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TECHNIQUES FOR DETERMINING SEEDLING WATER STATUS AND THEIR EFFECTIVENESS IN ASSESSING STRESS

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ABSTRACT--Three basic characteristics describe plant water status: (i) water content relative to that at full saturation, (ii) energy status of water in the cells, and (iii) osmotic potential. Techniques for measuring these parameters are assessed with regard to their relative ease of use, reliability, requirements in time and material, and value in predicting seedling performance. Standards for assessing plant water status are defined. Preliminary results of a study detecting membrane damage in shoots by measurement of the osmotic potential of expressed sap indicate that the technique may be used to detect the loss of membrane integrity caused by severe temperature stress.

3.1 INTRODUCTION

Because the degree of water saturation is crucial in determining plant response to the environment, we need reliable measures of plant water status that will indicate the severity of water stress. Unfortunately, many questions remain regarding what should be measured and how best to measure it. To answer these questions, we must consider not only the physiological aspects of water in plants but also the needs of the investigator or manager who frequently must make decisions on the basis of incomplete information. Kramer (1983) has outlined the criteria for ideal measurement parameters in a system for monitoring plant water status: they would show strong correlation between rates of physiological processes and the degree of water stress; have similar physiological significance for a wide range of plant materials; be simple, rapid, and inexpensive; and require only a small amount of plant material. Three parameters satisfy most of these characteristics: (i) water content relative to that at full saturation. (ii) water potential, and (iii) osmotic potential.

Duryea, M. L. (ed.). 1985. Proceedings: *Evaluating seedling quality: principles, procedures, and predictive abilities of major tests.* Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis. ISBN 0-87437-000-0

This chapter gives some of the underlying principles of plant water relations and describes in some detail how the three parameters can be used as indicators of plant water status. It summarizes current techniques of measurement and discusses the major limitations of each. For additional information, the reader may refer to Slatyer (1967). Barrs (1968). Boyer (1969). Slavik (1974), Richie and Hinckley (1975). Turner (1981), and Kramer (1983).

3.2 PRINCIPLES OF PLANT WATER RELATIONS

Water constitutes more than 80% of the fresh weight of actively growing shoots of woody plants. forming a continuous liquid phase from the root hairs to the leaf mesophyll cells. Living cells require a high degree of internal water saturation to function efficiently, and tissue water content can fluctuate only within narrow limits if growth and development are to continue unimpaired. A change in water content of 15% to 20% of the value at full hydration will. for most species, result in cessation of virtually all growth processes. A loss of only 1% to 2% of tissue water can trigger marked changes in physiological process. Both cell wall extension and protein synthesis, for example, are particularly sensitive to reductions in tissue water content.

"Water status" is a broad term referring to the general state of a plant in relation to water. It may refer to some aspect of the energy associated with it or to the actual quantity of water in the plant. These two aspects of water status are the basis for all current approaches to its measurement.

Movement of water from one place to another requires energy. Water, like any other substance, flows down gradients of energy. ,giving up energy as it moves. Plant biologists need to ask, "What property of water determines whether it will move from point A to point B within a plant, or from B to A? Under what conditions will it move?" The answers to these questions depend on the difference between the average energy content of the water molecules at the two points. The energy content is referred to as the "chemical potential" and is the free energy per mole of a substance. Chemical potential is a measure of the energy with which a substance will react or move. It dictates the direction of movement in much the same way that the temperature of an object dictates whether heat will flow toward it or away from it.

3.2.1 Water Potential

Water potential, **W** is the chemical potential of water. By definition, **W** of pure free water is zero, and any factor that decreases the free energy content of water thereby decreases **W**. Movement of water into and through a plant occurs along gradients of decreasing **W**. That is, the net movement of water is always from a region of higher t to a region of lower $_{p}$ (e.g., from -1 bar to -5 bars). This concept of energy status provides a convenient framework for describing plant water and is widely accepted. (For an early, comprehensive review of this subject, still unsurpassed for clarity, see Slatyer 1967.) Water potential is the most widely used indicator of plant water status because it is the major determinant for water movement through the plant and because it is easily measured.

To obtain a clearer picture of how water relations affect plant performance, it is useful to split **W** into measurable components that are known to exert direct effects on particular processes. The most important of these are osmotic potential (n) and pressure potential (P):

$$\psi = \pi + P.$$

The osmotic component represents the effect of dissolved solutes in the cell sap: it always acts to decrease 4'. The pressure component represents the effect on $_{w}$ of positive or negative pressure: its effect depends on whether the cells are living or dead.

The principle behind the operation of an osmometer. a laboratory device that measures osmosis, is useful for understanding the relation between n and P. In an osmometer, a membrane permeable to water but impermeable to a solution separates pure water from water containing solutes (Fig. 1). Water tends to diffuse toward the solution compartment, where it is less concentrated (i.e., where the chemical potential of water is lower). In Figure 1. the net movement of water across the membrane is toward the solution. This causes pressure to build and will, if permitted, expand the volume in the solution compartment. But an osmometer does not allow

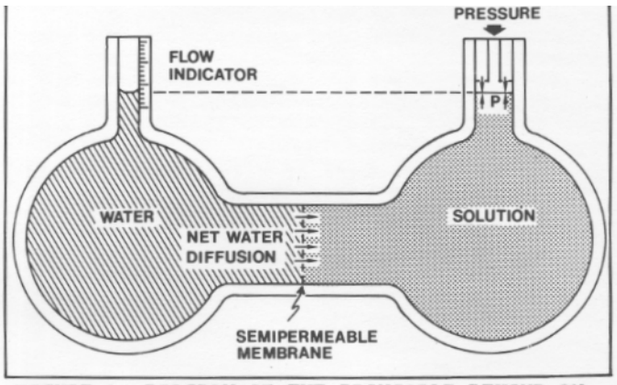


FIGURE 1. DIAGRAM OF THE PRINCIPLE BEHIND AN OSMOMETER. WATER MOVEMENT IS SHOWN BY A CHANGE IN WATER LEVEL IN THE FLOW INDICATOR. HYDROSTATIC PRESSURE (P) ON THE RIGHT-HAND COMPARTMENT PREVENTS THE NET DIFFUSION OF WATER INTO THE SOLUTION. THAT PRESSURE IS EQUAL TO THE OSMOTIC PRESSURE OF THE SOLUTION.

