Target Seedling Symposium

Chapter 9
Seedling Moisture Status

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ABSTRACT

The water status of nursery tree seedlings can be deter-
mined by measuring seedling water content, and by liquid
equilibration, psychrometric, and pressure chamber tech-
niques. The latter two techniques measure water poten-
tial, an expression of the free energy of water which is
closely related to physiological functions. Liquid equili-
bration methods are laborious, time consuming, and
imprecise. Water potential can be measured very accu-
rately with thermocouple psychrometers, but long equili-
bration times and other technical requirements make this
method best suited for laboratory use. The hydraulic leaf
press is easy to use and economical; however, endpoints
vary with the type of tissue and with the level of water
potential. The best choice for nursery work is the pressure
chamber. With it, measurements are fast, simple, and
accurate. It can be used to obtain estimates of osmotic
and turgor potential, measure the hydraulic conductivity
of root systems, and detect cold injury in roots. The pres -
sure chamber is being used to schedule irrigation and, in
some cases, to monitor water stress during lifting and
packing. During seedling growth, predawn water poten-
tials should be maintained above -0.5 MPa. Cold and
drought hardiness can be increased by exposure to mod-
erate water stresses (-0.5 to -1.0 MPa), but conditioning
procedures and responses have not been studied exten-
sively in northwest conifers. Available data indicate that
seedling water potentials down to -2.0 MPa during lifting
will not adversely affect seedlings, provided they are
moistened prior to storage. Interpretation of seedling
water potentials requires that consideration be given to
the magnitude of the water stresses, their duration, stage
of seedling growth or dormancy, the species involved,
and seedling vigor.

9.1 Introduction

The growth of plants probably is reduced more often by water deficits than by any other factor. In plants rooted in soil or other media, water deficits occur when water loss by transpiration exceeds water absorption through roots. In the case of bare-root nursery stock, water deficits can occur at any time from lifting to outplanting as a result of water loss from both shoots and roots. Whether in the nursery, cold storage or the field, conifer seedlings experience water deficits all the time, because moisture recharge never is complete. Thus water deficits are normal occurrences and become important only when they are large enough to adversely affect physiological processes, growth, or survival. Water deficits can affect practically every aspect of plant growth including anatomy, morphology, physiology and biochemistry (Kozlowski 1972, Hsiao 1973). Moderate water deficits can result in stomatal closure and reduced photosynthesis, while more severe deficits can damage the photosynthetic apparatus. Water deficits can affect respiratory and translocation processes, disrupt carbohydrate and protein metabolism, damage membrane structures of cells, and cause changes in enzyme activity. Also, water deficits often increase susceptibility to attacks by pathogens and insect—and severe desiccation, as a result of inadequate soil moisture, is a major cause of mortality of planted seedlings in the western United States. Currently, increased attention is being focused on all aspects of nursery culture of tree seedlings in attempts to improve seedling quality, and this has increased interest in the water relations of tree seedlings. This paper discusses water relations concepts and terminology, describes various methods of measuring and expressing water status in plants, and evaluates their usefulness for assessing the water status of nursery seedlings. For other reviews dealing with the water status of nursery seedlings, readers are referred to papers by Ritchie (1984), Joly (1985) and Landis et al. (1989).

9.2 Concepts and Terminology

9.2.1 Water content

The water status of a plant can be measured and expressed in a number of ways, all of which are useful for particular applications. The simplest method of determining water content involves measuring the fresh and ovendry weights of a plant part, and expressing the weight of water lost as a percent of ovendry weight. Dry weight, however, can undergo both short- and long-term changes, so attempts have been made to express leaf water content as a percentage of turgid or saturated weight. A commonly used version of this approach is Weatherley's (1950) Relative Water Content (RWC). The procedure involves weighing a leaf to obtain fresh weight, floating the leaf on water in the dark until it ceases to gain weight, and then weighing it to obtain turgid weight. The leaf is then ovendried, weighed again, and RWC calculated as:

$$RWC = \frac{\text{fresh wt. - ovendry wt.}}{\text{turgid wt. - ovendry wt.}} \times 100 \qquad (1)$$

In a fully turgid sample, RWC is 100%. A related method employing the same measurements can be used to express water content as water deficit (WD). Water deficit is calculated as:

$$WD = \frac{\text{turgid wt. - fresh wt}}{\text{turgid wt. - ovendry wt.}} \times 100 \qquad (2)$$

WD and RWC are related; RWC = 100 - WD, or RWC + WD = 100%. RWC and WD are more meaningful expressions of plant water status than water content as percent of dry weight because they relate field water content of foliage to the fully turgid condition, and thus provide a better correlation with physiological functions. Procedures most likely to give reliable results vary with species. A problem sometimes experienced with conifers is bringing the sample to full turgidity. Clausen and Kozlowski (1965) and Harms and McGregor (1962) found the use of entire needles satisfactory for several species of conifers. With proper calibration, RWC and WD can be related to plant water stress or water potential (explained below), but a calibration must be made for each species. With some species the calibration may be useful for only short-term studies, because the relationships can change with age of leaves and habitat (Knipling 1967).

9.2.2 Water potential

A meaningful assessment and expression of plant water deficit requires a quantitative measurement of water status that is directly related to physiological processes. The single most useful measurement is that of water potential because it is a measure of the chemical potential or free energy of water, it controls water movement in the soil-plant-atmosphere system, and it can be measured in plants and soil. 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 equivalent to pressure units; however, in SI (Systeme International) units (Incoll et al. 1977), pressure is expressed in pascals and 1 MPa (megapascal) 10 bars, 10 atm. or 150 psi. In this paper I will use the unit MPa which, in plant research, has largely supplanted the term "bars."

The water potential (Ψ_w) of the cells in a tree seedling is the sum of osmotic (Ψ_s), pressure or turgor (Ψ_p). matric (Ψ_m) and gravitational (Ψ_g) potentials. The influence of matric potentials is negligible and the gravitational potential becomes important only in tall trees, so that the equation for Ψ_w usually is expressed as:

$$\Psi_w = \Psi_s + \Psi_p$$

Table 9.1—*A comparison of units and descriptive terms for plant water potential (Y_w) and plant moisture stress (PMS). Y_w and PMS have the same value, but Y_w is expressed as a negative value, whereas PMS values are positive (Landis et al. 1989).*

Plant water potential Ψ_w				Plant moisture stress (PMS)		
Units		Relative	Relative	Units		Relative
MPa	Bars	rating	moisture	MPa	Bars	rating
0.0	0.0	High	Wet	0.0	0.0	Low
-0.5	-5.0			0.5	5.0	
-1.0	-10.0	Moderate		1.0	10.0	Moderate
-1.5	-15.0			1.5	15.0	
-2.0	-20.0	Low	Dry	2.0	20.0	High

where Ψ_s is a negative number and Ψ_p in turgid plants, is positive, so that Ψ_w in most situations is a negative number. Plant water potential becomes lower (more negative) as plants lose water and water deficit increases, and water movement both in plants and soils occurs along a gradient from high to low water potential. An in-depth discussion of water absorption and translocation processes in plants, which is beyond the scope of this paper, is adequately covered elsewhere for plants in general (Kramer 1983) and for containerized nursery seedlings (Landis et. al. 1989).

