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## Plant water status, hydraulic resistance and capacitance

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### 9.1 INTRODUCTION

Many excellent reviews have been written about plant water status and its measurement (e.g. Slatyer, 1967; Barrs, 1968; Boyer, 1969; Brown and van Haveren, 1972; Slavik, 1974; Turner, 1981). The reader is referred to these sources for a more complete review, particularly of the older literature. In this chapter, our major goals are to introduce the reader to the concept and measurement of plant water potential and its components, and then to discuss the consequences of gradients in these components within the plant. First, we describe the most commonly used techniques for measuring the water potential of higher plants growing under field conditions, specifically the psychrometric and pressure chamber techniques. Second, we describe methods for measuring the components of water potential, particularly turgor pressure and osmotic potential, and water content. Since the transpirational path of the plant can be regarded as a hydraulic resistor, transpirational fluxes occur only when gradients in the various components of water potential exist within the plant. Thus, the third concept that we introduce is hydraulic resistance. Techniques

for its calculation are described for steady-state transpiration, when root water absorption equals shoot evaporation. Nonsteady-state flux occurs when there is a net movement of water between the transpirational path and tissues adjacent to it. Such tissues are regarded as capacitors, the final topic of our discussion.

### 9.2 WATER POTENTIAL AND ITS COMPONENTS

The thermodynamic parameter commonly used to describe the energy status of water in plants is the water potential (Slatyer, 1967; Passioura, 1982; Nobel, 1983). This parameter, designated  $\Psi$ , is defined as:

$$\Psi = \frac{\mu_w - \mu_w^*}{\bar{V}_w} \quad (9.1)$$

where  $\mu_w$  is the chemical potential, or free energy per mole, of water at some point in the system at constant temperature and pressure,  $\mu_w^*$  is the chemical potential of pure water at the same temperature and at atmospheric pressure, and  $\bar{V}_w$  is the partial molal

volume of water. The quantity,  $\mu_w - \mu_w^*$ , in Equation 9.1 represents the work involved in moving one mole of water from some point in the system to a pool of pure water at the same temperature and at atmospheric pressure. In plants,  $\Psi$  varies from zero to negative values. The units of  $\Psi$  are those of pressure, with the common units being MPa (megapascals). Alternative units are bars (1 MPa = 10 bars) and  $\text{J m}^{-3}$  (1 MPa =  $10^6 \text{ J m}^{-3}$ ).

For a plant cell,  $\Psi$  may be expressed as the sum of three components:

$$\Psi = P + \pi + \tau \quad (9.2)$$

where  $P$  is the hydrostatic pressure (or turgor pressure),  $\pi$  is the osmotic potential and  $\tau$  is the matric potential (Tyree and Jarvis, 1982). At equilibrium,  $\Psi$  is the same across the heterogeneous phases of the cell, i.e. across the vacuole, cytoplasm and cell wall. However, the components of  $\Psi$  may differ markedly across these phases. For water in the vacuole and cytoplasm (the symplasmic water), the dominant components are usually  $P$  and  $\pi$ , with  $P$  usually having a positive value. In a cell at equilibrium,  $P$  and  $\pi$  are probably uniform throughout the symplasm, since it is unlikely that significant pressure gradients can exist across the bounding membranes of the vacuole and cytoplasmic organelles (Tyree and Jarvis, 1982). However, the particular solutes contributing to the reduction in  $\pi$  may differ between the various symplasmic compartments. For water in the cell wall (the apoplasmic water), the dominant component is usually  $P$ , with  $\pi$  and  $\tau$  contributing to  $\Psi$  mainly in the region immediately adjacent to the charged wall surface (Tyree and Karamanos, 1981). In this latter region, which is often less than 3 nm wide,  $P$  may be positive. For most of the water in the cell wall, however,  $P$  is negative. In some halophytes, both  $P$  and  $\pi$  may be important components of  $\Psi$  in the apoplasm.

Within a tissue, the symplasmic values of  $P$  and  $\pi$  may vary significantly from cell to cell, even at equilibrium. Given this variation, the

most appropriate parameters for describing the water relations of the tissue symplasm are the bulk, weight-averaged values of  $P$  and  $\pi$  (Tyree and Hammel, 1972). These weight-averaged values, designated  $\bar{P}$  and  $\bar{\pi}$ , respectively, are defined as:

$$\bar{P} = \sum_{i=1}^n \frac{w_s^i}{W_s} P^i \quad (9.3)$$

and

$$\bar{\pi} = \sum_{i=1}^n \frac{w_s^i}{W_s} \pi^i \quad (9.4)$$

where  $P^i$ ,  $\pi^i$ , and  $w_s^i$  are the turgor pressure, osmotic potential and weight of symplasmic water respectively in the  $i$ th cell in the tissue,  $W_s$  is the total weight of symplasmic water in the tissue and  $n$  is the number of cells in the tissue (Tyree and Jarvis, 1982). Thus, at equilibrium,  $\Psi$  in the tissue symplasm may be expressed as:

$$\Psi = \bar{P} + \bar{\pi} \quad (9.5)$$

(Compared to the symplasmic values of  $\bar{P}$  and  $\bar{\pi}$ , the symplasmic value of  $\tau$  is negligible.) Analogous weight-averaged values may also be defined for the components of  $\Psi$  in the tissue apoplasm. In addition to water in the walls of living cells, the tissue apoplasm includes water in the walls and lumina of dead cells, such as vessel elements, tracheids and fibers. The dominant component of  $\Psi$  in the tissue apoplasm is usually  $\bar{P}$ . At equilibrium,  $\Psi$  in the tissue apoplasm equals  $\Psi$  in the tissue symplasm.

A gravitational term is often included as a component of  $\Psi$ . As emphasized by Passioura (1982), however, the definition of  $\Psi$  in terms of  $\mu_w$  ignores external force fields. Hence, it is preferable to use a separate gravitational potential,  $\Psi_z$ , in evaluating the effects of gravity. The total water potential may then be defined as  $\Psi + \Psi_z$ . The value of  $\Psi_z$  increases with height at a rate of 0.0098 MPa

$\text{m}^{-1}$  (Nobel, 1983).

small, except in tall plants. Following the definition of  $\Psi$  for accurately measuring  $\Psi$  in higher plants, the units in higher plants are MPa. Under field conditions, numerical values of  $\Psi$  are often small, the ecological significance of these parameters for different habitats and exhibits. Recent studies, for example, on plants growing in a rain forest (Oberbauer et al., 1987), savannah (Munzinger et al., 1987), paramo (Goldstein et al., 1987), woodland and scrubland (Calkin and Pearcy, 1987), and temperate forest (Tyree et al., 1980, 1981; Parker et al., 1984; Barnes, 1985; Smith, 1982; Nilsen et al., 1982). In a salt marsh (Drake and Tyree, 1982),  $\Psi$  have documented significant differences in  $\Psi$  between species in these parameters. They have also shown that  $\Psi$  vary within individuals and are genetically, diurnal and

### 9.2.1 Techniques for measuring $\Psi$

Several techniques are available for measuring  $\Psi$  in plants. The principal methods for measuring  $\Psi$  in plants growing under field conditions are psychrometric and pressure chamber techniques.

(a) *Psychrometric techniques*  
Psychrometric techniques employ psychrometers are widely used for measuring  $\Psi$  in higher plants. For field conditions, psychrometers are useful primarily for measuring  $\Psi$  in excised tissue. The method is referred to as the psychrometric method. Recent advances in psychrometric facilities. Recent advances

$\text{m}^{-1}$  (Nobel, 1983). Thus, it is usually very small, except in tall trees.

Following the development of techniques for accurately measuring  $\Psi$  and its components in higher plants growing under field conditions, numerous studies have analyzed the ecological significance of variation in these parameters for plants growing in diverse habitats and exhibiting diverse growth forms. Recent studies, for example, have examined plants growing in habitats as varied as tropical rain forest (Oberbauer, 1983; Myers *et al.*, 1987), savannah (Meinzer *et al.*, 1983) and paramo (Goldstein *et al.*, 1984), Mediterranean woodland and scrub (Hinckley *et al.*, 1980; Calkin and Percy, 1984; Davis and Mooney, 1986), and temperate forest (Roberts *et al.*, 1980, 1981; Parker *et al.*, 1982), prairie (Knapp, 1984; Barnes, 1985), desert (Monson and Smith, 1982; Nilsen *et al.*, 1983, 1984, 1986), salt marsh (Drake and Gallagher, 1984) and coastal dune (Pavlik, 1984). These studies have documented significant variation among species in these parameters, particularly in relation to habitat water availability. They have also shown that these parameters may vary within individuals in response to ontogenetic, diurnal and seasonal factors.

### 9.2.1 Techniques for measuring $\Psi$

Several techniques are currently available for measuring  $\Psi$  in plant tissues (Turner, 1981). The principal methods used with higher plants growing under field conditions are the psychrometric and pressure chamber techniques.

#### (a) Psychrometric techniques

Techniques employing thermocouple psychrometers are widely used for measuring  $\Psi$  in higher plants. For plants growing under field conditions, psychrometric techniques are useful primarily under circumstances in which excised tissue samples can be transferred to temperature-controlled laboratory facilities. Recent advances in the use of these

techniques with attached organs such as leaves and stems may enhance their suitability under more remote circumstances as well. Excellent reviews of the theory, design and application of thermocouple psychrometers have been provided by Brown and van Haveren (1972) and Briscoe (1986).