***** PLEASE NOTE A bold **W** denotes the character: $\psi = \pi.$ And the second character depicted here is represented with “pi”.

expansion. Instead it permits the pressure to build by automatically applying equal and opposite pressure (such as from a piston) as soon as the volume of liquid begins to expand. As pressure increases on the walls of the solution compartment, **W** also increases. At some measurable applied pressure, the diffusion of water toward the solution compartment will cease. At that instant, **W** is the same on both sides of the membrane, and equilibrium is reached. The pressure that must be applied to prevent the movement of water into the solution compartment is called osmotic pressure.

A living plant cell is an osmotic system that functions according to the same principle. The plant cell membrane restricts movement of solute molecules more than it restricts movement of water and thus makes osmosis possible. During leaf expansion, for example, water molecules diffuse from a region of higher **W** on the outside of a cell to a region of lower **W** in the cell sap. The result is the same as in an osmometer; that is, pressure builds in the system until equilibrium is achieved. In this case, it is the rigid plant cell wall that supplies the opposing force and is thereby responsible for the increase of P inside the cell.

The pressure potential may be positive or negative. Adding pressure (by a piston in an osmometer or by the action of the cell wall in a living cell) results in positive P. often referred to as turgor pressure. But exerting tension on a column of water results in negative P. Pressure potential is usually positive in living cells, and large positive pressures (10 to 15 bars) can occur in woody plant cells because of their rigid walls. But P is almost always negative in conducting xylem elements. Osmotic potential is always negative. The addition of solutes always decreases **W**. Likewise, internal generation of solutes by plant cells results in lower (more negative) pi of the cell sap.

3.2.2 Plant Water Status as an Indicator of Stress

Which of the parameters of water status should be used to measure water stress? To answer this question, we must consider the cellular processes affecting growth and development. If a particular parameter can be shown to exert a strong and consistent influence on plant function, then it may be a good candidate for an indicator of internal stress.

Water potential is correlated with rates of many physiological processes, but there is no evidence that **W**, per se. directly affects these processes. Rather, it is increasingly evident that P and pi of the cell sap directly influence such processes as cell enlargement, photosynthesis, and growth (Weibe 1972, Hsiao et al. 1976). Although **W** gradients are the driving force for water transport within a plant, **W** is not necessarily a good indicator of water stress.

It is more desirable to measure pi and P. since they are more directly involved in specific responses to stress.

Both pi and P have important limitations as possible indicators of stress. Osmotic potential, measured separately, is an insensitive indicator because it is a direct function of the amount of solutes, which can change significantly within a relatively short time (12 to 15 days), regardless of the level of water stress imposed on the seedlings. Also. large differences due to seasonal changes in seedling physiology are superimposed on short-term changes (Joly and Zaerr, manuscript in preparation). Turgor pressure is closely related to water status but is not directly measurable in higher plant tissues; it can only be estimated as the difference between **W** and pi. Further, although P is crucial for leaf expansion, the lower threshold of P that leads to observable symptoms of water deficiency probably changes as a result of conditioning exposures to water stress.

Relative water content (RWC) has often been used to measure internal water stress. It is useful because it expresses the absolute amount of water required for full saturation. and is therefore important for calculating water requirements of a plant or stand. However, RWC cannot be used to predict the consequences of water loss in terms of changes in $_{W,}$ n, or P; therefore, a given value for RWC may have different physiological significance for different species, or even for different parts of one plant.

It is apparent that none of the parameters of internal water status (**W**, pi, P. or RWC), taken separately, is a totally sufficient indicator of water stress. A more complete description of plant water status can be obtained by determining the relation between RWC and **W**. Water potential is sensitive to changes in water content. How rapidly **W** changes as RWC decreases depends on pi of the cell sap and on the elasticity of the cell wall. Each of these factors, in turn, is influenced by the physiological age of the tissue, by seasonal growth patterns, and by the history of seedling exposure to water stress. The relation between RWC and **W**, often referred to as the moisture-release curve or waterpotential isotherm, is described by data obtained by the pressure-volume method. This method is widely used by plant physiologists to obtain a more precise description of water status than can be obtained from singleparameter estimates. Unfortunately, it does not meet the criteria of being simple, rapid, and inexpensive.

3.3 MEASUREMENT OF RELATIVE WATER CONTENT

The relative water content of plant tissue is determined as

$$RWC = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} .$$

For this method, leaf discs or needles are

placed in a sealed, preweighed vial immediately upon sampling. After the fresh weight is obtained, the tissue is floated on distilled water until it becomes fully turgid. It is then quickly blotted to remove surface moisture, returned to its vial, and reweighed. In order to prevent the loss of dry weight by respiration, the plant tissue is next killed and dried as quickly as possible in a forced-draft oven at constant temperature between 70° and 85°C. Twenty-four hours is adequate for most samples, but large samples may need more time.