The pressure potential (Ψ_p) or turgor pressure portion of Equation 3 is very important because of its direct influence on cell enlargement, guard cell movements and other processes dependent on changes in cell volume. It is usually assumed to be the difference between Ψ_w and Ψ_s and varies from zero in a flaccid cell to a value equal to that of the Ψ_s in fully turgid cells. The interrelationships of these factors can be illustrated in a Höfler (1920) diagram (Figure 9.1) which shows how the components of water potential shift with a change with seedling water content. When a seedling is fully turgid, Ψ_w is zero and Ψ_p is equal and opposite in sign to the value of Ψ_s. When water content decreases sufficiently to cause Ψ_p to decline to the zero turgor point, Ψ_w equals Ψ_s. The point of zero turgor, sometimes called the "wilting point," can be physiologically detrimental to the seedling; growth stops and if the conditions persists, cellular damage and death may occur.

Another term used to describe seedling water status, "plant moisture stress" (PMS), is so well established in the nursery literature and everyday jargon that there is little doubt that it will continue to be used. This presents no
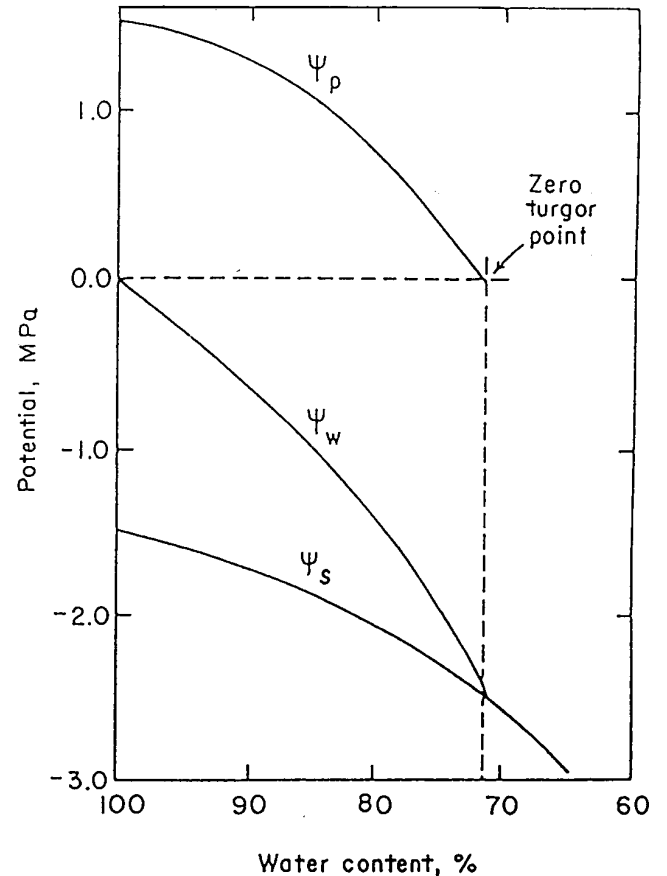

Figure 9.1—*A Höfler diagram showing the relationship between water potential (Y_w), osmotic potential (Y_s), and turgor (Y_p) over a range of water contents from full turgidity to the wilting point (Ritchie 1984).*

real problem since Ψ_w and PMS are dimensionally equivalent and differ only in sign (Table 9.1). Thus, as Ψ_w decreases (becomes more negative), PMS increases, i.e., a low Ψ_w of –2.0 MPa (-20 bars) is equivalent to a high PMS of 20 bars.

9.3 Water Potential Measurement Techniques

9.3.1 Liquid equilibration
This technique involves immersing weighed pieces of plant material in a series of solutions of known osmotic potentials (which in an unconfined solution equals 91Pw) made using sucrose, mannitol or polyethylene glycol of high molecular weight. After a suitable time period, the samples are removed, blotted and reweighed. Theoretically, the osmotic potential at which the sample neither gains nor loses water is equal to its water potential. Actually, weights are plotted over osmotic potentials

of the solutions and the water potential is taken as the value of osmotic potential where weight intersects the zero line.

A variation of the liquid equilibration method that avoids the need to weigh the sample involves measuring changes in density of the test solutions. The sample loses water to solutions with a lower water potential, diluting them, and absorbs water from solutions with a high water potential, concentrating them. The water potential of the sample is assumed to be equal to the osmotic potential of the solution which undergoes no change in density. Changes in solution concentration can be measured with a refractometer (Gaff and Carr 1964) or by observing the rise or fall of drops of dyed control solutions carefully introduced into the middle of test solutions from which samples have been removed. The dye method, first described in Russian by Shardakov (1948) and discussed in detail by Slavik (1974), has been used to measure needle water potential in several species of conifers (Brix 1966, Knipling and Kramer 1967, Cunningham and Fritts 1970). The dye method is simple, does not require expensive equipment, and can be used in both the laboratory and field, but problems can occur because of contamination of test solutions by cell sap and leaf surface residues. Its best use is to provide estimates of water potential rather than precise measurements. Leakage of solutes can be avoided by allowing weighed samples to equilibrate in air over solutions of known osmotic potentials, thereby avoiding direct contact with the solution (Slayter, 1958). While useful for some laboratory and field research, liquid and vapor equilibration techniques are too laborious and time consuming for operational nursery use.

9.3.2 Psychrometric methods
With the psychrometric method, a plant sample is enclosed in a small airtight chamber containing a fine wire chromel-constantan thermocouple and the chamber is brought to a constant temperature. The Spanner (1951) psychrometer (Figure 9.2) requires that sufficient time be allowed for both temperature equilibration and equilibration of vapor pressure of water in the air with water potential of the plant sample. A small current then is passed through the measuring junction cooling it (Peltier effect) sufficiently to condense water on the junction. After the cooling current is stopped, the rate of water evaporation from the measuring junction, and the magnitude of the resulting temperature depression, are functions of the humidity in the chamber. The voltage output from the thermocouple, recorded with a microvoltmeter, is a measure of the water potential of the sample.

The Richards and Ogata (1958) psychrometer originally was developed to measure the water potential of soil samples, but it quickly was adopted for measurement of plant water potential. A drop of water is placed on a small silver ring at the measuring junction, and voltage readings are taken when the rate of evaporation from the water droplet reaches a steady value indicated by a constant temperature depression of the thermocouple. Calibration with both types of psychrometers is performed by taking readings with salt solutions of known water potential in the chamber. Theoretical considerations for thermocouple psychrometers are discussed in detail by Rawlins (1966) and Dalton and Rawlins (1968), and much information is available in a review by Barrs (1965) and from books edited by Kozlowski (1968), Brown and Van Haveren (1972) and Slavik (1974).

The original versions of the Spanner and Richard and Ogata psychrometers have been modified in various ways to improve accuracy and reduce temperature sensitivity (Hsieh and Hungate 1970). Boyer and Knipling (1965), using a Richards and Ogata psychrometer, devised an isopiestic technique to avoid the problem of leaf resistance to diffusion of water vapor. A measurement is first made with water on the thermocouple, followed by another measurement with a solution whose water potential is close to that of the leaf sample. Voltage outputs then are graphed to determine the solution potential (equal to the sample potential) at which voltage output would be zero.