When a tissue sample is placed in an enclosed psychrometer chamber held at a constant temperature, the water in the sample will equilibrate with the chamber atmosphere. By measuring the equilibrium relative humidity ( $h$ ) of this atmosphere, thermocouple psychrometers enable the value of  $\Psi$  in the sample to be calculated as:

$$\Psi = \frac{RT \ln(h)}{\bar{V}_w} \quad (9.6)$$

where  $R$  is the universal gas constant and  $T$  is the Kelvin temperature (Turner, 1981; Nobel, 1983). A major assumption is that loss of water from the sample to the chamber atmosphere during the equilibration period does not affect the value of  $\Psi$  in the sample.

Three techniques are commonly available for measuring  $h$  in the psychrometer chamber. In the first technique, pure water is allowed to evaporate into the chamber atmosphere from the surface of a thermocouple junction. The resulting depression in the junction temperature (the so-called 'wet-bulb depression') is related to  $h$  by:

$$h = \frac{e_w - ac(T - T_w)}{e} \quad (9.7)$$

where  $a$  is the atmospheric pressure,  $c$  is the psychrometric constant,  $T$  is the psychrometer temperature (or dry-bulb temperature),  $T_w$  is the wet-bulb temperature,  $e$  is the saturation vapor pressure at  $T$ , and  $e_w$  is the saturation vapor pressure at  $T_w$  (List, 1968). In practice, the psychrometer is calibrated with solutions of known concentration to provide a conversion factor relating thermocouple output (in microvolts) to  $\Psi$ . Two procedures have been developed for placing pure water on the

thermocouple junction. One procedure uses a cooling current (the Peltier effect) to reduce the temperature of the junction below the dew point of the chamber atmosphere in order to condense water on it (Spanner, 1951). Another procedure involves manually placing a small amount of pure water on the junction prior to the measurement (Richards and Ogata, 1958). Because of its greater convenience, the procedure employing the Peltier effect has proven more adaptable for field use. Thermocouple psychrometers employing the Peltier effect are commercially available from JRD Merrill Specialty Equipment Corp. (Logan, Utah) and Wescor Corp. (Logan, Utah), while those employing a modified Richards and Ogata procedure are commercially available from Decagon Devices Corp. (Pullman, Washington).

The second technique, known as dew-point hygrometry, measures the dew-point temperature rather than the wet-bulb depression in order to determine  $h$  (Neumann and Thurtell, 1972; Campbell *et al.*, 1973). Dew-point hygrometers hold the thermocouple junction at the stable dew point by pulsed cooling currents. One advantage of this technique is that the dew-point depression is larger than the wet-bulb depression. Hence, the thermocouple signal is larger. Additionally, since no net movement of water from the thermocouple junction to the chamber atmosphere occurs at the dew point, the signal is stable for a long period, and the vapor equilibrium in the chamber is not disturbed. Calibration is the same as for the first technique. Thermocouple psychrometers capable of operating in the dew-point mode are manufactured by Wescor Corp. (Logan, Utah).

In the third technique, the change in the temperature of the thermocouple junction is measured when solutions with different  $\Psi$  values are placed on the junction (Boyer and Knipling, 1965). If a solution with a  $\Psi$  value equal to that of the sample is used, and if the sample has come to equilibrium with the

chamber atmosphere, then no net vapor movement will occur and the thermocouple output will be zero. This is the isopiestic point. In practice, since the relationship between the thermocouple output and the  $\Psi$  value of the solution is linear, only two solutions are used, typically distilled water and a solution whose  $\Psi$  value is close to that of the tissue sample. The isopiestic point is then determined by extrapolation. Although this technique involves more steps than the others, it has the same advantage as the dew-point technique in that the disturbance of vapor equilibrium in the chamber is minimized near the isopiestic point. According to Boyer and Knipling (1965), this minimizes the error associated with a high resistance to vapor exchange between the sample and the chamber atmosphere. The isopiestic technique has seen wide use in laboratory studies. Since it is more complicated than the other techniques, however, it has not been widely adopted as a practical technique for field use.

Thermocouple psychrometers have been used extensively to measure  $\Psi$  in excised tissue samples. The need for continuous nondestructive monitoring of  $\Psi$  in intact attached leaves has also promoted the development of *in situ* psychrometers (Boyer, 1972; Neumann and Thurtell, 1972; Campbell and Campbell, 1974). Partial dissolution or abrasion of the leaf cuticle (Neumann and Thurtell, 1972; Brown and Tanner, 1981; Savage *et al.*, 1984) and the use of compounds such as wax and lanolin to seal the psychrometer chamber to the leaf surface (Campbell and Campbell, 1974; Brown and Tanner, 1981) have helped to insure that vapor equilibrium is reached with *in situ* psychrometers. These innovations have also helped to reduce the initial equilibration period and to reduce the response time for changes in  $\Psi$  to minutes (Turner *et al.*, 1984). As a result, *in situ* leaf psychrometers appear to be suitable for the continuous monitoring of  $\Psi$  even in plants where  $\Psi$  fluctuates rapidly. *In situ*

stem (Michel, 1982) and root ( have also been u

In some cases, *in situ* leaf psych those measured (Boyer, 1967; correlations are  $\Psi$  are not transpir are not present w leaves and in se however, gradie the leaf, such the psychrometers r with pressure ch Shackel and Brin chrometers unav of the leaf to be transpiration in resistances to wa the leaf, this may  $\Psi$  (Brown and T 1983).

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stem (Michel, 1977; McBurney and Costigan, 1982) and root (Fiscus, 1972) psychrometers have also been used with some success.

In some cases, values of  $\Psi$  measured with *in situ* leaf psychrometers agree closely with those measured with pressure chambers (Boyer, 1967; Kikuta *et al.*, 1985). Good correlations are generally found when leaves are not transpiring and when gradients in  $\Psi$  are not present within the leaf. In transpiring leaves and in severely stressed large leaves, however, gradients in  $\Psi$  may occur across the leaf, such that measurements with *in situ* psychrometers may differ greatly from those with pressure chambers (Turner *et al.*, 1984; Shackel and Brinckmann, 1985). *In situ* psychrometers unavoidably cause some portion of the leaf to be shaded, thereby reducing transpiration in the area measured. If large resistances to water movement occur within the leaf, this may lead to overestimations of  $\Psi$  (Brown and Tanner, 1981; Savage *et al.*, 1983).

*In situ* leaf psychrometers are commercially available from Wescor Corp. (Logan, Utah), but often require some modifications to increase their thermal stability (Brown and Tanner, 1981; Savage *et al.*, 1983; Shackel, 1984). The precautions necessary for their use have been discussed at length by Shackel (1984).

The three most common sources of error associated with thermocouple psychrometers are: (1) vapor pressure disequilibria, (2) thermal gradients and instability, and (3) changes in  $\Psi$  in the sample due to growth and excision.

Vapor pressure disequilibria, except for those related to thermal gradients and instability or to the special problem of making a vapor seal with *in situ* psychrometers, are caused by water sinks within the psychrometer chamber and by the resistance to vapor diffusion from the sample to the chamber atmosphere. The first of these may be reduced by proper selection of chamber materials (Boyer, 1972; Dixon and Grace, 1982), scrupu-

lous cleanliness, and maximization of sample surface area per unit chamber volume. Salts and other foreign materials on the sample surface may also absorb water vapor. Washing the sample with distilled water and drying it thoroughly prior to measurement are good precautions. Techniques for reducing the resistance to vapor exchange from the sample, such as partial dissolution or abrasion of the leaf cuticle, have been used primarily with *in situ* psychrometers.

Thermal gradients and instability are caused by respiratory heat production from the sample (Barrs, 1964), by gradients or fluctuations in environmental temperature, and by absorption of solar irradiance. Thermal gradients and instability produce errors in the measurement of  $\Psi$  by causing the chamber atmosphere to differ in temperature from the sample or by causing electrical potentials to develop within the measuring circuit. The effects of respiratory heat production may be reduced by assuring good contact between the sample and the chamber and by using chamber materials with a high thermal conductivity. Gradients or fluctuations in environmental temperature may be minimized by performing operations in a controlled-temperature water bath if laboratory facilities are available. For *in situ* psychrometers, whose use has often been limited by the need for precise temperature control, Brown and Tanner (1981), Savage *et al.* (1983), and Shackel (1984) have discussed methods for increasing thermal stability. Absorption of solar irradiance may be reduced by using chamber materials with a high reflectivity.

The high resistance to vapor diffusion from the sample to the chamber atmosphere typically results in a long equilibration period, often on the order of hours. In an excised tissue sample, growth during the equilibration period will cause a reduction in  $\Psi$  since  $\bar{P}$  will decline in the absence of a water supply (Cosgrove *et al.*, 1984). Errors of this sort may be reduced by working at low temperature or with mature tissue. Changes in  $\Psi$  in an

excised tissue sample will also occur if water and solutes released from damaged cells at the cut surface are taken up by intact cells, thus altering their values of  $\pi$  and  $P$  (Barrs and Kramer, 1969; Nelsen *et al.*, 1978). Excision errors may be minimized by using samples with a low ratio of cut surface area to sample surface area. Errors resulting from water and solute release by damaged cells are eliminated with *in situ* psychrometers, provided that the epidermal cells are not damaged by partial dissolution or abrasion of the leaf cuticle.

(b) *Pressure-chamber technique*

Since its rediscovery by Scholander *et al.* (1965), the pressure-chamber technique has emerged as the most widely used method for measuring  $\Psi$  in higher plants growing under field conditions. This stems in large part from its simplicity and reliability, and its lack of requirement for precise temperature control (Turner, 1981). Excellent reviews of both the theoretical and practical aspects of this technique have been provided by Ritchie and Hinckley (1975) and Turner (1981, 1987).

