Chapter 1

Why Measure the Water Status of Plants and Soils?

Plant tissues contain large amounts of water, and even larger amounts must be supplied to replace the water lost in transpiration. A maize plant weighing about 800 g at tasseling contains about 700 g of water, and one must supply an additional 20,000 to 50,000 g to grow the plant to this stage. Such a large involvement of water makes it essential to understand how water is used in plant growth, especially since water is the largest input in agriculture. Irrigation has been practiced in agriculture for more than 5000 years and civilizations have fallen because of long dry spells or the failure of irrigation systems.

Historically, water management depended on measuring the time since the last rain or irrigation or occasionally on the extent of wilt of the leaves. These methods had the advantage of simplicity but were too crude to detect early losses in growth. Scientific investigations employed similar methods but were hampered by the difficulty in repeating conditions.

Part of the problem is that plants vary in their response to water. Those with deep roots may prosper when shallow-rooted individuals fail to grow. Early flowering or high water storage can fit some species for a desert existence that others cannot tolerate. Measuring the time since the last rain or the loss in soil water does not take these plant characters into account.

A better approach is to measure the water status of the plant. The differences in water use between species are then included in the measurements, and the varying effects of rainfall and evaporation are integrated as well. There is an increased predictability of plant performance and, for scientific purposes, experimental conditions are more easily measured and reproduced.

Methods of measuring plant water status can generally be classified in three categories. Those in the first category rely on concentrated solutions (osmotica) that cause water release from the tissue. Placing roots in a series of osmotica can indicate which solution

causes no water loss or gain, or which causes no change in tissue dimensions. The water status is then expressed in terms of the solution properties causing no change. While these are relatively simple methods, they suffer from the possibility of solute exchange with the tissue. If the membranes of the cells do not completely exclude the solute, the osmotic effectiveness of the solution is less than expected from the concentration and can differ in various tissues (Kramer and Boyer, 1995; Slatyer, 1967; Steudle, 1989). Also, tissue can release water and solute that can change conditions in the osmoticum (Knipling, 1967). Therefore, these methods are not often used.

The second category of water status methods is based on measures of plant water content that are informative when compared to other tissue properties. Typically, the water content is compared to the tissue dry weight or is expressed as a percentage of the maximum water the tissue can hold. The comparison gives a biological baseline or reference which is particularly valuable for determining whether sufficient water has been lost to alter enzyme activities or to concentrate cell constituents.

The third category is based on thermodynamic methods that determine the chemical potential of water in the tissue. These methods have the advantage that the water status is compared to a physically defined reference rather than a biological one, and the physical reference allows the chemical potential to be precisely reproduced at any time or place. The chemical potential has the further advantage that the forces moving water through the soil and plant can be measured.

The latter two categories of methods are the focus of this book, and emphasis is given to the last one because of the wealth of information that can be obtained and the large number of applications that can be made. Many scientific studies now employ measurements of the chemical potential or one of its components, and understanding the methods and their pitfalls is essential for joining this effort.

The development of thermodynamically based methods was given important impetus when Slatyer and Taylor (1960) suggested a new terminology for the water status of plants and soils. This was not the first such suggestion (e.g., see Kramer, 1985), but it was the first to use classical thermodynamics expressed in units already in frequent use.

It included terminology for all the forces in plants and soils, and the ideas were rapidly accepted.

The new terminology accelerated the development of new methods for measuring these forces. A pressure chamber was proposed to measure the tension in the xylem and apoplast of plants (Scholander *et al.*, 1965), and the equipment was used with a vapor pressure osmometer to measure the water potential of leaves soon afterward (Boyer, 1967a). Other methods employed vapor pressures (Monteith and Owen, 1958; Richards and Ogata, 1958; Spanner, 1951) to measure the water potential and were simplified and made more accurate (Boyer and Knipling, 1965). A microcapillary method was developed for directly measuring the turgor inside individual cells (Hüsken *et al.*, 1978). Each had the ability to indicate not only the water status of various parts of plants and soils but also the forces used to move water from place to place.

With the new methods, efforts have been increasingly directed to determining how water moves through the soil-plant system and how metabolism is affected. They show that water is directed to various parts of the soil-plant system according to the difference in water status between the parts (Boyer, 1985; Passioura, 1988). Cell water status often determines the rate of enlargement of the parts and thus is fundamental to the growth process. The forces moving water usually do not directly affect the activity of enzymes because other factors begin to alter enzyme activity before the water status becomes low enough to exert a direct effect (Kramer and Boyer, 1995). The important factors are the availability of products of photosynthesis, the movement of small regulatory molecules, changes in plant growth regulators, and differences in gene expression. As a result of this work, the control of plant metabolism with limited water is seen increasingly as a chemical problem that can be manipulated by altering the availability of particular regulators of enzyme activity and synthesis.