A significant innovation is the dew point hygrometer described by Campbell et al. (1973). It features an electronically maintained, constantly wet junction that produces a somewhat greater thermocouple output. Also, the very precise temperature control formerly considered necessary, now generally is not required so long as temperature remains constant during the time the measurement is being taken. Various forms of psychrometers have been used to measure water potential in conifers in detached needles (Brix 1962, Kaufmann 1968, Dosskey and Ballard 1980), attached roots (Nnyamah and Black 1977), and in tree trunks (Wiebe et al. 1970). Thermocouple psychrometers also have been modified to make *in situ* measurement of leaf water potential in aspen *(Populus tremuloides* Michx.) and in herbaceous plants (Hoffman and Hall 1976, Brown and McDonough 1977), but *in situ* leaf methods have not been used with conifers.

The psychrometer method has some distinct advantages. It is capable of making very accurate measurements of water potential, readings can be made with a small sample consisting of only one or two needles, and the system can be automated with data loggers (Stevens and Acock 1976). Also, the method permits assessment of the osmotic and turgor components of water potential. To accomplish this, a measurement of water potential is first made with an intact sample. The sample is then frozen and thawed to disrupt cell membranes and release cell sap, and another measurement is made to determine osmotic potential. Turgor potential is calculated as the difference between the water and osmotic potentials.
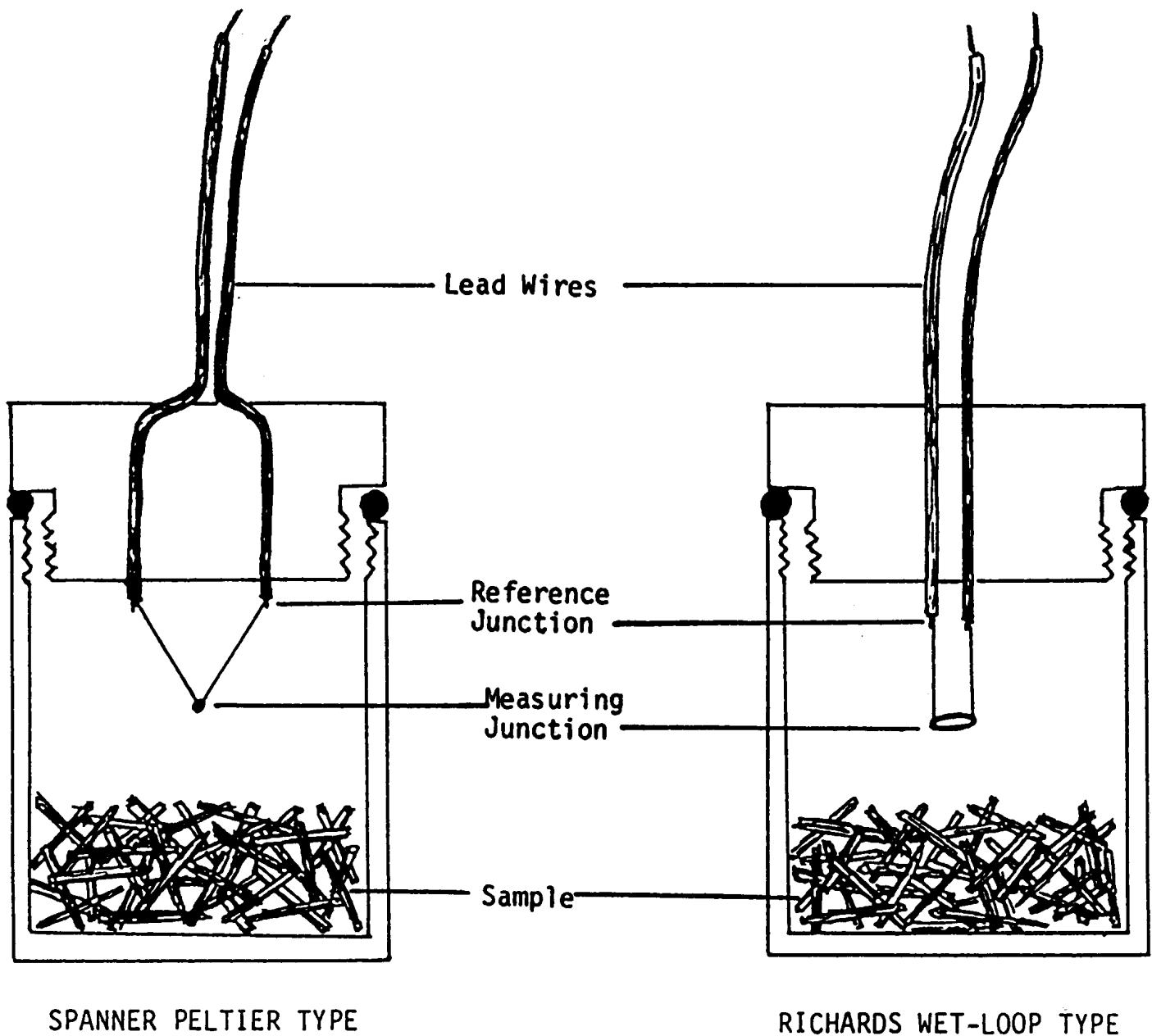
Figure 9.2—*Comparison of a Spanner and a Richards and Ogata thermocouple psychrometer. With the Spanner psychrometer, water is condensed on the measuring junction by Peltier cooling, whereas with the Richards and Ogata psychrometer, a drop of water is placed on the ring at the measuring junction.*

The psychrometric method has been very successful in the laboratory; however, certain considerations limit its usefulness in forest nurseries. Leaf surfaces and interiors of ample chambers must be kept clean, otherwise they tend to act as moisture sinks (Boyer 1972, Dixon and Grace 1982). Psychrometers need to be recalibrated periodically, and ambient temperature must be maintained fairly constant during measurements. Also, humidity equilibration with heavily cutinized conifer needles takes several hours, and cutting needles into segments can release resins (which tend to gum up the chamber) and extracel-

lular water which could result in erroneously high values of water potential. These problems have largely restricted the technique to laboratory use; however, further refinements may provide procedures applicable to some aspects of nursery research. For example, a unique temperature-corrected psychrometer now is available to continuously monitor water potential in intact plant stems (Dixon and Tyree 1984, Dixon et al. 1984). This psychrometer, which can be used with stem diameters down to about 7 mm (0.28 in), may provide a means of follow

ing changes in water potential in nursery seedlings for a period of days or weeks.