The validity of this method depends on the precision of estimates for each of three weight determinations, a reliable estimate of turgid weight being the most crucial. Procedures are given by Slatyer (1967), Barrs (1968), Catsky (1974), and Turner (1981), but the exact protocol most likely to give a reliable estimate of RWC varies with species. In all procedures, careful sampling and handling is paramount because large errors can be introduced by evaporative loss of water from the leaf samples. It is easy to underestimate the turgid weight and thereby inflate the value of RWC. If results are to be reliable, precautions should be taken to minimize the influence of these sources of error:

(1) The sample tissue may fail to resaturate to full hydration (i.e., $\psi = 0$). Leaf discs usually rehydrate in approximately 4 hours, but the process can take 20 hours or more in very dehydrated leaves. Complete elimination of the water deficit in conifer needles can take more than 24 hours (Millar 1966). During this time, the tissue may respire as much as several percent of its dry weight, yielding an overestimate of RWC. Error due to respiration can be minimized by maintaining the temperature at 2°C, but this may slow the rate of rehydration.

(2) In some cases, sample tissue may become oversaturated due to the injection and infiltration of water into the intercellular spaces along the margins of the leaf disc. This phenomenon is not detectable in some species but is important in others.

(3) An effective procedure for removing surface moisture is necessary. The particular protocol must be determined empirically for a given species, but it should be conducted under standard conditions of pressure such that surface water is removed but internal water is not forced from the leaf cells. Turner (1981) recommends that the drying be performed rapidly and in a humidified chamber to minimize evaporation.

3.4 MEASUREMENT OF TOTAL WATER POTENTIAL

3.4.1 Psychrometric Methods

Thermocouple psychrometry has been used for nearly 30 years to measure the water potential of soils and excised plant tissue. In the past, the technique was restricted to the laboratory because of the need for precise temperature control. But the introduction of temperature-compensated psychrometers and dewpoint hygrometers in the 1970's has greatly expanded its use for rapid, nondestructive measurement.

The thermocouple psychrometer employs the principle that the relative vapor pressure of a solution or piece of tissue is related to its water potential according to the equation:

$$\psi = \frac{R\,T}{\overline{V}} \ln p/p^o$$

where R is the universal gas constant, T is kelvin temperature, $\overline{V}$ is the partial molar volume of water (18 cm^3/mole), p is the vapor pressure in the sample chamber at T, and p^o is the vapor pressure of pure water at that temperature. For example, we know that the vapor pressure (p^o) developed inside a sealed chamber over pure water at 25°C is equivalent to the pressure required to raise a column of mercury (Hg) 23.7 mm high. If we add solutes to the liquid (or do anything to decrease its water potential), then fewer water molecules will have the energy to escape the liquid phase and enter the gas phase. As a result, the vapor pressure (p) measured at equilibrium and at the same temperature will be somewhat less than 23.7 mm Hg (say, 20.0 mm Hg). The ratio p/p^o will then be less than 1.0, and the natural logarithm of that ratio will be negative. In other words, adding solute molecules to pure water ($\psi = 0$) has decreased ψ to some value below zero. The effect of increasing water stress in plant tissues is exactly the same; that is, ψ is decreased. If we have an instrument of sufficient precision to measure the p/p^o ratio over a disk of plant tissue, then we can infer the ψ of that tissue.

When a sample of plant material is placed in a vapor-tight chamber (Fig. 2), the vapor pressure (relative humidity) of the air in the chamber eventually comes into equilibrium with the water potential of the tissue. The water potential of the enclosed volume of air can then be measured by determining the vapor pressure of that air. This is accomplished in many ways, depending on instrument design, but the most common infers ψ from the depression in wet-bulb temperature. Two thermocouples are built into the chamber. One has a relatively large mass and remains at the temperature of the air. An electric current is passed through the second thermocouple junction, causing it to cool slightly below the dewpoint temperature so that a minute drop of water condenses over it. This point of moisture acts as the wet bulb. The temperature difference between the wet and dry (air) thermocouples is a function of the vapor

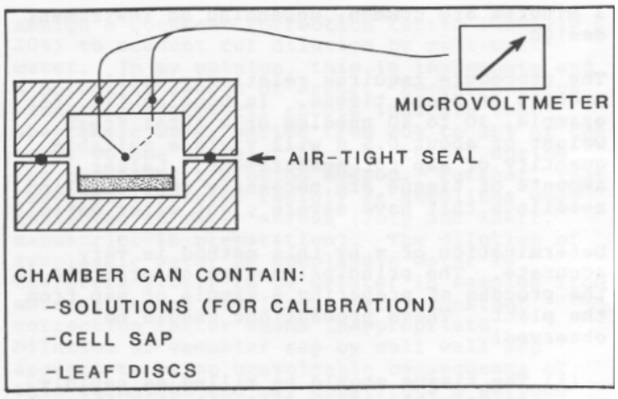


FIGURE 2. DIAGRAM OF A THERMOCOUPLE PSYCHROMETER FOR MEASURING WATER POTENTIAL OR OSMOTIC POTENTIAL. THE DEVICE MEASURES THE VAPOR PRESSURE OF THE ENCLOSED VOLUME OF AIR AFTER IT HAS COME INTO EQUILIBRIUM WITH THE SAMPLE.

pressure of the air in the chamber.

Several limitations arise from the use of this method:

(1) To ensure reproducible results, the temperature must be precisely controlled. Commonly available psychrometers should only be used in a room that can be regulated to plus or minus 1°C (Turner 1981).

(2) The long time required for equilibration of sample tissue with the chamber air space limits the number of samples that can be measured. It can take from 45 minutes to 4 hours for equilibration. depending on the amount of resistance to diffusion of water vapor into and out of the sample. This is an important limitation with leaf samples having waxy surfaces.

3.4.2 Pressure Chamber Method

The pressure chamber technique, devised in its modern form by Scholander et al. (1964. 1965). is the most widely used method for measuring t. It is reliable, simple to use, and it does not require fine regulation of temperature. The method has been summarized by Ritchie and Hinckley (1975). Cleary et al. (1978). and Cleary and Zaerr (1980).

