Chapter 3

Thermocouple Psychrometry

Thermocouple psychrometry is the most widely used method of measuring plant water status and probably is the most versatile. It can measure the water status of any plant part as well as soil or any substance containing water. The technique works over the entire range of water contents and, because it measures conditions in the gas phase, it does not require a continuous liquid phase for the measurement. The only requirement is that water be able to evaporate from the sample to the air. The method uses only a small sample which can be important for repeated measurements in the same plant or soil.

At the same time, psychrometry requires a good deal of care in its use. The sample is sealed in a closed container and the water in the sample evaporates to humidify the atmosphere. The measured humidity must accurately reflect the condition in the sample. The vapor pressures usually are high and approach saturation (relative humidity of 100%). With the humidity so near saturation, temperatures must be uniform so that condensation does not occur and lower the humidity. For the best accuracy, temperatures should not differ by more than 0.001°C anywhere in the chamber. Also, thermocouples are used to measure the humidity, but at high humidities they produce only a few billionths of a volt. Therefore, not only must temperatures be uniform, but small voltages must be accurately detected.

Instruments that incorporate these principles are available commercially. They use insulation and specially constructed and shielded circuits to provide the required stability. Depending on the specific design, measurements can be made with an accuracy that can exceed ±0.01 MPa. In this chapter, we will assume you have a commercial instrument (Appendix 3.1) and will describe the procedures for using it. However, the principles apply to any psychrometer and should be useful with custom-made units. For additional information, see Boyer (1969b) and Brown and van Haveren (1972).

Principles of the Method

When any liquid is sealed into a chamber containing air, it will evaporate until the partial pressure of the vapor equals the vapor pressure of the liquid. For example, liquid water will evaporate, causing the humidity to rise (Fig. 3.1A). Eventually the humidity becomes so high that the vapor condenses into the liquid at a rate that

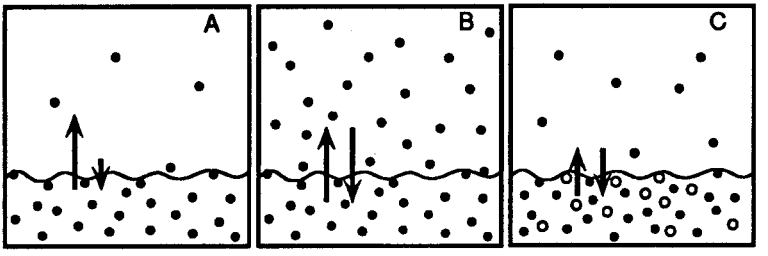

Figure 3.1. Evaporation of water in a closed container. A) Evaporation immediately after closing the container. The humidity is rising, and evaporation (upward arrow) is faster than condensation (downward arrow). B) At equilibrium, humidity has risen until evaporation occurs at the same rate as condensation. Humidity no longer changes. Relative humidity above pure water is 100%. C) For a solution, water molecules (solid circles) are excluded from the surface occupied by solute molecules (open circles). As a result, evaporation is slower and condensation slower (short arrows) and the humidity is lower than in B at equilibrium. The lower humidity (e_w /e_o) compared to B indicates a decrease in the free energy of the water molecules.

equals the evaporation (Fig. 3.1B). In this condition, the partial pressure of the vapor in the air equals the vapor pressure of water in the liquid and there is no further change in air humidity. The system in the chamber is in equilibrium because there is no net transfer of water within the system and the whole system remains stable with time. If we can measure the humidity of the air, we will know the vapor pressure of the liquid. As discussed in Chap. 1, the vapor pressure indicates the chemical potential of the water and thus the water potential.

Knowing the vapor pressure of the liquid tells us the water status of the liquid because the vapor pressure changes as various factors affect the molecules in the liquid. For example, adding solute to the water displaces some water from the space occupied by the solute (Fig. 3.1C). This will cause fewer water molecules to be exposed at the surface of the liquid and will decrease the rate of evaporation. A slower condensation rate will be required to equal the slower evaporation rate.