9.3.3 Hydraulic press
The J-14 hydraulic press was designed to provide a portable and inexpensive method for measuring plant water potential without the need for compressed gas. Thus it has some logistical and safety advantages over the pressure chamber. Hydraulic pressure beneath a flexible membrane is used to press a leaf or other tissue against a thick Plexiglass window until water appears at the cut edges or certain color changes occur. The pressure at this point is taken to be equal to the leaf water potential. Mixed results have been reported with the hydraulic leaf press. Cox and Hughes (1982), working with perennial grasses, found that predawn measurements with the leaf press correlated well with the pressure chamber under conditions of optimum soil moisture. Comparisons became erratic during periods of increased water stress, and large changes in water potential measured with the pressure chambers were measured as small changes with the leaf press. Shayo-Ngowi and Campbell (1980) report-ed that measurements of matric potential made using the hydraulic press with frozen tissue, including ponderosa pine, showed good agreement with matric potentials mea-sured with the pressure chamber. Brown et al. (1975) compared values obtained with thermocouple psychrom-eters and the leaf press for various plant parts including leaves and seeds, and found a poor correlation between the two methods. Sojka et al. (1990) compared measure-ment of water status made with the J-14 leaf press and a pressure chamber for tomato, rapeseed, corn, and soy-bean. The leaf press performed well with soybean but not with the other species, leading the authors to conclude that J-14 measurements are at best a relative indicator of water status in the absence of species-related calibrations. Grant et al. (1981) also obtained good results with the hydraulic press and soybeans.

Relatively few comparisons of the hydraulic press with other methods of measuring water potential have been made for conifers. The most extensive test of the hydraulic press with conifers appears to be the work done by Childs (1980) with Douglas-fir *(Pseuedotsuga menziesii* var. glauca [Beissn] Franco) seedlings. He found reasonable correlations with pressure chamber measurements, but satisfactory results required using several different end-points depending on the water potential of the sample, and calibrations with large numbers of samples. A similar comparison by Cleary and Zaerr (1980) with Douglas-fir produced poor results. A troublesome problem with the leaf press is correctly identifying the endpoint. Another is that the underlying theory is not as well established for the leaf press as it is for the pressure chamber method. Further work is needed before the leaf press can be rec-ommended for nursery use, but because of its low cost and simplicity it deserves further evaluation.

9.3.4 Pressure chamber
Since the description of the pressure chamber method by Scholander et al. (1965), and Waring and Cleary (1967), it has become the most widely used technique for measur-ing water potential in plants. It has been used to measure water potential in a wide variety of herbaceous and woody plants, including conifers, using samples of whole shoots and roots, individual leaves, fascicles of needles and single needles. Several types of pressure chambers are available commercially, and custom-built chambers or special methods of sealing the sample in the lid have been designed for use with conifer needles (Johnson and Nielsen 1969, Gifford 1972); wheat (Powell and Goggins 1985); sorghum (Blum et al.1973) and irregularly-shaped succulent samples (Simonelli and Spomer 1980).

Determinations made with the pressure chamber are rapid and simple, and measurement procedures have been described by numerous authors (Waring and Cleary 1967, Boyer 1967, Ritchie and Hinckley 1975, Cleary and Zaerr 1980). To make a measurement, a twig or shoot is cut from a plant, and if a conifer or hardwood is used, the bark and phloem are peeled back far enough to allow the twig to be inserted through a rubber stopper or similar type of compression seal. The sample is placed in the chamber with the cut end of the shoot protruding through the lid of the chamber and exposed to atmospheric pres-sure (Figure 9.3). Chamber pressure is slowly increased with nitrogen from a high pressure tank until water is forced back to the cut surface. That pressure, indicated on a pressure gauge, is taken as the water potential of the sample. A bleed-off valve allows nitrogen to be exhausted rapidly from the system following a determination. Certain precautions are required to obtain reliable results with the pressure chamber. These are discussed in detail by Ritchie and Hinckley (1975) and will not be repeated here, other than to emphasize that readings should be made quickly to avoid sample desiccation, needle removal should be kept at a minimum so that a large pro-portion of the foliage is enclosed in the chamber, and pressure should be increased at a moderate rate (about $0.07 \text{ MPa sec}^{-1}$). Recognizing the endpoint (the point at which water is observed on the cut surface) can be a problem with some species, particularly pines, because resin exuding from the cut surface may be mistaken for water. One solution is to wipe away the resin. McGilvray and Barnett (1988) suggest holding a small piece of brown paper towel against the cut stem, so that water exuding from the cut surface can be detected as a wet spot darken-ing the paper.

When a twig is cut from an intact branch, negative pres-sure or tension in the water conducting element is released, and water retreats from the cut surfaces. The general assumption is that the positive pressure required to force water back to the cut surface is equal to the nega-tive pressure which existed in the intact twig prior to exci
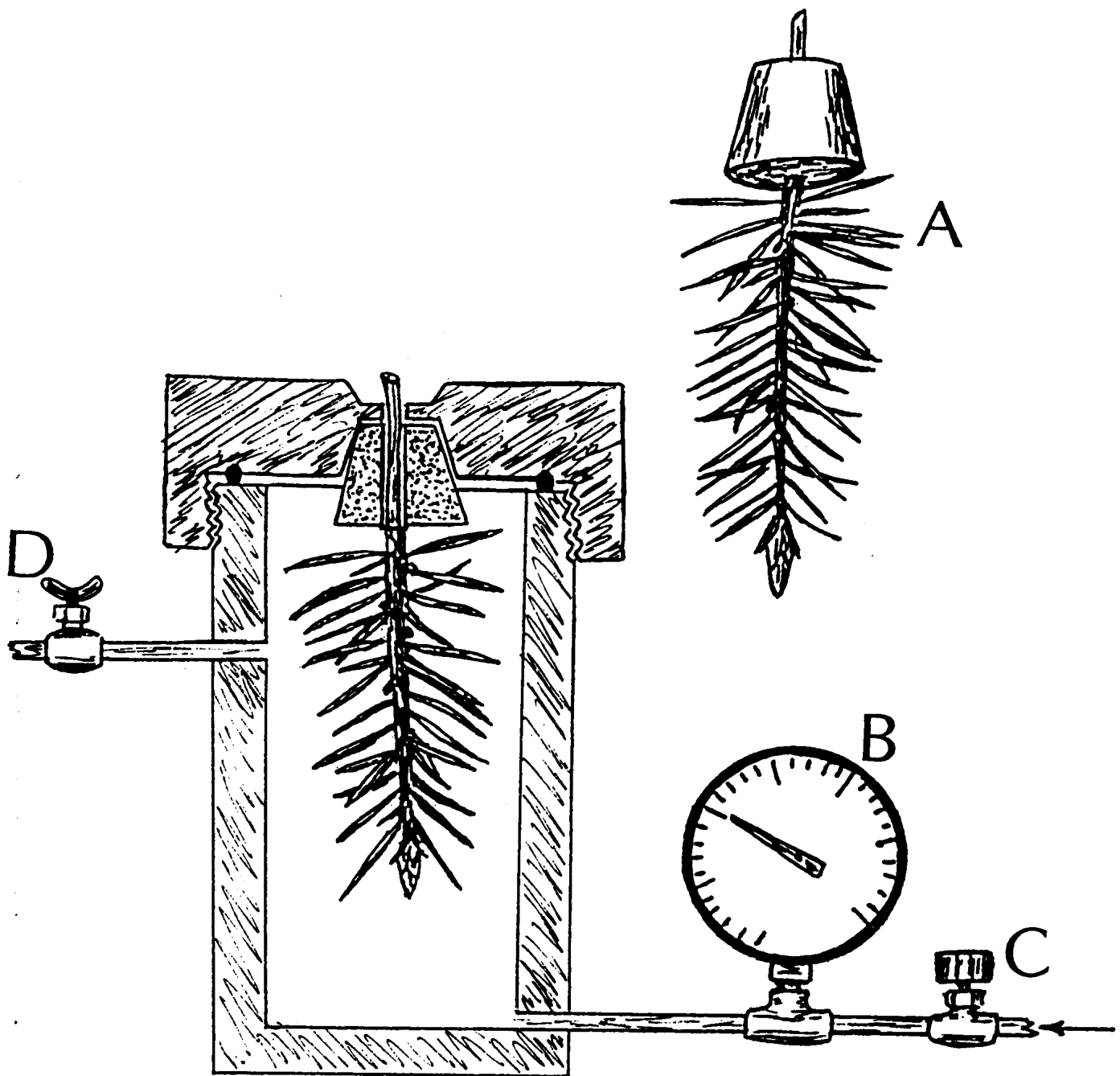
Figure 9.3—*Diagram of a pressure chamber showing (A) a conifer twig with the cut end protruding through a rubber stopper, (B) pressure gauge, (C) pressure increase needle valve, and (D) pressure release valve.*

