Chapter 23
Assessing Seedling Quality

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Abstract

Characteristics of planting stock which reflect quality (defined here as performance potential) are categorized as either "performance" attributes or "material" attributes. Performance attributes, such as root-growth potential, cold hardiness, and stress resistance, are assessed by subjecting whole seedlings to certain environmental regimes and evaluating their response. Because performance attributes are integrators of all or many seedling subsystems, they often correlate well with seedling performance potential; however, they tend to require laborious and time-consuming procedures. Material attributes, such as dormancy status, water relations, nutrition, and morphology, are assessed by measuring the attribute in question by any number of direct or indirect methods. Although material attributes are often more easily and rapidly measured than performance attributes, the former generally yield little definitive information on seedling quality unless values fall well outside of some established range. Of the Northwest nurseries responding to the OSU Nursery Survey, many reported using various methods to assess seedling conditions. However, most methods were used to indicate the desirability of carrying out certain cultural operations, such as irrigation or lifting, rather than to measure seedling quality itself.

23.1 Introduction

The final test of a forest-tree seedling is its performance after outplanting. Every observer of plantation establishment is aware that survival and adequate early growth of planted seedlings cannot be taken for granted. Some seedlings survive and prosper even on difficult sites, whereas others die soon after planting or remain in check for several years. These differences in performance reflect differences in factors which collectively make up what is known as "seedling quality." As defined at the New Zealand IUFRO workshop, "Techniques for Evaluating Planting Stock Quality" (August 1979), the quality of planting stock is the degree to which it realizes the objectives of management—"Quality is fitness for purpose." If the purpose of planting stock is to become established and grow successfully in a plantation, then fitness is a function of survival and growth potential. Seedling quality, then, is defined in these terms in this chapter.

Seedling quality is prerequisite to intensive forest practice because upon it depends the initial architecture of the forest. Hence, it has been the subject of much research and several recent reviews. Bunting [7] discussed morphological and physiological aspects of seedling quality. Jaramillo [53] evaluated several electrical and chemical indicators of planting-stock condition. Chavasse [15] reviewed cultural techniques for maintaining seedling quality with emphasis on New Zealand production systems and species. Schmidt-Vogt [97] reviewed much of the European work, and Cleary et al. [18] gave a brief overview pertinent to Northwest nurseries. A special issue of the *New Zealand Journal of Forestry Science* (vol. 10, no. 1) is dedicated entirely to the subject of planting-stock quality. Finally, Sutton

In Duryea. Mary L., and Thomas D. Landis (eds.). 1984. Forest Nursery Manual: Production of Bareroot Seedlings. Martinus Nijhoff/Dr W. Junk Publishers. The Hague/Boston/Lancaster, for Forest Research Laboratory, Oregon State University. Corvallis. 386 p.

[114] presented an especially thoughtful, yet concise, synthesis of the subject. All of the above make excellent reading.

Seedling quality reflects the integration of a multitude of physiological and morphological characteristics of the seedling—much as human health reflects a vast array of human physiological and morphological properties—and an instructive analogy can be drawn here. When examined by a physician, the patient is subjected to a battery of measurements—some simple and others highly sophisticated. It is from the collective results of these tests, not just one test alone, that the physician is able to characterize the patient's general health. As there is no one index of human health, there is no one yardstick of seedling quality. Furthermore, the likelihood of finding one is low. Like the physician, we have at our disposal an array of procedures which can be applied to develop information on certain aspects of seedling quality. From these tests and the informed interpretation of their results, it is possible to predict, with some reliability, the survival and growth potential of any seedling on any site.

For this review, attributes of seedling quality are grouped into two categories. **Performance attributes** are measured by subjecting whole seedlings to some test condition and measuring their performance; examples are root-growth potential and stress resistance. These attributes integrate the combined functioning of many physiological and morphological subsystems within the seedling. **Material attributes** include certain of these subsystems; examples are root starch concentration, leaf osmotic potential, and shoot:root ratio. These attributes, taken in mass, ultimately determine seedling performance but, considered individually, have relatively low predictive value unless they fall far outside some normal range. The relationship among material and performance attributes, and their influence on seedling quality, are illustrated in Figure 1.

In this chapter, I review in detail techniques proposed for assessing seedling quality towards defining the state-of-the-art of this technology, contrast current practices in Northwest nurseries with the state-of-the-art, and present practical information for forest-nursery and regeneration personnel.

Unfortunately, providing balanced coverage of the various seedling attributes discussed is not always possible. For example, a detailed section on frost-hardiness testing is followed by a brief page on stress testing. This apparent lack of balance does not necessarily indicate the relative importance of the former and unimportance of the latter, but rather reflects the simple fact that the scientific literature on frost hardiness is vast whereas that on stress testing is limited.

Finally, much of the quantitative information presented here was developed in research on Douglas-fir *[Pseudotsuga menziesii* (Mirb.) Franco]. This, again, reflects the nature of the available literature. A solid data base for this very important species is highly desirable. Such a data base is also needed, however, for many other important species, particularly the interior pines (*Pinus* spp.), which have not been the subject of such intensive investigation. However, the biological similarities among conifers native to the Northwest are generally strong enough to render this review relevant to most, if not all, commercially important species.

23.2 Performance Attributes

23.2.1 Root-growth potential

A key to seedling survival and establishment is rapid resumption of water and mineral uptake after outplanting. Resumption depends on the rate at which seedlings renew intimate soil-root contact by initiating and elongating roots into the soil matrix. Stone [108] first reported that tree seedlings vary widely in their ability to regenerate new roots after planting into an optimum environment—which depends upon their physiological status. This ability, called root-growth potential (RGP) [85], is a key seedling-quality attribute for the above reason; it is also a good general indicator that all systems in the seedling are functioning properly. High RGP is often correlated with high field survival [e.g., 85; also 71].

A seedling develops RGP while it is growing in the nursery. If seedlings are not to be stored, RGP should be measured immediately after lifting. However, because RGP can change dramatically during storage [47, 69, 84, 140], it should be measured after storage as well as before. Expression of RGP is mediated by conditions on the planting site, especially soil moisture and temperature. This sequence, recently reviewed by Ritchie and Dunlap [85], is summarized in Figure 2.

23.2.1.1 Standard measurement method

The standard method of measuring RGP is similar to that first described by Stone et al. [112, 113]. After all white root tips are removed, seedlings are potted in a light soil or potting mix (peat:vermiculite forestry mix is recommended) and held for a specific period, usually 28 days, under conditions favorable for root growth. Though these conditions vary somewhat for different species, 20°C air and soil temperature and 16-hour photoperiods are often used. Seedlings are then carefully washed out of the pots and new roots measured, counted, or both. Three pots of five seedlings each per treatment are normally sufficient to give valid statistical comparisons.

Test conditions can be tailored to species (e.g., boreal conifers may have lower optimum soil temperatures), but it is particularly important that conditions be consistent among tests. Most critical are soil temperature and moisture, air temperature, humidity, and photoperiod [85, 115], each of which can affect test results.

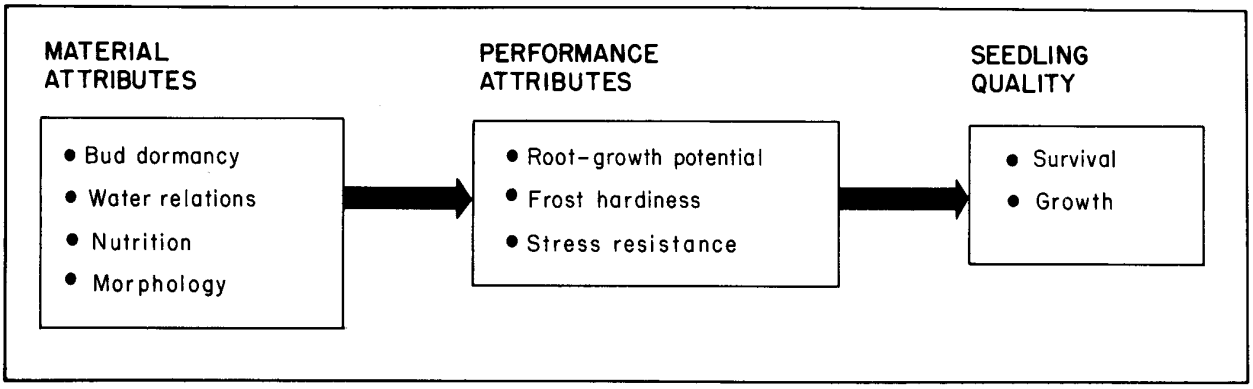

Figure 1. Seedling quality can be assessed in terms of measurable performance attributes which, in turn, reflect the sum of innumerable material attributes. Performance attributes are normally better predictors of seedling survival and growth than material attributes.

23.2.1.2 Short cuts

The standard method just discussed (23.2.1.1) has three major disadvantages: (1) it requires substantial quantities of potting soil and considerable greenhouse space, (2) root measuring and counting are laborious and time consuming, and (3) results are not available for 1 month. Several approaches that circumvent these problems follow:

Hydroponic growing.—RGP tests need not necessarily be carried out in pots of soil mix. We have had good results with aerated water baths made from 38-liter (10-gal.) fish aquariums, painted black, and covered with plywood lids into which 5.5-cm (2.2-in.) holes had been drilled. Seedlings were suspended into the tanks through #12 rubber stoppers drilled and slit radially and placed in the holes. Baths were filled with tap water, which was continuously aerated with a small aquarium pump and bubble stone. No nutrients were added, but a copper penny was placed in each tank to impede algae and mold growth. When held in a greenhouse next to seedlings in standard root-growth trials, seedlings in the baths produced nearly the same length and number of new roots as those in the pot t rials in 11 separate tests.

Some advantages of hydroponic growing are: (1) less space is required, (2) there is no need for pots or potting mix, (3) root temperature and moisture conditions are readily controlled and remain nearly constant, (4) roots are neither broken nor lost during extraction, (5) roots are clean and very easily measured, and (6) root growth can be observed during the test.

Shortening testing time.—Several workers have experimented with reducing testing time of the standard method from 1 month to only 1 or 2 weeks. According to Burdett [pers. commun., 9], 1- and 2-week results are well correlated with 4-week results in some species, hence greatly reducing the time needed for testing. Burdett's test conditions, which accelerate root growth, are:

Day temperature	30 ± 0.5°C
Night temperature	25 ± 0.5°C
Daily photoperiod	16 hours
Light intensity	11,000 ± 1,000 lux
Relative humidity	75 ± 5%

It has been our experience with coastal Douglas-fir (var. *menziesii) that* new roots do not appear until near the end of the second week at 20°C air and soil temperature. It may be possible to accelerate this process with forcing conditions such as Burdett describes. Stone [unpubl. data, 110] has tried accelerated conditions with white fir [*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.] with only limited success.

Streamlining measurement procedures.—Typically, number and total length of new roots per seedling are measured to estimate RGP. Number gives an estimate of initiation rate, and length an estimate of elongation rate. Both are normally needed for detailed physiological studies but may not be necessary for gross estimates of RGP.

Some short cuts are available: (1) counting the number of roots which exceed some crit ical length (e.g., 1 cm); (2) measur-