The simplest method of measuring the humidity is to place a solution of known vapor pressure into the atmosphere (Boyer and Knipling, 1965). If it evaporates, its vapor pressure is higher than the

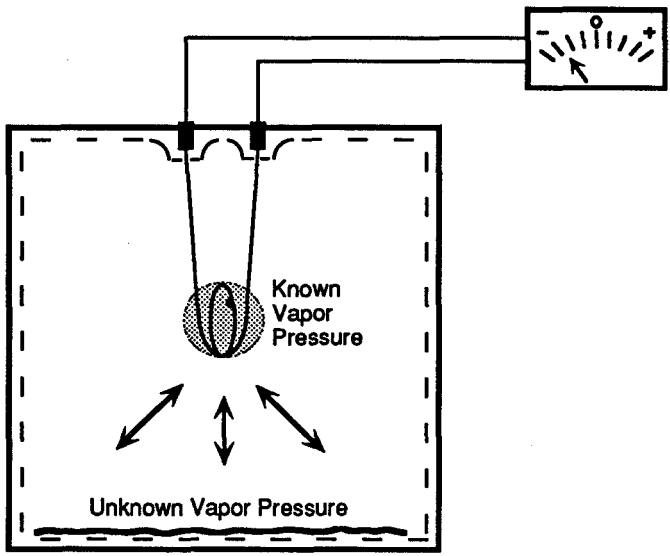

Figure 3.2. Evaporation from a thermocouple solution to a liquid of unknown vapor pressure inside plant tissue (wavy solid line on bottom). The thermocouple is wound in a spiral to form a loop where a droplet of solution can be placed (shaded area). The sensing junction is the black dot. If the thermocouple solution has a vapor pressure higher than that of the unknown, water evaporates from the solution and condenses in the unknown, cooling the solution, decreasing the voltage of the sensing junction, and decreasing the voltage on the meter, which remains steady as long as evaporation is steady.

humidity of the atmosphere. If it condenses water from the air, its vapor pressure is lower. Evaporation or condensation can be detected from the temperature. If evaporation occurs, the solution cools. If condensation occurs, the solution warms. The temperature can be detected with a thermocouple (Fig. 3.2). When the solution cools, the voltage produced by the thermocouple is low. When the solution warms, the voltage is high. When the voltage is the same as that of a dry thermocouple, which is neither cooled by evaporation nor warmed by condensation, the vapor pressure of the solution on the thermocouple is the same as the humidity of the atmosphere and in turn the vapor pressure of the solution in the chamber. The solutions are isopiestic, that is they have the same vapor pressure, and the isopiestic condition identifies the vapor pressure and thus the chemical potential of the solution in the chamber.

The isopiestic condition also is isothermal because the thermocouple solution and the sample solution have the same temperature, which is the same as the uniform temperature surrounding the chamber. As pointed out earlier, an isothermal condition is necessary before a relationship can be seen between the vapor pressure and the water potential.

Other variations of this method are available and will be described but all of them enclose a small sample in a vapor chamber and all use thermocouples to determine the humidity of the air above the sample. The main differences are in the voltage measurement for the thermocouple and whether calibration is required.

Theory of Psychrometry

As discussed in Chap. 1, there is a relationship between the vapor pressure of a solution and its water potential Ψ_w (Eq. 1.5). The relationship can be simplified to

$$\Psi_w = 137.2 \ln \frac{e_w}{e_o} \qquad (3.1)$$

at 25°C which shows that vapor pressure is a sensitive indicator of the water potential. For example, the vapor pressure of pure water gives a relative humidity of 100% when it is sealed in a chamber kept at a uniform temperature (e_w / e_o is 1), and $\Psi_w = 0$. When the water is not pure, e_w / e_o is less than 1 and $\ln(e_w / e_o)$ is negative, and Ψ_w is negative. For a relative humidity of 99.3%, Ψ_w is -1.0 MPa. Clearly, humidities are high above most biological samples and soils even when the water potential is quite negative!

From the process of evaporation, it can be seen that water vapor moves toward regions of lower vapor pressure and thus toward more negative water potentials. In the psychrometer chamber, the vapor moves similarly and most solutes are nonvolatile, so the thermocouple solution acts as though it is separated from the tissue by a differentially permeable barrier -- the atmosphere in the vapor chamber -- that allows only water to move. The movement can be opposed by an equal vapor pressure in the same way that the movement of liquid water through a membrane can be opposed by an external pressure. The potential measured by the opposing vapor pressure expresses not only the work that the water can do but also the direction the water will move (Chap. 1). The movement of vapor toward more negative potentials is thus an expression of the same phenomenon in the liquid and is the key to water acquisition by plants.

