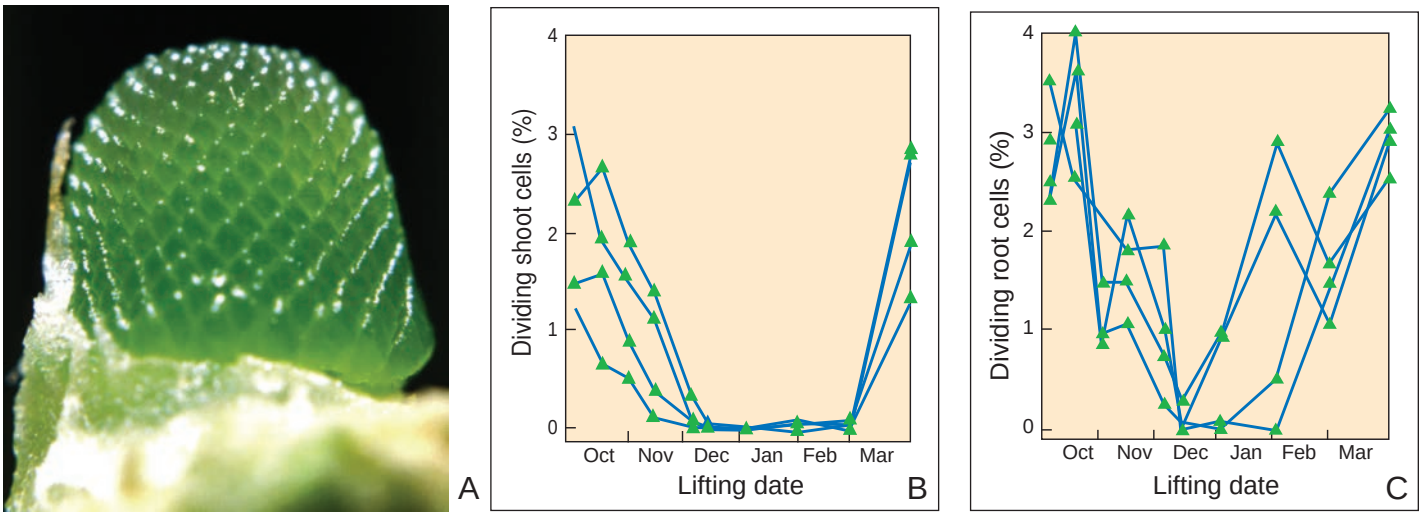


Figure 7.2.35 —*Measuring cell division rates in buds (A) is a laboratory measure of dormancy. Shoot activity over 4 years shows a characteristic pattern of inactivity during winter (B), but roots (C) continue to grow whenever conditions are favorable (modified from O'Reilly and others 1999).*

Measuring mitotic index. In our definition of dormancy, we stressed that dormancy referred only to buds or other plant meristems (fig. 7.2.29). Laboratory techniques have been developed to measure the number of meristematic cells that are dividing at any given time (fig. 7.2.35A). Although primarily used for research purposes, these measurements also illustrate dormancy patterns.

For example, the tips of terminal shoots and long roots of bareroot Douglas-fir seedlings were excised and, after examining meristematic cells with a 400X microscope, a mitotic index was calculated (O'Reilly and others 1999). The results indicate that terminal bud activity shows a definite seasonal pattern; cell division slows gradually in fall and stops completely during winter. With warmer temperatures and longer days in late winter and early spring, cell division begins to increase rapidly (fig. 7.2.35B). This is in direct contrast to the patterns of root meristem activity, showing that roots never become truly dormant but will grow whenever soil temperatures permit (fig. 7.2.35C). Although useful to researchers, this test is too time consuming to be used operationally.

Bud size and development. Although bud size and development are not, in themselves, indicative of the intensity of bud dormancy, they have traditionally been viewed by nursery managers as an indicator of plant quality. For example, a bud length measuring protocol was developed by the Ontario Ministry of Natural Resources as part of their former quality testing service. The process involves cutting buds in half and counting needle primordia. At the end of the hardening phase, low numbers of primordia were interpreted to indicate stressful conditions and increased susceptibility of overwinter damage. Conversely, seedlots having buds with large numbers of needle primordia were rated as being of higher quality (Colombo and others 2001).

Dormancy: Summary. Although the term "dormant plants" is common in nursery jargon, dormancy refers only to meristematic tissues of the shoot: buds and lateral cambium. Bud dormancy has been studied most intensively and is of major interest to plant producers and users.

Forest and conservation nursery crops, like all perennial plants, undergo an annual cycle of activity. In late summer, shortening photoperiods trigger plants to begin the bud dormancy process that culminates in late fall. This condition is known as deep dormancy and can be released by exposure to a period of low temperatures. This process is known as satisfying the chilling requirement, and temperatures in the range of about 3 to 5 °C (37 to 41 °F) are most efficient. By late winter, the chilling requirement has been met and buds will break whenever temperatures permit.

Unfortunately, bud dormancy cannot be quickly or easily measured. The only reliable method is to conduct a bud

break test by bringing samples of plants into a forcing greenhouse at regular intervals throughout winter and recording the days required for the buds to break (DBB). After the relationship between DBB and chilling has been developed for a nursery, it can be used to establish harvesting windows and to estimate the dormancy intensity of crops during subsequent winters.

A useful index of dormancy intensity, the dormancy release index, makes the DBB information more practical by converting the data to a straight line.

Although we lack a rapid test for bud dormancy, it can be estimated from the known relationship between chilling and dormancy intensity as measured by DBB. Nurseries can measure the chilling requirement for their various crops and use this information to monitor the release of bud dormancy.

7.2.5.2 Stress resistance

In the previous section, we indicated that dormancy is closely related to stress resistance (SR). From an operational standpoint, we will introduce some techniques that nursery managers can use to estimate the relative SR of a crop at any point during the harvesting-to-outplanting process.

The concept of stress resistance. Plants are subjected to a variety of stresses (mechanical stresses, root exposure, rough handling, and desiccation, to name just a few) from the time they are harvested in the nursery to when they are outplanted. Nursery managers use a variety of cultural techniques, collectively termed "hardening-off," to prepare their stock to tolerate these stresses. Realizing its importance and practical applications, plant physiologists have been studying SR for almost 40 years.

Hermann (1967) determined that SR was related to root system function in bareroot stock, and Lavender (1984) showed that SR varies seasonally, reaching a midwinter peak after bud dormancy intensity has begun to decline (fig. 7.2.36). The data for this seasonal curve came mainly from outplanting trials, which is why it corresponds exactly with the traditional midwinter lifting season.