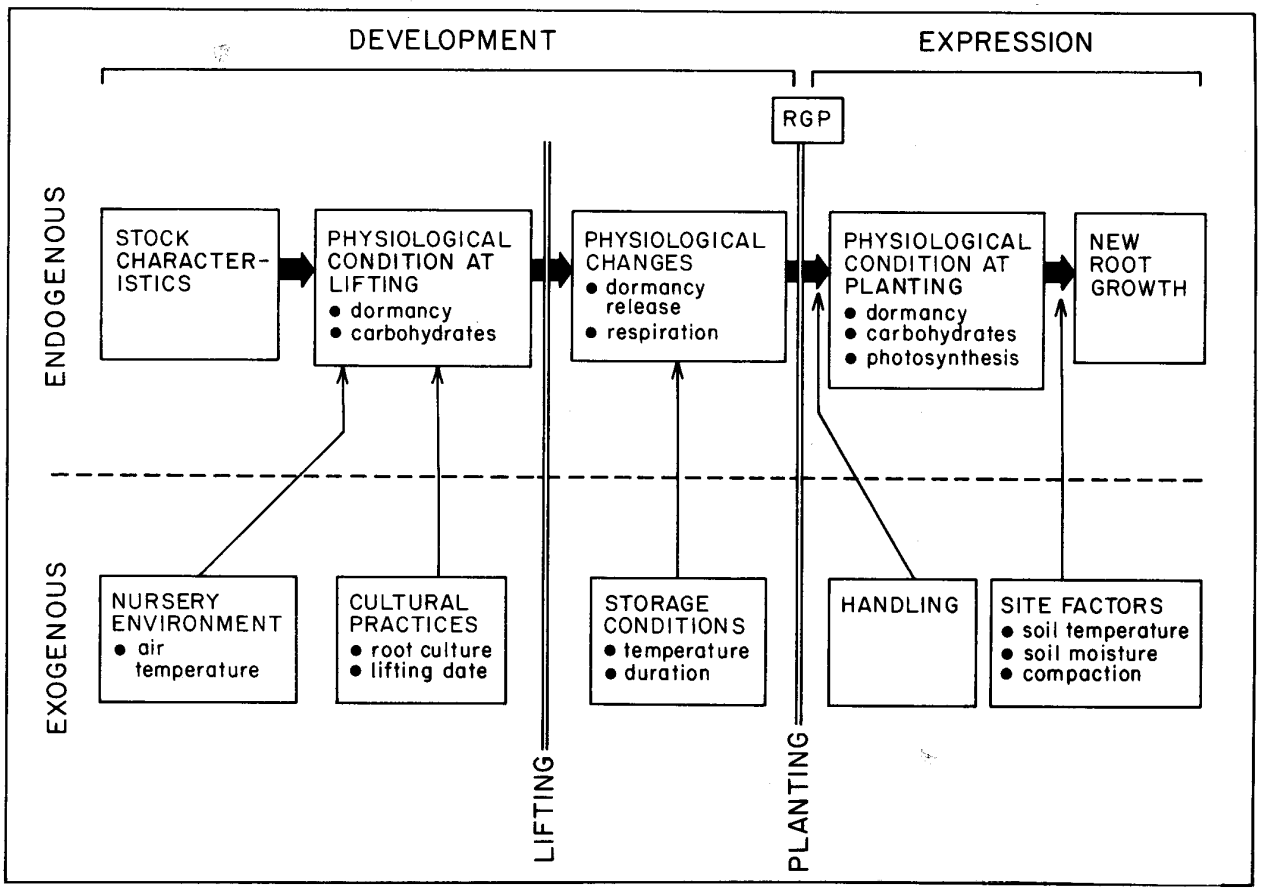

Figure 2. Development and expression of root-growth potential (RGP). Development is affected by endogenous (internal) seedling properties which reflect exogenous (external) forces; these forces act upon the seedling during nursery growth and storage. After planting, expression is limited by factors at the planting site. The most appropriate point at which to measure RGP is immediately before planting (adapted from [85]; reproduced with permission from the New Zealand journal of Forestry Science).

ing the length of only the three longest roots; (3) clipping, drying, and weighing the new roots; (4) developing a scoring index based upon numbers of roots exceeding a certain length; (5) developing a set of "reference" photographs of root systems of known lengths for visual comparisons: and (6) measuring root volume before and after the 30-day test [8]. Burdett [pers. commun., 9] recommends a scoring system based on the following scale:

Class	Description
0	No new root growth
1	Some new roots, but none over 2 cm long
2	1 to 3 new roots over 1 cm long
3	4 to 10 new roots over 1 cm long
4	11 to 30 new roots over 1 cm long
5	More than 3 5 new roots over 1 cm long

Each of these methods is useful, but information content usually falls 'with measurement cost. It is important to design the measurement strategy with objectives and resource constraints clearly in view.

23.2.2 Frost hardiness

Frost hardiness may be defined as the minimum temperature at which a certain percentage of a random seedling population will survive or will sustain a given level of damage [102, 121, 128]. The term LT_{50} (lethal temperature for 50% of a population) is commonly used to define the hardiness level.

During the growing season, tree seedlings are normally killed by temperatures near freezing. During fall, hardiness increases rapidly in response to changing photoperiod, low temperatures, and other factors [136] and reaches a seasonal minimum in midwinter. For coastal Douglas-fir, this minimum is around -25°C; in many timberline species, it is near -40°C [3]; and in boreal species such as spruces (*Picea* spp.) and firs (*Abies* spp.), it may be -70°C or lower [93]. With a return to springlike conditions, hardiness is rapidly lost.

If tree seedlings are subjected to temperatures below their hardiness limit after planting, mortality will be substantial. Hence, frost hardiness can be a major factor affecting survival and establishment [134] and must be regarded as a key seedling-performance attribute.

The mechanisms of frost hardiness are very complex and involve many interacting factors, including (1) the ability of plant tissues to (a) avoid or tolerate freeze desiccation, (b) prevent lethal intracellular ice-crystal formation, and (c) withstand nonlethal extracellular ice-crystal formation, and (2) the propensity of cell water to reach subfreezing temperatures without freezing, i.e., supercooling [67, 136]. Elaboration of these mechanisms is beyond the scope of this chapter, but for the interested reader, Mazur [68] and Levitt [65] offer thorough analyses, Weiser [136] gives a concise review pertinent to woody plants, and Glerum [34] and Brown [6] review aspects of frost hardiness in forest trees.

Assessing frost hardiness has two steps: (1) subjecting plant material to subfreezing temperatures and (2) evaluating the effect of this treatment. Frost-hardiness determination can then usefully be applied in the nursery (1) as a guide to providing frost protection during autumn and spring and (2) as an indicator of stock hardiness at planting time. Because the effects of cold storage on hardiness are poorly understood, hardiness rating of seedlings when lifted may not be valid after storage.

23.2.2.1 Freezing treatments

The classical procedure for freeze testing is to (1) randomly select a sample of seedlings from the population of interest, (2) place them into a freeze chamber of some type, (3) lower the temperature at a given rate until the test temperature is reached, (4) hold the test temperature for a given time period, (5) then return at a given rate to the starting temperature. This is repeated across a range of temperatures believed to bracket the hardiness of the seedlings.

Several aspects of this procedure warrant attention. First, sample size should be carefully determined because seedlings (and transplants) vary genetically with respect to hardiness development and phenology. Generally, between 20 and 40 plants are used depending upon species and experience of the evaluator. Second, the rate of temperature decrease should be monitored. Timmis [unpubl. data, 119] recommends that a 5°C/hour temperature decrease not be exceeded because higher rates may compound injury induced by the minimum temperature. Note, however, that the rate of temperature increase can exceed that of temperature decrease, e.g., 20°C/hour vs. 5°. Third, duration of the minimum temperature also is important because longer exposures normally increase damage. Two hours at minimum temperature is common. Most crucial is that, for results to be comparable, all tests must be carried out in precisely the same manner [65]. Repeated freezing can result in increased damage, especially when the minimum temperature is low enough to cause injury [36]. It is also important, when using whole seedlings, to insulate the roots because they are likely to be far less hardy than the shoots [39, 80].

Numerous types of freezing chambers are available, ranging from simple units which can be taken to the field and placed over seedlings [e.g., 35] to sophisticated laboratory chambers with precise programmable temperature controllers [e.g., 101]. Such chambers include radiation [2] and advective [89] frost chambers and freezing bars [92] which provide temperature gradients. Advantages and disadvantages of the various types of units are discussed in a comprehensive review by Warrington and Rook [134].

23.2.2.2 Evaluating frost damage

The only procedure for unequivocally evaluating damage after freezing tests is to hold the seedlings in a greenhouse or growth chamber for several weeks and then visually to inspect them, including roots, for damage. It is also critical to understand which tissues are likely to be least hardy, which varies seasonally. Menzies and Holden [73] recommend the following index to evaluate freeze damage in Monterey (*Pinus radiata* D. Don) and bishop (*Pinus muricata* D. Don) pines and Douglas-fir seedlings:

Index value	Damage
0	None
1	Buds undamaged, needles reddening
2	Buds may be damaged, 10 to 30% of needles killed
3	40 to 60% of needles killed
4	70 to 90% of needles killed
5	All needles killed, stem dead

The obvious and formidable disadvantage to this approach is the often excessive time required for damage to become apparent, during which seedlings must be cared for and observed.

Several methods have been proposed for avoiding this waiting period by indirectly assessing frost damage immediately after the freezing test. Most are based upon measuring the degree of inactivation of enzymatic or metabolic functions or measuring changes in membrane properties. Timmis [117] has critically evaluated the applicability of five such techniques to tree seedlings: (1) direct measurement of photosynthesis, (2) leaf-segment flotation on phosphate buffer solution as an estimate of photosynthetic rate [122], (3) dehydrogenase en-

zyme activity assessed with the tetrazolium chloride test [106], (4) changes in membrane ion permeability detected by electrical impedance [5, 33, 125, 126], and (5) plant water potential measured with a pressure chamber [4]. Timmis found that each method was useful to some degree in detecting freezing damage. However, accuracy of the determination depended upon the stage of hardiness of the tissue when freeze tested. On balance, the electrical impedance method gave the most reliable results across all levels of hardiness, confirming findings of van den Driessche [126]. Differences in electrical impedance ratio in the upper stem predicted survival after freezing with 87% accuracy and enabled LT_{50} values to be predicted within 2°C at all phases of hardening and dehardening.

Impedance ratio measurement.—The following method is recommended for coastal Douglas-fir nurseries [unpubl. data. 120]. The meter used, designed by W. D. Perry, Weyerhaeuser Co., is enclosed in a small hand-held plastic box.[1] Impedance ratios (IR) obtained on freeze-treated seedlings are interpreted differently according to the stage of hardening or dehardening when seedlings are frozen. During early stages of hardening (until late November), LT_{50} values can be estimated within 1°C if an IR of 3 is used to discriminate between live and dead seedlings. That is, seedlings with ratios lower than 3 will be dead and those with higher than 3 will survive. After November the discriminating ratio increases gradually to about 5 in late January. IR values are less reliable in midwinter because low temperatures tend to kill buds before stems and because bud injury is not detected by stem impedance ratios. Therefore, to estimate freeze damage during this period, seedlings should be held in a warm greenhouse for 3 days and the buds then cut open and examined for obvious browning in relation to (1) uninjured buds and (2) buds definitely killed by deep freezing (lower than - 30°C). The extent of bud mortality in the test seedlings is then judged and classified.

In April, or in prematurely dehardened seedlings, the meter again gives good estimates of LT_{50} values if a discriminating ratio of 2.5 is used.

Diffusate conductivity method.—This widely used method—possibly more accurate, but also more laborious, than measuring IR—is based upon the principle that freeze-injured cells contain damaged membranes which allow cell fluid to escape into the xylem. Cell fluid contains dissolved materials and therefore has higher electrical conductivity than xylem water, which is relatively pure. Comparing the conductivity of xylem diffusate from among uninjured, injured, and dead seedlings provides an estimate of the amount of injury, if any, that occurred. The method, pioneered by Dexter et al. [21, 22], has been used successfully on a number of woody plant species [e.g., 107, 12 5, 128, 139].

In the following procedure (after [36]), stem segments 2.5 cm long are collected from freeze-treated seedlings immediately below the apical bud. These are placed into capped glass vials containing 15 ml of distilled water and held in a water bath at 25°C for 24 hours. They are then shaken, and the conductivity of the water (and xylem diffusate) is measured with a suitable device. The stem segments are then killed (frozen at - 15°C for 24 hours), replaced into the 25°C water bath for 24 hours, and remeasured. Relative conductivity, R_t, is calculated as

$$R_t = L_t/L_k \quad (1)$$

where L_t is the specific conductivity of the diffusate from the ample subjected to temperature (t), and L_k is the specific conductivity of the diffusate from the sample frozen at temperature and then killed. The R_t of frozen seedlings can be confounded, however, by changes in the R, of unfrozen seedlings. To eliminate this source of error [31], an injury index, h, must be calculated:

$$I_t = 100 (R_t - R_o) / (1 - R_o) \quad (2)$$

where R_o is the relative conductivity of the control (unfrosted) seedling given by L_o/L_d, Lo is the conductance of diffusate from controls, and L_d is the conductance of diffusate from controls killed as indicated above.

Green and Warrington [36] reported excellent results with this method on Monterey pine. R_t values determined 3 days after freezing treatments accurately predicted freezing damage as assessed visually 1 month later. This correlation was improved to $r^2 = 0.92$ with the I_t value. Green and Warrington determined that an R_t value of 0.5 or greater indicated seedling death was imminent. van den Driessche [128] applied the diffusate conductivity method to Douglas-fir seedlings with some success but was not able to identify a critical index of injury, as were Green and Warrington. Nevertheless, the method predicted well ($r^2 = 0.77$) the lethal temperature of whole plants subjected to freezing temperatures.

23.2.3 Stress resistance

A simple technique for assessing a seedling's overall "physiological soundness" has been pioneered at Oregon State University [46][2] and is currently offered by the university as a service. Sixty seedlings are randomly selected from a lot and divided into two equal groups. The first group (controls) is planted directly into 25- x 25-cm (10- x 10-in.) fiber pots, 10 per pot, placed in a greenhouse or growth room, and watered. The second group is washed, blotted to remove excess water from the roots, and then suspended in a growth cabinet for 15 minutes at 30% relative humidity and 32°C (90°F). Following this stressing treatment, seedlings are removed and their roots soaked in water for 5 minutes. They are then potted and placed alongside the controls, where both groups are watered regularly and maintained under fairly constant 20°C (68°F) temperature and a 16-hour photoperiod.

Seedlings are evaluated after 2 weeks, 1 month, and 2 months. Mortality is noted when it occurs. After 2 months, seedlings are classified as follows:

Mortality among stressed stock, %	Classification
0-10	Excellent
11-20	Good
21-30	Fair
31-100	Poor

If there is mortality in the control group or if abnormal budbreak is noted, these classifications can be modified. The length of time required for stress damage to become apparent varies. Some seedlings will show no damage for 4 weeks, then begin to turn brown and die: others will begin to show damage after 10 days. Generally, lots in the poorest condition will show damage symptoms earliest.