sion. Theoretical considerations of forces involved in water movement during a measurement with a pressure chamber are discussed by Boyer (1967) and Ritchie and Hinckley (1975). In brief, the pressure chamber method measures the pressure necessary to raise the potential of water in the leaf cells to the point at which it equals or slightly exceeds the potential of the xylem sap at atmospheric pressure.

Pressure chamber measurements, however, do not include the osmotic component in the xylem sap; therefore, the values obtained are only estimates (rather than actual values) of leaf water potential and are referred to by most researchers as "xylem pressure potentials," although again, the more general term "plant moisture stress" is acceptable. Because the osmotic component usually is negligible, it is assumed that pressure chamber readings approximate leaf water potential in many species.

In spite of the considerable literature on the pressure chamber method, it is difficult to precisely assess the accuracy of measurements made with a pressure chamber. Early investigators (Boyer 1967, Kaufmann 1968), comparing pressure chamber readings in conifers with those made with thermocouple psychrometers, found that at low water potentials, pressure chamber values could be as much as 0.5 MPa more negative than those obtained with psychrometers. The closest agreement occurs at high and moderate water potentials. Roberts (1977) found good agreement between pressure chamber and psy-chrometer readings for needles of Scots pine *(Pinus sylvestris* L.). Surprisingly, there appear to be only two such comparisons for western conifers. One was by Waring and Cleary (1967) with Douglas-fir in which pres-sure chamber readings were found to agree within + 0.1 MPa of those determined with a vapor equilibrium tech-nique. In a more recent test (Hardegree 1987) with pon-derosa pine *(Pinus ponderosa* Doug. ex Laws), values obtained with a pressure chamber were about -0.5 MPa lower than those measured with a Richards and Ogata-type psychrometer. In any case, absolute accuracy is not a prerequisite for nursery work so long as standard guide-lines for relative values are recognized and reasonably reflect seedling condition.

Pressure chamber measurements can easily be made with fascicles of needles from long-needled species such as ponderosa pine and lodgepole pine *(Pinus contorta* Dougl. ex Loud.). The advantages of needle measure-ments are that repeated measurements can be made on small seedlings, gas consumption is reduced and, theoret-ically at least, readings with needles should more closely approximate needle water potential than measurements with shoots. Johnson and Nielson (1969) found that nee-dle water potential was nearly identical to that measured on the branch from which needles were taken in several species of pines. They also found that if the needle fasci-cle is stripped off so that the xylem trace remains attached, there is no problem with resin obscuring the endpoint. Resin exudation was a problem, however, if a single pine needle was used. Ritchie and Hinckley (1971) also found similar water potentials in needle fascicle and shoots of lodgepole pine and Jeffrey pine *(Pinus jeffreyi* Grey, and Balf.) seedlings, but in Douglas-fir, Pacific sil-ver fir *(Abies amabilis* [Dougl.] Forbes), and noble fir *(Abies procera* Rehd), needle values were up to 0.4 MPa higher than equivalent branch values. On the other hand, Kelliher et al. (1984), working in a young Douglas-fir stand, found that values of needle xylem water pressure potential obtained with a pressure chamber were similar to twig xylem water pressure potential. Measurements with individual small needles such as those of Douglas-fir require that the needle be held in a rubber stopper modi-fied in such a way to assure that a large portion of the needle remains exposed within the chamber (Ritchie and Hinckley 1971). Kelliher et al. (1984) reported that break-

age of needles and the minute size of the needle xylem make measurement of needle xylem potential quite diffi-cult. Only about 40 percent of their measurements were successful. While useful for research studies, single-nee-dle measurements normally are not needed in nursery work.

Pressure chamber guidelines usually specify that samples be measured quickly after detachment to avoid desicca-tion; however, with proper precautions excised conifer foliage can be stored for several hours with minimal change in xylem pressure potential. Kaufmann and Thor (1982) found that excised branch tips of Engelmann spruce *(Picea engelmannii* Parry ex Engelm.) and sub-alpine fir *(Abies lasiocarpa* [Hook] Nutt.), and fascicles of lodgepole pine needles stored in cool, humid vials exhib-ited very little change in xylem pressure potential over a four-hour period. Myers (1988), employing a similar tech-nique, harvested fascicles of radiata pine *(Pinus radiata* D. Don) before dawn, stored them in test tubes on ice, and measured xylem pressure potential two or three hours later. Samples stored for measurement later should be placed quickly in sealed containers kept humid and cool, and the cut ends of the samples should not be allowed to contact and absorb free water.