Obviously, nursery managers want to maximize SR in their crops and maintain this condition until they are shipped to

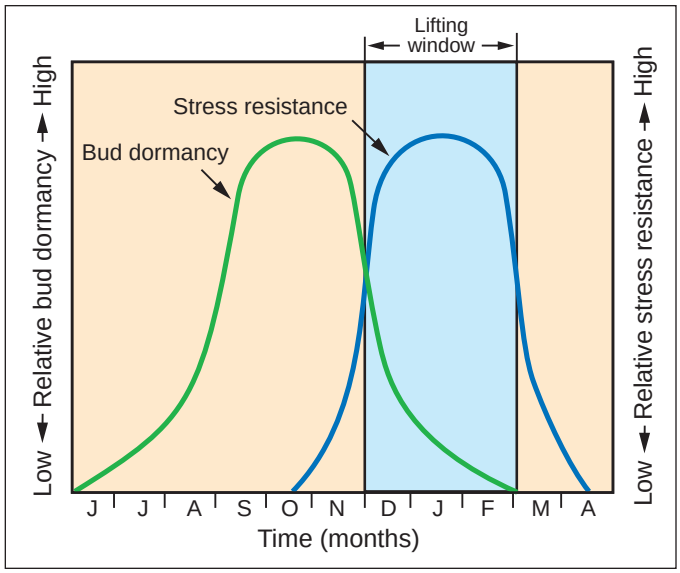


Figure 7.2.36—*This classic illustration shows that bud dormancy and stress resistance follow similar bell-shaped trajectories but occur at different times. Comparison to the traditional midwinter lifting window shows that stress resistance is a better indicator of when to harvest (lifting window) and store nursery crops (modified from Lavender 1984).*

their customers for outplanting or transplanted back into the nursery. But how can they measure or estimate SR, and how can they culture their crops to achieve maximum SR?

Measuring stress resistance. A quick and easy way to measure the SR of nursery stock would be an invaluable tool, and there have been many attempts to develop a test to ascertain this important aspect of quality.

Stress tests. During the 1970s and 1980s, several attempts were made to develop quick tests of SR. For example, a stress test was developed at Oregon State University (McCreary and Duryea 1984) that consisted of harvesting plants, potting them, and exposing them to stressful conditions, mainly high temperature, low humidity, and low soil moisture. After a predetermined time, plants would then be moved into a greenhouse and, after several weeks, be assessed for survival, root growth, bud break, and other indicators of vigor (fig. 7.2.37). Despite some promising early results, the outcomes of literally hundreds of such tests proved difficult to interpret and not very repeatable. Accordingly, this quality test was abandoned.

Figure 7.2.37—*Stress tests involve harvesting seedlings and exposing them to a stressful environment. At Oregon State University, the stress was a dry, hot greenhouse.*

Another more elaborate and more time-consuming, but more accurate, method of measuring SR involves a procedure similar to cold hardiness testing (Ritchie 1986). It consists of three sequential steps:

1. Exposing plants to a controlled stress treatment. The most commonly used stress treatments employ some sort of controlled trauma to root systems. This might involve exposure to high or low temperatures, prolonged drying, or a simulation of rough handling, such as dropping or tumbling.

2. Outplanting stress-treated plants into a natural environment where their growth response to the treatment can be expressed. By "natural," we mean the plants should be growing in soil and exposed to the ambient outdoor environment, but they must be able to express growth potential without confounding effects of browsing, water stress, or weed competition. A bareroot nursery bed that is watered regularly and kept weed free is ideal. The test plants are set out in replicated blocks along with nonstressed controls of similar initial size from the same seedlots or families.

3. Evaluating the impact of the stress treatment by comparing the performance of the stressed plants to that of nonstressed controls after a predefined time period, typically one complete growing season. The assessment can be as simple as measuring shoot growth or as complicated as destructively sampling the entire plant and measuring total biomass. We have found that removing the shoot of the plant and determining its dry weight is a good basis for comparison. In this approach, SR is characterized as the difference in growth between the stressed plants and nonstressed controls. A helpful way of expressing this difference numerically is by calculating a stress injury index (SII) using the first-year shoot growth of the stressed (Gs) and nonstressed control seedlings (Gc):

$$SII = 100 - (Gs/Gc \times 100)$$

The SII expresses the percentage reduction in top growth resulting from stress injury, and so, the lower the value, the higher the stress resistance of the test plants (Ritchie and others 1985).

Using cold hardiness tests to estimate overall stress resistance. Decades of nursery experience have shown that, when plants are at their maximum state of hardiness, they are the most resistant to the many stresses of harvesting, handling, storage, shipping, and outplanting. In fact, recent genetic research has revealed that some of the same (dehydrin) gene complexes that are involved in cold acclimation also play a key role in resistance to water stress (Wheeler and others 2005).

Container nurseries in western Canada use a "storability test" to determine if plants are physiologically ready for harvesting, packaging, and cold storage (Simpson 1990). Essentially, if plants are cold hardy to a threshold temperature of –18 °C (0 °F), then they are ready to withstand the stresses of storage. A more recent modification that uses chlorophyll fluorescence (see Section 7.2.4.4) to determine

Table 7.2.10—*Seedling quality classes based on dormancy release index (DRI) and stress resistance (SR) (modified from Ritchie 1989)*

Quality class	DRI value	Degree of SR
Class 2	< 0.25	Seedlings are below peak SR but are increasing.
Class 1	0.26 to 0.40	Seedlings are at peak SR.
Class 3	> 0.40	Seedlings are beyond peak and SR is decreasing.

if tissue damage has occurred and produces results up to 6 days earlier than visual evaluation (L'Hirondelle and others 2007). Because this method tests plant samples directly, it has proved to be a reliable predictor of outplanting performance (Kooistra 2003). A similar storability test based on FIEL is used in container nurseries in Ontario (Colombo 2009). To use this test in a more temperate or coastal area, a higher temperature threshold would need to be determined.

Using chilling hours to predict stress resistance. It is intuitive that SR is very closely related to dormancy, and this has been verified by plant physiology research (Ritchie 1986, 1989; Ritchie and others 1985). As dormancy intensity weakens through winter in response to chilling, SR gradually increases to a midwinter high. Then it falls rapidly as dormancy is fully released and spring approaches (fig. 7.2.38). The physiological mechanisms behind this relationship are not fully understood, but it is repeatable from year to year with different crop types (bareroot and container) and species (Douglas-fir, pines, spruces, some hardwoods) and across nurseries (Burr and others 1989; Cannell and others 1990; Ritchie and others 1985). This means that if you can track the dormancy status of a crop through winter, this information can be used to estimate SR without measuring it directly.