The goal of the testing procedure is ultimately to predict field survival, hence the testis designed so that mortality of the stressed trees should correspond roughly with expected field mortality. Under normal conditions, "poor" lots should not be planted at all, and "fair" lots should be planted only in areas where severely stressful conditions will not be encountered.

Tests have been administered to over 1,000 seedling lots representing virtually all important Northwest conifers during the past 4 years. Unfortunately, it has not been possible to quantitatively assess the accuracy of all test predictions.

[1] Circuit design and operating procedure are available from the author on request.

[2] For more information, contact Douglas McCreary, Department of Forest Science, Oregon State University, Corvallis, Oregon 97331.

However, during 3 years of testing in cooperation with the Bureau of Land Management, field performance correlated well with lots displaying either very high or very low test survival. Correlations were not as strong in intermediate lots [pers. commun., 70].

There have apparently been no published attempts to relate performance in the stress test with other performance attributes. Possibly, peak periods of stress resistance may not coincide with those of other properties such as RGP.

In the future, those using this test procedure may be asked to furnish information on the history of each lot submitted for testing (e.g., lifting date, storage time and temperatures, etc.). In time, and with this information, developing valuable correlations among these variables and stress resistance may be possible.

Finally, the physiological mechanisms underlying stress resistance are not well understood. They may be related to the seedling's ability to grow roots, to control water loss, to increase water uptake, to endure internal water deficits, or to other mechanisms (see analysis of [118]). This may be a profitable area for future research.

23.3 Material Attributes

23.3.1 Bud dormancy

Perennial plants which have evolved in regions with strongly seasonal climates can adapt to a wide range of environmental temperature and moisture regimes with changing seasons. Plants "anticipate" these changes by keying in on reliable environmental cues such as photoperiod and soil temperature. As seasons change, plants cycle through various physiological states, each adaptively tuned to ambient conditions; this is referred to as the dormancy cycle and has been a major area of inquiry in plant-biology research ([e.g., 81, 90, 95, 131]: see also chapter 14, this volume).

In conifer seedling crops, the dormancy cycle comprises several "stages" [18]. Dormancy is **induced** from midsummer to late summer (dates given by Cleary [18] are specific to western Oregon) as overwintering buds are formed. These may break and form lammas shoots if seedlings are fertilized, given long photoperiods or heavy irrigation, or experience heavy late summer or early autumn rain after a droughty period. Dormancy **deepens** in late summer and early fall. During this period, buds will not flush if exposed to favorable conditions, but seedlings are not yet resistant to frost or lifting damage and cannot be successfully cold stored [47, 64]. Dormancy **peaks** (true dormancy) in early winter, when it is characterized by (1) an almost total absence of growth anywhere on the seedling and (2) a requirement for several hundred-hours of low temperatures (0 to 10°C) before buds can break in response to higher temperatures [81]. This **chilling requirement** [133] is an adaptive mechanism which ensures against buds breaking during a midwinter warm spell and being subsequently killed by a return of cold weather.

The length of the chilling requirement has been determined experimentally for coastal Douglas-fir by Wommack [141], Lavender and Hermann [63], and van den Driessche [127]; for interior Douglas-fir (var. *glauca)* by Wells [137] and van den Driessche [127]; for western hemlock *[Tsuga heterophylla* (Raf.) Sarg.] by Nelson and Lavender [74]; and for several spruces by Nienstadt [75, 76]. The chilling requirement of these species is generally fulfilled by exposure to temperatures at or below 5°C for 2,000 hours and may also be fulfilled by cold storage. After this requirement has been satisfied, buds will break rapidly once exposed to springlike conditions; in this state, seedlings are called **postdormant.** The interactions among chilling, flushing temperature, photoperiod, and time required for budbreak have been elegantly demonstrated for Douglas-fir by Campbell [10] and Campbell and Sugano [12].

Of most interest to the forest-nursery manager is the stage of **true dormancy.** It is generally felt that seedlings lifted before or after the period of true dormancy are high risk and prone to suffer serious damage from cold storage [47]. If so, it is important to know when true dormancy begins and ends. Beyond this, Hermann [42, 43, 44] demonstrated, in a series of important experiments, that Douglas-fir seedlings vary greatly with respect to their ability to withstand environmental stresses as they pass through the stage of true dormancy itself. Hence, it is not only important to know when seedlings enter true dormancy, but also to know the intensity of dormancy at any point in time. Because seedlings do not change visibly from late summer to early spring, determining their exact dormancy status (or intensity) has been troublesome and the subject of much experimentation. Some suggested techniques follow. Four of these—dry-weight fraction, mitotic index, hormone analysis, and electrical resistance—if developed and verified, would offer rapid, inexpensive methods of assessing dormancy status and clearly deserve further study.

23.3.1.1 Budbreak tests

The most reliable measure of the intensity of dormancy is the time required for terminal buds to break in a forcing environment [50]. In practice, this is determined by bringing a sample of seedlings indoors, potting them in a suitable medium, and holding them in a standard test environment simulating springlike conditions (e.g., 12- to 14-hour days, 20°C air temperature). Seedlings are checked daily; when terminal bud scales part to expose new needles, the date is recorded. After terminal buds have broken on all seedlings, the average number of days to terminal budbreak (DBB) is calculated. Because dormancy intensity weakens as winter chilling accumulates, buds will break faster the later in winter seedlings are forced (Fig. 3).

23.3.1.2 Dormancy release index

The relationship between DBB and chilling sum (see 23.3.1.3) can be fitted with a reciprocal function (i.e., 1/DBB) (Fig. 3). Campbell and Sugano [11]employed this relationship to quantify dormancy intensity in Douglas-fir seedlings. They devel-

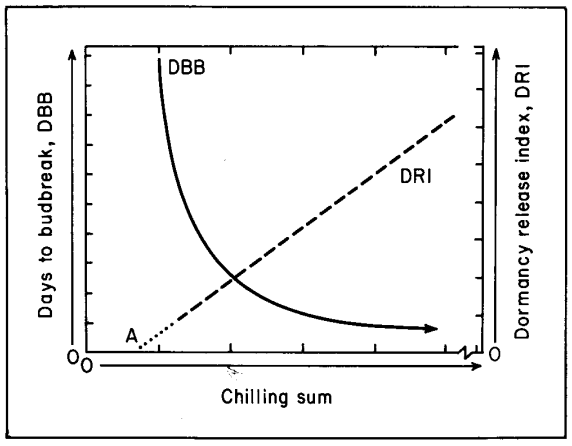

Figure 3. Chilling sum at time of lifting is related to days to terminal budbreak, or DBB (solid curve), of coastal Douglas-fir seedlings under 16-hour photoperiod and 20°C day and night temperature. Resulting values can be expressed as a dormancy release index, or DRI (straight dashed line); for Douglas-fir, DRI = 10/DBB. Extrapolation of DRI to point A on the x-axis may provide an estimate of the earliest date to begin lifting for storage.

oped the term DARD (daily average rate of development), calculated as:

$$DARD = 100/DBB \quad (3)$$

which gives an estimate of the developmental rate of the seedling at any time during dormancy release.

This concept has been extended [85] into what is called a dormancy release index (DRI):

$$DRI = DBB_r/DBB \quad (4)$$

where DBBI is the number of days required for budbreak in a fully chilled seedling.[3] For coastal Douglas-fir, DBB, is 10; hence, for this species, DRI = 10/DBB. The value of DBB_r must be determined experimentally for each species but probably does not vary among species by more than a few days. Dormancy release can be compared among species with DRl because it always varies from 0 (in a seedling just entering true dormancy) to 1 (in a seedling fully released from dormancy). DRI values for stored and unstored Douglas-fir seedlings have been shown to be good indicators of physiological condition in our own unpublished experiments.

23.3.1.3 Chilling sums

The disadvantage of DRI as a nursery manager's tool is the excessive time required to get results. Seedlings lifted early in winter may not break bud in the test environment for 100 days or more. However, we have found with Douglas-fir that the relationship between DRl and chilling sum (number of hours a seedling spends at $\leq$ 5°C) does not vary appreciably among seedlots at a given nursery from year to year. Therefore, once this relationship has been empirically established for a given species and nursery, dormancy status during winter can be accurately predicted from monitoring chilling sums.

The chilling sum is determined simply by monitoring air temperature at about 1 m above the ground and summing the hours during which the temperature is within some range known to be effective at releasing dormancy in the species of interest. Our experience has been that the range 0 through 5°C is useful for northern conifers. In California nurseries, however, the range 0 through l0°C may be more appropriate. Once hourly temperature data have been collected, chilling sums may be tallied within any temperature range desired. Data collection should begin around October 1 in coastal nurseries and in early to mid-September in more northern or interior regions.

This approach has been highly refined for predicting time of budbreak in fruit crops, especially in regions where late frosts are common. By taking into account the relative efficiencies of different chilling temperatures and the effects of warm interruptions (which can negate chilling) and other factors, chilling equations are available which predict budbreak time with an error of only ±2 days for some crops [27, 83]. Whether this level of accuracy is warranted in forest-seedling crops, however, is yet to be established.

By calculating chilling sums, it may also be possible to estimate the earliest date at which lifting for storage can begin. In Figure 3, extrapolation of the DRI curve to the x-axis (point A) indicates that the first several hundred hours of autumn chilling do not actually contribute to physiological dormancy release. Point A generally coincides with the last week in November in western Washington, which is viewed by some nursery personnel as the earliest date on which successful lifting for storage can occur. However, this relationship needs to be developed for other species and regions.

[3] Determined experimentally with seedlings lifted from the nursery in late winter, stored at -l°C for 6 months, then tested for budbreak at 20°C under a 16-hour photoperiod.

23.3.1.4 Oscilloscope technique

Zaerr [143] reported the interesting observation that square-wave electrical signals are propagated differently through living plant tissue than through dead tissue. The form of this propagation can be determined with an oscilloscope. Following up on this work, Ferguson et al. [29] tested a wide range of species, including some conifers, at different times of year and found that the types of oscilloscope waves observed seemed to be related to periods of plant activity and inactivity. This finding led to speculation that the oscilloscope technique may be the long-awaited "dormancy meter," and a number of investigators set out to verify Ferguson's results.

Disappointingly, this work has not been very successful [1, 50, 53, 79] due to lack of reproducibility, interspecific variability, and artifacts produced by touching or moving sample branches. These problems probably reflect the unknown complexity of plant-tissue circuitry, and it is likely that a dormancy-related change in a particular capacitive or resistive component will have only a very small effect on the overall response. Furthermore, changes in properties unrelated to dormancy will also influence tissue electrical properties [116]. Though this technique may hold promise with further development, its present operational usefulness for assessing seedling dormancy status is limited [50, 53].

23.3.1.5 Dry-weight fraction

The dry-weight fraction (DWF) of seedling shoots may be a simple, rapid method of assessing dormancy. Dry-weight fraction is calculated as

$$DWF = DW/TW \quad (5)$$

where DW is the oven-dry weight of the seedling shoot, and TW is its turgid weight.

Dry-weight fraction changes annually in a predictable manner in many woody plant species. In Douglas-fir seedlings, DWF increases gradually during fall and early winter, peaks in January, then falls rapidly during spring [88]. If this pattern reflects seedling physiological condition and is relatively independent of weather, then it might be used as an indirect measure of seedling dormancy status during winter. DWF is now used routinely in some Swedish seedling nurseries to determine when to begin lifting [pers. commun., 91].

23.3.1.6 Mitotic index

During autumn, mitotic activity in conifer buds declines rapidly as dormancy deepens [77, 78]. This phenomenon has been exploited by Carlson et al. [13] as a tool for determining when Douglas-fir seedlings have become dormant. Using a squash and stain technique, they microscopically ascertain the percentage of cells in the terminal meristem which show mitotic figures. This "mitotic index" (MI) declines steadily throughout autumn, reaching zero apparently at about the time seedlings enter dormancy. Hence, it might serve as an indicator of the onset of dormancy. But because MI remains near zero until mid-March, it would not be useful in assessing the progress of dormancy release.

23.3.1.7 Hormone analysis

Dormancy induction and release are hormone-mediated processes. In principle, then, it should be possible to assess the status of dormancy by measuring concentrations, or ratios of concentrations, of various dormancy-regulating hormones. Good correlations have been observed, for example, between free abscisic acid concentration and apparent dormancy intensity throughout winter in buds of European beech (*Fagus sylvatica* L.) trees [142]. Hatch and Walker [38] were able to assess the dormancy intensity of peach and apricot buds on the basis of the concentration of gibberellic acid required to make them

break. Zaerr and Lavender [144] have evaluated the potential for using hormone tests as a litmus for dormancy status in seedlings and concluded that rapid advances in analytical techniques might make this a real possibility in the future—but not now.