Questions that are now attracting attention include: What conditions lead to changes in regulatory behavior at the molecular level? Is decreased transport of regulatory molecules from the soil a result of the decreased water in the soil or a property of the plant? What signal causes changes in levels of plant growth regulators and gene expression in plant tissue? Answers to these questions will

continue to require repeatable water status measurements. For an expanded treatment of these questions, the reader is directed to Kramer and Boyer (1995) and to a symposium volume edited by Close and Bray (1993).

A Little Thermodynamics

Let us think about how the later physically based methods originated. When we consider molecules of any kind, all of them contain energy in their atoms and chemical bonds that can be exchanged with the surroundings by their motions, chemical reactions, and radiational exchanges (here we assume that the isotopic composition remains stable). The energy exchanges always result in a rearrangement of chemical or atomic structure that in itself requires energy (Fig. 1.1). Thus, a fraction of the energy goes to the rearrangements and a fraction to the surroundings, and the latter fraction can be made to do work. The rearrangement energy is the entropy and the energy available for work is the free energy (Fig. 1.1).

It readily can be seen that the amount of work is determined by the number of molecules exchanging energy. Doubling the number of molecules doubles the work, all other factors remaining constant. Often, however, it is more desirable to know the work per molecule or per mole of molecules than the total work. J. Willard Gibbs (1931) recognized this and defined the term "potential" and symbol μ as the way to describe the work that a mole of molecules can do.

The work is not known in absolute terms because the total amount of energy in molecules is not known. Therefore, the work is determined by comparing the chemical potential of the system with a reference potential. For liquid water, the reference has been chosen to be pure unrestrained water at atmospheric pressure, a defined gravitational position, and the same temperature as the system being compared. If we define the chemical potential of the system to be measured as μ_w and the chemical potential of the reference as μ_o, $(\mu_w - \mu_o)$ is the comparison we wish to make. When the system is not pure water, the μ_w is lower than μ_o, and $(\mu_w - \mu_o)$ is negative. When the system is pure water, $(\mu_w - \mu_o)$ is zero. When the system is varied in pressure or gravitational position in a water column, $(\mu_w - \mu_o)$ can be positive or negative.

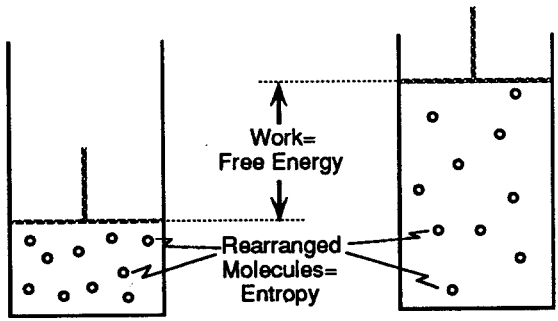

Figure 1.1. Molecular changes occurring when work is done by a gas and piston. The rise in the piston represents work which is the force of the piston times the distance moved. On the left, the molecules are close together and on the right they are far apart. The work cannot occur unless this molecular rearrangement takes place. The energy consumed in the rearrangement is the entropy. The remaining energy raises the piston, does the work, and is the free energy.

The $(\mu_w - \mu_o)$ is the energy state of the molecules. It does not matter how the molecules get to that state, the energy is the same whenever $(\mu_w - \mu_o)$ is at the same level. The energy represents the maximum work that can be done if the molecules are part of an ideal machine. Pure water moving through a selective membrane into a solution on the other side is a machine allowing work to be done as molecules on one side escape from the bulk and pass through the membrane to the other side. If the membrane allows water to pass but not solute (the membrane reflects solute), more water will move to the solution side than to the other side because the free energy of the pure water is higher than in the solution. The work is determined by the potential difference on the two sides of the membrane and the net volume of water moved. The work can be measured by opposing this movement with a chemical potential that counters the movement.

If the membrane is not reflective for solute, the volume of water moving into the solution is the same as the volume of water and solute moving in the opposite direction. No work is done because there is no net volume change. Nevertheless, at the beginning, the $(\mu_w - \mu_o)$ is the

same as when the reflective membrane was present. Thus, the ability to do work is identical but the work actually done depends on the characteristics of the machine.

This example illustrates that the $(\mu_w - \mu_o)$ is an intrinsic property of the molecules. The membrane simply determines the work extracted from the molecules. The reflectiveness of the membrane is usually described by the reflection coefficient which is 1 for a perfectly reflective membrane but 0 for a nonreflective one. This is important for anyone studying water movement in plants and soils. The osmotic effectiveness of a solution is determined by the membrane reflectiveness from the beginning even though large concentration differences exist on the two sides of the membrane. This is one reason why methods of measuring water status with osmotica may not give accurate data unless the membranes have a reflection coefficient of 1.