The pressure-chamber technique measures the apoplastic value of  $\bar{P}$  (Scholander *et al.*, 1965). If the apoplastic values of  $\bar{\pi}$  and  $\bar{\tau}$  are negligible, then this technique also measures the apoplastic value of  $\Psi$ . If the tissue is at equilibrium, then the apoplastic value of  $\Psi$  will equal its symplasmic value.

The apoplastic value of  $\bar{\pi}$  is usually higher than  $-0.1$  MPa and is often higher than  $-0.02$  MPa, though it can be much lower in halophytes (Ritchie and Hinckley, 1975; Turner, 1981). Thus, the apoplastic value of  $\bar{\pi}$  is often negligible. As a precaution, however, this value should be checked for the species of interest in a given study, particularly for species growing under conditions of high salinity. (The technique for measuring the apoplastic value of  $\bar{\pi}$  is discussed in Section 9.2.3.)

The apoplastic value of  $\bar{\tau}$ , which results from the interaction between the water dipole and the electric field associated with the

charged cell wall surface, is negligible unless substantial intrusion of air-water interfaces occurs in the cell wall pores (Tyree and Jarvis, 1982). For most cell walls, this intrusion does not occur until  $\Psi$  drops below  $-14$  MPa, which is well below the lowest  $\Psi$  values experienced by most higher plants.

In a transpiring leaf with a finite resistance to water flow between the apoplasm and symplasm, the apoplastic and symplasmic values of  $\Psi$  will not be equal (Turner, 1981). Once transpiration stops, however, the apoplastic and symplasmic  $\Psi$  values will converge (Passioura, 1982). This convergence will occur quite rapidly in most leaves, with any disequilibrium between the apoplasm and symplasm disappearing during the time period between excising a leaf and making a measurement with the pressure chamber (Turner, 1981). The equilibrium  $\Psi$  value will be influenced by the apoplastic and symplasmic capacitances (Passioura, 1982) (see Section 9.4). Since the capacitance of the symplasm is usually much greater than that of the apoplasm, the equilibrium  $\Psi$  value will closely reflect the symplasmic  $\Psi$  value at the time of leaf excision.

Thus, in theory, the pressure-chamber technique is capable of measuring the symplasmic value of  $\Psi$  under most conditions. The technique itself is elegant in its simplicity (Scholander *et al.*, 1965; Turner, 1981, 1987). A leaf (or branch) is excised from the plant with a sharp razor blade, and is inserted into the pressure chamber with the cut surface of the petiole (or stem) protruding slightly through the rubber sealing gasket. Pressure is then increased gradually in the chamber by adding nitrogen or air from a compressed gas source. The pressure is increased until water in the xylem first appears at the cut surface of the petiole. The accurate determination of this end point is aided by the use of a dissecting microscope or high-magnification hand lens. The balance pressure in the chamber at this end point, taken as a negative value, equals the apoplastic value of  $\bar{P}$  in the

leaf, which in turn equals the value of  $\Psi$  under

Important procedures for measuring  $\Psi$  with the pressure-chamber technique have been described by Turner (1981, 1987).

1. Immediately prior to excising the leaf from the plant, the leaf is placed in a plastic sheath (around the petiole) to prevent desiccation. The measurement point is sealed to remain on the leaf when it is placed in the pressure chamber. In rare cases, Turner and Lorrain (1981) report significant decreases in  $\Psi$  following excision. To avoid this, closing the leaf in a plastic sheath prior to excision is recommended. The leaf should not be enclosed in the chamber longer than 1–2 min before it begins to increase in  $\Psi$ .
2. The petiole should be cut as close to the leaf as possible. When the leaf is excised from the plant, the petiole is cut, and the leaf is placed in the chamber, which is under vacuum. The distance from the cut surface to the measurement point (Scholander *et al.*, 1965). The vacuum is applied to the air-water interface between the petiole and the xylem elements. The petiole has been cut only once, and the xylem is emptied. The measurement point is reached in a few seconds, resulting in no noticeable change in the tissue  $\Psi$ . The petiole has been cut more than once, and the movement of water from the xylem will occur, which will result in a noticeably high measurement. The measurement will be greatest in the xylem elements and in the petiole.  $\Psi$  values.
3. The length of petiole in the pressure chamber should be kept constant both to prevent desiccation of the exposed petiole and to

leaf, which in turn equals the symplasmic value of  $\Psi$  under most conditions.

Important precautions to be taken in measuring  $\Psi$  with the pressure-chamber technique have been nicely summarized by Turner (1981, 1987). These include:

1. Immediately prior to being excised from the plant, the leaf should be enclosed in a plastic sheath (except for the protruding petiole) to prevent water loss during the measurement period. The sheath should remain on the leaf inside the pressure chamber. In rapidly transpiring leaves, Turner and Long (1980) measured significant decreases in  $\Psi$  within the first 10–30 s following excision, and recommended enclosing the leaf in a plastic sheath 1–2 s prior to excision. The attached leaf should not be enclosed in the sheath for much longer than 1–2 s, however, as  $\Psi$  may begin to increase.
2. The petiole should not be recut after the leaf is excised from the plant. When the petiole is cut, the water in the xylem, which is under tension, will recede a short distance from the cut surface (Scholander *et al.*, 1965). The water will recede until the air–water interface is unable to pass through the pores in the end walls of the xylem elements. As long as the petiole has been cut only once, water will refill the emptied xylem elements when the end point is reached in the pressure chamber, resulting in no net water movement into the tissue symplasm. If the petiole has been cut more than once, however, a net movement of water into the symplasm will occur, which will result in an erroneously high measurement of  $\Psi$ . This error will be greatest in species with large xylem elements and in species experiencing low  $\Psi$  values.
3. The length of petiole protruding from the pressure chamber should be minimized, both to prevent evaporation from the exposed petiole and to avoid exclusion

errors (Millar and Hansen, 1975).

4. The rubber sealing gasket should fit tightly around the petiole such that no gas escapes from the pressure chamber. Damage to herbaceous petioles resulting from constriction by the gasket may be minimized by the use of a quick-setting epoxy resin (Nobel and Jordan, 1983).
5. The rate of pressurization in the chamber should be very slow to prevent the development of  $\Psi$  disequilibria within the tissue symplasm. Turner (1981) recommends a rate of 0.003–0.005 MPa s<sup>-1</sup>. Internal  $\Psi$  disequilibria may be particularly problematical in branches, compound leaves and severely stressed large leaves, within which resistances to water transport may vary significantly from point to point (Turner *et al.*, 1984).

The extent to which these precautions need to be followed will depend on the species of interest in a given study. With some species, for example, the petiole can be recut several times without significantly affecting the measured value of  $\Psi$ . In addition, it is possible with some species to use a moderately rapid initial rate of chamber pressurization, followed by a slow rate of pressurization near the end point. The latter method enables more leaf samples, and hence a larger number of individuals within the population, to be processed in a short period of time. Exploratory measurements with each new species of interest will aid in determining the optimal procedures to be used.

Even when these precautions are stringently followed, however, the ease with which  $\Psi$  can be measured with the pressure-chamber technique may vary from species to species. In most species, the end point is very well defined, enabling  $\Psi$  to be measured with a high degree of precision. In some species, however, determination of the end point is complicated by resin exudation from ducts in the xylem or fluid exudation from the pith and cortex (Ritchie and Hinckley, 1975).

Removal of these exudates from the cut surface by repeated blotting with lintless tissue during chamber pressurization, or by constriction of the stem, may aid with the end point determination (Turner, 1981, 1987).

In the US, pressure chambers are commercially available from PMS Instrument Co. (Corvallis, Oregon) and Soil Moisture Equipment Corp. (Santa Barbara, California).

### 9.2.2 Techniques for measuring the symplasmic values of $\bar{\pi}$ and $\bar{P}$

Pressure-chamber and psychrometric techniques are also the principal methods for measuring the symplasmic values of  $\bar{\pi}$  and  $\bar{P}$  in higher plants growing under field conditions. The pressure-probe technique, which enables  $P$  to be measured directly in the cells of intact tissues, has recently been adapted successfully for use with mesophytic plants under precisely controlled laboratory conditions (Husken *et al.*, 1978; Zimmermann *et al.*, 1980; Brinckmann *et al.*, 1984; Cosgrove *et al.*, 1984; Shackel and Brinckmann, 1985). With appropriate modifications, this technique may eventually be suitable for use with plants growing under field conditions.

#### (a) Pressure-chamber technique

The most widely used method for measuring the symplasmic values of  $\bar{\pi}$  and  $\bar{P}$  in intact living tissues is the pressure-chamber technique. This technique was originally developed by Scholander *et al.* (1964, 1965) and has subsequently been refined by Tyree and co-workers (Tyree and Hammel, 1972; Tyree, 1981; Tyree and Karamanos, 1981; Tyree and Richter, 1981, 1982; Tyree and Jarvis, 1982). The simplicity of the technique enables it to be used even in remote field sites.