A valuable feature of the pressure chamber is that it can be used to estimate osmotic and turgor potential by the "pressure-volume" method (Tyree and Hammel 1972, Roberts and Knoerr 1977, Ritchie and Roden 1985, Schulte and Hinckley 1985). A cut twig is placed in a pressure chamber and subjected to increasing increments of pressure, and the volume of sap exuded with each increment is measured, usually by weighing the expressed sap. Finally, the branch is weighed, dried and reweighed. The procedure is described in detail by Ritchie (1984). Ritchie and Shula (1984), using the pressure-volume method, showed that considerable seasonal changes in tissue water relations occur in Douglas-fir seedlings, par-ticularly in the shoots. In a modified version of this method, tissue water content is reduced by allowing the foliage to transpire between successive measurements with the pressure chamber (Ritchie and Roden 1985). From these data a "pressure-volume" (P-V) curve repre-senting the relationship of reciprocal water potential $(1/\Psi_w)$ with water content can be plotted (Figure 9.4). The upper portion of the line is curvilinear for a small decrease in water content, while the lower portion becomes linear with further decrease in water content. The osmotic potential at full turgor can be determined by extrapolating the linear portion of the curve back to point A on the y-axis. The osmotic potential at zero turgor, which occurs where the curvilinear and linear regions meet, can be determined by extrapolating horizontally to point B. This value is the same as the water potential since at zero turgor (the wilting point), water potential equals the osmotic potential. Colombo et al. (1984) sug-
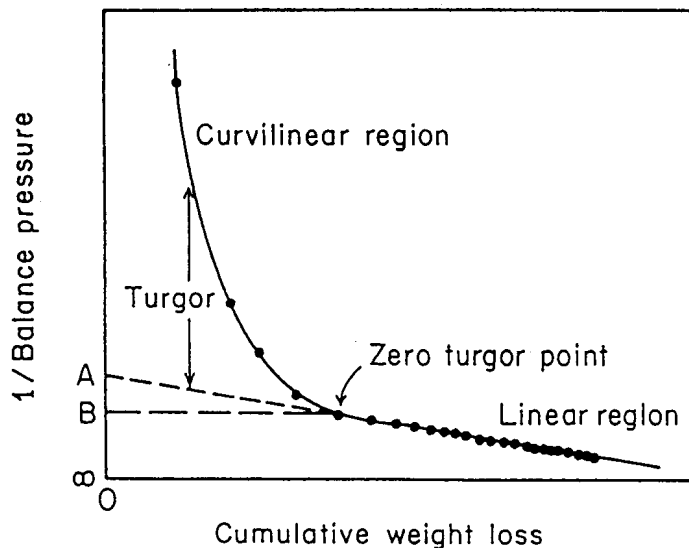
Figure 9.4—*A pressure-volume (P-V) curve showing extrapolation of the linear region to point A to obtain an estimate of the osmotic potential at full turgor, and horizontally to point B for an estimate of the osmotic potential and water potential at zero turgor.*

gested that since cell expansion ceases at zero turgor, the water potential at the wilting point, determined from a P-V curve, is a critical water potential for growth and thus could be used as an index of seedling quality. Thus, the water potential at zero turgor can be considered a "target" in that seedling water potentials should be kept above this point to maintain normal seedling functioning and growth. This "critical water potential," however, is not *fixed,* but varies seasonably (Ritchie, 1984). It should also be noted that while the osmotic component does influence seedling hardiness, it is only one of the factors determining seedling quality. Osmotic and turgor potentials also can be obtained with a pressure chamber used in combination with a thermocouple psychrometer (Livingston and Black 1987). Water potential is measured with a pressure chamber, osmotic potential of frozen and thawed tissue or expressed sap measured with a psychrometer, and turgor pressure is calculated as the difference between the water and osmotic potentials.

The pressure chamber also has several other interesting applications. These include measuring the hydraulic conductance of roots (Johnson et al. 1988, Smit and Stachowiak 1988), and detecting some types of seedling damage such as cold injury in conifer roots which damages cell membranes. To measure hydraulic conductance, a root system is immersed in water in a pressure chamber with the cut stump protruding through the lid. Pressure in the chamber is raised to create a pressure gradient from

the root surface to the cut stump forcing water through the roots. Rates of water movement per unit of pressure per unit of root material (surface, weight) are then used to calculate hydraulic conductance. Procedures are discussed in detail by Markhart and Smit (1990). The application to cold injury is based on the observation that under pressure more water can be expressed from cold damaged tissue than from healthy tissue (Ritchie 1990). A recent review of various applications of pressure chambers, thermocouple psychrometers, and other methods of measuring plant water status is that by Hanks and Brown (1987).

9.4 Operational Applications

It should be remembered that seedling water relations are by nature dynamic, and that a single measurement of water potential, by whatever method, represents only the water potential present at the time the measurement was taken. It does not provide any information on the magnitude or duration of previous moisture stresses. If severe and of long duration, such previous stresses could affect present growth behavior. Also, tree seedlings typically exhibit diurnal variations in water potential (Figure 9.5) related to environmental conditions (McDonald and Running 1979), thus timing of measurements needs to be considered. If measurements are being taken to follow seedling drying trends in nursery beds, then predawn measurements are preferred because water potentials at that time approach equilibrium with soil water potentials, and thus provide the most stable basis for day-to-day comparisons. For some purposes, a midday or early afternoon measurement also is useful because it provides an indication of maximum water stress, which together with predawn values shows daily minimum and maximum water stresses experienced by seedlings.

The pressure chamber can be used to schedule irrigation, but there is little information available on the effects of plant water deficits on the growth of seedlings of western conifers. Consequently, there are few published water potential standards for seedlings available to guide nursery managers. One study with Douglas-fir indicates that shoot elongation in Douglas-fir seedlings can occur at plant water potentials more negative than -0.5 MPa (Zaerr and Holbo 1976). In any case, because of the dynamic nature of water relations, it is impractical to specify what seedling water potentials or osmotic potentials ought to be (i.e., "targets") at any given time. Instead, there are general guidelines, based on studies with two species (Douglas-fir and ponderosa pine) that suggest stress limits that should not be exceeded. Some general criteria for containerized seedlings based on predawn water potentials are given in Table 9.2. A detailed description of procedures recommended for maintaining non-stressful water potentials in containerized seedlings and growing media is presented by Landis et al. (1989). According to these authors, a general rule for container seedlings is to irrigate
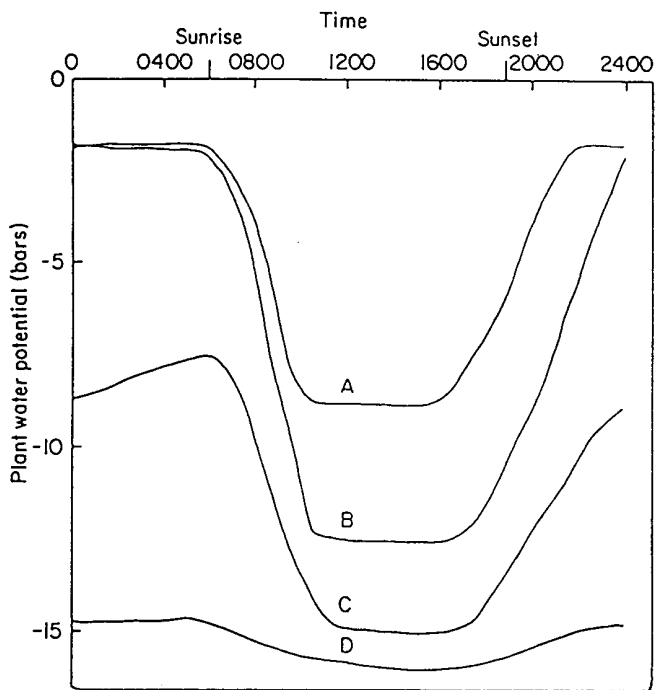
Figure 9.5—*Diurnal patterns of plant water potential for a nursery seedling under varying conditions of soil and atmospheric water stress. A - high soil water potential and low evaporative demand; B - high soil water potential and high evaporative demand; C - low soil water potential and high evaporative demand; D - extreme plant water stress (McDonald and Running 1979).*

when predawn water potential drops below -0.5 MPa, and water potential should not be allowed to decrease below -1.0 MPa unless reduced growth or dormancy induction is desired. The same guidelines apply to bareroot stock growing in nursery beds, although water potentials can be expected to decrease more slowly in bareroot seedlings because the roots of these seedlings exploit a greater mass of soil than container seedlings.