A leaf or stem sample is placed in a chamber with the cut end protruding through a rubber stopper sealing the chamber (Fig. 3). The atmospheric pressure in the chamber is then gradually increased by means of an external source of compressed air or nitrogen until sap just appears at the cut ends of the xylem elements. At that instant the hydrostatic pressure inside the xylem elements is exactly balanced by the applied external pressure, and the chamber pressure is recorded.

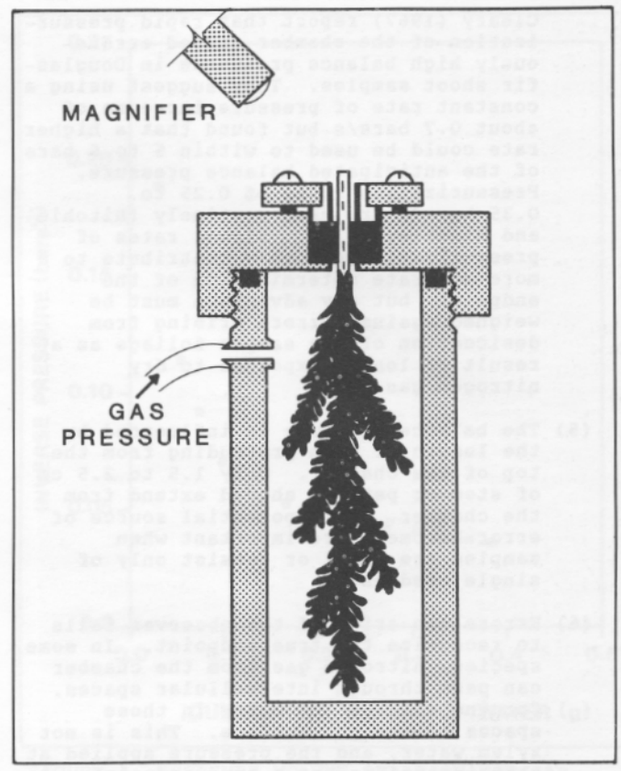


FIGURE 3. PRESSURE CHAMBER FOR MEASURING PLANT WATER POTENTIAL.

Several precautions should be followed to ensure safe operation of the chamber and to maximize reliability of this technique.

(1) The user should never place his or her face over the chamber as the seal may break under high pressure and force the stem from the chamber.

(2) Water loss by transpiration from leaves must be minimized between sampling and measurement. Turner and Long (1980) report that loss of water by rapidly transpiring sorghum leaves lowered the water potential by 2 to 7 bars in as little as 30 seconds. Covering the leaf or shoot with a plastic sheath at the time of sampling reduces error to an acceptable level.

(3) The twig or petiole should be excised with a sharp razor blade or knife, and only one cut should be made. Subsequent cutting to provide a better fit in the chamber or to create a cleaner cross section surface can result in erroneously low balancing pressures (Barrs 1968, Ritchie and Hinckley 1975).

(4) The balance pressure is influenced by the rate of pressure increase. If pressure is increased too rapidly. significant errors occur. Waring and

Cleary (1967) report that rapid pressurization of the chamber caused erroneously high balance pressures in Douglasfir shoot samples. They suggest using a constant rate of pressure increase of about 0.7 bars/s but found that a higher rate could be used to within 5 to 6 bars of the anticipated balance pressure. Pressurization rates of 0.25 to 0.35 bars/s are used routinely (Ritchie and Hinckley 1975). Slower rates of pressurization probably contribute to a more accurate determination of the endpoint, but any advantage must be weighed against errors arising from desiccation of the sample foliage as a result of longer exposure to dry nitrogen gas.

(5) The balance pressure is influenced by the length of stem protruding from the top of the chamber. Only 1.5 to 2.5 cm of stem or petiole should extend from the chamber. This potential source of error becomes more important when samples are small or consist only of single needles.

(6) Errors can arise if the observer fails to recognize the true endpoint. In some species, nitrogen gas from the chamber can pass through intercellular spaces. forcing some of the water in those spaces to the cut surface. This is not xylem water, and the pressure applied at that instant is not a true endpoint. A similar problem occurs with some coniferous species because resin exudation may make detection of the endpoint difficult (Ritchie and Hinckley 1975). Drying the cut surface several times with filter paper will remove the resin and help the observer to distinguish between resin and xylem water. A binocular microscope, a clean, well-lit cut surface, and experience will also help the observer to make the distinction.

3.5 MEASUREMENT OF OSMOTIC POTENTIAL

3.5.1 Psychrometric Method

The psychrometric method measures the change in the relative vapor pressure of water caused by the presence of solutes. It employs the same principle described for measuring •of leaf samples. In order to measure only the effect of cell solutes on vapor pressure, the pressure (turgor) in cells must be reduced to zero. This is usually done by rapidly freezing and thawing the tissue, a procedure that produces ice crystals that rupture cell membranes. At this point, **n** is equal to +. The tissue sample is then crushed. and a small quantity of solution placed on a filterpaper disk is inserted into the psychrometer. Equilibration of the solution with the air in the sample chamber takes less time than that necessary for determining ,p of leaf samples. Equilibration times of 1.5 to 3 minutes are common, depending on instrument design.

The procedure requires relatively small amounts of plant tissue. In Douglas-fir, for example, 30 to 40 needles or a total fresh weight of about 0.5 g will yield a suitable quantity of sap for measurement. Larger amounts of tissue are necessary when sampling seedlings that have severe plant water deficit.

Determination of **n** by this method is very accurate. The principal source of error is in the process of preparing a sample of sap from the plant. These precautions should be observed:

(1) The tissue should be killed as rapidly as possible after collection of the sample. If samples are collected in the field, they should be stored on dry ice until they can be placed in a freezer at -10° to -20°C.