23.3.1.8 Electrical resistance

Cyclic seasonal changes in the electrical resistance of the inner bark of maple (*Acer* spp.), oak (*Quercus* spp.), and pine trees have been reported [20]. In some cases, these changes were dramatic, with resistance decreasing from spring to summer and increasing from summer through autumn. Unfortunately, the period of greatest interest to the nursery manager—December through March—was not sampled due to frozen stems.

23.3.2 Water relations

Most aspects of seedling physiology influence, and are influenced by, seedling water status (see also chapter 12, this volume). Its effects on plant growth and function and the technology available for measuring it are subjects of a voluminous and complex literature [e.g., 56-61, 66, 103, 104, 123]. Here, a few central concepts are briefly reviewed, and then several measurement methods that may be of practical value to the nursery grower are summarized.

23.3.2.1 Water potential

The status of water (W) in a seedling reflects the imbalance between the rate at - which water is absorbed by its roots (A) and the rate at which it is transpired (T) through the leaves:

$$W \simeq (A - T + S) \qquad (6)$$

where S, a relatively small term, represents the storage of water within the seedling itself. During the day, and sometimes at night, T exceeds A so that the water in the seedling comes under tension, or "stress." When stress is sufficiently great or prolonged, growth and photosynthesis cease, metabolic systems break down, and mortality follows.

As used above, W represents the water content of the seedling. But to be physiologically precise, water status should be quantified in terms of its free energy, or "water potential," Ψ_w. Water potential is defined thermodynamically as the ability of water to do work in comparison to free, pure water at standard pressure and temperature, whose water potential is zero. Units of water potential are dimensionally equivalent to pressure units; therefore, Ψ_w can be expressed in pounds per square inch, atmospheres, or bars. In the metric system, the appropriate units are joules per kilogram or Pascals. Here I will use the unit megaPascal, MPa, which is recommended for plant research [52].[4]

23.3.2.2 Components of water potential

The water potential of a seedling has several component potentials. Here, we are interested primarily in two, osmotic potential (Ψ_π) and turgor potential (Ψ_p), which are related to Ψ_w as follows:

$$\Psi_w = \Psi_p + \Psi_\pi$$

Turgor potential is a positive force exerted inward on the cell contents by the rigid cell wall, much as the skin of a balloon exerts a force on the air inside the balloon. As the cell loses water, the force weakens. Osmotic potential is a negative force resulting from the effect of dissolved solutes (e.g., sugars, salts) and other materials on the free energy of water. As solute concentration increases, osmotic potential decreases: in pure water, it is zero.

[4] 1 MPa = 10 bars ~ 10 atm ~ 150 psi.

Turgor potential is a **very important** property. Virtually every physiological process in the seedling is sensitive to turgor such that a turgor drop below a given level, if sustained, can result in death [51].

These concepts are integrated into a diagram (Fig. 4), originally conceived by the German scientist Karl Hofler [49], illustrating the manner in which the components of water potential change as the seedling gains or loses water. When a seedling is fully hydrated (100% water content), Ψ_w is zero, by definition, and the value of Ψ_p is equal and opposite in sign to the value of Ψ_π (due to Equation 7). As a net loss of water is experienced, solutes are trapped in the cells by the cell membrane while water escapes into the cell walls and xylem. Thus, cell solute concentration increases and osmotic potential decreases. Turgor also falls because the cells lose volume. Therefore, water potential falls and stress increases. In the example shown in Figure 4, with a loss of, say, 10% water content, Ψ_w is - 0.5 MPa, Ψ_π is - 1.7 MPa, and Ψ_p is 1.2 MPa. When water loss is 30%, Ψ_p becomes zero and Ψ_w is - 2.5 MPa. This value of water potential at zero turgor (Ψ_z) is a **critical point** because it presumably indicates at what stress level death is imminent.

These concepts underline the central point that seedling water status cannot be adequately described by measuring water potential alone. Complete assessment must also include estimates of its components. Having said that, I now turn to a survey of available techniques, only two of which, psychrometry and the pressure chamber, are capable of determining the value of component potentials.

23.3.2.3 Measurement techniques

Gravimetric methods.—Gravimetric methods of measuring seedling water status yield information on water content only and not on water potential. However, if other alternatives

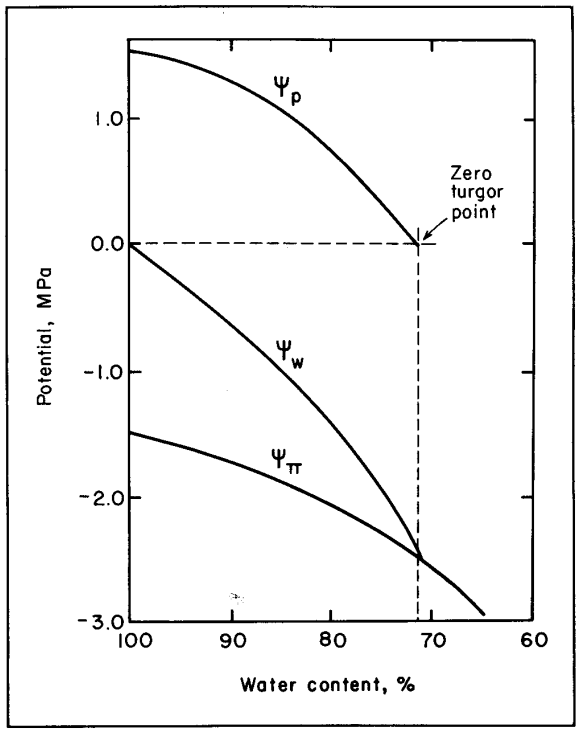

Figure 4. A Hofler diagram showing the manner in which water potential, Ψ_w, and its component potentials (osmotic, Ψ_π, and turgor, Ψ_p), change with respect to a change in cell water content.

are not available, or if calibrations between water content and potential have been established, then measuring seedling water content may be useful.

A widely used measure is Weatherley's [135] Relative Water Content (RWC). It is determined by weighing a sample (normally a leaf) immediately after it is collected and again after it has been brought to full turgidity by floating on water in the dark until it ceases to gain weight. The sample is then oven dried for 48 hours and weighed again. RWC is calculated as:

$$RWC = \frac{\text{fresh wt. - dry wt.}}{\text{turgid wt. - dry wt.}} \times 100 \quad (8)$$

In a fully turgid sample, RWC is 100%. A corollary measure is the "water deficit," in which the same steps are performed, and water deficit (WD) is calculated:

$$WD = \frac{\text{turgid wt. - fresh wt.}}{\text{turgid wt. - dry wt.}} \quad (9)$$

A particular disadvantage of using these methods on conifer seedlings is the difficulty sometimes experienced in bringing sample material to full turgor.

Psychrometric methods.—Psychrometric techniques of estimating water potential are based on the principle that if a tissue sample is placed in a small chamber, the humidity of the air in the chamber will come to equilibrium with tissue Y_w. Hence, with appropriate calibration, a measure of humidity gives an estimate of water potential [26, 105, 138]. Though this principle has been understood for many years, only recently have affordable devices been developed for accurate, reproducible measurement.[5] They generally consist of a small chamber containing a thermocouple psychrometer (to measure humidity) and the associated electronics to generate, read, and transmit an electrical signal.

A significant advantage of psychrometry is that it permits separate estimates of the osmotic and turgor components of water potential. To do this, Y_w is measured in the normal way; then the tissue sample is frozen and remeasured. Freezing destroys the cell membranes, eliminating turgor potential. Therefore, the value obtained on the frozen sample is equal to osmotic potential. Turgor can then be calculated as the difference between water and osmotic potentials.

Success of this method with conifers has been mixed. There are several sources of difficulty: (1) conifer needles come to equilibrium very slowly in the sample chamber due to their waxy surface and propensity to tightly close stomata, (2) resins exuded from cut needles tend to gum up the chamber and thermocouples, and (3) cutting needle tissue releases extracellular water which dilutes the sample and yields water-potential values that are erroneously high. Although psychrometry is the technique of choice for determining water potential in the laboratory [104], it has not yet found use as an operational nursery tool.

Density method.—The density method (also called the dye method) was first described in Russian by Shardakov [100] and later in English by Knipling [55]. A series of graded water solutions having a range of osmotic potentials is prepared. Each of the solutions is then divided in two parts and a dye (e.g., methylene blue) introduced into one part. Next, the solutions are placed into a series of test tubes, each pair containing a solution of known osmotic potential, one clear and one dyed. A small sample of plant tissue is placed into each of the clear solutions and held there for several minutes. Samples with lower osmotic potentials than the solution will take up water; therefore, the density of the solution will increase due to its increased solute concentration. Samples with higher osmotic potentials than the solution will lose water, thus diluting the solution and decreasing its density. The samples are then removed, and a drop of the dyed solution is introduced into the middle of each test tube containing the clear solutions. The solution having the same osmotic potential as the sample will not have changed density; thus, the drop of dye will remain in the middle of the tube.

This method is economical, portable, and rapid. It requires neither electricity nor gas pressure and can be made with very simple parts. But it does not provide estimates of water-potential components, and there is little, if any, information on its applicability to conifers.

Freezing-point depression method.—The freezing point of a solution is a function of its osmotic potential, a measure of the former providing an estimate of the latter. Cary and Fisher [14] and Fisher [30] describe an inexpensive, portable device capable of accurately measuring the freezing point of plant sap, provided that appropriate temperature corrections are made.

The major limitation to this method is in obtaining the sample of plant sap for analysis. Squeezing tissue yields a sap sample which is nearly pure because it contains extracellular and filtered cellular water. Grinding or blending plant material to obtain sap contaminates cell water with extracellular water and raises the osmotic potential. Freezing-point tests on sap collected by these different methods from the same tissue sample have yielded results which differ by as much as 50% [94]. Because of this problem, and because data on conifers are very limited, the technique is not recommended for nursery use.

J-14 hydraulic press.—A relatively recent innovation in rapid water-potential determination is the hydraulic press.[6] In principle, the device uses hydraulic pressure to press a sample of plant material against a clear plexiglass screen. As pressure increases, water is exuded from the cut tissue or leaf edges, or the tissue changes color. Childs [17] evaluated the press against the pressure chamber technique (see below), reporting good correlations (r^2 ranged from 0.66 to 0.90) in calibrations with bareroot and container stock and field-grown seedlings. However, similar comparisons by Cleary and Zaerr [19] gave poor results. More work is needed before this technique can be recommended for nursery use.

Pressure chamber.—Reintroduction of the Dixon pressure chamber by Scholander et al. [99] has provided an ingenious and invaluable tool for measuring plant water potential. Since then, considerable experience has been gained with the pressure chamber, much of it summarized by Ritchie and Hinckley [87]. The apparatus and procedures required to use this technique with forest-tree seedlings have been recently detailed elsewhere [18, 19] and will not be repeated here. Rather, I will focus on aspects of pressure-chamber use not addressed by the above papers—specifically, on measuring roots and individual needles and on generating "pressure-volume" curves.

Although not given much attention in the literature, the root system is an integral part of a seedling's anatomy and is generally far more susceptible to cold and desiccation than the shoot. Hence, the physiological condition of the root system should be assessed as part of overall seedling quality. The pressure chamber can be used to develop such information.

We have successfully measured root water potential using apparatus and procedures identical to those described for shoots [19]. Normally, the seedling is severed at the root

5 One such unit can be purchased from Wescor, Inc., Logan, Utah 84321.

6 Available from Campbell Scientific, Inc., Logan, Utah 8432l.

collar; then the entire root system, with soil removed, is placed in the chamber for measurement. With larger seedlings, such as 2+1s, it may be necessary to remove a major lateral root for measurement; its value will be nearly identical to that of the entire root system. Values of root water potential are normally much higher than those of shoots and exhibit far less seasonal and diurnal fluctuation [88].

Measuring water potential of leaves (needles) rather than that of branches enables repeated determinations on a seedling and greatly reduces compressed gas consumption. To measure needles directly, the rubber gland (#6 rubber laboratory stopper) holding the sample in the chamber lid must be modified (Fig. 5). Note that the stopper has been slit to the radius on one side so that a needle can be placed into the central hole without pushing it through the stopper. Note also that a portion of the underside of the stopper has been hollowed out with a cork borer so that the needle is not crushed during pressurization.

To prepare pine samples for measurement, collect a fascicle of needles and strip off the fascicle sheath. Then sever the base of the fascicle crosswise with a razor blade: this will cut the xylem traces and permit sap to escape during measurement. For conifers other than pines, the needle should be cut crosswise just above its point of attachment to the branch. The measurement is then performed as on a small branch, except that viewing should be with a 15 or 20x magnifier and a light.

Needle water potential was nearly identical to that measured on the branch from which needles were taken in several pine species by Johnson and Nielson [54] and in ponderosa (*Pinus ponderosa* Dougl. ex Laws.) and Jeffrey (*Pinus Jeffreyi* Grev. & Balf.) pines by Ritchie and Hinckley [86]; however, in Douglas-fir, Pacific silver fir (*Abies amabilis* Dougl. ex Forbes), and noble fir (*Abies procera* Rehd.), needle values were higher than equivalent branch values. Calibrations for leaf and stem water potential of these species are [86]:

Species	Equation[1]	r^2
Pacific silver fir	$Y_S = -0.59 + 1.48\ Y_1$	0.91
Noble fir	$Y_S = -0.47 + 1.34\ Y_1$	0.82
Douglas-fir	$Y_S = -0.77 + 1.28\ Y_1$	0.92

[1] Y_S = stem water potential (bars).
Y_1 = leaf water potential (bars).