Several investigators have shown that controlled water potentials can be used to condition seedlings to better tolerate adverse conditions following planting. Many species acclimate morphologically and physiologically when exposed to sublethal water stress. Increased moisture stress can be used to induce seedling dormancy during the summer (Zaerr et al. 1981). Blake et al. (1979) found that exposing Douglas-fir seedlings to a mild stress of -0.5 to -1.0 MPa during late summer improves cold hardiness, while a moderate stress (-1.0 to -1.5 MPa) retarded lammas growth and reduced cold hardiness. Timmis and Tanaka (1976), working with container-grown Douglas-fir seedlings, also found that moisture stress increased cold

Table 9.2—*Growth response and cultural implications of inducing moisture stress in conifer seedlings in northwest nurseries (Landis et al. 1989).*

Plant water potential (predawn) MPa	Moisture stress rating	Seedling response/ cultural implications
0.0 to −0.5	Slight	Rapid growth
-0.5 to −1.0	Moderate	Reduced growth/ best for overall hardening
-1.0 to −1.5	High	Restricted growth/ variable hardening results
-1.5 to −2.5	Severe	Potential for injury
< -2.5	Extreme	Injury or mortality

hardiness. Seedlings also can be conditioned for increased drought hardiness. Christersson (1976) showed that subjecting pot-grown Scots pine *(Pinus silvestris* L.) and Norway spruce *(Picea abies* [L] Karst.) seedlings to a period of moisture stress enabled seedlings to tolerate a drought stress of -3.5 MPa, compared to a drought stress of -2.5 MPa for unhardened seedlings. Other effects of moisture-stress conditioning also have been noted. Seiler and Johnson (1985, 1988) reported that moisture-stress conditioning of loblolly pine *(Pinus taeda* L.) seedlings resulted in acclimation of photosynthesis to low water potentials, lowered osmotic potential, reduced transpiration, and increased water-use efficiency. Results, however, varied with species. For example, red spruce *(Picea rubens* Sarg.) seedlings exposed to sublethal water stress did not become more drought tolerant, undergo osmotic adjustment, or show photosynthetic or stomatal acclimation to water stress (Seiler and Cazell 1990).

Some nurseries measure seedling water potential during lifting and packing operations. Low water potentials can occur during lifting of seedlings because of low soil moisture content, cold soils (Lopushinsky and Kaufmann 1984, Lopushinsky and Max 1990), or high evaporative demand. These concerns have led to the establishment of guidelines based on pressure chamber readings (Day and Walsh 1980, Scholtes 1989) in an attempt to avoid lifting and packing seedlings with low water potentials. Generally, seedlings are not lifted when water potentials drop below -1.5 or -2.0 MPa, and water potentials are not allowed to fall below -0.5 MPa during grading and packing. These limits appear to be arbitrarily set because little is known about the relationship of water potentials in seedlings during lifting and processing to subsequent field survival and growth. Cleary (1971) found that in Douglas-fir and ponderosa pine seedlings, photosynthesis drops at water potentials between -1.0 and -2.0 MPa, and below -2.0 MPa vigor presumably continues to decline. But

these criteria, or those listed in Table 9.2, cannot be used to predict responses of seedlings which have been allowed to recover from water stress, kept in cold storage, and in many cases, planted months later.

Occasionally, bare-root seedlings in storage bags dry out during cold storage. Depending on the extent and duration of moisture stress, such drying may or may not affect seedling performance after planting. Daniels (1978) found that when bare-root Douglas-fir seedlings with a water potential of -2.0 MPa were lifted and cold stored for 55 days, field survival and growth declined. But he also found that the adverse effects of low water potentials at lifting were eliminated by spraying the trees with water immediately after lifting. In another study, water potentials as low as -1.7 MPa during storage of Douglas-fir seedlings were found to have no effect on subsequent survival (Hermann et al. 1972), and in spruce survival decreased only when water potentials were less than -2.0 MPa at the time seedlings were planted (Ruetz 1976). In a recent study with white spruce *(Picea glauca* [Moench] Voss), seedlings lifted in October at two levels of water potentials, above -0.1 MPa and below -0.11 MPa, and freezer stored for seven months, showed no adverse effects of pre-storage moisture stress on timing of budburst or height growth (Rose 1 990). The absence of adverse effects probably can be explained by the fact that the "high stress" treatment was relatively mild, i.e., an average water potential of only -0.135 MPa, and that the roots of seedlings in both stress treatments were dipped in water prior to storage.

9.5 Interpretation of Water Potential Values

Probably the most difficult problem associated with measurement of seedling water potentials is interpreting the significance of lowered water potentials for seedling growth and survival, particularly with mid-range values from about -1.0 to -2.5 MPa. When assessing seedling responses, consideration must be given not only to the magnitude of water stresses, but also to their duration, the stage of growth or dormancy at which stresses occur, the species involved, and seedling vigor. Certainly, seedlings which have desiccated to water potentials below -4.0 MPa for prolonged periods of time very likely will exhibit reduced growth and survival, but what about seedlings with a water potential of -2.0 MPa? A water potential of –2.0 MPa measured at midday during the summer in nursery beds which show high predawn potentials (0 to -0.5 MPa) will, with most species, have little or no effect on seedling growth in the nursery or subsequently in the field. A water potential of -2.0 MPa measured before dawn, on the other hand, is a cause for concern. Low predawn seedling water potentials develop as the result of a gradual increase in soil water stress over a considerable period of time. Thus, the seedlings would have been subjected to a low water potential, during both nighttime and

daytime periods, for an extended period of time. A predawn potential of -2.0 MPa is not likely to result in seedling mortality, however, it will prevent normal stomatal opening during the daytime, greatly reduce photosynthesis, and severely suppress or stop seedling growth. Following irrigation, seedling water potentials will increase. Normal growth rates may or may not resume, however, depending on the duration of the water stress, the sensitivity to stress of the species involved, and other factors. A water potential of -2.0 MPa measured predawn or in early morning hours during lifting also is also a cause for some concern, but in a different sense. Since the seedlings are dormant, suppression of current growth is not a problem. Also, it has been shown that during winter and early spring, Douglas-fir seedlings are at their highest level of resistance to water stress (Hermann 1967, Ritchie 1984, Lavender 1985). If the moisture stresses are only temporarily high, or can be relieved by delaying the lifting or by moistening the seedlings after lifting, it is unlikely that measurable survival or growth effects will be observed. On the other hand, unmoistened seedlings with a water potential of -2.0 MPa at the time they enter storage, or seedlings which have desiccated to -2.0 MPa during storage, very likely will experience some reduction in survival and growth.

A factor that needs to be taken into account is the relative sensitivity of different species to water stress. Differences in drought resistance are recognized, but it is not known, for example, to what extent the elongation of terminal shoots in Douglas-fir seedlings is reduced by a given water stress, compared to bud elongation in ponderosa pine or lodgepole pine. Finally, the overall vigor status of seedlings also needs to be taken into account, because it is likely that seedlings low in vigor from other causes will be affected to a greater degree by water stress than seedlings with high vigor.