(2) Samples should be thawed at a constant room temperature for not less than 30 minutes and not more than 1 hour. Long thawing can cause quantitative changes in the composition of the cell sap owing to the hydrolysis of starch to sugars.

(3) Frozen-thawed tissue should be crushed by a standardized procedure because the results depend on the pressure applied. The pressing device should apply a standard pressure in excess of 50 bars.

(4) Expressed sap should be stored in the same container in which the tissue was crushed. Very small volumes of liquid are obtained, and concentration of the solution as a result of evaporation should be avoided as it can result in serious error. The liquid sample should be loaded rapidly onto a filter-paper disk and into the psychrometer chamber. the whole procedure taking no more than 1 or 2 seconds.

(5) The instrument should be calibrated against a series of standard solutions at the beginning of each measurement period and readjusted as necessary. Samples containing high concentrations of solutes tend to contaminate the thermocouple quickly.

There is still considerable discussion among plant physiologists regarding the correspondence of measured pi with the actual pi of vacuolar sap in intact, living cells. The cell sap obtained from frozen-thawed tissue is a mixture of solutions from the apoplasm (water in the cell walls and in xylem elements) and from the symplasm (water inside cell membranes). The process of measuring n of the liquid inside plant cells invariably results in a mixing of that solution with apoplasmic water.

Nevertheless, the psychrometric method is in frequent. current use. Investigators simply

assign a constant correction factor (usually 20%) to account for dilution by cell-wall water. In my opinion, this is inadequate and a potential source of error. Recent observations of Douglas-fir shoots indicate that apoplasmic water varied from 20% to 35% of the total volume of water at full turgor during the course of a growing season. Further, that fraction increased rapidly as seedlings were subjected to water stress (Joly and Zaerr. manuscript in preparation). The dilution of symplasmic water by apoplasmic water is therefore likely to be greater in samples from water-stressed seedlings, and a constant correction factor seems inappropriate. Dilution of vacuolar sap by cell wall sap appears to be an unavoidable consequence of this technique and may constitute a serious error, especially for woody species.

3.5.2 Pressure-Volume Method

The pressure-volume (P-V) technique, as developed by Scholander et al. (1964, 1965) and Tyree and Hammel (1972). has been useful in studying water relations of higher plants. Water potential of a leaf and stem sample are measured during a period in which the tissue is progressively dehydrated to well past the point of turgor loss (P = 0). Water potential of the tissue is measured as a function of the volume (or weight) of water lost, and a set of paired data points, the cumulative volume of water expressed and the chamber pressure required to express that volume, is developed during the pressure-induced dehydration (Fig. 4). The resulting P-V curve may then be analyzed to estimate several measures of tissue water status, including n and P as a function of water content.

As water is lost from the sample tissue during pressure-induced dehydration, turgor is at some point forced to zero. As water content is further reduced, the hydrostatic pressure measured by the chamber is due solely to the contribution of the osmotic constituents of the cells, and $\Psi = \pi$. Beyond the point of turgor loss. the cumulative weight of water expressed from the sample (W_e) is related to the applied pressure (P_a):

$$1/P_C = (W_S - W_e)/RTN_S$$

where W_s is the weight of symplasmic water (i.e., the total weight of water in the protoplasm and vacuoles of all living cells), R is the universal gas constant, T is kelvin temperature, and N_s is the number of moles of solutes in the symplasmic water (Scholander et al. 1965. Tyree and Hammel 1972). The relation between 1/PC and W_e should, in theory, become linear when turgor pressure becomes zero. A regression analysis of the data points in this linear region is performed in order to determine the line of best fit. The Y-intercept of that line is an estimate of n at full turgor.

The technique involves placing a shoot sample in a pressure chamber with the cut end of the stem just protruding from the chamber, as previously described. The flow of nitrogen gas into the chamber is set at approximately 0.05 bars/s until fluid just appears at the cut surface of the stem. The initial m (balance pressure) is recorded, the chamber pressure is reduced to slightly below that pressure, and the stem is fitted with a preweighed sap-collection assembly. The chamber is then slowly pressurized to 5.0 bars above the balance point. Fluid exudes from the cut surface and is absorbed by paper strips inside the sap collector. The elevated pressure is maintained for 10 to 30 minutes. depending on the species being measured, then slowly reduced 1 to 2 bars below the anticipated new balance pressure. The sap-collection assemblies are removed, and the chamber is slowly pressurized until fluid returns to the severed ends of the xylem elements. The balance pressure can then be determined within t 0.05 bars by examining the cut surface under a binocular microscope. These steps are then repeated 15 to 20 times in order to obtain sufficient pairs of P-V data in the zero turgor region.

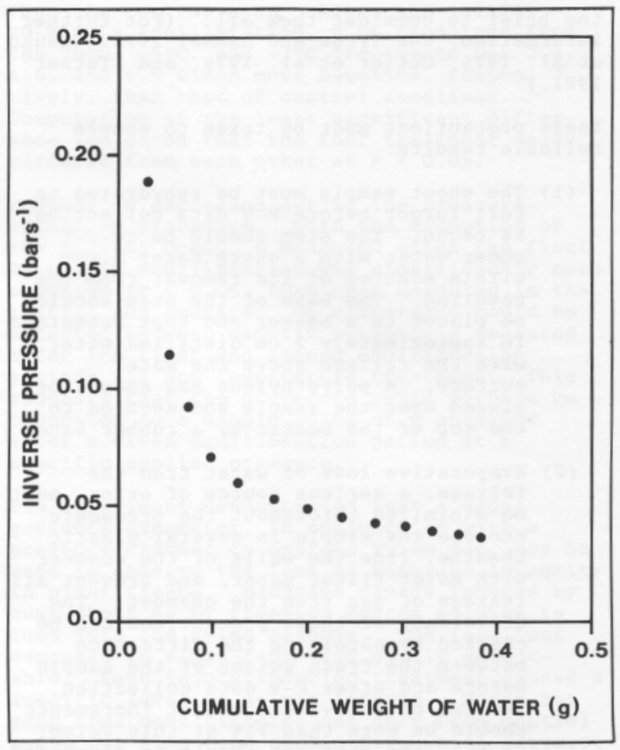


FIGURE 4. PRESSURE-VOLUME RELATION FOR SAP EXPRESSED FROM A TYPICAL DOUGLAS-FIR SHOOT.