A highly valuable feature of the pressure chamber is that it enables osmotic and turgor potentials (Equation 7) to be measured through generation of what is called a "pressure-volume" (P-V) curve [16, 37, 40, 124]. A simplified procedure for generating a P-V curve is given in Appendix 1, this chapter. A P-V curve represents the relationship of reciprocal water potential ($1/Y_W$) with water content. The curve has two distinctly different regions, one that is curvilinear and one that is linear (Fig. 6). The linear region can be extrapolated to the y-axis (to point A) with a straight line to give the osmotic potential when the seedling is at full turgor. The point where the linear and curvilinear regions meet is the water potential at which turgor is lost, or the "zero turgor point." Its value can be determined by extrapolating horizontally to point B.

An important finding has been that both these properties change dramatically from month to month in Douglas-fir seedlings [88]. Water potential at zero turgor in shoots and roots was lowest (seedlings tend to be more drought tolerant) in midwinter and late summer and highest in spring over the course of a year (Fig. 7). This may partly explain why seedlings are so sensitive to handling and planting when lifted in March and April.

This technique also has considerable potential for detecting certain types of hidden seedling damage. For example, frost-damaged seedlings typically have membrane lesions which result in solute leakage from cells (see 23.2.2.2). This disrupts

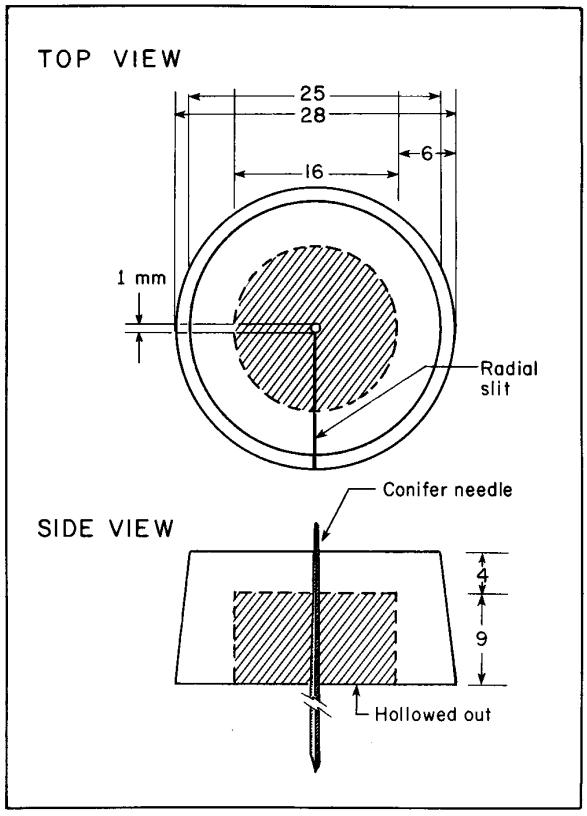

Figure 5. Diagram of a #6 rubber laboratory stopper modified to accept a conifer needle for pressure-chamber measurement. All dimensions are in millimeters.

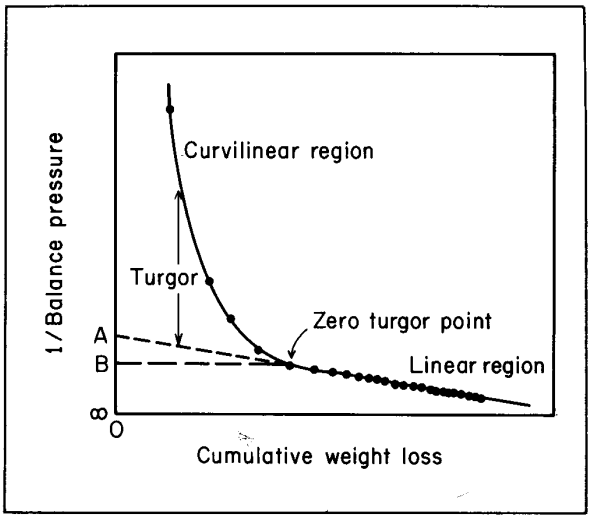

Figure 6. A pressure-volume (P-V) curve. The linear region represents the relationship between cell volume and osmotic potential when turgor equals zero. Extrapolation to point A gives an estimate of the osmotic potential at full turgor, to point B an estimate of the osmotic potential at zero turgor (see Appendix 1, this chapter).

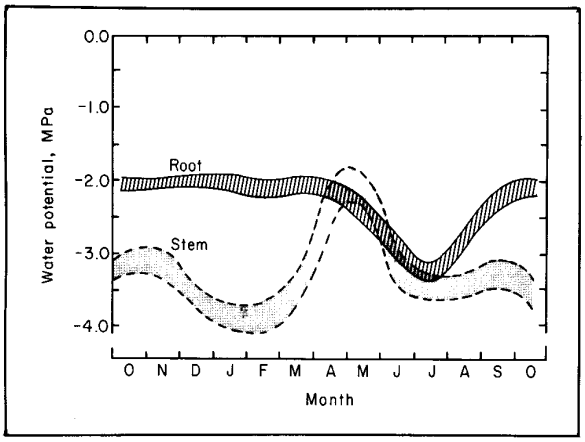

Figure 7. Seasonal changes in the "critical water potential" for a Douglas-fir seedling. This value is approximately equal to the water potential at which turgor becomes zero (zero turgor point; see Fig. 6).

tissue osmotic properties. A P-V curve from such a seedling would not show a well-defined linear region. Or seedlings with severely depleted carbohydrate reserves resulting, say, from long-term storage would have abnormally high osmotic potentials at full turgor.

Cleary and Zaerr [19] have suggested some general guidelines for interpreting pressure-chamber readings on tree seedlings. For bareroot stock from a nursery bed, water potential should not fall below -1.0 MPa and, ideally, should be above -0.5 MPa; if it falls below -2.0 MPa, the seedling may suffer severe physiological damage. Figure 7, however, indicates that the above values are not fixed. Seedlings in midwinter are apparently far more tolerant of low water potentials than they are in spring.

23.3.3 Nutrition

The literature on plant nutrition in general is voluminous. Our interest, however, is plant nutrient analysis as a direct indicator of seedling quality. The literature on this topic, unfortunately, is weak. Two aspects of nutrition are considered here-mineral nutrients and food reserves.

23.3.3.1 Mineral nutrients

All physiological processes, as well as morphological ones, are influenced by mineral nutrition [114]. There has been hope, then, that some simple measure of seedling nutrient status might be developed as an index of seedling quality [e.g., 96].

In reviewing nutrient status of Northwest conifer seedlings, van den Driessche [129] found that potassium (K), phosphorus (P), and nitrogen (N) affect various species differently with respect to frost hardiness. Increased K generally improves hardiness, whereas excess P has been shown to decrease hardiness in some species. N can improve hardiness if applied late in summer after height growth has ceased; if applied earlier, N can prolong shoot growth, retard dormancy development, and delay the onset of hardiness (see chapter 7, this volume).

Stress resistance also may be affected by nutritional status. For example, N and K can reduce transpiration rate, whereas P tends to increase it. N and K may also improve tissue water relations by enhancing turgor maintenance through osmotic adjustment. As to the effects of mineral nutrition on RGP, the data are too limited to warrant discussion [85].

van den Driessche [130] was able to show an improvement in survival of Douglas-fir seedlings following N fertilization in the nursery. It was not clear, however, whether this effect was direct or due to a general increase in seedling size (see 23.3.4) brought about by the extra N.

Menzies [72] analyzed foliar nutrient content of Douglas-fir seedlings grown at a large Northwest coastal nursery. December-lifted seedlings had adequate to low N, low to very low P, and adequate to low K, according to van den Driessche's [125] classification. By March, all nutrients had fallen to low or very low concentrations. Yet these seedlings had 98% survival and excellent growth 2 years after outplanting. It may be that, except in cases of severe deficiency, growth and performance reflect the intricate interplay between seedling nutrition and other factors governing physiology, morphology, and site conditions. Because the effects of mineral nutrition on seedling physiology are complex and interacting, no consistent relationship has yet been demonstrated between any aspect of seedling nutrient content and seedling quality, except in cases of severe deficiency.

23.3.3.2 Food reserves

Seedlings store food reserves in the form of sugars, starch, hemicelluloses, proteins, fats, oils, and other compounds (for discussion, see [62] and also chapter 14, this volume). The sugars and, especially, starch are key forms. Many workers have stressed the importance of adequate food reserves to seedling performance [see reviews by 48, 132], and some have suggested that a measure of starch content might be used as an indicator of seedling vigor [28, 32].

Hellmers [41] noted a correlation between a decline in root starch during storage (determined by iodine staining) and reduced survival in ponderosa pine seedlings after outplanting. Winjum [140] suggested that concentration of reducing sugar might serve as an index of seedling quality in Douglas-fir and noble fir. Puttonen [82] mentioned the possibility of using the carbohydrate pool as a measure of seedling physiological condition. Others [e.g., 109, 111] have proposed a cause-effect relationship between carbohydrate reserves and RGP, although more recent evidence [84] does not support this view.

Unfortunately, this relationship does not seem to have been examined by systematic, rigorous experimentation. This is disappointing because some carbohydrate components (e.g., starch) are easily determined and because the idea that "food reserves" are critical to seedling quality seems sound. However, carbohydrate chemistry is exceedingly complex. Interconversions among carbohydrates occur continuously, and the various metabolites function in different ways at different times. Therefore, although carbohydrate assessment would seem to hold promise as a future tool for indicating seedling quality, such technology is not now available.

23.3.4 Seedling morphology

In a strict biological sense, morphology means form and structure. In practice, however, any seedling characteristic that can be readily observed or measured is normally construed as morphological. The most commonly cited morphological properties are those that are most easily measured: shoot height and weight, root-system weight or volume, root fibrosity (often subjectively assessed), stem diameter at the root collar, bud "set," foliage color, and various ratios such as shoot:root weight or top heightatem diameter (sturdiness ratio). Each of the above characteristics can be manipulated to some extent in the nursery by controlling seedbed density, undercutting and wrenching, transplanting, top mowing, irrigation, and nutrient management (see chapter 15, this volume). Because they are relatively easy to control and measure, morphological characteristics have been used extensively over the years to define seedling quality [7, 114]. Indeed, some European nations have adopted legislation establishing morphological grading standards for tree seedlings [98].

More recently, however, considerable research attention has been focused on "physiological grading" of planting stock (as previously discussed). Results of this work indicate that: (1) seedling physiological condition exerts a strong influence on seedling survival and growth potential; (2) components of physiological condition are numerous, change rapidly over time, and can change independently of one another; and (3) physiological condition cannot be visually determined.

It follows from this that comparisons of seedling performance based upon **morphological** traits are valid only when seedlings are in the same **physiological** condition when tested. This simple deduction probably invalidates much of the published research on the effects of morphology on seedling performance and accounts for the inconsistency and variability which pervade this literature (see, e.g., [45]). I know of no published work on seedling morphology and performance in which the condition of physiological homogeneity has been quantitatively satisfied. Therefore, the following comments on the relation between morphology and performance are based upon generalizations and personal observation and, as a result, must be viewed as qualitative and biased.

Operational experience tends to indicate that, other factors equal, seedlings with large stem calipers tend to outperform those with smaller calipers [15, 18, 25, 98, 114]. Furthermore, stem caliper tends to be well correlated with other seedling size characteristics, as illustrated by unpublished data from four Douglas-fir stock types (Table 1). Note, however, that stem caliper was not well correlated with shoot:root ratio in these seedlings (see also chapter 24, this volume).

Dobbs [24] examined the relationship between mass (fresh weight) and field performance of white spruce [*Picea glauca* (Moench) Voss] and lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) seedlings and transplants in interior British Columbia. Large individuals tended clearly to outperform small ones regardless of species or stock type. Furthermore, the size advantage was amplified by site characteristics: differences in performance were more pronounced on unscarified sites. Although not quantitatively documented, his seedlings seem to have been physiologically similar.

Table 1. Linear relationships between stem caliper (diameter, mm) and five morphological characteristics of Douglas-fir seedlings of four stock types from a single Twin Harbors, Washington, seedlot.

Characteristics	Stock type[1]	r^2
Height (cm) and stem caliper	2+0S	0.41
	2+0L	0.31
	1+1	0.26
	2+1	0.45
Root dry weight (g) and stem caliper	2+0S	0.80
	2+0L	0.78
	1+1	0.69
	2+1	0.82
Shoot dry weight (g) and stem caliper	2+0S	0.89
	2+0L	0.81
	1+1	0.71
	2+l	0.85
Total dry weight (g) and stem caliper	2+0S	0.88
	2+0L	0.83
	1+1	0.76
	2+1	0.87
Shootroot ratio (dry) and stem caliper	2+0S	0.00
	2+0L	0.00
	1+l	0.03
	2+1	0.01

[1] Standard bed density 2+0 seedlings (2+0S) were grown at ~ 209/m^2 and low density 2+0 seedlings (2+0L) at ~ 143/m^2; transplants were grown at ~ 209/m^2, transplanted at ~ 55/m^2.