The idea of Slatyer and Taylor (1960) was to express the chemical potential in pressure units to make it simpler to apply to plant and soil systems. This was done by dividing $(\mu_w - \mu_o)$ by the partial molal volume of liquid water $\bar{V}_w$ to give the water potential Ψ_w:

$$\Psi_w = \frac{(\mu_w - \mu_o)}{\bar{V}_w}. \qquad (1.1)$$

Because the units for $(\mu_w - \mu_o)$ are energy per mol and for $\bar{V}_w$ are volume per mol, the units of Ψ_w are energy per volume = force per unit area = pressure. The pressure is usually expressed in megapascals (MPa) where 1 megapascal = 10^6 pascals = 10^6 newtons$\cdot$m^{-2} = 1 joule$\cdot$m^{-3} = 10 bars = 9.87 atmospheres or 145 pounds per square inch.

The $\bar{V}_w$ is the volume of a mole of liquid water mixed with other molecules in the system and is nearly a constant 18 cm$^3\cdot$mol^{-1} over most of the temperatures and water contents of cells and soils. Therefore, the Ψ_w is simply $(\mu_w - \mu_o)$ divided by a constant. In concentrated solutions, dry soils, and other systems of low water content, this simplification may not hold because interactions between water and the other molecules can be so extensive that 1 mol of water no longer occupies 18 cm^3. In this case, the proportionality breaks down, and $(\mu_w - \mu_o)$ should be used whenever Ψ_w is below about -10 MPa.

The chemical potential can be measured from the relationship between pressure and volume. In Fig. 1.1, work is done by the expansion of the gas against the piston. The work is the distance the piston moves times the force exerted by the piston, which is described by $dV \cdot P$ and has units of m^3 x force$\cdot m^{-2}$ = force$\cdot$distance. The work can be measured by holding the volume constant and measuring the change in pressure or by holding the pressure constant and measuring the change in volume. Of the two the former is easier so that at constant temperature

$$(\mu_w - \mu_o) = \int dV \cdot P = \bar{V}_w \int_o^P dP \qquad (1.2)$$
$$= \bar{V}_w \cdot (P - 0),$$

where the volume is held constant. Notice that, because the pressure is in the *liquid* water whose volume is essentially incompressible, the volume of 1 mol of water is a constant $\bar{V}_w$. The constant does not enter the integration and the equation gives the maximum work that 1 mol of liquid water molecules can do. From Eq. 1.2, the water potential is

$$\Psi_w = \frac{(\mu_w - \mu_o)}{\bar{V}_w} = P, \qquad (1.3)$$

which indicates that the water potential is equivalent to a pressure, usually negative. To measure this pressure, we create a counteracting pressure that prevents work, i.e., prevents water from moving in the plant or soil system. The measuring pressure equals P. Because the measuring pressure counteracts P, it is an equilibrium measurement.

Measuring work with vapor pressures follows a similar procedure. The pressures are applied to water vapor in the *gas* phase. In this case, the volume of a mole of water is no longer constant. From the gas law, the volume of a mole of gas molecules is $v = RT/e$ where we use lower case v and e to indicate the volume and pressure in the gas phase. The chemical potential in the gas phase is

$$(\mu_w - \mu_o) = \int dv \cdot e = RT \int_{e_o}^{e_w} \frac{de}{e} = RT \ln \frac{e_w}{e_o}. \quad (1.4)$$

Here, R is the gas constant (8.3143×10^{-6} $m^3 \cdot MPa \cdot mol^{-1} \cdot K^{-1}$), and T is the Kelvin temperature (K), which is held constant. Therefore, RT does not enter the integration. The water potential is

$$\Psi_w = \frac{RT}{\bar{V}_w} \ln \frac{e_w}{e_o}, \quad (1.5)$$

which expresses the water potential in the usual way by dividing the chemical potential by the partial molal volume of *liquid* water.

One may visualize that just as the chemical potential affects the ability of liquid water molecules to escape through a membrane, it will affect the ability of liquid water molecules to escape into the gas phase (evaporate). If we can measure the ability to evaporate, we have a measure of the chemical potential in the liquid. To measure the ability to evaporate, we need only to create a partial pressure for water in the gas that matches the vapor pressure of water in the liquid, preventing evaporation. This is the equilibrium vapor pressure, and the Ψ_w in the gas equals the Ψ_w in the liquid. Equation 1.4 tells us that $(\mu_w - \mu_o)$ of the gas is related to the ability to evaporate according to the ratio of the vapor pressure of the system (e_w) to the vapor pressure of the reference (e_o), i.e., the relative humidity at the temperature of the system.