Used with care, this technique provides the best estimate of the osmotic potential of cell sap of any current method. However, the technique is very sensitive to experimental conditions, and many sources of error exist. The outline of the procedure given here is far

too brief to consider them all. (For further information, see Tyree and Hammel 1972, Cheung et al. 1975. Cutler et al. 1979, and Turner 1981.)

These precautions must be taken to ensure reliable results:

(1) The shoot sample must be rehydrated to full turgor before P-V data collection is begun. The stem should be re-cut under water with a sharp razor blade within minutes of its removal from the seedling. The base of the stem should be placed in a beaker and kept submerged in approximately 2 cm distilled water, with the foliage above the water surface. A polyethylene bag should be placed over the sample and secured to the top of the beaker by a rubber band.

(2) Evaporative loss of water from the foliage, a serious source of error, must be minimized throughout the procedure. Enclose the sample in several plastic sheaths, line the walls of the chamber with moist filter paper, and prevent all leakage of gas from the chamber. The efficiency of these precautions can be checked by obtaining the difference between the fresh weight of the sample before and after P-V data collection. The sum of individual weight increments should be more than 95% of this value; if not, the data set should be discarded.

(3) The balance pressure should be measured only after equilibrium has been reached within the sample tissue. The length of time necessary for equilibration varies by species and stage of growth and must be determined empirically by the investigator. This can be done by making a balance-pressure determination, backing the pressure off about 1 bar, waiting 5 minutes, and making a second determination. If the first and second measurements agree within about 0.07 bars (i.e., about 1 lb/in.2), then the sample has had sufficient time to reach equilibrium (Tyree et al. 1978). Appropriate exchange times for rice are approximately 2 to 5 minutes. but 10 to 15 minutes or more are required for woody plants.

Measurement of n by the P-V method requires considerable time, 4 to 5 hours for a single P-V curve. But the information derived from the data analysis is often worth the effort. One can obtain accurate estimates not only of n. but also of cell wall elasticity, water content at the point of turgor loss, and the proportion of apoplasmic water. Nevertheless, the time requirement is a serious constraint when many samples must be measured; therefore, although the technique is an important research tool, it is unlikely to become important for assessing nursery stock quality unless an abbreviated version is developed.

3.6 WATER STATUS AS A PREDICTOR OF SEEDLING PERFORMANCE

Environmental stresses result in physical and chemical stresses within living cells. If the stress is prolonged or severe, subcellular mechanical structures or metabolic functions are damaged. Both high and low temperature stress may impair the semipermeable properties of membranes. Some damage can be sustained and repaired, but if membrane disruption is widespread, death will follow.

Loss of membrane integrity means that cell solutes leak from the protoplasts into the xylem. A means of accurate measurement of the extent of leakage may allow development of a procedure for evaluating tissue damage and for predicting the probability of survival. Preliminary development of such a procedure was undertaken to test this hypothesis.

3.6.1 Detecting Membrane Injury

Studies were conducted on 2-year-old Douglasfir seedlings grown from a bulk mixture of open-pollinated seeds from a Corvallis, Oregon seed source. These were grown in raised nursery beds and transplanted to 1-gallon pots 6 weeks before treatments. Seedlings were 25 to 35 cm tall at the time of treatment; all had stopped growing and had a well-developed terminal bud with firm bud scales. In August 1984, 12 pots containing three seedlings each were arranged in a split-plot design in order to compare four temperature regimes: the control, $-5^{\circ}C$ for 1 hour, $-7^{\circ}C$ for 1 hour. and $70^{\circ}C$ for 3 minutes. After exposure to each of the three lethal temperature regimes, pots were removed from the treatment chamber and allowed to stand on a greenhouse bench for 2 hours before sampling.

Samples consisted of about 15 cm of the terminal portion of a shoot and all branches on the shoot. Each sample was severed from the seedling with a razor blade and quickly placed inside a polyethylene sheath. The stem protruded through a small hole at one end of the sheath.

A pressure chamber (PMS Instruments Co.. Corvallis, Oregon) was used to express fluid from the samples. It was fitted with a head modified to accommodate three samples, so that all three seedlings from a pot could be analyzed simultaneously. The statistical analysis was conducted on the basis of pot means.

After the initial balance pressure was recorded, the chamber pressure was gradually increased to about 10.5 bars, and a solutefree paper disk was placed on top of the cut end of each stem. When a disk became saturated with expressed sap, it was quickly transferred to the sample chamber of a vapor pressure osmometer (Wescor Inc., Logan. Utah) for determination of sap osmolarity (i.e.. total concentration of dissolved particles). The osmometer measures dewpoint temperature

depression within the chamber. a colligative property of the solution that is a function of solution vapor pressure. Measurement proceeds automatically until osmolarity is displayed after 90 seconds. It is then converted to **n** according to pi= cRT, where c is concentration in m moles/kg. R is the universal gas constant, and T is kelvin temperature.

When the initial volume of sap for determining 4 was collected, the chamber pressure was increased to approximately 17 bars and held for 20 minutes. Another set of paper disks was then placed on the cut stems and n was determined a second time. Finally, the chamber pressure was increased to about 24 bars and held for an additional 20 minutes. Saturated disks were obtained as before, and the osmolarity of the sap was determined.