The theoretical aspects of the pressure-chamber technique have been discussed in detail by Tyree and Jarvis (1982). In brief, the symplasmic value of  $\bar{\pi}$  is approximated by:

$$\bar{\pi} = - \frac{\phi \rho RTN}{W_s} \quad (9.8)$$

where  $\phi$  is an osmotic coefficient (to account for the nonideality of solute behavior),  $\rho$  is the density of water in the symplasm, and  $N$  is the number of moles of solutes in the symplasm (ionic species counted separately). As the water content of an initially saturated tissue decreases, the symplasmic value of  $\bar{P}$  will decrease and eventually will reach zero. If negative values of  $\bar{P}$  do not occur in the tissue symplasm, then at all lower water contents,  $\Psi$  equals  $\bar{\pi}$  (see Equation 9.5). Thus, from Equation 9.8:

$$\frac{1}{\Psi} = \frac{1}{\bar{\pi}} = - \frac{W_s}{\phi \rho RTN} \quad (9.9)$$

The tissue relative water content ( $R'$ ) is defined as:

$$R' = \frac{W_s + W_a}{W_s^o + W_a^o} \quad (9.10)$$

where  $W_a$  is the weight of apoplasmic water,  $W_s^o$  is the weight of symplasmic water at full hydration, and  $W_a^o$  is the weight of apoplasmic water at full hydration. Thus:

$$W_s = R'(W_s^o + W_a^o) - W_a \quad (9.11)$$

Substituting into Equation 9.9 the expression for  $W_s$  in Equation 9.11 yields:

$$\frac{1}{\Psi} = \frac{1}{\bar{\pi}} = \frac{W_a}{\phi \rho RTN} - \frac{R'(W_s^o + W_a^o)}{\phi \rho RTN} \quad (9.12)$$

For values of  $R'$  less than that at which  $\bar{P}$  reaches zero, Equation 9.12 describes a linear relationship between  $1/\Psi$  (or  $1/\bar{\pi}$ ) and  $R'$ , assuming that  $W_a$  and  $\phi$  are constant. Hence, a plot of  $1/\Psi$  versus  $R'$  yields a straight line in the region where  $\bar{P} = 0$  (Fig. 9.1). For values of  $R'$  greater than that at which  $\bar{P}$  reaches zero, the relationship between  $1/\Psi$  and  $R'$  is no longer linear, since  $\Psi = \bar{P} + \bar{\pi}$ . However, the relationship between  $1/\bar{\pi}$  and  $R'$  remains

linear, assuming  $\bar{P}$  constant in this region. In Equation 9.12, it is possible that  $R'$  reaches zero. The relationship is then represented by a dashed line, which reduces to:

$$\frac{1}{\bar{\pi}} = - \frac{W_s^o}{\phi \rho RTN}$$

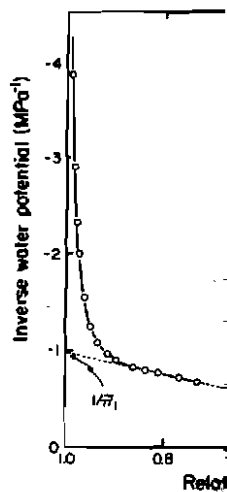


Fig. 9.1 Relationship between tissue water potential ( $\Psi$ ) and tissue water content ( $R'$ ) (see Equation 9.12). The point where  $\bar{P} = 0$  exists at  $1/\Psi = -1/\bar{\pi}$ . The dashed line defines the relationship between the reciprocal ( $1/\bar{\pi}$ ) and  $R'$ . The intercept on the  $y$ -axis yields the inverse of the water potential at full hydration ( $\bar{P} = 0$ ). In this example it is  $-1$ . The dashed line with the slope  $1/\bar{\pi}$  is the relationship between  $1/\bar{\pi}$  and  $R'$  in this region where  $\bar{P} = 0$ . Together with the calculated  $1/\bar{\pi}$  and  $R'$ , it is possible to determine  $\bar{P}$  from Equation 9.5, since  $\Psi = \bar{P} + \bar{\pi}$ . It is also possible to determine  $\bar{P}$  from Equation 9.3). The data are for *Dubautia paleata*.

linear, assuming that  $W_a$  and  $\phi$  remain constant in this region. Hence, from Equation 9.12, it is possible to calculate  $1/\bar{\pi}$  for all values of  $R'$  greater than that at which  $\bar{P}$  reaches zero. The latter relationship is illustrated by a dashed line in Fig. 9.1. At full hydration, where  $R' = 1$ , Equation 9.12 reduces to:

$$\frac{1}{\bar{\pi}} = - \frac{W_s^o}{\phi \rho RTN} \quad (9.13)$$

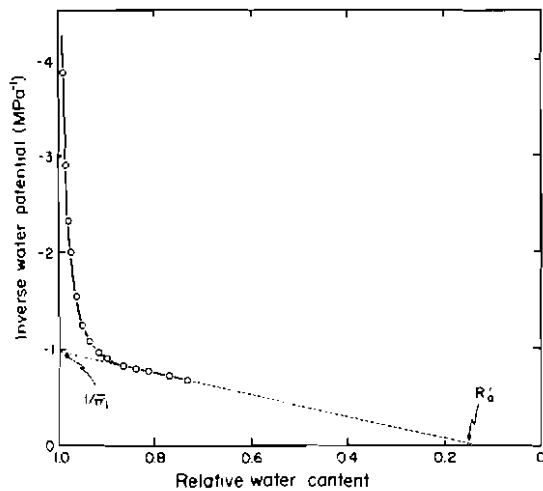


Fig. 9.1 Relationship between the reciprocal of tissue water potential ( $1/\Psi$ ) and tissue relative water content ( $R'$ ) (solid line). An additional data point exists at  $1/\Psi = -7.41 \text{ MPa}^{-1}$  and  $R' = 0.997$ . The dashed line defines the calculated relationship between the reciprocal of tissue osmotic potential ( $1/\bar{\pi}$ ) and  $R'$ . The intercept of the dashed line with the  $y$ -axis yields the reciprocal of tissue osmotic potential at full hydration ( $1/\bar{\pi}_i$ ). The value of  $\bar{\pi}_i$  in this example is  $-1.02 \text{ MPa}$ . The intercept of the dashed line with the  $x$ -axis yields the relative water content of the tissue apoplasm ( $R'_a$ ). The value of  $R'_a$  in this example is 0.14. Given the measured relationship between  $1/\Psi$  and  $R'$ , together with the calculated relationship between  $1/\bar{\pi}$  and  $R'$ , it is possible to calculate the relationship between tissue turgor pressure ( $\bar{P}$ ) and  $R'$  from Equation 9.5, since  $\bar{P} = \Psi - \bar{\pi}$  (see Fig. 9.2). It is also possible to calculate the relationship between  $\bar{P}$  and  $\Psi$  in a similar manner (see Fig. 9.3). The data are for the Hawaiian bog species, *Dubautia palcata*.

The right side of Equation 9.13 thus equals the reciprocal of the tissue osmotic potential at full hydration ( $\bar{\pi}_i$ ).

Given the calculated relationship between  $1/\bar{\pi}$  and  $R'$ , together with the measured relationship between  $1/\Psi$  and  $R'$ , it is also possible to calculate relationships between  $\bar{P}$  and  $R'$  (Fig. 9.2) and  $\bar{P}$  and  $\Psi$  (Fig. 9.3) using Equation 9.5. Species (or individuals) may differ markedly in their capacities for maintaining high  $\bar{P}$  values as both  $R'$  and  $\Psi$  decline. These differences in turgor maintenance capacity may result from two separate phenomena (Robichaux, 1984). First, the maximal value of  $\bar{P}$  may increase, reflecting a decrease in the value of  $\bar{\pi}$  at full hydration ( $\bar{\pi}_i$ ). Second, the rate at which  $\bar{P}$  declines with decreasing  $R'$  (or  $\Psi$ ) may decrease. The

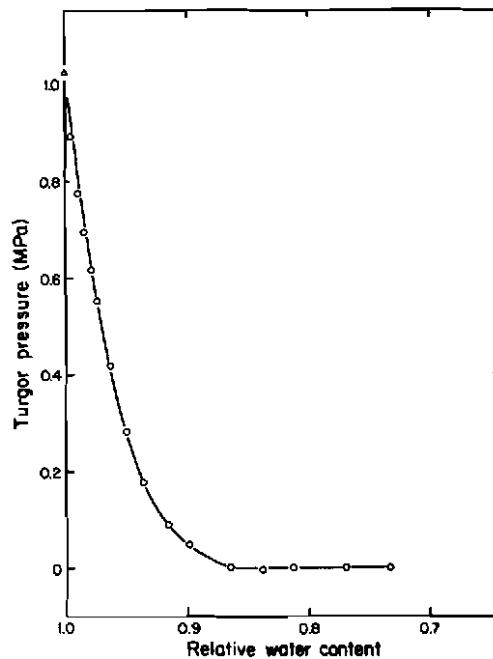


Fig. 9.2 Relationship between tissue turgor pressure and tissue relative water content. The relationship was obtained from the data in Fig. 9.1. The maximal value of  $\bar{P}$ , denoted by the triangle, was obtained from the calculated value of  $\bar{\pi}_i$ , which is equal in magnitude but opposite in sign.