As discussed in the previous section, bud dormancy peaks in fall and is released gradually during winter as plants are exposed to low temperatures—the "chilling

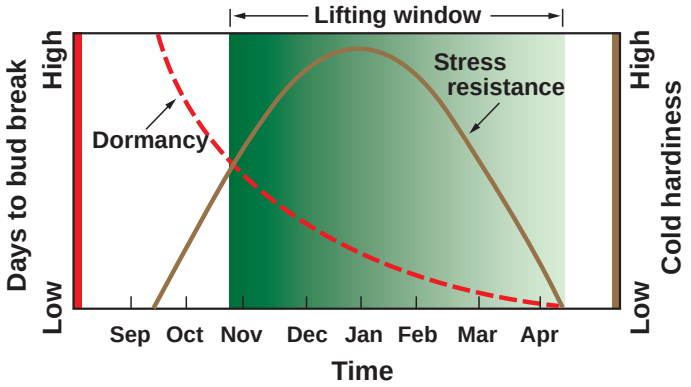


Figure 7.2.38—*Both bud dormancy, as measured as days to bud break (DBB), and stress resistance, as measured by cold hardiness tests, can be used to determine the best time to harvest nursery stock (lifting window). Cold hardiness tests, however, are so much quicker and easier that they have become the standard test for lifting and subsequent refrigerated storage.*

requirement." Transforming this curvilinear relationship into a linear dormancy release index (DRI) makes it much easier to use. The DRI is 0 at peak dormancy in fall, and approaches 1 as dormancy is released in spring.

Research with Douglas-fir has revealed a consistent relationship between DRI and SR (Ritchie 1986). In early winter, when DRI is in the range between 0 and about 0.25, SR is low but increasing. Between DRI 0.26 and 0.40 (midwinter), SR reaches a seasonal high, but when DRI exceeds 0.40 (early spring), SR declines and plants become very susceptible to damage. These results lead to the definition of three seedling quality classes based on dormancy intensity and SR (table 7.2.10).

After the relationship between chilling and DRI has been established for a given species in a given nursery, it can be used to estimate SR at any point during the winter for subsequent crops at that nursery. Let us say, for example, that it is late December and your nursery chilling sum is about 1,000 hours. Using figure 7.2.39, you would estimate that DRI was approaching 0.2. From table 7.2.10, we see that stock at this time is in SR Class 2—not yet peaked, but will improve with more chilling. Now, let's say it is February and you have about 2,000 hours of chilling at your nursery. DRI is about 0.38, indicating that SR is in the seasonal high range but will soon begin to decline.

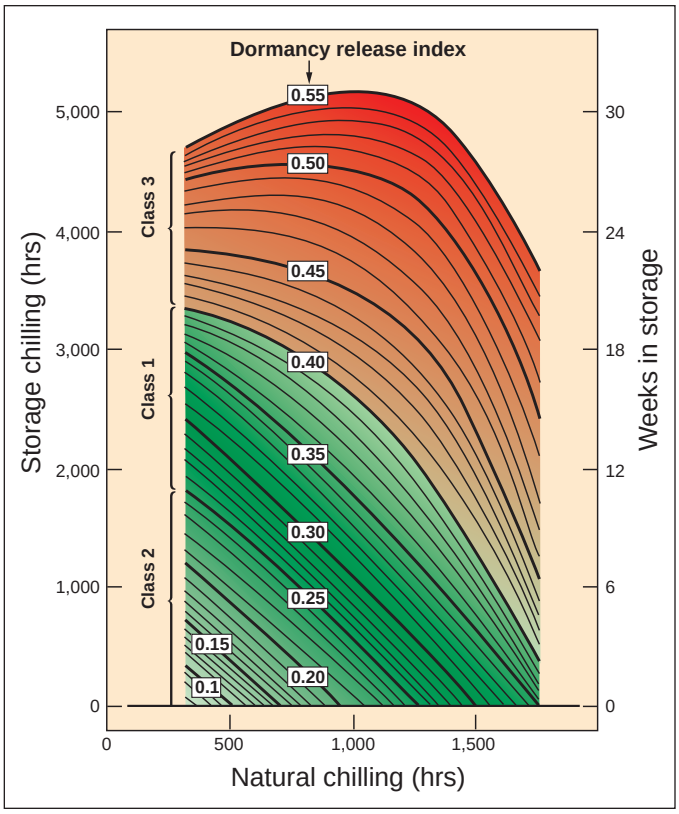


Figure 7.2.39—*Graph showing how the chill sum at time of lifting, combined with time in cooler or freezer storage, can be used to predict the dormancy release index (DRI) and stress resistance class (table 7.2.10), of planting stock. The graph is entered on the X-axis at the nursery chill sum at which seedlings were placed into storage. The storage duration is found on the Y-axis. These lines intersect at the DRI value of the seedlings at that time. Their quality class can then be read from the Y-axis (modified from Ritchie 1989).*

Adjusting for the added effect of refrigerated storage. For crops transplanted or outplanted without cooler or freezer storage ("hot-planted"), DRI is very useful. You simply look at the chilling sum at any point and, from it, estimate stress resistance. But many nursery crops are refrigerated from a few weeks to several months before transplanting or outplanting. So, how does that affect SR?

The low temperatures in refrigerated storage are within the chilling range; hence, they contribute to dormancy release. They do so inefficiently, however, because storage temperatures are below the optimum chilling temperature (Ritchie 1984a, van den Driessche 1977). Therefore, *refrigerated storage has the effect of slowing the release of dormancy.* This means that plants harvested and placed into refrigerated storage will pass through SR Classes 2, 1, and 3 more slowly than they would if left in open container storage (see Chapter 7.4). Plants that are kept in freezer storage accumulate very little chilling because temperatures are well below optimum. These plants must have already accumulated an adequate level of chilling prior to being placed in storage.

To use the graph, select total ambient chilling hours from a nursery on the X-axis. For this example, let us use 1,000 hours. At this point, the stock will have a DRI value of about 0.20, placing it in Quality Class 2 (table 7.2.10). Now, if the plants are held in refrigerated storage for about 4 weeks, they will enter Class 1 and have even higher SR. However, if these same plants had been held in the nursery for a few more weeks until they accumulated over 1,300 hours of chilling, they would exceed the DRI limit of 0.25 and enter Class 1 and have maximum SR. Then, if they were placed in freezer storage, they could be held for at least 15 weeks (right axis) before their DRI approached 0.40 and their quality dropped to Class 3. (Note: as a rule of thumb, cooler storage should not exceed 6 weeks. If storage longer than 6 weeks is needed freezer storage should be used—see Chapter 7.3.)

On a practical basis, figure 7.2.39 integrates the effect of both harvesting date and storage duration on DRI and, hence, stress resistance. If the chill sum at the time of harvesting is known, then storage duration can be planned to deliver stock when it is at maximum SR: Class 1. If the planned outplanting date is known, then lift date and time in storage can be prearranged to deliver stock

to the outplanting site so it will be in Class 1. This graph illustrates the very important point that, for outplanting sites that cannot be accessed until late, early winter lifting with overwinter freezer storage is preferable to late spring lifting with or without storage.