Osmotic potential of the expressed sap at each of the three applied pressures was plotted (Fig. 5) for each of the four temperature treatments. Osmotic potential of control seedlings was low (approximately 0.63 bars) and, not unexpectedly, remained constant as pressure was increased. Xylem sap has a low concentration of solutes. As external pressure is increased, water is gradually forced out of living cells, across cell membranes, and into the xylem. In healthy seedlings. solutes are filtered by the membrane and remain in the protoplasm of the cells. Thus, n should, in theory. remain low.

The mean **n** of the exuded sap from each injury-inducing treatment was more negative

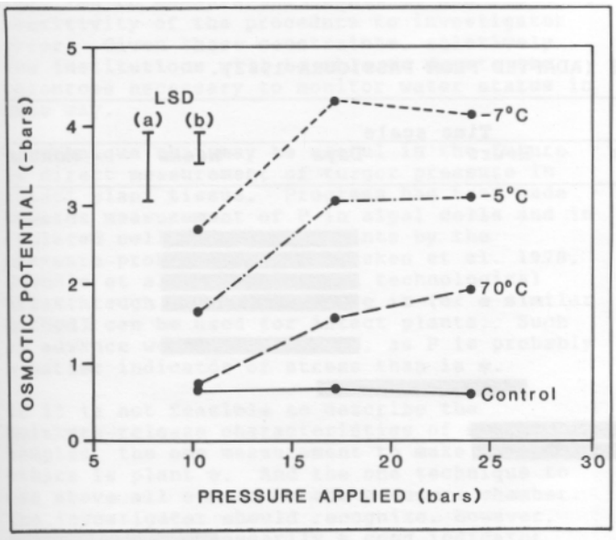


FIGURE 5. THE OSMOTIC POTENTIAL OF XYLEM SAP EXPRESSED AT DIFFERENT PRESSURES FROM DOUGLAS-FIR SEEDLINGS SUBJECTED TO FOUR TEMPERATURE TREATMENTS. LEAST SIGNIFICANT DIFFERENCES (LSD) ARE SHOWN FOR (a) TEMPERATURE TREATMENTS WITHIN PRESSURES AND (b) PRESSURES WITHIN TEMPERATURES.

than the control mean. After the initial 20-minute equilibration. **n** of seedlings from the 70°C, -5°C, and -7°C treatments was 2.4. 4.6, and 6.5 times more negative, respectively. than that of control seedlings. Computation of the least significant difference indicates that the four treatment means differed from each other at $P < 0.05$.

Further, **n** decreased during the pressure - exudation procedure. With the exception of the control group, **n** measured after the first 20-minute equilibration was significantly more negative ($P < 0.05$) than that measured in the initial sap exudate. No difference could be detected between osmotic potentials measured after the first and second equilibration periods for any temperature treatment. This suggests that the procedure could perhaps be truncated to a single value of **n** obtained after a given equilibration period at a specific applied pressure.

The experiment demonstrated that a vapor pressure osmometer, in conjunction with a pressure chamber to express xylem sap, may be used to observe the loss of membrane integrity in plant tissue. Membrane damage induced by heat shock or by freezing causes solutes to leak into the xylem pathway. The resultant decrease in **n** was rapid and easily detectable. Each injury-inducing treatment caused a specific level of solute leakage. Means for each treatment differed not only from control means but also from each other, which suggests that the extent of leakage may be proportional to the extent of injury.

The results are encouraging, but because they are preliminary, they must be interpreted with caution. The vapor pressure osmometer can be used to measure **n** with a high degree of precision, but we need to know whether or not the technique can be used to predict fine differences in injury. It will be necessary to study the relation between **n** of the xylem sap and the extent of physical damage to foliage or cambial tissue caused by a particular treatment. Ideally, a quantitative relation will be established between the extent of leakage and the probability of seedling survival.

As a tool for assessing membrane integrity in seedlings, the technique could be useful where stress has been sufficient to cause lesions in subcellular structures and functions and loss of membrane semi-permeability. It must be noted, however, that many other physiological attributes are important in evaluating seedling quality and that seedling mortality can be caused by many events other than membrane disruption.

3.6.2 Physiological Effects of Plant Water Deficit

The parameters of plant water status discussed here are transient; **w**, pi, P. and RWC are each highly responsive to changes in the seedling environment. A look at processes in

the plant and the environment that affect plant water status serves to emphasize that plant water relations are in dynamic equilibrium with the environment (Table 1). Given this dynamic equilibrium, can the water status of a seedling be used to predict its future quality and long-term productivity?

The current water status of a plant strongly reflects recent loss of water by transpiration and the rate of flow of water into and through the plant. Measurement of W. n, and P are crucial to understanding plant behavior at a particular time and under particular conditions of stress. But plants actively modify their internal water status, adjusting to changing internal or external conditions on time scales ranging from nearly instantaneous to weeks or months. Thus, none of these measurements, taken either singly or together is likely to be useful as a predictor of future performance unless it falls considerably outside some "normal" range. Nevertheless, an accurate description of seedling water status indicates the physiological condition of a seedling at a point in time. In that sense, the parameters described here provide an opportunity to monitor (but not necessarily predict) seedling quality.

Virtually all aspects of seedling growth are sensitive to water deficits; leaf anatomy. shoot growth, leaf expansion, bud formation. cambial growth, and root growth are affected by even mild levels of internal stress. The principal advantage of monitoring seedling water status is that it provides an objective assessment of stress severity during production and handling. Maintenance of appropriate target ranges of $_{ip.}$ n. P. and RWC is a quantitative approach to culture and increases the probability of producing seedlings of desired morphology and vigor.