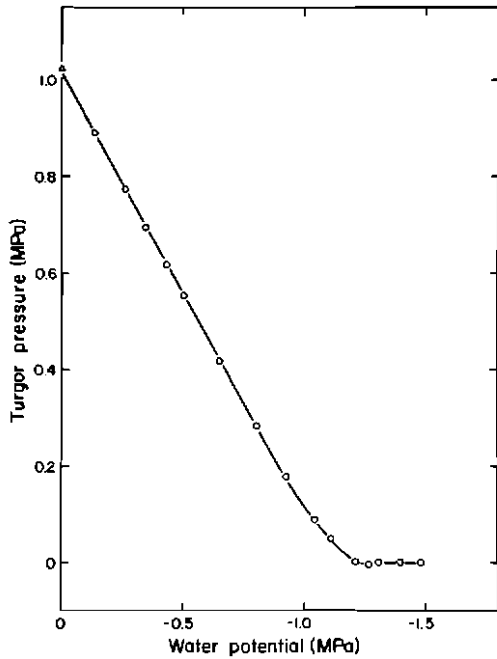


Fig. 9.3 Relationship between tissue turgor pressure and tissue water potential. The relationship was obtained from the data in Fig. 9.1. The maximal value of  $\bar{P}$ , denoted by the triangle, was obtained from the calculated value of  $\bar{\pi}_i$ .

latter change reflects an increase in the degree of tissue elasticity.

The bulk tissue elastic modulus ( $\bar{E}$ ) is defined as the change in  $\bar{P}$  for a given fractional change in the weight of symplasmic water (Tyree, 1981; Tyree and Jarvis, 1982). Hence:

$$\bar{E} = \frac{d\bar{P}}{dW_s} W_s \tag{9.14}$$

If  $W_a$  remains constant as  $W_s$  decreases, then:

$$\bar{E} = \frac{d\bar{P}}{dR'} (R' - R'_a) \tag{9.15}$$

where  $R'_a$  is the relative water content of the tissue apoplast, or the apoplasmic fraction (Robichaux *et al.*, 1986). In practice,  $\bar{E}$  may be calculated using the finite form of Equation

9.15. To calculate the value of  $\bar{E}$  near full hydration ( $\bar{E}_i$ ), for example, one obtains  $\Delta\bar{P}/\Delta R'$  from the linear slope of the first four to five points of the  $\bar{P}$  versus  $R'$  relationship (Fig. 9.2) and  $R'$  from the mean value for these four to five points. One obtains  $R'_a$  from the original plot of  $1/\Psi$  versus  $R'$ , by extrapolating Equation 9.12 to the point at which  $1/\Psi = 0$  (Fig. 9.1). At this point:

$$R' = R'_a = \frac{W_a}{W_s^0 + W_a^0} \tag{9.16}$$

From Equation 9.15, it follows that an increase in the degree of tissue elasticity is reflected in a decrease in  $\bar{E}$ . In addition to calculating  $\bar{E}_i$ , it is important to calculate the relationship between  $\bar{E}$  and  $\bar{P}$  when comparing the tissue elastic properties of different species or individuals (Tyree and Jarvis, 1982; Robichaux, 1984; Robichaux and Canfield, 1985). To

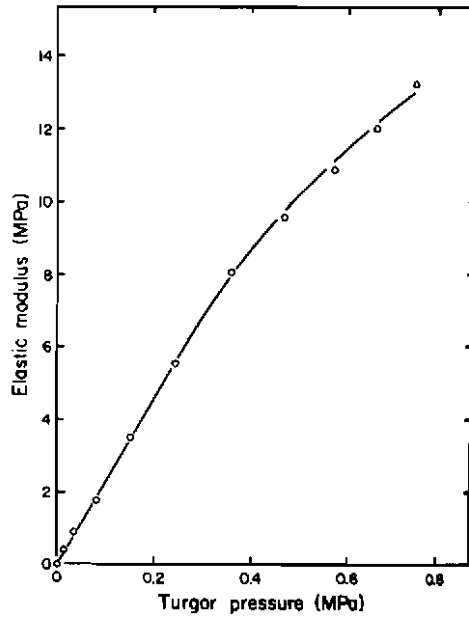


Fig. 9.4 Relationship between the tissue elastic modulus and tissue turgor pressure. The relationship was obtained from the data in Fig. 9.2. The tissue elastic modulus is defined as the change in  $\bar{P}$  for a given fractional change in the weight of symplasmic water (see Equations 9.14 and 9.15).

determine this relationship for each successive  $\bar{P}$  versus  $R'$  relationship, the procedure outlined in words, one calculates 3-6, 4-7, etc., beginning with the mean  $\bar{P}$  value for the first point (Fig. 9.4). (We have chosen the relationship as optimal when the relationship between the  $\bar{P}$  versus  $R'$  relationship is high. When fewer data points may be calculated for three points.)

Data obtained with this technique may be used to study changes in  $\bar{P}$  in plants under various conditions. For comparison, one may measure the diurnal changes in  $\bar{P}$  and compare them to the procedures described in the preceding section and the relationship between  $\bar{P}$  and  $\Psi$  (Fig. 9.3). Given the relationship between  $\bar{P}$  and  $\Psi$  for a given day, one may then study changes in  $\bar{P}$ . We assume a diurnal relationship between  $\bar{P}$  and  $\Psi$  (Tyree *et al.*, 1979) and for conditions of low water potential (Tyree and Roberts, 1985). This should be checked for a particular study. Three procedures may be used to study the relationship between  $\bar{P}$  and  $\Psi$  in three procedures collected in the field. The first procedure returned to the field where the petiole (or stem) is cut to eliminate the water to eliminate the initial cut can be made. If the plant is harvested, then the petiole (or stem) should be cut and as much water as possible should be removed during this procedure. The potential for error

determine this relationship, one calculates  $\bar{E}$  for each successive set of four points in the  $\bar{P}$  versus  $R'$  relationship (Fig. 9.2), following the procedure outlined above for  $\bar{E}_i$ . In other words, one calculates  $\bar{E}$  for points 1-4, 2-5, 3-6, 4-7, etc., beginning near full hydration. The mean  $\bar{P}$  value for each set of four points is then used in plotting  $\bar{E}$  as a function of  $\bar{P}$  (Fig. 9.4). (We have found four points to be optimal when the number of data points in the  $\bar{P}$  versus  $R'$  relationship is moderately high. When fewer data points are available,  $\bar{E}$  may be calculated for each successive set of three points.)

Data obtained with the pressure-chamber technique may be used to estimate diurnal changes in  $\bar{P}$  in plants growing under field conditions. For comparable tissues, one measures the diurnal changes in  $\Psi$  according to the procedures discussed in Section 9.2.1, and the relationship between  $\bar{P}$  and  $\Psi$  according to the procedures discussed in this section (Fig. 9.3). Given the assumption that the latter relationship does not change during the day, one may then calculate the diurnal changes in  $\bar{P}$ . We emphasize that the assumption of a diurnally constant relationship between  $\bar{P}$  and  $\Psi$  may not always be valid, particularly for herbaceous species (Acevedo *et al.*, 1979) and for species growing under conditions of low water availability (Bowman and Roberts, 1985). Thus, this assumption should be checked for the species of interest in a particular study.

Three procedures are available for generating the relationship illustrated in Fig. 9.1. In all three procedures, a leaf (or branch) is collected in the field, sealed in a plastic bag and returned to the laboratory or field station, where the petiole (or stem) is recut under water to eliminate air in the xylem. (If the initial cut can be made under water when the leaf is harvested, there is no need to recut the petiole.) Care should be taken to keep the leaf blade and as much of the petiole as possible dry during this procedure. This minimizes the potential for errors associated with non-

symplasmic water loss. The leaf is allowed to rehydrate by storing it in a cool dark humid chamber with the end of its petiole submerged in distilled water. As a general rule, the rehydration period should not extend much beyond the point at which  $\Psi$  approaches zero. This minimizes the potential for errors associated with metabolic changes within the leaf. Once the leaf is fully rehydrated, its saturated weight is measured, preferably with a balance having 0.1 or 1 mg accuracy. Prior to measuring the saturated weight, it is advisable to recut the petiole above the level to which it was submerged during the rehydration. This further minimizes the potential for errors associated with nonsymplasmic water loss. Following the saturated weight determination, the initial  $\Psi$  value is measured with the pressure chamber. This initial  $\Psi$  value should be higher than  $-0.1$  MPa if the leaf has fully rehydrated.

At this point, the three procedures diverge. In the first procedure, the leaf is allowed to dry under ambient conditions on the laboratory bench (Hinckley *et al.*, 1980; Robichaux, 1984; Kikuta *et al.*, 1985). At periodic intervals, the fresh weight and water potential of the leaf are measured. For each  $\Psi$  measurement, two fresh weight measurements are obtained, one immediately preceding and one immediately following the  $\Psi$  determination. The average of these two fresh weight measurements is used in the subsequent calculation of  $R'$ . To prevent a significant weight change during the measurement, the leaf may be enclosed in a plastic sheath while it is in the pressure chamber. The precautions discussed in Section 9.2.1 should be followed in measuring  $\Psi$  with the pressure chamber at each point. In addition, the rate at which the pressure is released from the chamber after each reading should be very slow, typically on the order of  $0.003$ – $0.005$  MPa  $s^{-1}$ . A rapid release rate will result in a rapid decline in leaf temperature, which may cause condensation on the leaf surface, damage to the leaf membranes or even freezing of the leaf.

In the second procedure, the leaf loses water by overpressurization in the pressure chamber rather than by evaporation on the laboratory bench (Wilson *et al.*, 1979; Wilson and Ludlow, 1983). After the saturated weight and initial  $\Psi$  value are obtained, the leaf is exposed to a pressure 0.2–0.3 MPa in excess of the initial balance pressure. This overpressurization forces water out of the leaf more rapidly than it would be lost by evaporation. The expressed water is blotted from the petiole and discarded. After 4–10 min of overpressurization, the pressure is slowly released from the chamber, the leaf is removed and its fresh weight is measured. The leaf is then reinserted into the pressure chamber and the corresponding  $\Psi$  value measured. Following the  $\Psi$  determination, the leaf is exposed to another period of overpressurization, and the measurement cycle is repeated. Thus paired measurements of fresh weight and  $\Psi$  are obtained at periodic intervals as the leaf water content declines.