Application to other species and regions. The data that were used to produce figure 7.2.39 came from coastal Douglas-fir seedlings from four different seedlots (high and low elevation lots in both Washington and Oregon) that were grown in two different coastal nurseries (Washington and Oregon). These results have been operationally tested with Douglas-fir crops from other seedlots and during other growing seasons with consistent results. Therefore, for West Coast nurseries raising Douglas-fir, figure 7.2.39 is a very handy way of estimating SR from chilling hours.

For interior or northern nurseries, however, the relationship between chilling and DRI may be quite different. This was tested in an interior west Canadian nursery with lodgepole pine and interior spruce (Ritchie and others 1985). The results showed that chilling began to accumulate earlier in fall and that more chilling accumulated throughout winter. The results also suggested that these species may require more chilling hours for full dormancy release than coastal Douglas-fir, similar to results with ponderosa pine (*Pinus ponderosa*) (Wenny and others 2002). Nevertheless, the overall relationships (if not the same numbers) shown in figure 7.2.39 were similar to what has been found with Douglas-fir. Therefore, to accurately predict SR from chilling hours for other species and nurseries, a chilling-DRI "calibration curve" needs to be developed.

Stress resistance: Summary. Stress resistance (SR) is an important, but elusive, performance attribute that describes a plant's ability to tolerate the stresses associated with harvesting, handling, storing, and outplanting. SR varies seasonally; it is low in fall, high in midwinter, and low in spring.

SR is very laborious to measure, so no operational test is currently being used. However, because the seasonal pattern of SR closely coincides with the pattern of cold hardiness, standard cold hardiness tests can provide quick and useful estimates of SR.

Studies have shown that SR is related to dormancy intensity expressed as a dormancy release index (DRI). When DRI is in a range between 0 and about 0.25, SR is low but improving. Between DRI 0.25 and 0.40, SR is at a seasonal high. Above DRI 0.40, SR is declining. Most important, this relationship tends to be consistent whether or not plants have been stored.

Because cooler and freezer storage slows the release of dormancy, storage prolongs the period of high SR. These relationships can be used to schedule harvesting and storage in order to deliver stock to the planting site that has very high resistance to stress. Although most of this research was done with bareroot stock of commercial conifers, the basic principles should apply to container plants of other species.

7.2.5.3 Root growth potential

Although Wakeley (1954) published the first account of the relationship between new root growth and plant quality, it was Stone (1955) who, after experimentation, coined the term "root regenerating potential" to describe his new indicator of seedling physiological quality.

Basing their effort on Stone's original research, other workers began developing and using this method of plant assessment (for example, Burdett 1979; Jenkinson 1975). A comprehensive review of root growth potential (RGP) by Ritchie and Dunlap (1980) was responsible for a flurry of new research and adoption of RGP as the first performance quality test used operationally in forest nurseries. Because of this wide interest, a chapter on Assessing Seedling Quality in the *Forest Nursery Manual* (Duryea and Landis 1984) featured a discussion and strong endorsement of RGP (Ritchie 1984b). Further reviews (Duryea 1985; Ritchie 1985; Ritchie and Tanaka 1990) made this test the most popular and widely used quality test (fig. 7.2.40A). RGP tests have been employed worldwide and have been the subject of much discussion (Binder and others 1988; Landis and Skagel 1988; Sutton 1983) and even debate (Simpson and Ritchie 1997).

RGP test procedure. The RGP test consists of placing a random sample of plants into an environment that promotes rapid root growth. After 7 to 28 days, the plants are evaluated for new root growth. In the following section, we examine each step in the process.

Sampling. As with all tests, if sampling is biased (not random), test results will be meaningless. The number of plants used in a typical RGP test is quite small and should be randomly selected from the population at large in order to be as representative as possible. A sample of 60 seedlings, which is the number usually required by testing laboratories, is only 0.12 percent of a moderately sized seedlot of 50,000 seedlings. A 25- to 30-plant sample would be a minimum number to evaluate.

It is simple in principle to collect a random sample when plants are still in containers or on the grading table, but sampling becomes more difficult after stock has been packaged and stored. When cooler stored, it is operationally difficult to sample from bagged plants, because a number of bags must be accessed, opened, and the sample collected from throughout the bag, not just from the top layer of plants. Sampling during freezer storage requires special packaging (Landis and Skagel 1988).

Time of sample collection. Tests performed on plants at the time of harvesting are useful to evaluate nursery cultural practices but may not reflect the condition of the plant at time of outplanting. If you are interested in outplanting performance, then the best time to sample is as close to the time of outplanting as operationally possible (Landis and Skagel 1988).

Test environment. The testing environment is particularly important because it must provide conditions that are near "optimum" for root growth (Landis and Skagel 1988). The temperature should be 19 to 25 °C (66 to 77 °F). The rooting medium should be well aerated and watered, and there should be adequate light and long days. Because these factors will affect test results, it is important to maintain consistent conditions across tests, although this can be difficult.

Three types of test environments have been used:

Pots in greenhouse—Most quality-testing facilities use this method, in which plants are potted in 3.8 to 7.6 liter (1 to 2 gal) containers filled with a well-drained artificial growing medium. The pots are kept well irrigated in a greenhouse (fig. 7.2.40B) for the duration of the testing period (Ritchie 1985; Tanaka and others 1997). After 7 to 28 days, the growing medium is washed from the roots (fig. 7.2.40C) and the amount of new root growth is rated.

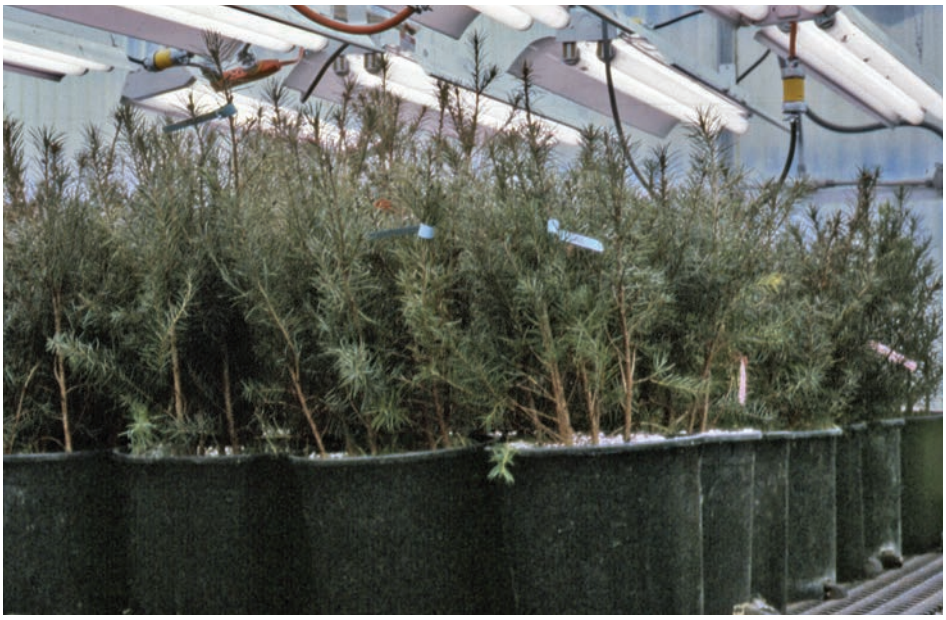
Hydroponic—Plants are suspended with their roots in warm, aerated water, such as in an aquarium. This method has found use with several deciduous hardwood species (Wilson and Jacobs 2006).