Of course, temperature has large effects on the vapor pressure of water but Eq. 1.5 compares e_w and e_o at the same temperature and e_w/e_o responds only to nonthermal effects (concentration, pressure, and so on). Temperature has its effect mostly on T (which decreases at lower temperatures) and slightly on $\bar{V}_w$ (which decreases, then increases at lower temperatures). As T decreases to absolute zero, Ψ_w approaches zero. Similarly, P in Eq. 1.3 shows no thermal response because of the isothermal nature of the measurement, but it will respond to the change

in potential according to T in the measured system. Appendix 3.2 is a practical demonstration of these facts as the osmotic potentials of sucrose solutions become less negative when the Kelvin temperatures decrease. As a consequence, the cell changes slightly in potential as T varies, and the e_w or P used to measure the potential will vary accordingly. The response is not large because there is only a narrow range of Kelvin temperatures in which biological systems exist, and the e_w or P measurements respond similarly and predictably.

This book will treat pressure and vapor pressure methods of measuring plant water status. The pressure chamber and pressure probe use pressures to measure Ψ_w and thus Eqs. 1.2 and 1.3 apply (Chaps. 2 and 4). The thermocouple psychrometer uses vapor pressures to measure Ψ_w and Eqs. 1.4 and 1.5 apply (Chap. 3). The equal signs in the equations indicate that the measurements are made at equilibrium, termed thermodynamic equilibrium.

The Value of Thermodynamic Equilibrium

Thermodynamics tells us that it is simplest to measure the energy state of molecules by using a system that prevents any work from being done, that is, by preventing the molecules from changing to a lower energy state during the measurement. In practical terms, this is done by using a measuring system to counterbalance the tendency of the molecules to do work. In this case, the measuring system is in thermodynamic equilibrium with the molecules being measured. For pressure in liquids, we counterbalance the pressure with an opposing but equal pressure. For vapor pressures, we create a vapor pressure that equals the vapor pressure of the molecules, preventing evaporation and again counterbalancing the pressure being measured.

Thermodynamic equilibrium is valuable first because it allows molecular energies to be determined without changing the molecules. If measurements are not at equilibrium, the measured molecules change energy and the measurement is affected by all the factors that affect energy change: the size of the energy differences that drive the process, the resistances to energy change imposed by the apparatus, the position of the exchanging molecules relative to each other, and so on. Measuring pressure without using a counterbalancing pressure on liquids, for example, allows flow to occur. While the flow rate can give

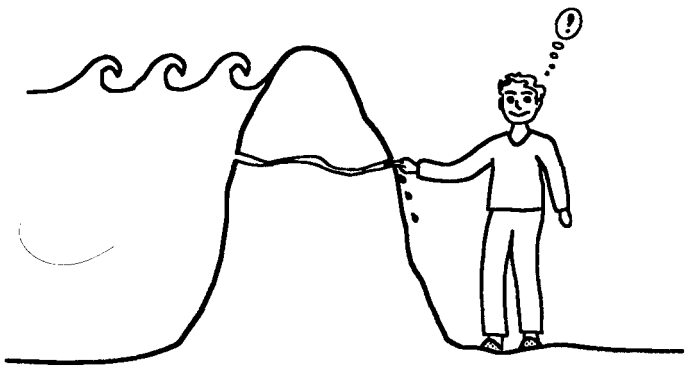

Figure 1.2. Accurate measurements of the pressure in this pore are easiest when there is no flow!

information about the pressure, one also must know the pressure difference between the measured molecules and the measuring instrument, the resistance to flow between the two systems, and many other factors. The pressure measuring instrument can be calibrated if conditions can be precisely controlled and repeated, but additional complexity is added. Therefore, it is preferable to measure at equilibrium where there is no flow.

The second reason thermodynamic equilibrium is important is that energy standards are readily available. Reference pressures are precisely known for the atmosphere or at the base of a column of water. Vapor pressures of solutions are well known and standards are readily available in the laboratory. As a result, equilibrium methods need little if any calibration, which is a great simplification. Moreover, because the factors affecting energy exchange between the molecules and measured system do not affect the measurement, determinations have less variability (see Chap. 5, Fig. 5.1 for an example). All in all, more accurate measurements are the result (Fig. 1.2).

Additional Readings

For readers interested in pursuing these concepts further, a number of papers and reviews treat various aspects of measuring plant and soil water status. Particularly recommended are papers by Slatyer and Taylor (1960), Ritchie and Hinckley (1971), Tyree and Hammel (1972), and Brown and Oosterhuis (1992), reviews by Boyer (1969b) and Zimmermann and Steudle (1978), and the symposium proceedings published by Brown and Van Haveren (1972) and by Hanks and Brown (1987). A review by Barrs (1968) is useful for a historical description of older methods but contains several errors concerning more recent methods.