In the third procedure, the leaf also loses water by overpressurization in the pressure chamber (Cheung *et al.*, 1975; Ritchie and Roden, 1985). In this case, however, the expressed water is collected and weighed, while the leaf remains enclosed in the pressure chamber. After the saturated weight and initial  $\Psi$  value are obtained, the leaf is exposed to a pressure 0.2–0.3 MPa in excess of the initial balance pressure for 10 min, during which the expressed water is collected in a preweighed section of plastic tubing filled with dry tissue. (The tubing should fit tightly on the exposed end of the petiole to minimize evaporative water loss.) After 10 min, the tubing is removed and weighed rapidly. The weight of expressed water is subtracted from the leaf saturated weight to obtain the leaf fresh weight. The pressure in the chamber is lowered by 0.2–0.3 MPa for approximately 15 min, after which a new  $\Psi$  value is measured. The new  $\Psi$  value will depend on the amount of water lost during the previous overpressurization period. Fol-

lowing the new  $\Psi$  determination, the leaf is exposed to another period of overpressurization, and the measurement cycle is repeated. The leaf should be enclosed in a plastic sheath while it is in the pressure chamber, since evaporative water loss will introduce a significant error into the fresh weight calculation. In addition, a partial pressure of approximately 0.2 MPa of compressed air should be maintained in the pressure chamber throughout the measurement period, since continuous exposure of the leaf to pure nitrogen gas for a prolonged period may cause cell membrane damage (Cheung *et al.*, 1975).

In all three procedures, the measurements are carried out until the water content of the leaf has declined to 55–65% of its original saturated value. The leaf is then dried in an oven at 70–80°C for 18–24 h. For each  $\Psi$  measurement, the corresponding  $R'$  value is calculated as:

$$R' = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \quad (9.17)$$

A plot of  $1/\Psi$  versus  $R'$  yields the relationship illustrated in Fig. 9.1. The region over which this relationship is linear may be determined graphically with the aid of a stepwise linear regression program. When the data exhibit more scatter than that shown in Fig. 9.1, more sophisticated analytical methods may be used to determine the linear region (Powell and Blanchard, 1976; Sinclair and Venables, 1983). At high  $R'$  values, points will deviate from linearity because of positive  $\bar{P}$  values. In some cases, points may also deviate from linearity at very low  $R'$  values (Wilson *et al.*, 1979). The latter deviation appears to result from cell death (Tyree *et al.*, 1973), together with the associated leakage of solutes into the apoplasm.

The three procedures offer different practical advantages. The first procedure offers the major advantage that six to seven leaf samples may be processed concurrently by

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one individual working with one pressure chamber. In the second and third procedures, in contrast, several pressure chambers are required for processing multiple samples, since the pressure chamber is used both for expressing water from the leaf and for measuring  $\Psi$ . The latter procedures, however, offer the advantage of being more rapid in many instances.

In principle, the three procedures should yield the same result. However, a recent analysis by Ritchie and Roden (1985) suggests that this may not be true in all cases. For those cases in which different results are obtained, there is currently no consensus as to which procedure yields the more accurate result.

Regardless of the procedure used, the pressure chamber technique has four major assumptions. These are: (1) the symplasmic value of  $\Psi$  is accurately measured by the pressure chamber (see Section 9.2.1), (2) negative values of  $\bar{P}$  do not occur in the symplasm (i.e.  $\bar{P} \geq 0$ ), (3) the net water loss from the tissue is entirely from the symplasm (i.e.  $W_a = \text{constant} = W_a^0$ ), and (4) as water is lost from the symplasm, the concentration of solutes increases in an ideal manner (i.e.  $\phi = \text{constant}$ ). The consequences of violating these assumptions have recently been analyzed by Tyree and Karamanos (1981), Tyree and Richter (1981, 1982), Tyree and Jarvis (1982), Cortes and Sinclair (1985) and Robichaux *et al.* (1986).

The pressure-chamber technique is also subject to several other potential sources of error (Tyree and Karamanos, 1981; Tyree and Richter, 1981). These include: (1) gradual changes in  $\bar{\pi}$  over the time course of the measurements, (2) plastic deformation of the cell walls over the time course of the measurements, (3) systematic and random errors in the measurement of  $\Psi$ , and (4) internal  $\Psi$  disequilibria. As discussed in Section 9.2.1, internal  $\Psi$  disequilibria may be particularly problematical in branches, compound leaves and severely stressed large leaves. According to Tumer *et al.* (1984), allowing the leaf to lose

water by evaporation rather than overpressurization may minimize the existence of internal  $\Psi$  disequilibria.

#### (b) Psychrometric techniques

Several techniques employing thermocouple psychrometers are also available for measuring the symplasmic values of  $\bar{\pi}$  and  $\bar{P}$  (Turner, 1981). The value of  $\Psi$  in a tissue sample is first measured with the psychrometer. Sap is then extracted from the sample with a small press. The sample may be pressed either in the fresh state (Markhart and Lin, 1985) or following freezing and thawing (Turner, 1981). The latter procedure aids in membrane disruption. The sap extract is then immediately placed on a filter paper disc in the psychrometer chamber and its value of  $\bar{\pi}$  determined (Turner, 1981). Alternatively, the tissue sample may be frozen and thawed and its  $\Psi$  value measured a second time (Acevedo *et al.*, 1979). Since  $\bar{P}$  is zero following freezing,  $\Psi$  equals  $\bar{\pi}$  in the second measurement. Once  $\Psi$  and  $\bar{\pi}$  are known, the value of  $\bar{P}$  in the sample prior to pressing and freezing is calculated with Equation 9.5.

With these techniques, the symplasmic values of  $\bar{\pi}$  and  $\bar{P}$  may be measured much more rapidly than with the pressure-chamber technique. The psychrometric techniques are subject to a major source of error, however, in that the symplasmic water is diluted with apoplasmic water following membrane disruption by pressing or freezing (Tyree and Jarvis, 1982). Hence, the symplasmic value of  $\bar{\pi}$  may be significantly overestimated with these techniques, resulting in erroneously low  $\bar{P}$  estimates. In addition, ion exchange between symplasmic ions and exchange sites in the cell wall following membrane disruption may result in incorrect  $\bar{\pi}$  and  $\bar{P}$  estimates (Tyree and Jarvis, 1982).

#### 9.2.3 Technique for measuring the apoplasmic value of $\bar{\pi}$

A major assumption of the pressure-chamber

technique for measuring  $\Psi$  is that the apoplasmic value of  $\bar{\pi}$  is negligible (see Section 9.2.1). The latter value may be determined by placing a leaf in a pressure chamber, expressing water (or sap) from the xylem by overpressurization (see Section 9.2.2), and then measuring the  $\bar{\pi}$  value of the expressed water (or sap) with a thermocouple psychrometer. It is best not to use the water that is first expressed, since it may be contaminated by solutes released from damaged cells at the cut surface of the petiole.

### 9.3 WATER CONTENT

In certain instances, decreases in tissue water content may be more important than decreases in  $\Psi$  or  $\bar{P}$  in terms of influencing plant growth (Sinclair and Ludlow, 1985; Ludlow, 1987). For example, Kaiser (1982) has demonstrated a strong correlation between changes in leaf protoplast volume and changes in leaf photosynthetic activity. Thus, it is often important to measure both water content and  $\Psi$  for plants growing under field conditions.

Tissue water content may be expressed in several ways, including the amount of water per unit dry weight, per unit fresh weight and per unit weight of water at full hydration (Slatyer, 1967). The third expression, which equals  $R'$ , is the most satisfactory in terms of quantifying tissue water deficits, since it is not influenced by changes in tissue dry weight (Slatyer, 1967; Turner, 1981).

Diurnal changes in  $R'$  either may be estimated using data obtained with the pressure chamber technique or may be measured directly. In the former case, the procedure is analogous to that described previously for estimating diurnal changes in  $\bar{P}$ . Similar precautions also hold for estimating  $R'$  in this manner.

In contrast to  $\bar{P}$ ,  $R'$  may also be measured directly for higher plants growing under field conditions. Leaf samples (often as small

discs) are collected in the field and placed immediately into hermetically sealed tared containers (Turner, 1981). (Any evaporative water loss introduces a significant error into the  $R'$  calculation.) After their fresh weights are obtained, the samples are rehydrated to obtain their saturated weights, then oven-dried to obtain their dry weights. Values of  $R'$  are then calculated with Equation 9.17.

As a modification of this method, some workers have used a  $\beta$ -ray absorption technique (Nakayama and Ehrler, 1964) to monitor the water content of a given leaf repeatedly, then harvested the leaf to obtain saturated and dry weights for the  $R'$  calculations (e.g. Schulze *et al.*, 1972). To be operational, however, the  $\beta$ -ray absorption technique has to be calibrated initially against direct measurements of leaf water content.

### 9.4 HYDRAULIC RESISTANCE AND CAPACITANCE

Even in the absence of soil water deficits, diurnal reductions in shoot water potential occur as a consequence of water loss in transpiration (Kozlowski, 1968; Ehrler *et al.*, 1978). The cause of these reductions in shoot water potential is the resistance to liquid water flux (hydraulic resistance) in the soil/plant transpirational path. Increased plant hydraulic resistance has been shown to be the mechanism by which some fungal pathogens cause wilting (Duniway, 1977; Olsen *et al.*, 1983). The measurement of plant hydraulic resistance has also been used to assess the functional significance of xylem type (Calkin *et al.*, 1985) and to help evaluate the factors limiting plant water uptake (Elfving *et al.*, 1972; Bates and Hall, 1982). Plant hydraulic resistance may also play a role in the determination of habitat, especially in relation to stomatal behavior. For example, Koppers (1984) found that the combination of high hydraulic resistance and low stomatal response to humidity was possibly responsible

for limiting *Ribes*. When leaves w... levels, desiccation... soil and the plan... resistance of the... the contributions... here.