Aeroponic—Plants are suspended in a closed chamber while warm water mists the roots (fig. 7.2.40D). Forest Service nurseries have used this technique with good results (Rietveld and Tinus 1990). One benefit is that the rack of plants can be easily removed from the misting chamber to monitor root development during the test period (fig. 7.2.40E).

Evaluation. After the test is completed, new root growth must be quantified. Researchers have attempted to shortcut this tedious process using photography, dyes, root volume measurements, and other approaches. Despite this, the tried-and-true "root count" technique has prevailed. This involves visually estimating the number of new roots greater than 1 cm (0.4 in) long on the plant. An experienced technician can do this in a few minutes. This count can be reported as a raw number (for example,120 roots per plant) or transformed into an index such as reported by Burdett (1979) and modified by Tanaka and others (1997) (table 7.2.11). Root numbers and total root length are usually well correlated.

RGP as a predictor of ouplanting performance. Interpretation of the results of RGP tests remains challenging. A common misconception has been to assume that RGP results directly predict outplanting performance. In other words, high RGP always ensures high survival, while low RGP always ensures low survival (fig. 7.2.41A). At best, RGP is positively correlated with survival only about 75 percent of the time (Ritchie and Dunlap 1980, Ritchie and Tanaka 1990). Sometimes these correlations are weak, sometimes strong. Binder and others (1988) found no correlation between RGP and outplanting mortality in 8,600 operational trials in British Columbia. This is because the outplanting environment (which is usually very different from the RGP testing environment) has an overriding influence on performance (Binder and others 1988; Landis and Skagel 1988; Simpson and Ritchie 1997; Sutton 1983). Performance of low-RGP stock on harsh sites and of high-RGP stock on mild sites is usually predictable. However, performance of low-RGP stock on mild sites and high-RGP stock on harsh sites is not (fig. 7.2.41B).

A



B

C

E

D

Figure 7.2.40—*Because the relationship between new roots and outplanting success is intuitively important (A), the root growth potential test quickly became the most popular and widely used assessment of plant quality. One testing procedure involves growing test plants in pots in a greenhouse (B), washing roots (C), and then rating the amount of new root growth. In the second procedure, test plants are supported in a mist chamber (D) and then measured for the length and number of new roots (E).*

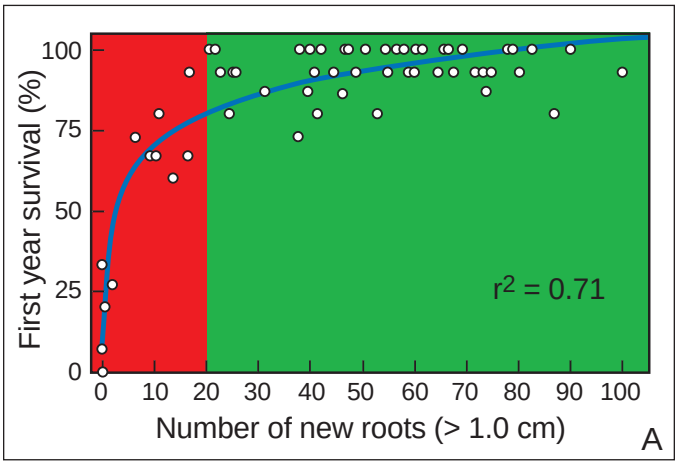
Table 7.2.11—*Root growth index (RGI) scale developed by Tanaka and others (1997) to quantify root growth following a root growth potential (RGP) test*

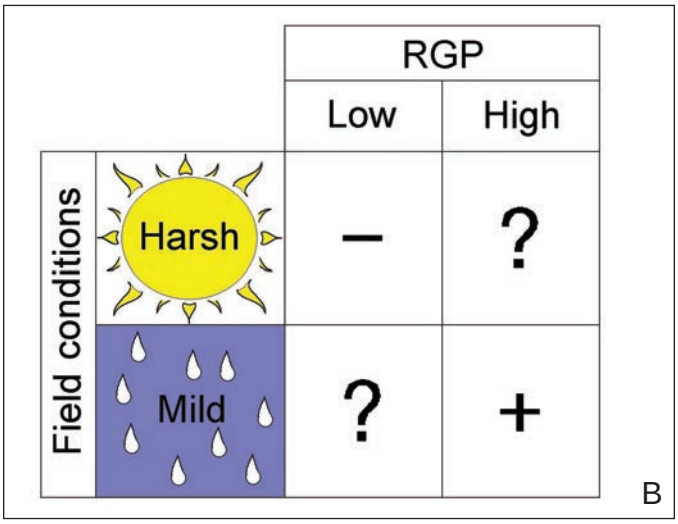
Root growth index (RGI)	Number of new roots 1 cm or longer
0	None
1	Some roots but none > 1 cm
2	1–3
3	4–10
4	11–30
5	31–100
6	101–300
7	More than 300

It seems intuitive that for a newly outplanted plant to survive and grow, it must rapidly regenerate new roots in order to maintain an adequate water balance. This logic has been used to explain why RGP can be expected to predict survival. Simpson and Ritchie (1997), however, point out that newly planted stock is almost never able to grow roots after outplanting because, although soil moisture may be high, soil temperature during the winter or early spring planting season in most places is far below the threshold temperature for root growth (fig. 7.2.41C). Under these conditions, the existing root system is adequate to supply water to the plant until the soil warms and roots begin to grow (McKay 1998). Therefore, whether or not new root growth occurs immediately after planting is of little consequence to field performance.

Why RGP often works. The discovery that many conifer seedlings, especially Douglas-fir, require mainly current photosynthate for new root growth (van den Driessche 1987, 1991) has provided a rationale for interpreting RGP test results. For a plant to grow new roots in the test environment, the foliage must be photosynthesizing (fig. 7.2.42). Therefore, the stomata must be open, the leaves must be healthy, and the photosynthetic apparatus must be functioning properly. Photosynthate must move to the root system, so the phloem pathway to the roots must be intact, and the roots themselves must be metabolizing normally. If any of these systems have been compromised by, say, cold damage, water stress, disease, photodamage, or other agents, a depression of RGP will result.