Cleary et al. [18] also have reported that on sites where animal browsing, brush competition, and snow press were severe, larger seedlings had an advantage, presumably because they can sustain browsing and can grow above weeds more effectively than smaller seedlings. However, largeness can be disadvantageous under some conditions. For example, on high-elevation sites where brush competition is not excessive but desiccating winds are prevalent, a large foliar surface would tend to place greater transpirational demand on the root system (see 23.3.2.1); in that case, smaller seedlings may be preferred.

But size alone is not meaningful if the seedling is out of balance [18, 72]. A large top requires a large root system to supply water and nutrients; hence, some measure of shoot: root ratio balance is indicated. The pitfall here is that root weight or volume is not a very good indicator of the root system's ability to provide water and minerals. Total surface area of the root system or some measure of root-system fibrosity or absorption capacity is needed, but, unfortunately, such quantities are difficult and costly to determine. Furthermore, the root surface must effectively contact the soil after planting.

Dickson et al. [23] attempted to develop an integrated approach to quantifying morphological quality by formulating an index which included several morphological features. Their quality index (QI) was calculated as:

$$QI = \text{seedling dry wt. (g)} / \left[\frac{\text{height (cm)}}{\text{diameter (mm)}} + \frac{\text{top wt. (g)}}{\text{root wt. (g)}} \right] \quad (9)$$

where the higher the index, the better the seedling. When I applied this index to Douglas-fir seedlings of four stock types (plug, 2+0, 1+1, and 2+1), it gave values of 0.99, 1.79, 1.88. and 2.30, respectively. This ranking corresponds quite closely (and perhaps coincidentally) with observed performance rankings of these stock types in many of our field trials.

In conclusion, morphological characteristics probably exert the ultimate influence on seedling performance only when physiological characteristics do not differ significantly among seedlings.

23.4 Current Practice

Of the nurseries surveyed in the OSU Nursery Survey (see chapter 1, this volume), all reported making some routine measurements of seedling quality. Each was contacted by letter or telephone to obtain detailed information on the nature, application, and interpretation of the tests used. A synthesis of this information follows.

23.4.1 Root-growth potential

Four nurseries reported using RGP to assess seedling quality. One uses the standard measurement method developed by Stone, described in 23.2.1.1, and three use the more rapid scoring system developed by Burdett, described in 23.2.1.2. One nursery indicated that RGP was measured only for troubleshooting and not routinely. Another indicated that RGP was used initially to establish the optimum lifting window and later only as an annual spot check.

23.4.2 Frost hardiness

Nine nurseries reported assessing frost hardiness on a more or less routine basis. All use the test to determine when frost protection is needed in fall and spring, and all but one reported testing l +0s only. The most commonly used method is to place potted seedlings in an on-site freezer chest and reduce the temperature to some level, then remove the seedlings and assess damage after a few days. Temperatures used

generally bracket the expected LT_{50} value. Methods of assessing damage ranged from odor, to general visual appearance, to cutting buds, to scraping bark to detect dead cambium. One nursery reported using frost hardiness as an indicator of when to begin fall lifting, but none reported using it as an indicator of seedling quality before shipping stock to customers.

23.4.3 Stress resistance

Only three nurseries measure stress resistance. They use the services of Oregon State University and the test methods described in 23.2.3. One nursery reported that results of stress tests did not agree well with results of RGP tests and that RGP correlated better with seedling survival in the field. Most stress tests are conducted for reforestation personnel rather than for nurseries.

23.4.4 Dormancy

Seven nurseries assess seedling dormancy. Three use the oscilloscope, as described by Ferguson (see 23.3.1.4). One uses visual appearance of buds and foliage color; another performs a budbreak test. Two coastal nurseries monitor chilling sums and begin lifting when cumulative hours of air temperature below 6°C approach 600. One reported experimenting with chilling sums. All of the above assess dormancy only as a guide to determine when to begin lifting, and none use it as a measure of seedling quality itself.

23.4.5 Water relations

Water status was the most common measure of seedling quality. Thirteen nurseries routinely measure water status with a pressure chamber. The most common application is monitoring stress buildup in seedlings during lifting, grading, packing, and storage.

Each nursery has guidelines regarding acceptable levels of stress. Generally, nurseries do not lift when stress exceeds 1.0 or 1.5 MPa and do not permit stress to exceed 0.5 MPa when grading and packing. Some nurseries use predawn pressure-chamber measurements to indicate the need for irrigation and to manage development of late-summer stress for dormancy induction. But none reported measuring stress in root systems or on individual needles or using P-V curves or other more advanced techniques, although one Oregon nursery is beginning some preliminary work in this area.

23.4.6 Nutrition

Eight nurseries reported monitoring seedling foliar nutrient content. In all but one, this is restricted to the 1+0 crop. In all cases, samples are sent to a regional laboratory (either the Ministry of Forests Laboratory in Victoria or Oregon State University in Corvallis) for testing and interpretation. Most samples are taken in late summer or fall and the results used to fine-tune fertilizer prescriptions for the following year. Many foliar analyses are used in conjunction with soil nutrient analyses (see chapter 8, this volume). Again, in no case was nutrient content used as an index of seedling quality itself. No mention was made of carbohydrate analysis.

23.4.7 Morphology

Virtually all nurseries grade seedlings based upon their morphological characteristics. In almost every case, stem caliper at the root collar and shoot height (from the root collar to the terminal bud) are the characteristics measured. Also, seedlings showing any visible signs of damage such as torn roots, scarred bark, or broken tops are normally culled. Cull standards vary with nursery and species and are often determined by the buyer of the stock.

23.5 Summary

Attributes of seedling quality are categorized as either performance attributes (RGP, frost hardiness, stress resistance) or material attributes (bud dormancy, water relations, nutrition, morphology). Performance attributes are assessed by placing samples of seedlings into specified controlled environments and evaluating their responses. Although some effective short-cut procedures are being developed, performance tests tend to be time consuming; however, they produce results on whole-plant responses which are often closely correlated with field performance. Material attributes, on the other hand, reflect only individual aspects of seedling makeup and are often poorly correlated with performance.

Bud dormancy status seems to be correlated, at least phenologically, with the three performance attributes. Unfortunately, no rapid method of measuring dormancy intensity is yet available, although several are promising. Nursery chilling sums seem to offer a good method of indirectly estimating dormancy in some species.

Seedling water status also is related to all three performance attributes, but in complex and interacting ways. Although several methods are available for measuring water status, the pressure chamber is the method of choice because it (1) is rapid, accurate, and simple to use, (2) measures water status in energy terms, and (3) permits estimation of the turgor and osmotic components of water potential.

Seedling nutrition affects all aspects of seedling performance. However, measurements of nutritional status (usually made on foliage) are poor indicators of seedling quality.

Seedling morphology is a widely used grading criterion. More often than not, larger seedlings tend to outperform smaller seedlings on many sites. However, physiological factors generally override size effects.

Of 21 Northwest nurseries surveyed, all reported using at least one of the above measurement techniques. However, with the exception of morphology, these techniques were generally not used to assess seedling quality itself, but rather to monitor the effects of some cultural operation (e.g., lifting, grading, wrenching) on the seedling crop or to determine the optimum manner in which to perform such operations.

23.6 Recommendations

23.6.1 Operations

It is not realistic to recommend that nurseries routinely monitor the physiological condition of their planting stock, given the complex, time-consuming nature of the available methods. With respect to performance attributes, which give the most useful predictions, test results would be available too late to be of much practical use. Nearly all nurseries do monitor seedling morphology, and this practice is certainly worthwhile. Emphasizing root quality as well as traditional height and caliper standards also might be desirable.

For troubleshooting, when seedling damage is suspected, a good approach seems to be the accelerated RGP test (see 23.2.1.2). Any serious damage would generally be expected to show up under the forcing conditions described, although there are never any guarantees. Of course, this assumes that nurseries have access to controlled-environment chambers. Measurements of water potential, by pressure chamber or any other method, seem to be of limited use because a dead seedling can have either high or low water potential. Cold damage to the stem can sometimes be detected by sectioning buds, and cold damage to the roots by scraping bark to detect dead cambium. A more laborious but more definitive method seems to be the P-V curve, where freeze-caused cell lesions

are evidenced by abnormally high osmotic potentials or the lack of a linear portion of the curve. However, the relationship **between degree** of damage and impact on performance has yet to be established.

A broader and perhaps ultimately more useful approach to assessing seedling quality has two parts:

(1) Nurseries should systematically collect air temperature data beginning in early autumn so that, over 3 or more years, a typical chilling curve for that nursery could be developed. From this information, plus a record of the lifting date and time in storage for each stock order, nursery managers could infer the degree of stock dormancy. Such data should accompany each stock order shipped, along with any other information that might bear on the performance potential of that stock (e.g., cold-storage temperature, climatic abnormalities during lifting, etc.).

(2) In planning the performance tracking for each year's plant, regeneration personnel should select for tracking stock that spans a range of lift-store combinations. Performance of this stock-whether successes or failures—should be systematically reported back to the nursery each year. In addition, woods personnel should note site weather conditions and any abnormalities which might have affected stock performance (e.g., inadvertent overheating of stock, poor performance of planting crew, etc.). With nursery and woods personnel cooperating in such a manner, it should be possible over time to build a data base to assist nursery managers not only in fine-tuning their lift-store operations but also in accurately predicting, rather than directly assessing, stock quality.

23.6.2 Research

In my judgment, past research on assessing seedling quality has overemphasized developing a "black box" which could be used to give an immediate, categorical evaluation of a given seedling based upon some measurable property. Had this work been successful, this chapter could have been written on one page. Considering the complexity of the seedling, the planting site, and seedling-site interactions, it is doubtful that such a black box will ever be developed.

A seemingly more intelligent approach would be to establish empirical relationships between seedling quality (assessed as cold hardiness, RGP, and some measure of drought resistance) and seedling history. It is already well known that these properties change seasonally in predictable ways, all three tending to be low in fall, high in winter, and low again in spring. Hence, winter-lifted seedlings tend always to be of the best physiological condition. Although it has not yet been rigorously demonstrated, these properties are probably related to the bud-dormancy intensity of the seedling as it weakens through winter in response to chilling. Exploring the relationships between chilling history and the above properties seems a potentially valuable avenue for research.

One complicating factor is the effect of cold storage on these performance attributes. It is known, for example, that cold storage affects RGP. Depending upon the lifting date. RGP can increase, decrease, or remain constant in storage. Why does this happen? Are there predictable patterns? Could RGP be predicted from chilling history? The same questions apply to cold hardiness and drought resistance. With these empirical relationships established for given regions and species, it would be possible operationally to make educated predictions of seedling quality without ever examining the seedlings themselves.

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References

1. Askren, C. A., and R. K. Hermann. 1979. Is the oscilloscope technique suitable for predicting survival of planting stock? Tree Planters' Notes 30:7-12.
2. Aston, M. J., and D. M. Paton. 1973. Frost room design for radiation frost studies in Eucalyptus. Australian J. Botany 21:193-199.
3. Becwar, M. R., C. Rajashekar, K. J. Hansen Bristow, and M. J. Burke. 1981. Deep undercooling of tissue water and winter hardiness limitations in timberline flora. Plant Physiology 68:111-114.
4. Bixby, J. A., and G. N. Brown. 1974. Rapid determination of cold hardiness in black locust seedlings using a pressure chamber. 3rd North American Forest Biology workshop, Boulder, Colorado. Abstr. No. 12.
5. Blazich, F. A., D. R. Evert, and D. E. Bee. 1974. Comparison of three methods of measuring winter hardiness of internodal stem sections of *Forsythia intermedia* "Lynwood." J. American Society for Horticultural Sci. 9:211-214.
6. Brown, G. N. 1980. Control of frost hardiness in tree shoots. Pages 297-304 *in* IUFRO Proc., joint workshop on xylem and shoot growth physiology (C. H. A. Little, ed.). Fredericton, New Brunswick, Canada.
7. Bunting, W. R. 1980. Seedling quality: growth and development—soil relationships, seedling growth and development, density control relationships. Pages 21-42 *in* Proc., North American forest tree nursery soils workshop (L. P. Abrahamson and D. H. Bickelhaupt, eds.). State Univ. New York, Coll. Environ. Sci. and Forestry, Syracuse.
8. Burdett, A. N. 1979. New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. Can. J. Forest Res. 9:63-67.
9. Burdett, A. N. 1981. Personal communication, B. C. Ministry of Forests, Victoria.
10. Campbell, R. K. 1978. Regulation of bud-burst timing by temperature and photoregime during dormancy. Pages 19-33 *in* Proc., V. North American forest biology workshop (C. A. Hopis and A. E. Squillace, eds.). Univ. of Florida, Gainesville.
11. Campbell, R. K., and A. I. Sugano. 1975. Phenology of bud-burst in Douglas-fir related to provenance, photoperiod, chilling, and flushing temperature. Botanical Gazette 136:290-298.
12. Campbell, R..K., and A. I. Sugano. 1979. Genecology of bud-burst phenology in Douglas-fir: response to flushing temperature and chilling. Botanical Gazette 140:223-231.
13. Carlson, W. C., W. D. Binder, C. O. Feenan, and C. L. Preisig. 1980. Changes in mitotic index during onset of dormancy in Douglas-fir seedlings. Can. J. Forest Res. 10:371-378.
14. Cary, J. W., and H. D. Fisher. 1969. Plant moisture stress: a portable freezing point meter compared with the psychrometer. Agronomy J. 61:302-305.
15. Chavasse. C. G. R. 1980. Planting stock quality: a review of factors affecting performance. New Zealand J. Forestry 25:144-171.
16. Cheung, Y. N. S., M. T. Tyree, and J. Dainty. 1975. Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. Can. J. Botany 53:1342-1346.
17. Childs, S. (Date unknown.) Evaluation of a rapid technique to estimate plant water potential for applications in forestry. Unpublished manuscript, Washington State Univ., Pullman. 7 p.
18. Cleary, B. D., R. D. Greaves, and P. W. Owston. 1978. Seedlings. Pages 63-97 in Regenerating Oregon's forests (B. D. Cleary, R. D. Greaves, and R. K. Hermann, eds.). Oregon State Univ. Ext. Serv., Corvallis.