A related issue which deserves consideration here is the extent to which measurements of water potential or PMS can be used to assess seedling quality. The importance of plant water status to seedling growth and survival, and the ease with which measurements of water potential now can be made with pressure chambers, have tended to foster the belief that a measurement of water potential or PMS can be used as an index of seedling quality. In a very limited sense it can, as for example, in the case of seedlings with extremely high stresses, or those subjected to prolonged desiccation during storage. And, as mentioned earlier, the pressure chamber can be used to check for cold injury in roots. Generally, however, factors known to influence seedling quality such as root growth potential, stored carbohydrate level, cold resistance, size of seedlings, and size of root systems, have no direct relationship to water potential. Clearly, seedlings can be so deficient in some or a combination of the above attributes

that prospects of good growth and survival are poor, yet can be moist enough to exhibit a low water stress.

9.5.1 Allowable water potential limits

Given the above considerations, what then are allowable water potential limits (targets) for nursery seedlings during the growth stage, and during lifting and storage? For seedlings during the growth stage, appropriate stress limits are those shown in Table 9.2, i.e., predawn water potentials should be kept above -0.5 MPa to maintain growth, and in the range of -0.5 to -1.0 MPa to limit growth, induce dormancy or increase cold-hardiness. During lifting and processing, seedling water potentials ought to be maintained above -1.0 MPa, with seedlings moistened as required to reduce stresses to this level. Seedlings about to be placed in storage also should have water potentials above -1.0 MPa. Seedlings with water potentials between -1.0 and -2.0 MPa that have been moistened before being placed in storage probably will not experience significantly reduced survival and growth, mainly because in sealed bags, the seedlings will equilibrate to higher water potentials. On the other hand, placing unmoistened seedlings with a water potential of -2.0 MPa or less in cold storage has been shown to result in reduced seedling performance. The actual falldown in performance will vary for different lots, depending on the influence of other factors that also affect seedling vigor.

During cold storage, seedlings kept in sealed storage bags typically will have water potentials above -0.7 MPa (most often above -0.5 MPa), and will not exhibit problems related to water stress. Water potentials in the range of -0.7 to -2.0 MPa increase the likelihood of adverse effects. Moistening such seedlings, and allowing time for water stress to decline will reduce, but may not entirely eliminate, adverse effects. Stored seedlings with water potentials below -2.0 MPa can be expected to show reduced field performance. Again, moistening such seedlings will reduce the water stress, but probably not restore seedling performance to normal levels. Actual performance will vary, depending on the duration of the exposure to water stress, and the influence of other vigor-related seedling factors.

The foregoing discussions emphasize that, properly used, measurements of seedling water potential can provide valuable information that will help nursery personnel produce high quality stock. Conversely, improper measurements and interpretations of water potentials can result in unnecessary work and precautions and can lead to less than effective nursery management.

9.6 Summary

Information on the water status of nursery seedlings is important because water deficits affect practically every aspect of plant growth. The water status of tree seedlings can be determined by measuring seedling water content, and by liquid equilibration, psychrometric, and pressure chamber techniques. The last three methods are preferred because they measure water potential, an expression of the free energy of water, which is more directly related to physiological functions in plants than is water content. Liquid equilibration methods are laborious, time consuming, and yield estimates rather than precise values of water potential. Water potentials can be measured most accurately with thermocouple psychrometers which also can be used to measure osmotic potentials, but long equilibration times, temperature sensitivity and other technical considerations make this method better suited for use in the laboratory than in forest nurseries. The J-14 hydraulic leaf press is easy to use and economical, but endpoints vary with the type of tissue and with the level of water potential. So far it has not found wide acceptance for use with conifers.

The method of choice for nursery work is the pressure chamber because it is fast, simple and accurate. It can provide estimates of osmotic and turgor potential, and it also can be used to measure the hydraulic conductivity of root systems and to detect cold injury in roots. The pressure chamber also is useful for scheduling irrigation. To maintain growth, seedlings should be irrigated when predawn water potential drops below -0.5 MPa. Conditioning seedlings in the nursery by exposure to moderate moisture stresses can cause osmotic adjustments and other physiological changes that increase cold and drought hardiness in seedlings, but conditioning procedures and effects have not been thoroughly studied in northwest conifers. In some nurseries, the pressure chamber also is being used to monitor seedling water potentials during lifting and packing. Limited data indicate that during lifting, seedling water potentials down to -2.0 MPa will not adversely affect seedlings, provided that seedlings are moistened to relieve stresses prior to storage. Storing seedlings with a water potential of -2.0 MPa or less, however, likely will result in reduced survival and growth after outplanting.

9.7 Research Needs

Additional research related to the water status of nursery seedlings is needed in several areas. More research is needed on the effects of plant water deficits on all aspects of seedling growth, including bud and shoot extension, needle elongation, stem diameter, and root growth. Seedling water status is a major determinant of seedling growth, yet water potential guidelines presently available are only general in nature, and do not adequately reflect stress-related growth responses for many important species or provenances of species. Better information in this area is needed to permit nursery managers to tailor irrigation schedules more closely to the requirements of specific species.

More research also is needed to determine how moisture-tress conditioning can be used to acclimate seedlings to better tolerate adverse conditions. Such conditioning may be particularly feasible with container-grown seedlings since environmental factors can be closely controlled in container facilities (greenhouses). Pressure-volume curves obtained with a pressure chamber provide a means of monitoring osmotic adjustments during such condition-ng.

Another research need is related to the concern about seedling water potentials during lifting and packing. Research is needed to determine what, if any, relationship exists between low water potentials during lifting and packing of seedlings and subsequent performance in the field. Seedling water potential generally increases in the precooler during processing and during cold storage (J. Scholtes 1990, R. Rose 1990, personal communication). These observations, and the ability to eliminate moisture stresses by moistening seedlings prior to storage, suggests that a temporary low water potential during lifting and processing is not a serious problem, but data in this area are lacking.

More information is needed about the ways in which moisture stress and seedling vigor interact, and how these interactions affect seedling performance. It is well known that seedling vigor can vary considerably as a result of different lifting dates, time in storage, and other factors. So the question arises, "To what extent do low water potenials affect survival and growth of seedlings of low vigor compared to those with high vigor?"

Additional research also is needed to determine whether the hydraulic leaf press can be used to measure water potentials in nursery conifer seedlings. There are indications that the endpoint is easily observed at high water potentials (Childs 1980), suggesting that the method may provide a quick and easy way to check seedling moisture stress during grading and packing when moisture stresses usually are relatively low.

Finally, though not directly applicable to routine nursery operation, more research is needed on the effects of water stress at the molecular level in tree seedlings. It is known, for example, that water stress can cause changes in the kinds and concentrations of growth substances in the root that affect shoot metabolism and growth (Itai and Vaadia 1965, Livne and Vaadia 1972). To better understand water stress-growth interactions, more emphasis needs to be placed on the effects of water stress on the balance of growth regulators and on other enzyme-mediated pro-esses because the effects of water deficits cannot be explained fully by decrease in water content or water potential.

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