Taken in that light, then, a more realistic view is that RGP testing is analogous to seed testing, which provides a snapshot of seed viability at the time seeds are tested. No one would expect seeds that had 95 percent laboratory germination to always give 95 percent emergence in the nursery. But if the test gave an abnormally low value, it would indicate poor seed viability. This is the model to use when interpreting RGP test results. The RGP test is a "red flag" test that identifies stock lots that, for whatever reason, are not up to par.





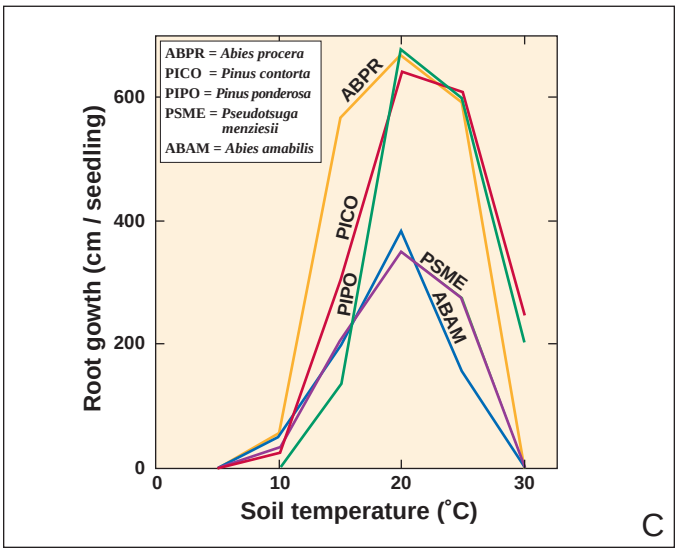


Figure 7.2.41—*Although a good relationship between root growth potential (RGP) test values and outplanting success sometimes exists (A), limiting factors on the outplanting site often prevents good predictability. Performance of low-RGP stock planted on a harsh site or high-RGP stock on a mild site is generally predictable. However, performance of high-RGP stock on a harsh site, or low-RGP stock on a mild site is not (B). One frequent problem is that soil temperatures on the outplanting sites are much lower than the ideal temperatures used in the testing environments (C) (A, modified from Grossnickle 2000; C, modified from Lopushinsky and Max 1990).*

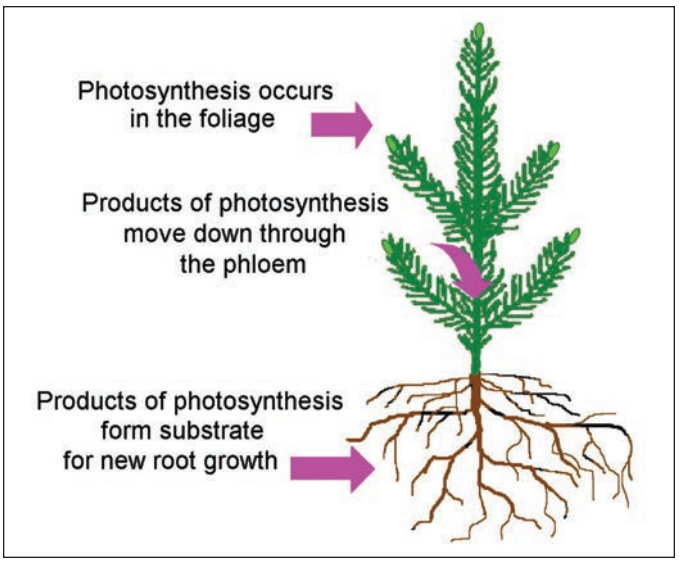


Figure 7.2.42—*Root growth in many conifers depends on a supply of current photosynthate from the shoot (van den Driessche 1987, 1991). Any factors that depress photosynthesis or impede the flow of photosynthate from leaves to roots will result in reduced root growth potential.*

Root growth potential: Summary. RGP remains the most popular quality test because it is intuitive, robust, and simple. Like any test, however, RGP has its limitations. The major drawback of the RGP test is the long testing period and the limited predictive ability. RGP tests provide only a "snapshot in time," because plant physiological quality can change right up until the stock is outplanted.

RGP sometimes predicts survival and other times does not. This is because site conditions, which are very different from the testing conditions, can override stock quality. RGP does not predict root growth after outplanting, and root growth after outplanting generally has little to do with survival.

The RGP test is a valuable test of viability—that is, it determines whether plants are alive and functional at the time the test is conducted. RGP test results integrate many physiological systems in plants, such as stomatal function, the photosynthetic mechanism, phloem integrity, root viability, seedling nutrition, and so on. If any of these systems have been compromised, it will show up as a depression of RGP.

Regardless of their predictive value, RGP tests have been done long enough to show that nursery stock with high RGP values will have great survival and growth (Maki and Colombo 2001). Results of an RGP test should be interpreted in the same way as results of a seed germination test. It is a "red flag" test that identifies sub-par lots and may or may not predict field performance.

7.2.6 Correlating Combinations of Plant Quality Tests To Predict Outplanting Performance

As you should have deduced by now, nursery plant quality is a complicated subject. So, instead of trying to predict outplanting performance with just one variable, it makes sense to attempt correlations with two or more plant quality indices. Research to develop a comprehensive approach that uses a battery of tests has been done (Grossnickle and others 1991) but has not been adopted operationally. Recent research in British Columbia measured root growth potential, chlorophyll fluorescence, and stomatal conductance of conifer seedlings and then correlated them singly and in combination with survival and growth after outplanting (L'Hirondelle and others 2007). They found that, while survival was highly correlated with root growth potential ($R^2 = 0.72$), the combination of root growth potential and chlorophyll fluorescence was a good predictor of survival plus shoot growth as measured by dry weight (fig. 7.2.43). We hope more research will be done in this area to further refine our ability to mathematically predict nursery stock quality.