19. Cleary, B. D., and J. B. Zaerr. 1980. Pressure chamber techniques for monitoring and evaluating seedling water status. New Zealand J. Forestry Sci. 10:133-141.
20. Davis, W., A. Shigo, and R. Weyrick. 1979. Seasonal changes in electrical resistance of inner bark in red oak, red maple, and eastern white pine. Forest Sci. 25:282-286.
21. Dexter, S. T., W. E. Tottingham, and L. F. Graber. 1930. Preliminary results in measuring the hardiness of plants. Plant Physiology 5:215-223.
22. Dexter, S. T., W. E. Tottingham, and L. F. Graber. 1932. Investigations of the hardiness of plants by measurement of electrical conductivity. Plant Physiology 7:63-78.
23. Dickson, A., A. L. Leaf, and J. F. Hosner. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. Forestry Chronicle 36:10-13.
24. Dobbs, R. C. 1976. Effect of initial mass of white spruce and lodgepole pine planting stock on field performance in the British Columbia interior. Can. Forestry Serv., Dep. Environ. Rep. BC-X-149. 14 p.
25. Edgren. J. W. 1980. The reforestation system—a team effort. Pages 12-20 in Proc., North American forest tree nursery soils workshop (L. P. Abrahamson and D. H. Bickelhaupt, eds.). State Univ. New York, Coll. Environ. Sci. and Forestry, Syracuse.
26. Ehlig, C. F. 1962. Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiology 37:288-290.
27. Erez, A., G. A. Couvillon, and C. H. Hendershott. 1979. The effect of cycle length on chilling negation by high temperatures in dormant peach leaf buds. J. American Society for Horticultural Sci. 104: 573-576.
28. Farmer, R. E., Jr. 1978. Seasonal carbohydrate levels in roots of Appalachian hardwood planting stock. Tree Planters' Notes 29:22-24.
29. Ferguson, R. B., R. A. Ryker, and E. D. Ballard. 1975. Portable oscilloscope technique for detecting dormancy in nursery stock. U.S.D.A. Forest Serv., Intermountain Forest and Range Exp. Sta., Ogden, Utah. Gen. Tech. Rep. INT-26. 16 p.
30. Fisher, H. D. 1972. An inexpensive method of determining plant moisture stress using freezing-point depression. Soil Sci. 113:383-385.
31. Flint, H. L., B. R. Boyce, and D. J. Beattie. 1967. Index of injury—a useful expression of freezing injury to plant tissues as determined by the electrolytic method. Can. J. Plant Sci. 47:229-230.
32. Gilmore, A. R. 1962. Root growth of transplanted loblolly pine (*Pinus taeda* L.) seedlings in relation to chemical root reserves. Illinois State Academy of Sci. Trans. 55:38-41.
33. Glerum, C. 1970. Vitality determinations of tree tissue with kilocycle and megacycle electrical impedance. Forestry Chronicle 46:63-64.
34. Glerum, C. 1976. Frost hardiness of forest trees. Pages 403-420 *in* Tree physiology and yield improvement (M. G. R. Cannell and F. T. Last, eds.). Academic Press, New York.
35. Glerum, C., J. L. Farrar, and R. L. McLure. 1966. A frost hardiness study of six coniferous species. Forestry Chronicle 42:69-75.
36. Green, L. M., and I. J. Warrington. 1978. Assessment of frost damage in radiata pine seedlings using the diffusate conductivity technique. New Zealand J. Forestry Sci. 8:344-350.
37. Gross, K. 1980. The pressure-volume technique as an aid to the characterization of freshness of forest planting stock. Pages 137-151 in Proc., Characterization of plant material (H. Schmidt-Vogt, ed.). IUFRO meeting, Div. 1, Freiburg i. Br., Federal Republic of Germany.
38. Hatch, A. H., and D. R. Walker. 1969. Rest intensity of dormant peach and apricot leaf buds as influenced by temperature, cold hardiness and respiration. J. American Society for Horticultural Sci. 94:304-307.
39. Havis, J. R. 1976. Root hardiness of woody ornamentals. HortScience 11:385-386.
40. Hellkvist, J., G. P. Richards, and P. G. Jarvis. 1974. Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. J. Applied Ecology 11:637-667.
41. Hellmers, H. 1962. Physiological changes in stored pine seedlings. Tree Planters' Notes 53:9-10.
42. Hermann, R. K. 1962. The effect of short-term exposure of roots on survival of 2-0 Douglas-fir stock. Tree Planters' Notes 52:28-30.
43. Hermann, R. K. 1964. Effects of prolonged exposure of roots on survival of 2-0 Douglas-fir seedlings. 1. Forestry 62:401-403.
44. Hermann, R. K. 1967. Seasonal variation in sensitivity of Douglas-fir seedlings to exposure of roots. Forest Sci. 13:140-149.
45. Hermann, R. K., and D. P. Lavender. 1976. Large stock: is it really superior? IUFRO, 14th World Congress. Contributed paper, Working Group 1.05.04. Mimeo. 8 p.
46. Hermann, R. K., and D. P. Lavender. 1979. Testing the vigor of coniferous planting stock. Oregon State Univ., Forest Res. Lab., Corvallis. Res. Note 63. 3 p.
47. Hermann, R. K., D. P. Lavender, and J. B. Zaerr. 1972. Lifting and storing western conifer seedlings. Oregon State Univ., Forest Res. Lab., Corvallis. Res. Pap. 17. 8 p.
48. Hocking, D., and R. D. Nyland. 1971. Cold storage of coniferous seedlings: a review. State Univ. New York, Coll. Forestry, Syracuse. Applied Forestry Res. Institute Rep. 6. 70 p.
49. Höfler, K. 1920. Ein Schema für die osmotische Leistung der Pflanzenzelle. Deutsche Botanische Gesellschaft, Berlin 38:288-298.
50. Holbo, H. R., C. A. Askren, and R. K. Hermann. 1981. Does the oscilloscope technique reveal the dormancy status of Douglas-fir and ponderosa pine? Forest Sci. 27:405-412.
51. Hsaio, T. C., E. Acevedo, E. Fereres, and D. W. Henderson. 1976. Stress metabolism: water stress, growth and osmotic adjustment. Philosophical Trans. of the Royal Society of London 273:479-500.
52. Incoll, L. D., S. P. Long, and M. R. Ashmore. 1977. SI units in publications in plant science. Current Advances in Plant Sci. 28:331-343.
53. laramillo, A. 1980. Review of techniques used to evaluate seedling quality. Pages 84-95 *in* Proc., Intermountain Nurseryman's Assoc. and Western Forest Nursery Association. U.S.D.A. Forest Serv., Intermountain Forest and Range Exp. Sta., Ogden, Utah. Gen. Tech. Rep. INT-109.
54. Johnson, N. E,, and D. G. Nielson. 1969. Pressure chamber measurements of water stress in individual pine fascicles. Forest Sci.15:452-453.
55. Knipling, E. B. 1967. Measurement of leaf water potential by the dye method. Ecology 48:1038-1040.
56. Kozlowski, T. T. (ed.). 1968. Water deficits and plant growth. Vol. I. Development, control, and measurement. Academic Press, New York. 390 p.
57. Kozlowski, T. T. (ed.). 1968. Water deficits and plant growth. Vol. II. Plant water consumption and response. Academic Press, New York. 333 p.
58. Kozlowski, T. T. (ed.). 1972. Water deficits and plant growth. Vol. III. Plant responses and control of water balance. Academic Press, New York. 368 p.
59. Kozlowski, T. T. (ed.). 1976. Water deficits and plant growth. Vol. IV. Soil water measurement, plant responses, and breeding for drought resistance. Academic Press, New York. 383 p.
60. Kozlowski, T. T. (ed.). 1978. Water deficits and plant growth. Vol. V. Water and plant disease. Academic Press, New York. 323 p.
61. Kozlowski, T. T. (ed.). 1981. Water deficits and plant growth. Vol. VI. Woody plant communities. Academic Press, New York. 582 p.
62. Kramer, P. J., and T. T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York. 811 p.
63. Lavender, D. P., and R. K Hermann. 1970. Regulation of the growth potential of Douglas-fir seedlings during dormancy. New Phyfologist 69:675-694.
64. Lavender, D. P., and P. F. Wareing. 1972. Effects of daylength and chilling on the responses of Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) seedlings to root damage and storage. New Phytologist 71:1055-1067.
65. Levitt, J. 1980. Responses of plants to environmental stresses. Vol. I. Chilling, freezing and high temperature stresses. Academic Press, New York. 497 p.
66. Levitt, J. 1980. Responses of plants to environmental stresses. Vol. II. Water, radiation, salt and other stresses. Academic Press, New York. 607 p.
67. Li, P. H. 1978. Plant cold hardiness research. HortScience 13:222-224.
68. Mazur, P. 1969. Freezing injury in plants. Annual Review of Plant Physiology 20:419-448.
69. McCracken, I. 1978. Carbon dioxide uptake of pine seedlings after cool storage. Forest Sci. 24:17-25.