#### 9.4.1 Steady-state transpirational pa

The most appropri... plant hydraulic res... state water flux to... water movement a... tance. Thus:

$$F = \frac{(\Delta\bar{P} + c}{$$

where  $F$  is the water...  $\Delta\bar{P}$  is the hydrostatic... the resistor,  $\bar{\sigma}$  is... coefficient of the re... potential gradient a... the gravitational pot... resistor and  $R_h$  is t... The resistor may be... any part thereof... the hydrostatic and... respectively. Water... plant body, from sc... involve several parall... are dominated by hy... and some of which... driving forces. If  $\bar{\sigma}$  is... osmotic driving force... It should be emphat... is valid only for ste... whole plant, this w... water flux into the ro... to the water flux lea... strict steady-state co... occurs in the field bec... water between tissu... (ors) and the transp... (see Section 9.4.2). If

for limiting *Ribes uva-crispa* to shaded habitats. When leaves were exposed to high light levels, desiccation occurred rapidly. Both the soil and the plant contribute to the overall resistance of the transpirational path. Only the contributions of the plant are discussed here.

#### 9.4.1 Steady-state flux: calculation of the transpirational path hydraulic resistance

The most appropriate equation for calculating plant hydraulic resistance relates the steady-state water flux to the driving forces for liquid water movement and to the hydraulic resistance. Thus:

$$F = \frac{(\Delta\bar{P} + \bar{\sigma}\Delta\bar{\pi}) + \Delta\Psi_z}{R_h} \quad (9.18)$$

where  $F$  is the water flux through the resistor,  $\Delta\bar{P}$  is the hydrostatic pressure gradient across the resistor,  $\bar{\sigma}$  is the effective reflection coefficient of the resistor,  $\Delta\bar{\pi}$  is the osmotic potential gradient across the resistor,  $\Delta\Psi_z$  is the gravitational potential gradient across the resistor and  $R_h$  is the hydraulic resistance. The resistor may be the entire plant body or any part thereof.  $\Delta\bar{P}$  and  $\bar{\sigma}\Delta\bar{\pi}$  represent the hydrostatic and osmotic driving forces, respectively. Water flux through the entire plant body, from soil to atmosphere, may involve several parallel paths, some of which are dominated by hydrostatic driving forces and some of which have significant osmotic driving forces. If  $\bar{\sigma}$  is 0 (as in the xylem), no osmotic driving forces are present.

It should be emphasized that Equation 9.18 is valid only for steady-state flux. For the whole plant, this would require that the water flux into the root system be equivalent to the water flux leaving the canopy. This strict steady-state condition probably rarely occurs in the field because a net movement of water between tissue water sources (capacitors) and the transpirational path can occur (see Section 9.4.2). It is probably true, how-

ever, that for the many plants for which the magnitude of the total water flux is much greater than that contributed by capacitors, the dynamics of water flux may be described by a series of steady states.

Despite the appropriateness of Equation 9.18 for describing water fluxes within the plant body, its utility is extremely limited, particularly under field conditions. This is primarily because of our current inability to measure accurately the component driving forces. If we assume  $\bar{\sigma} = 1$  (most plant membranes have reflection coefficients of 1 for most pertinent solutes), then Equation 9.18 reduces to:

$$F = \frac{\Delta\Psi + \Delta\Psi_z}{R_h} \quad (9.19)$$

where  $\Delta\Psi$  is the water potential gradient across the resistor. Although some theoretical situations have been described for which Equation 9.19 would not be appropriate (Fiscus *et al.*, 1983), many data sets are adequately handled by this equation. This is true because at moderate to high fluxes, osmotic forces probably play only a small role in water movement. Our inability to measure accurately  $\Delta\bar{P}$ ,  $\bar{\sigma}$  and  $\Delta\bar{\pi}$  for field-grown plants makes necessary the use of Equation 9.19, at least as a first approach. Except in tall trees, Equation 9.19 may be further simplified by ignoring  $\Delta\Psi_z$ .

In many cases, a linear relationship between  $\Delta\Psi$  and  $F$  is evident, particularly at moderate to high fluxes (Fig. 9.5). The slope of the linear region is taken to indicate  $R_h$ .

In the field,  $F$  through intact plants can be measured using a porometer or other gas-exchange apparatus (Roberts and Knoerr, 1978; Bates and Hall, 1982; Nobel and Jordan, 1983; Meinzer *et al.*, 1983; Kupperts, 1984), heat-pulse or heat-flow apparatus (Kucera *et al.*, 1977; Allaway *et al.*, 1981; Cohen *et al.*, 1983), or energy balance equations (Abdul-Jabbar *et al.*, 1984). Schulze *et al.* (1985) compared the use of the porometer and heat-

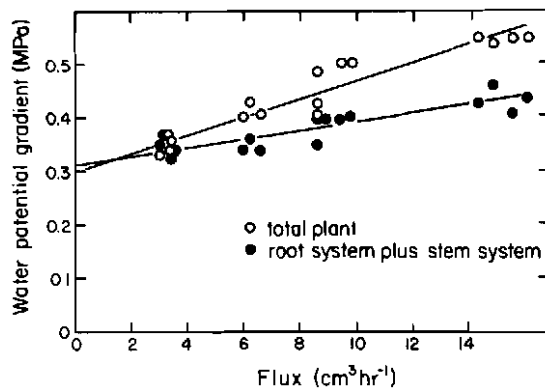


Fig. 9.5 Relationships between the water potential gradient ( $\Delta\Psi$ ) and the steady-state water flux ( $F$ ) in 8-week-old sunflower plants grown in soil in pots. Flux was measured gravimetrically. The soil was kept moist and was assumed to be at  $\Psi = 0$  MPa at the time of the measurements. For the total plant,  $\Delta\Psi$  was calculated from the soil to the transpiring leaf. Leaf  $\Psi$  was measured with a pressure chamber. The equation for the linear regression is  $\Delta\Psi = 0.0171(F) + 0.297$ . For the root system plus stem system,  $\Delta\Psi$  was calculated from the soil to the stem. Stem  $\Psi$  was measured using covered (i.e. non-transpiring), fully-expanded leaves. The equation for the linear regression is:  $\Delta\Psi = 0.0079(F) + 0.313$ .

flow techniques. They found that the two techniques yield similar results in the estimation of water flow through the plant, though careful attention must be paid to the boundary layer resistance within the potometer cuvette. A high boundary layer resistance can lead to severely underestimated transpiration rates. For water flow through rootless plants, potometers can be used to measure  $F$  (Running, 1980).

Measurement of  $\Delta\Psi$  can be accomplished using the psychrometric or pressure chamber techniques discussed in Section 9.2.1. Usually, the  $\Psi$  of transpiring leaves is measured to obtain one end of the  $\Psi$  gradient (the downstream end). Although the leaf system  $R_h$  may be large (Tyree and Cheung, 1977; Black, 1979; Koide, 1985a), in some cases it is the root system plus stem system  $R_h$  that is of most interest (Duniway, 1977; Koide 1985b).

In these cases, it is sufficient to measure the  $\Psi$  of nontranspiring leaves to obtain the downstream end of the  $\Psi$  gradient. Plastic or aluminum foil wrappings are often used for this purpose (Begg and Turner, 1970; Black, 1979; Koide, 1985b). Values of root system plus stem system  $R_h$  have been calculated in this fashion for intact sunflower plants (Fig. 9.5). Alternatively, stem  $\Psi$  may be measured with stem thermocouple psychrometers (McBurney and Costigan, 1982), although problems associated with thermal gradients under field conditions may limit thermocouple psychrometer utility (see Section 9.2.1).

Measurement of  $\Psi$  at the upstream end of the  $\Psi$  gradient can be difficult. In theory, it is the average  $\Psi$  at the surface of the absorbing roots that is used in the calculation of root system or whole plant  $R_h$ . As mentioned previously, root psychrometers have been used (Fiscus, 1972), but it is usually easier to arrive at an average root surface  $\Psi$  by other means. The bulk soil  $\Psi$  measured with soil psychrometers has been used for this purpose (Allen *et al.*, 1981). There is some justification for doing so in comparative studies if it can safely be assumed that the  $\Psi$  gradients from bulk soil to roots are the same for all treatments. However, if the plants that are compared have markedly different transpiration rates or different rooting densities, and particularly if they grow in very dry light-textured soils, large differences in the  $\Psi$  gradients from bulk soil to roots might be expected to occur (Faiz and Weatherley, 1977). Jones (1983) showed that the root surface  $\Psi$  of field-grown water-stressed plants could be estimated from the leaf  $\Psi$  and leaf vapor conductances of the stressed plants and the well-watered controls. Other workers have measured pre-dawn leaf  $\Psi$  to estimate root surface  $\Psi$ , but upon initiation of transpiration, the  $\Psi$  of the rooting zone may be somewhat lower than it was before dawn.