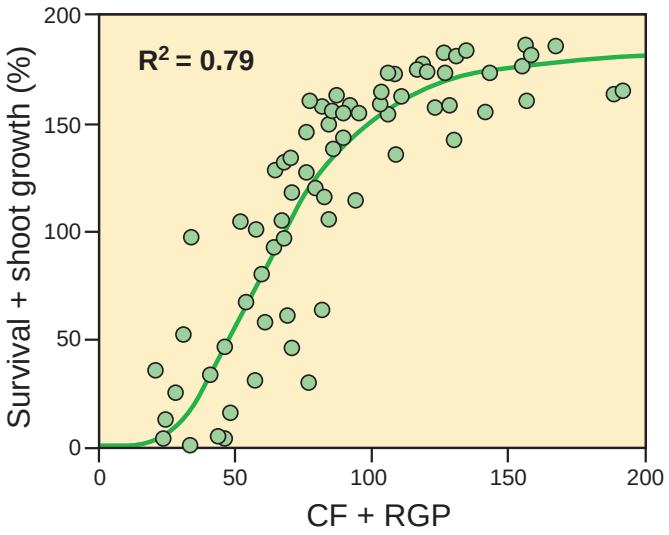


Figure 7.2.43—*Measuring root growth potential and chlorophyll fluorescence proved to be a good predictor of total outplanting performance (survival + shoot growth) of conifer seedlings (modified from L'Hirondelle and others 2007).*

7.2.7 Limitations of Plant Quality Tests

7.2.7.1 Timing

Each plant quality test we have discussed should be done at a particular time in the nursery-through-outplanting cycle. Morphological attributes change as the crops grow in the nursery but remain constant after harvesting. Physiological and performance attributes, however, vary considerably depending on when the measurements are taken. For instance, plant moisture stress has a pronounced diurnal pattern whereas cold hardiness increases during the fall and can be lost during refrigerated storage (Sundheim and Kohmann 2001). Root electrolyte leakage and chlorophyll fluorescence (CF) are used mainly to detect damage following a stress event. Therefore, they should be measured immediately after the event, while keeping two important considerations in mind. First, to know whether test results are "normal," baseline information on these variables must be available. That often calls for routine monitoring of these variables in healthy crops prior to the stress event. The second, and very important, point is that plants may require time to exhibit stress symptoms and also have the ability to recover from stress. So, for example, CF values measured the day after a cold event may not give an accurate picture of the damage sustained by the crop or of its longer-term response.

Both nursery managers and seedling users can use plant quality tests but would do so at different times. For example, a nursery manager would use plant moisture stress to schedule irrigation and cold hardiness tests to determine lifting windows and storability, whereas a seedling user might use plant moisture stress to ensure that nursery stock was not moisture-stressed prior to outplanting and cold hardiness tests to indicate overall stress resistance before outplanting (fig. 7.2.44).

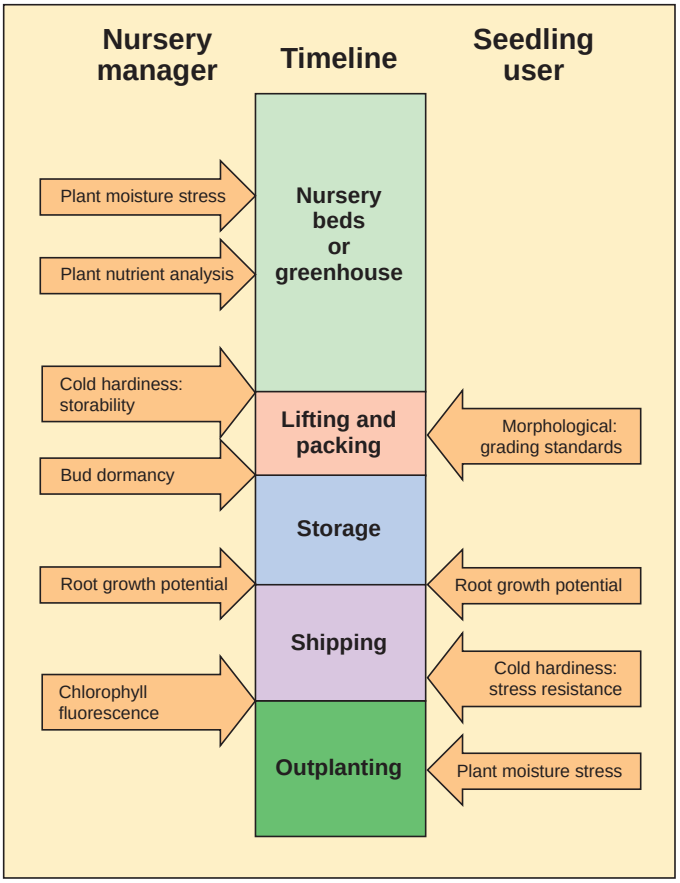


Figure 7.2.44—*Plant quality testing can be done by both nursery managers and seedling users. The timing of the various tests will vary with the desired interpretation.*

7.2.7.2 Sampling

Proper sampling is critical to effective seedling quality testing. If the sample is biased, the test results will be biased and therefore worthless. One wonders how many of the quality tests that failed to predict field performance were conducted on samples that did not adequately represent the populations from which they were drawn. It is important to follow the "three Rs" of sampling: random, replicated, and representative. Multiple samples collected randomly from throughout a given crop will yield the most useful data. Many growers are resistant to spending the time or money to collect and test samples in this manner. If you think about it, however, spending a relatively small amount of time and money on a single, biased test is simply wasted time and money to generate meaningless data, whereas spending a little more time and money using a three R sampling protocol produces valuable data which can assist with management decisions.

7.2.7.3 Unreasonable expectations

It is important that plant producers and users employ the right test at the right time and that they remain aware of the pitfalls of reading too much into test results. A discussion of this topic can be found in Simpson and Ritchie (1997) who propose the following conceptual model of field performance:

Field performance = f(SC, PM, SR, PV)

where:

SC = site conditions (all physical, chemical, and biological characteristics of the site during and after planting),

PM = plant morphological attributes (stem diameter and height, shoot-to-root ratio, root quality, and so on),

SR = stress resistance (ability to withstand stresses associated with harvesting, storage, handling, and planting), and

PV = plant viability (freedom from disease, injury, or stress-induced disorders); plant "functional integrity" (Grossnickle and Folk 1993) is a good way to express this idea.

Obviously, quality testing does not provide information on SC, but it can yield detailed information on PM and can offer insights on SR through monitoring of cold hardiness and dormancy intensity. PV can also be approximated using root growth potential, chlorophyll fluorescence, root electrolyte leakage, and, to some extent, plant moisture stress.

With this "package" of available quality tests and protocols, nursery managers have sufficient tools to make more than an educated guess about the quality of any given stock lot at any given time. But, it should be remembered that quality must be viewed within the context of site conditions that can never be fully predicted.

7.2.8 Commercial Plant Quality Testing Laboratories

Several of the tests enumerated above can be administered on the nursery site (for example, root electrolyte leakage, root growth potential, chill sum accumulation). Certain tests (for example, cold hardiness and chlorophyll fluorescence), however, require elaborate and expensive equipment. Seedling quality laboratories typically use equipment such as growth chambers, which generate more uniform, replicable test conditions. Using a testing service has the added benefit of providing an independent assessment of seedling quality. Over time, these assessments can be organized in a database to reveal patterns that might not otherwise be apparent (Colombo 2009).