70. McCreary, D. 1982. Personal communication, Department of Forest Science, Oregon State Univ., Corvallis.
71. McMinn, R. G. 1980. Root growth capacity and field performance of various types and sizes of white spruce stock following outplanting in the central interior of British Columbia. Pages 37-41 *in* Proc., Characterization of plant material (H. Schmidt-Vogt. ed.). IUFRO meeting, Div. I, Freiburg i. Br., Federal Republic of Germany.
72. Menzies, M. I. 1980. Effect of nursery conditioning on the water relations of two year old Douglas-fir seedlings after lifting and outplanting. Ph.D. thesis. Univ. of Washington, Seattle. 208 p.
73. Menzies, M. I., and D. G. Holden. 1981. Seasonal frost tolerance of *Pinus radiata*, *Pinus muricata* and *Pseudotsuga menziesii.* New Zealand J. Forestry Sci. I 1:92-99.
74. Nelson, E. A., and D. P. Lavender. 1979. The chilling requirement of western hemlock seedlings. Forest Sci. 25:48 5-490.
75. Nienstaedt, H. 1966. Dormancy and dormancy release in white spruce. Forest Sci. 12:374-384.
76. Nienstaedt, H. 1967. Chilling requirements in seven *Picea* species. Silvae Genetica 16:65-68.
77. Owens, J. N.1968. Initiation and development of leaves in Douglas-fir. Can. J. Botany 46:271-278.
78. Owens, J. N., and M. Molder. 1973. A study of DNA and mitotic activity in the vegetative apex of Douglas-fir during the annual growth cycle. Can. J. Botany 51:1395-1409.
79. Parmelee, K. 1979. The relationship of electrical impedance to vegetative maturity and dormancy in red-osier dogwood. M.S. thesis, Oregon State Univ., Corvallis. 87 p.
80. Pellett, N. E., and D. B. White. 1969. Soil-air temperature relationships and cold acclimation of container-grown *Juniperus chinensis* "Hetzi." J. American Society for Horticultural Sci. 94:453-456.
81. Perry, T. O. 1971. Dormancy of trees in winter. Science 171:29-36.
82. Puttonen, P. 1980. Effect of temporary storage temperature on carbohydrate levels in Scots pine seedlings and planting success. Pages 169-179 *in* Proc., Characterization of plant material (H. Schmidt-Vogt, ed.). IUFRO meeting, Div. 1, Freiburg i. Br., Federal Republic of Germany.
83. Richardson, E. A., S. D. Seeley, and D. R. Walker. 1974. A model for estimating the completion of rest for "Redhaven" and "Elberta" peach trees. HortScience 9:331-332.
84. Ritchie, G. A. 1982. Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can. J. Forest Res. 12:905-912.
85. Ritchie, G. A., and J. R. Dunlap. 1980. Root growth potential: its development and expression in forest tree seedlings. New Zealand J. Forestry Sci. 10:218-248.
86. Ritchie, G. A., and T. M. Hinckley. 1971. Evidence for error in pressure-bomb estimates of stem xylem potentials. Ecology 52:534-536.
87. Ritchie, G. A., and T. M. Hinckley. 1975. The pressure chamber as an instrument for ecological research. Advances in Ecological Res. 9:165-254.
88. Ritchie, G. A., and R. G. Shula. 1983. Seasonal changes of tissue water relations in shoots and root systems of Douglas-fir seedlings. Forest Sci. (in press).
89. Robotham, R. W., J. Lloyd, and I. J. Warrington. 1978. A controlled environment room for producing advective white or black frost conditions. J. Agric. Engineering Res. 23:301-311.
90. Romberger. J. A. 1963. Meristems, growth and development in woody plants. U.S.D.A. Forest Serv., Washington, D.C. Tech. Bull. 1293. 214 p.
91. Rosvall-Ahnebrink, G. 1982. Personal communication, Institution f6r Skogsforyngring Sveriges Lantbruksuniversitet, Garpenberg, Sweden.
92. Rowley, J. A., C. G. Tunnicliffe, and A. O. Taylor. 1975. Freezing sensitivity of leaf tissue of C4 grasses. Australian J. Plant Physiology 2:447-451.
93. Sakai, A. 1978. Low temperature exotherms of winter buds of hardy conifers. Plant and Cell Physiology 19:1439-1446.
94. Salisbury, F. B., and C. W. Ross. 1978. Plant physiology. Wadsworth Publishing Co., Inc., Belmont, California. 438 p.
95. Samish, R. M. 1954. Dormancy in woody plants. Annual Review of Plant Physiology 5:183-204.
96. Sandvik, M. 1980. Environmental control of winter stress tolerance and growth potential in seedlings of *Picea abies* (L.) Karst. New Zealand J. Forestry Sci. 10:97-104.
97. Schmidt-Vogt, H. (ed.). 1980. Characterization of plant material. Proc., IUFRO meeting, Div. 1. June 23-26. Freiburg i. Br., Federal Republic of Germany. 193 p.
98. Schmidt-Vogt, H. 1981. Morphological and physiological characteristics of planting stock: present state of research and research tasks for the future. Pages 433-446 *in* Proc., IUFRO XVII World Congress. Kyoto, Japan.
99. Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339-346.
100. Shardkov, V. W. 1948. New field method for the determination of the suction pressure of plants (in Russian). Doklady Akademii Nauk. SSSR 60:169-172.
101. Shepherd, K. R. 1980. The use of controlled environments in forestry research. New Zealand J. Forestry Sci. 10:105-115.
102. Siminovitch, D. 1963. Evidence from increase in ribonucleic acid and protein synthesis in autumn for increase in protoplasm during the first frost hardening of black locust bark cells. Can. J. Botany 41:1301-1308.
103. Slatyer, R. O. 1967. Plant-water relationships. Academic Press, New York. 366 p.
104. Slavik, B. 1974. Methods of studying plant water relations. Springer-Verlag. New York. 449 p.
105. Spanner, D. C. 1951. The Peltier effect and its use in the measurement of suction pressure. J. Experimental Botany 11:145-168.
106. Steponkus, P. L., and F. O. Lanphear. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Plant Physiology 42:1423-1426.
107. Stergios, B. G., and G. S. Howell. Jr. 1973. Evaluation of viability tests for cold stressed plants. J. American Society for Horticultural Sci. 98:325-330.
108. Stone, E. C. 1955. Poor survival and the physiological condition of planting stock. Forest Sci. 1:90-94.
109. Stone, E. C. 1970. Variation in the root-growth capacity of ponderosa pine transplants. Pages 40-46 *in* Regeneration of ponderosa pine (R. K. Hermann, ed.). Oregon State Univ., Corvallis.
110. Stone, E. C. 1980. Unpublished data. Univ. of California, Berkeley.
111. Stone, E. C., and J. L. Jenkinson. 1970. Influence of soil water on root growth capacity of ponderosa pine transplants. Forest Sci. 16:230-239.
112. Stone, E. C., J. L. Jenkinson, and S. L. Krugman. 1962. Root regenerating potential of Douglas-fir seedlings lifted at different times of the year. Forest Sci. 8:288-297.
113. Stone, E. C., G. H. Schubert, R. W. Benseler, F. J. Baron, and S. L. Krugman. 1963. Variation in the root regenerating potentials of ponderosa pine from four California nurseries. Forest Sci. 9:217-225.
114. Sutton, R. F. 1979. Planting stock quality and grading. Forest Ecology and Management 2:123-132.
115. Thompson, B. E., and R. Timmis. 1978. Root regeneration potential in Douglas-fir seedlings: effect of photoperiod and air temperature on its evaluation and control. Pages 86-109 *in* Proc., IUFRO symp. on root physiology and symbiosis (A. Riedacker and J. Gagnaire-Michard, eds.). Nancy, France.
116. Timmis, K. A., L. H. Fuchigami, and R. Timmis. 1981. Measuring dormancy: the rise and fall of square waves. HortScience 16:200-202.
117. Timmis. R. 1976. Methods of screening tree seedlings for frost hardiness. Pages 421-435 *in* Tree physiology and yield improvement (M. G. R. Cannell and F. T. Last, eds.). Academic Press, New York.
118. Timmis. R. 1980. Stress resistance and quality criteria for tree seedlings: analysis, measurement and use. New Zealand J. Forestry Sci. 10:21-53.
119. Timmis, R. 1980. Unpublished data, Weyerhaeuser Technology Center, Tacoma, Washington.
120. Timmis, R. 1981. Unpublished data, Weyerhaeuser Technology Center, Tacoma, Washington.
121. Timmis, R., and J. G. Worrall. 1974. Translocation of dehardening and bud-break promoters in climatically 'split' Douglas-fir. Can. J. Forest Res. 4:229-237.
122. Truelove, B., D. E. Davis, and L. R. Jones. 1974. A new method for detecting photosynthesis inhibitors. Weed Sci. 22:15-17.
123. Turner, N. C., and P. J. Kramer (eds.). 1980. Adaptation of plants to water and high temperature stress. John Wiley and Sons. New York. 482 p.

124. Tyree, M. T., and H. T. Hammel. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J. Experimental Botany 23:267-282.
125. van den Driessche, R. 1969. Influence of moisture supply, temperature, and light on frost-hardiness changes in Douglas-fir seedlings. Can. J. Botany 47:1765-1772.
126. van den Driessche, R. 1973. Prediction of frost hardiness in Douglas-fir seedlings by measuring electrical impedance in stems at different frequencies. Can. J. Forest Res. 3:2 56-264.
127. van den Driessche, R. 1975. Flushing response of Douglas-fir buds to chilling and to different air temperatures after chilling. B.C. Forest Serv., Victoria. Res. Note 71. 22 p.
128. van den Driessche, R. 1976. Prediction of cold hardiness in Douglas-fir seedlings by index of injury and conductivity methods. Can. J. Forest Res. 6:511-5l5.
129. van den Driessche, R. 1980. Health, vigor and quality of conifer seedlings in relation to nursery soil fertility. Pages 100-120 *in* Proc., North American forest tree nursery soils workshop (L. P. Abrahamson and D. H. Bickelhaupt, eds.). State Univ. New York, Coll. Environ. Sci. and Forestry, Syracuse.
130. van den Driessche, R. 1980. Effects of nitrogen and phosphorus fertilization on Douglas-fir nursery growth and survival after out-planting. Can. J. Forest Res. 10:65-70.
131. Vegis, A. 1964. Dormancy in higher plants. Annual Review of Plant Physiology 15:185-224.
132. Venn, K. 1980. Winter vigor in Picea abies (L.) Karst. VII. Development of injury to seedlings during overwinter cold storage. A literature review. Reports of the Norwegian Forest Res. Institute 35.9:485-529.
133. Wareing, P. F. 1969. The control of bud dormancy in seed plants. Symposia of the Society of Experimental Biology 23:241-262.
134. Warrington, I. J., and D. A. Rook. 1980. Evaluation of techniques used in determining frost tolerance of forest planting stock: a review. New Zealand J. Forestry Sci. 10:116-132.
135. Weatherley, P. E. 1950. Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. New Phytologist 49:81-97.
136. Weiser, C. J. 1970. Cold resistance and injury in woody plants. Science 169:1269-1278.
137. Wells, S. P. 1979. Chilling requirements for optimal growth of Rocky Mountain Douglas-fir seedlings. U.S.D.A. Forest Serv., Intermountain Forest and Range Exp. Sta., Ogden, Utah. Res. Note INT-254.9 p.
138. Wiebe, H. H., G. S. Campbell, W. R. Gardner, S. L. Rawlins, J. W. Cary, and R. W. Brown. 1971. Measurement of plant and soil water status. Utah Agric. Exp. Sta. Bull. 484. 71 p.
139. Wilner, J. 1960. Relative and absolute electrolytic conductance tests for frost hardiness of apple varieties. Can. J. Plant Sci. 40:630-637.
140. Winjum, J. K. 1963. Effects of lifting date and storage on 2+0 Douglas-fir and noble fir. J. Forestry 61:648-654.
141. Wommack, D. E. 1964. Temperature effects on the growth of Douglas-fir seedlings. Ph.D. thesis, Oregon State Univ., Corvallis. 176 p.
142. Wright, S. T. C. 1975. Seasonal changes in the levels of free and bound abscisic acid in blackcurrant (*Ribes nigrum*) buds and beech (Fagus sylvatica) buds. J. Experimental Botany 26:161-174.
143. Zaerr, J. B. 1972. Early detection of dead plant tissue. Can. J. Forest Res. 2:105-1 l0.
144. Zaerr, J. B., and D. P. Lavender. 1980. Analysis of plant growth substances in relation to seedling and plant growth. New Zealand J. Forestry Sci. 10:186-195.

Appendix 1: Simplified Procedure for Constructing Pressure-Volume Curve

(l) Prepare in advance about 40 sections of 3- or 5-mm inside-diameter plastic tubing by cutting it into 5-cm-long sections and filling each section with dry tissue paper.

(2) In late afternoon or evening, select the seedling to be tested and sever it at the root collar. The shoot should be small enough that it can be placed into a pressure chamber. With a large seedling, use only the terminal portion.

(3) Partially submerge the shoot in room-temperature water overnight so that it becomes saturated (reaches full turgor).

(4) Early the following morning, remove the shoot and surface-dry it with a soft towel.

(5) Remove the bark from the basal 1 cm of the shoot and enclose the foliage in a plastic bag. The bag should be perforated and tied to the stem near the base.

(6) Place the bagged shoot into the pressure chamber and measure the balance pressure (P*); balance pressure is the same as plant moisture stress or shoot water potential. Record this value in space "A_1" on the data sheet (Fig. A1-1). If the pressure is greater than 0.1 MPa (1 bar), it indicates that the shoot is not at full turgor: it must be discarded and another sample selected.

(7) Weigh a piece of tissue-filled tubing to the nearest 0.001 g and record this value in space "B," (Fig. A1-1). Place the tubing over the end of the shoot which is protruding from the pressure chamber so that the dry tissue is in contact with the xylem surface.

(8) Increase the chamber pressure 0.5 MPa (5 bars) and hold it constant for 10 minutes. Because the time period is important, it is desirable to use a laboratory timer.

(9) After 10 minutes, remove the tube and record its weight in space "C_1." The weight gain in grams is due to the weight of the sap absorbed by the tissue and equals the incremental volume of sap lost in cubic centimeters at that pressure.

(10) Slightly reduce the chamber pressure to draw any sap away from the cut surface; then slowly increase the pressure, determine a new balance pressure, and record it in space "A_2."

(11) Weigh another piece of plastic tube, record its weight in space "B_2," and place it atop the cut stem as in step (7).

(12) Repeat steps (7) through (11) about 2 5 times.

The data sheet will then contain a series of P* values, along with pairs of corresponding initial and final tube weights. Calculate the reciprocal of each pressure (1/P*) and the tube-weight difference at each pressure increment. Then calculate the cumulative tube-weight differences beginning at the first pressure and at each successive pressure to the end.

The pressure-volume (P-V) curve is constructed by plotting the values of 1/P* against the corresponding cumulative weight-loss value and should resemble the curve shown in Figure 6 of the text.

Note: The same procedure may be used on root systems if 0.3 MPa pressure increments are substituted for 0.5 MPa increments in step (8).

Seedling number _____ Date _____

Root or shoot _____ Name _____

P*	1/P*	Tubing weight, g Initial	Tubing weight, g Final	Difference	Cumulative wt. loss, g
A_1		B_1	C_1		
A_2		B_2	C_2		
…		…	…		
…		…	…		
…		…	…		
A_{25}		B_{25}	C_{25}		

Figure A1-1. Sample pressure-volume data sheet.