If it can be assumed that the root surface  $\Psi$  does not change with time or transpiration rate (such as in moist soils of high hydraulic

conductivity), then we can know the actual  $\Delta\Psi$  and the actual flux. Instead of the relationship between leaf  $\Psi$  and  $F$ , we have extrapolated intercepts that equal the effective  $R_h$  that  $\bar{\sigma} = 1$  and steady-state  $F$ .

Richter (1973) and others, have pointed out that these methods, which are based on the assumption that  $\Psi$  is not confused with resistances, are based on flux densities (rather than being that of leaves). Resistivities are not resistances, but they are worthwhile for comparison, particularly for organs of different size (Newman, 1977; 1979). Calculation of  $R_h$  on an area basis may also be possible, but it can be linearly related to the sectional area supply (Running, 1978).

#### 9.4.2 Nonsteady-state water flux and capacitance

Under many natural conditions, water flux cannot be measured at steady state; that is, the amount of water taken up by the root system is not equal to the amount transpired by the leaves. This is particularly true in meristematic and young plants. In a steady-state water flux experiment, the expansion of growing tissue is measured. In other cases, a net flux of water is measured out of or into nongrowing tissue. This is viewed as part of the water balance of each tissue water source. The water balance is called water capacitance. Any tissue that is not actively transpiring can serve as a water source. The capacitance can serve as a buffer for water balance on a seasonal basis (Running, 1978).

conductivity), then it may not be necessary to know the actual  $\Delta\Psi$  driving the transpirational flux. Instead, the slope of the relationship between leaf  $\Psi$  and  $F$  should be  $R_h$ . The extrapolated intercept on the ordinate will equal the effective root surface  $\Psi$ , provided that  $\bar{\sigma} = 1$  and steady-state flux prevails.

Richter (1973) and Jarvis (1975), among others, have pointed out that resistances, which are based on fluxes ( $\text{cm}^3 \text{s}^{-1}$ ), are often confused with resistivities, which are based on flux densities ( $\text{cm}^3 \text{s}^{-1} \text{cm}^{-2}$ ), the area being that of leaves, roots or membranes. Resistivities are not serially additive as are resistances, but their calculation may be worthwhile for comparative purposes, particularly for organs such as roots that differ in size (Newman, 1973; Fiscus and Markhart, 1979). Calculation of resistivities on a leaf area basis may also be justified since leaf area can be linearly related to the xylem cross-sectional area supplying the leaf (Salleo *et al.*, 1985).

#### 9.4.2 Nonsteady-state flux: calculation of capacitance

Under many natural circumstances, plant water flux cannot be considered to be steady state; that is, the amount of water absorbed by the root system is not equal to that transpired by the canopy. In plants with meristematic and young tissue, some nonsteady-state water flux occurs owing to the expansion of growing cells (Boyer, 1985). In other cases, a net flux of water can occur out of or into nongrowing tissues not normally viewed as part of the transpirational path. Such tissue water sources (or sinks), whether living (symplasmic) or nonliving (apoplasmic), are called water capacitors.

Any tissue that is not separated from the transpirational path by an infinite hydraulic resistance can serve as a capacitor. Water exchange from capacitors occurs on a diurnal and seasonal basis (Kozlowski, 1968; Waring and Running, 1978). The shrinking and swell-

ing of tissues are the result of symplasmic capacitors losing and gaining water (Kozlowski, 1968). Tissues such as leaves (Meidner, 1952), stems, fruits (Kozlowski, 1968) and roots (Huck *et al.*, 1970) have been shown to change physical size as a consequence of water exchange. Root shrinkage may be important to overall plant hydraulic resistance because it can reduce the contact surface area between root and soil (Faiz and Weatherley, 1982). Wood may serve as an apoplasmic capacitor, although cavitation, not shrinkage, is the result of water loss (Siau, 1971; Waring and Running, 1978).

In some cases, capacitor water can make a significant contribution to transpiration. In *Agave deserti*, stored water can sustain maximal transpiration for up to 16 h (Nobel and Jordan, 1983). Many trees also store water that can contribute significantly to transpiration (Jarvis, 1975; Waring and Running, 1978; Waring *et al.*, 1979; Schulze *et al.*, 1985). In other cases, capacitor water may make only a slight contribution to transpiration. Nevertheless, any exchange of water between plant tissues and the transpirational path may have an effect on the local tissue  $\Psi$ . The amount of change in  $\Psi$  for a given amount of water exchange depends on the capacitance of the tissue.

The tissue capacitance ( $C$ ) is defined as the change in tissue water volume ( $V$ ) for a given change in tissue  $\Psi$  (Nobel, 1983). Hence:

$$C = \frac{\Delta V}{\Delta \Psi} \quad (9.20)$$

Clearly, the capacitance of a tissue consisting of 100 cells would be 10 times greater than that of a tissue consisting of 10 cells, assuming cell size and all else to be equal. Thus, for comparing differences in capacitors that are not solely related to differences in total water volume, a volume-normalized capacitance ( $\bar{C}$ ) can be defined as:

$$\bar{C} = \frac{\Delta V}{\Delta \Psi} \frac{1}{V_0} \quad (9.21)$$

where  $V_0$  is the tissue water volume at full hydration. Since  $V/V_0$  equals  $R'$ ,  $\bar{C}$  can be expressed as:

$$\bar{C} = \frac{\Delta R'}{\Delta \Psi} \quad (9.22)$$

(Nobel and Jordan, 1983).

The principal method for measuring  $\bar{C}$  in higher plants growing under field conditions is the pressure-chamber technique. This technique is very similar to that discussed in Section 9.2.2 for measuring the symplasmic values of  $\bar{\pi}$  and  $\bar{P}$ . Instead of plotting the relationship between  $1/\Psi$  and  $R'$  (Fig. 9.1), one simply plots the relationship between  $\Psi$  and  $R'$  (Fig. 9.6). The reciprocal of the slope of the latter relationship equals  $\bar{C}$ . The various procedures for generating this relationship are discussed in detail in Section 9.2.2.

The rate of water exchange between the capacitor and the transpirational path depends on the gradient in  $\Psi$  and on the

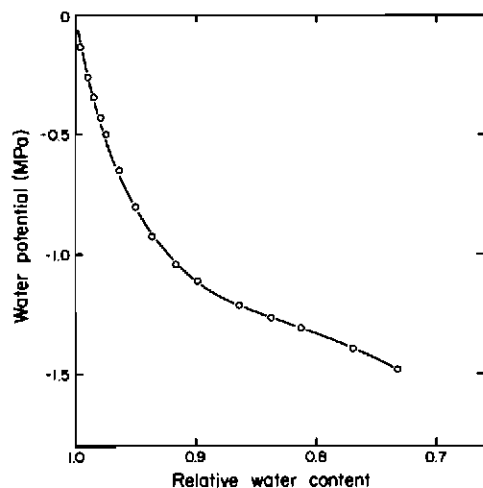


Fig. 9.6 Relationship between tissue water potential and tissue relative water content. The data are the same as in Fig. 9.1. The volume-normalized capacitance ( $\bar{C}$ ) of the tissue equals the change in  $R'$  for a given change in  $\Psi$  (see Equation 9.22). Thus,  $\bar{C}$  equals the reciprocal of the slope of this relationship.

hydraulic resistance to water exchange (the transfer hydraulic resistance). By analogy to electrical circuits, Nobel (1983) showed that the time constant ( $\tau'$ ) for water exchange between the capacitor and the transpirational path equals  $R_H^t \bar{C}$ , where  $R_H^t$  is the transfer hydraulic resistance.

The pressure-chamber technique for measuring  $\tau'$ , and thus  $R_H^t$ , has been discussed in detail by Nobel and Jordan (1983). A leaf (or other organ) is placed in the pressure chamber and an initial  $\Psi$  value ( $\Psi_a$ ) is obtained. The pressure in the chamber is then increased to a value (corresponding to  $\Psi_b$ ) that is 0.2 MPa in excess of the initial balance pressure. Thus,  $\Psi_b = \Psi_a - 0.2$ . (For some species, a value other than 0.2 MPa may be more appropriate for this overpressurization.) The water expressed from the leaf by this overpressurization is blotted from the petiole and discarded. At various time intervals, the pressure in the chamber is briefly lowered to the point at which exudation just ceases. Thus, for each time interval ( $t$ ), a corresponding  $\Psi$  value ( $\Psi_t$ ) is obtained, with  $\Psi_a > \Psi_t > \Psi_b$ . The value of  $\tau'$  is then calculated with the equation:

$$\Psi_t = (\Psi_a - \Psi_b)e^{-t/\tau'} + \Psi_b \quad (9.23)$$

If  $\bar{C}$  is known for this same range of  $\Psi$  values, then  $R_H^t$  may be calculated as  $\tau'/\bar{C}$  (Nobel and Jordan, 1983). The precautions associated with this technique are similar to those discussed for the pressure-chamber techniques of Sections 9.2.1 and 9.2.2.

## 9.5 CONCLUSION

In this chapter, we have provided a brief introduction to the theory and measurement of plant water status, hydraulic resistance and capacitance. In conclusion, we emphasize that we currently seem limited more by the accuracy and precision of our measurements than by the theoretical framework supporting them. For example, while the steady-state

water flux equation is theoretically correct, hydraulic resistance measurements measure the component of water flux differently. Thus, while the techniques discussed are useful frameworks, the limitations of our measurements should always be kept in mind.

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water flux equation (Equation 9.18) is theoretically correct, its utility for calculating hydraulic resistance is limited by our inability to measure the component driving forces accurately. Thus, while the concepts and techniques discussed in this chapter should serve as a useful framework for field research, the limitations of our current methods should always be kept in mind.

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