Although there are important gaps in our knowledge, we have made progress in the last two decades in defining the physiological consequences of plant water deficit. We know, for example, that:

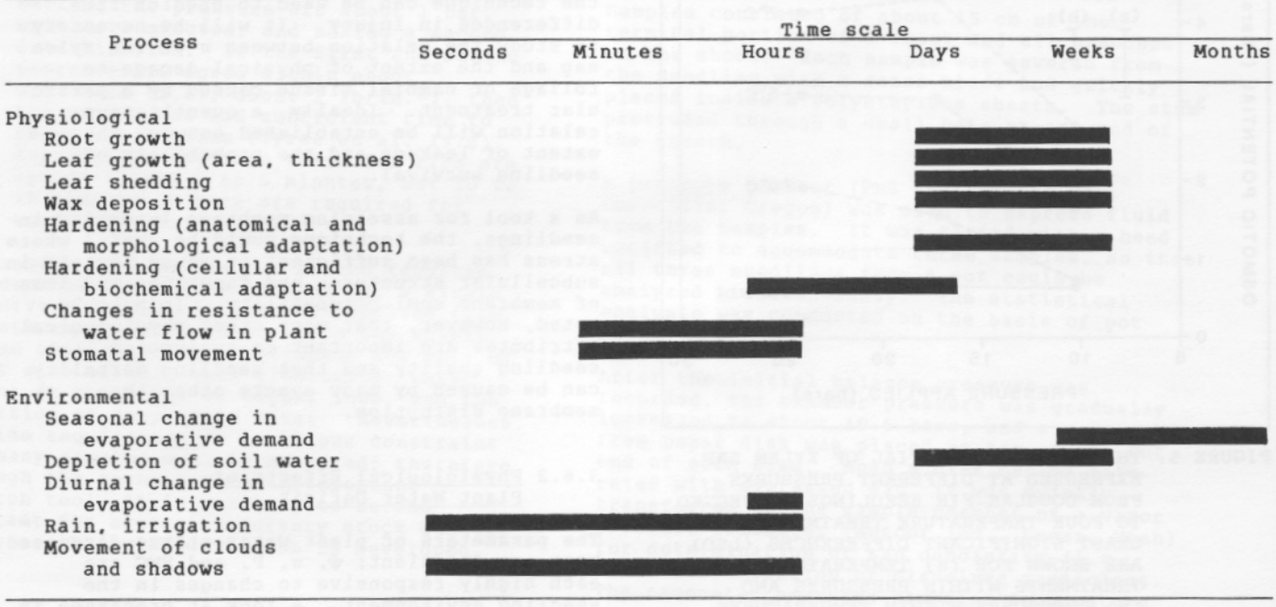
(1) Seedling dormancy can be induced by increasing plant moisture stress during the early- to mid-summer period (Zaerr et al. 1981).

(2) Increased cold hardiness can be induced by mild stress (-5 to -10 bars) applied during midsummer (Blake, Zaerr, and Hee 1979).

(3) Frequent irrigation in late summer can delay initiation of dormancy and disrupt the sequence of physiological events necessary to develop frost hardiness (Lavender and Cleary 1974).

We also know that irrigation scheduling must be tailored precisely to particular situations. Crop sensitivity to internal water deficit depends on the species and stage of growth. Further, water-retention characteristics of the nursery soil, average rainfall pattern, solar radiation, and vapor-pressure deficit each influence seedling water status. The timing and amount of water applied must be based on an experimental approach that takes into account the internal water status of the particular crop under production.

TABLE 1. PROCESSES AFFECTING PLANT WATER RELATIONS (ADAPTED FROM PASSIOURA 1982).



3.7 RECOMMENDATIONS

Water stress is an imprecise term not easily defined. Nevertheless, it is useful to define broad classes of stress in terms of ψ. Hsiao (1973) identifies three classes for a typical cell: mild stress--ψ is lowered by a few bars; moderate stress--ψ is lowered by more than a few bars but less than 12 to 15 bars; and severe stress--ψ is lowered by more than 15 bars. Most nurseries have developed guidelines and procedures in order to keep ψ within acceptable limits; these generally reflect Hsiao's assessment of stress categories. Few nurseries lift seedlings when ψ is lower than -10 to -15 bars, and precautions are generally taken to keep ψ above -5 bars during grading and packing. It is important to note, however, that xylem ψ of (for example) -13 bars recorded in each of several plants may have very different consequences for the individual plants. Depending on the extent of cellular and metabolic adjustment that has taken place during and before soil drying, ψ of -13 bars may result in serious metabolic disturbance or it may reflect only moderate stress. The outcome depends on many internal factors, including the relative efficiency of turgor-regulation mechanisms.

The most complete approach to determining water status is concurrent measurement of water content and ψ. By determining this relationship, we may accurately assess the role of π and P in specific physiological processes. However, there are significant limitations, the most important being the time required to measure the seedlings and the sensitivity of the procedure to investigator error. Given these constraints, relatively few institutions will be able to devote the resources necessary to monitor water status in this way.

A technique that may be useful in the future is direct measurement of turgor pressure in intact plant tissue. Progress has been made towards measurement of P in algal cells and in isolated cells of higher plants by the pressure-probe technique (Husken et al. 1978, Steudle et al. 1983). But a technological breakthrough is needed before it (or a similar method) can be used for intact plants. Such an advance would be valuable, as P is probably a better indicator of stress than is ψ.

If it is not feasible to describe the moisture-release characteristics of seedling samples, the one measurement to make above all others is plant ψ. And the one technique to use above all others is the pressure chamber. The investigator should recognize, however, that ψ is not necessarily a good indicator of water stress and that plant reactions to stress cannot be interpreted as simple responses to changes in ψ.

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