At the time of this writing (2009), we are aware of four laboratories in North America that provide quality testing services. They are listed in appendix 7.2.1.

7.2.9 Summary and Conclusions

Plant quality is divided into three broad categories of attributes: morphological, physiological, and performance. Morphological attributes are easy to see and measure and do not change readily after plants are harvested and stored. Container size and plant density have the most pronounced effects on morphology. Although many characteristics may be measured (for example, shoot height, stem diameter, biomass) and ratios of those characteristics can be calculated (for example shoot-to-root ratio), shoot height and stem diameter are the most frequently measured morphological traits and the most commonly used grading criteria. Initial shoot height tends to be correlated with height growth after outplanting, whereas initial stem diameter is better correlated with survival.

Physiological attributes are not readily visible and require specialized equipment and testing to ascertain. Evaluations of plant moisture stress, cold hardiness, root electrolyte leakage, and chlorophyll fluorescence are most common.

Plants lose water more rapidly through transpiration than they can absorb from the soil, putting the plants under "plant moisture stress" (PMS). This level of stress can be quantified by using a pressure chamber. Although a direct correlation between PMS and any of the classical plant quality indicators is lacking, nursery managers can use pre-dawn PMS measurements to schedule irrigation and to monitor stress during hardening, harvesting, and outplanting.

Development of cold hardiness in nursery stock is triggered by changes in photoperiod in late summer and increases rapidly in late fall and early winter as plants experience lower temperatures. For temperate zone plants, peak hardiness occurs in January and is quickly lost as photoperiods lengthen and temperatures increase. Different plant parts may have different cold hardiness levels; buds are generally most cold hardy while roots are the least. Cold hardiness levels can be determined using a whole plant freeze test, freeze-induced electrolyte leakage (FIEL), or analysis of genetic indicators. Results from testing can be used by nursery managers to decide safe windows for harvesting, to provide necessary frost protection, and as a surrogate for estimating overall stress resistance.

Assessing root electrolyte leakage (REL) is similar to FIEL, but it is broader because this test looks at potential loss of root viability from many factors, such as disease, rough handling, and desiccation, and not just at damage from cold temperatures. It is difficult to correlate REL with plant survival because many factors other than root damage can affect REL.

Chlorophyll fluorescence provides a means of determining a plant's ability to efficiently photosynthesize. Stresses, whether they are short term, subtle, long term, or severe, can impair this important physiological process. This measurement can identify when significant damage to the photosynthetic system has occurred, indicating a plant's performance may be compromised. More work is needed before this test will be an operational quality test.

Performance attributes integrate both morphological and physiological attributes. Although testing performance attributes has great value, these tests can be laborious and expensive. Measures of dormancy, stress resistance, and root growth potential (RGP) are the common tests.

Although nursery managers talk about dormant plants, dormancy only refers to meristematic tissues, and only bud dormancy has been extensively studied. Shoots may cease to elongate and form buds in response to changing environmental conditions that are less favorable to growth (quiescence), or in response to reduced photoperiod (deep dormancy) that culminates in fall. Once deeply dormant, buds require a specific duration of exposure to cold temperatures (chilling) before shoot growth will resume. The chilling requirement is the length of exposure to cold temperatures that buds need before they are once again quiescent and ready to resume growth when temperatures permit. The only reliable way to estimate the intensity of bud dormancy is to measure how much chilling buds have been exposed to, and then record how many days are required for those buds to resume shoot growth (days to bud break—DBB) when they are returned to favorable growing conditions. The relationship between chilling and DBB is curvilinear, but a simple dormancy release index (DRI) can be used to convert the data to a straight line and make it easier to use; for example, in establishing harvesting windows and estimating the dormancy intensity of crops during subsequent winters.

Measuring stress resistance (SR) can be a very laborious process, but an important one because it describes a plant's ability to tolerate the stresses associated with the harvesting-to-outplanting process. Because the seasonal pattern of SR closely coincides with the pattern of cold hardiness, standard cold hardiness tests can provide quick and useful estimates of SR. Moreover, SR is related to dormancy intensity expressed as a dormancy release index. Because refrigerated storage slows the release of bud dormancy, storage prolongs the period of high SR.

Root growth potential (RGP) is the most popular performance test. This test provides an indication of the overall viability of the plant at the time of testing because many integrated physiological processes in plants are responsible for new root production. This test provides only a snapshot-in-time evaluation of the plant; it is important to remember that physiological quality can change right up until the stock is outplanted. RGP may or may not be well correlated with survival because site conditions can override stock quality, but plants having low RGP should be further evaluated with respect to potential site conditions.

In general, morphological attributes, because they seldom change during the harvest-to-outplanting process, may be measured any time. Physiological attributes, because they can change frequently, however, provide only a momentary analysis of plant quality. Testing plant moisture stress at different stages of the harvest-to-outplanting process can ensure that plant stress is minimized. Chlorophyll fluorescence and root electrolyte leakage tests may be used immediately after an unexpected stress event to ascertain damage levels or recovery from those events. Cold hardiness testing can be done to determine proper harvesting windows and prior to outplanting to ensure that stress resistance is still high. Performance attributes such as stress resistance may be done anytime during the harvesting-to-outplanting process, but root growth potential is probably best done immediately prior to outplanting to ensure overall plant viability.

None of these plant quality tests will yield meaningful results unless the population of plants is sampled randomly and thoroughly. Plant producers and users must be cognizant of what each test does and does not infer about plant quality and must be mindful that test results must be considered within the context of expected, but never fully predicted, site conditions.

7.2.10 Literature Cited

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7.2.11 Appendix

Appendix 7.2.1—*Seedling quality testing facilities and their procedures*

Company	Address	Types of tests offered			
		Morphology	Root growth potential	Cold hardiness	Others
Nursery Technology Cooperative	Oregon State University Dept. of Forest Science Richardson Hall 321 Corvallis, OR 97331 Tel: 541-737-6576 Fax: 541-737-1393 http://ntc.forestry.oregonstate.edu/sqes	X		X	
KBM Forestry Consultants	SQA Coordinator 349 Mooney Avenue Thunder Bay, ON P7B 5L5 Tel: 807-345-5445 ext. 34 Fax: 807-345-3440 E-mail: sgellert@kbm.on.ca	X	X	X	X
Laboratory for Forest Soils and Environmental Quality	Tweeddale Centre for Industrial Forest Research 1350 Regent Street Fredericton, NB E3C 2G6 Tel: 506-458-7817 Fax: 506-453-3574 E-mail: jestey@unb.ca	X	X	X	X
Franklin H. Pitkin Nursery	Center for Forest Nursery and Seedling Research College of Natural Resources University of Idaho Moscow, ID 83844–1137 Tel: 208-885-7023 Fax: 208-885-6226 E-mail: seedlings@uidaho.edu	X	X	X	X