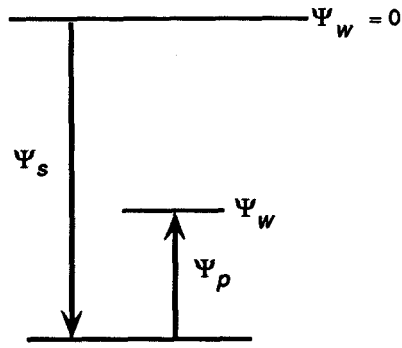

Figure 3.3. Free energy diagram showing the effect of solute (Ψ_s) and positive pressure (e.g., turgor pressure, Ψ_p) on the water potential of a solution inside a cell (Ψ_w). Solute decreases the free energy while positive pressure increases it. The top of the diagram shows the Ψ_w of pure, free water at the same temperature, which is the reference with a vapor pressure equivalent to 100% relative humidity and a water potential of zero.

Equation 3.1 is written in terms of the energy in the liquid-air system and not how fast the energy is achieved. The equal sign indicates that e_w / e_0 will give an unambiguous measure of the energy when the measurement is made at equilibrium with an opposing vapor pressure. The equilibrium is achieved when the sensor (the liquid on the thermocouple) has the same energy, that is vapor pressure and temperature, as the liquid in the sample.

Vapor pressures change with temperature but in equilibrium measurements e_w and e_0 are at the same temperature and the only effect is on the coefficient in Eq. 3.1. The coefficient 137.2 decreases to 131.6 when the temperature of the measurement decreases from 25°C (298 K) to 12°C (285 K), which is the same as the temperature dependence for the pressure chamber and pressure probe (see Chaps. 1, 2, and 4) and for solutions (see Appendix 3.2).

The vapor pressure develops at the surface of the solution in the sample. For plant tissue, this surface is in the cell walls and for soils it is on the surface of the soil particles. As discussed in Chap. 2, the water potential of the cell walls or apoplast is determined by the osmotic potential and matric potential of the apoplast solution, $\Psi_{w(a)} = \Psi_{s(a)} + \Psi_{m(a)}$, and a similar relation holds for the soil. The components are different for the protoplasts which have their own osmotic potential and a turgor pressure as shown in Fig. 3.3: $\Psi_{w(p)} = \Psi_{s(p)} + \Psi_{p(p)}$. Each protoplast is essentially in equilibrium with its cell wall: $\Psi_{w(a)} = \Psi_{w(p)}$.

The vapor pressure of the surface solution is affected by each of these components as water moves between the apoplast and protoplasts. Each component can be measured under the appropriate conditions in a psychrometer.

How Thermocouples Work

If two dissimilar metallic conductors are joined to make a circuit as in Fig. 3.4A, a voltage is generated at each junction (Seebeck thermoelectric effect). The voltage varies with temperature, but if the temperature is uniform, there is no current because the voltages in the circuit are the same and oppose each other (note that, in moving around the circuit, the sequence of metals at the first junction is opposite that at the other junction and thus the voltages are opposing). It is possible to insert a voltmeter to measure the voltage in the circuit (Fig. 3.4B). As long as the temperature remains uniform throughout the circuit, the thermoelectric properties of the voltmeter do not affect the circuit (the two contacts with the voltmeter generate equal and opposing voltages).

When the thermocouple junctions have different temperatures, the voltmeter will show the voltage difference between the two junctions with a sign that depends on which junction is warmer. In thermocouple psychrometers, one junction is held at the temperature of the surroundings, i.e., the temperature of the chamber in which the sample is located (T_1 in Fig. 3.4C), and is termed the reference junction (Fig. 3.5). The other junction is termed the measurement junction (Fig. 3.5) and is exposed to the chamber atmosphere where the junction is warmed (T_2 in Fig. 3.4C) or cooled (T_0 in Fig. 3.4D) by water condensing to or evaporating from the junction.

The reference junction usually consists of a pair of copper posts sealed into the top of the vapor chamber. The voltmeter is inserted between the two posts (Fig. 3.5). Because the posts have the same temperature (T_1), their effect on the voltages is equal but opposite and cancels. Thus, the circuit indicates the difference between the reference and measurement junctions as if the voltmeter was not there.

Thermocouples having their junctions at different temperatures absorb or liberate heat at the junctions in proportion to the current produced in the circuit (Peltier effect). The amount of heat absorbed or liberated can be increased by inserting a battery to increase the current flow around the circuit. The heat liberated at one junction equals the heat absorbed at the other junction, and reversal of the current flow reverses the liberation/absorption of heat at each junction. The Peltier

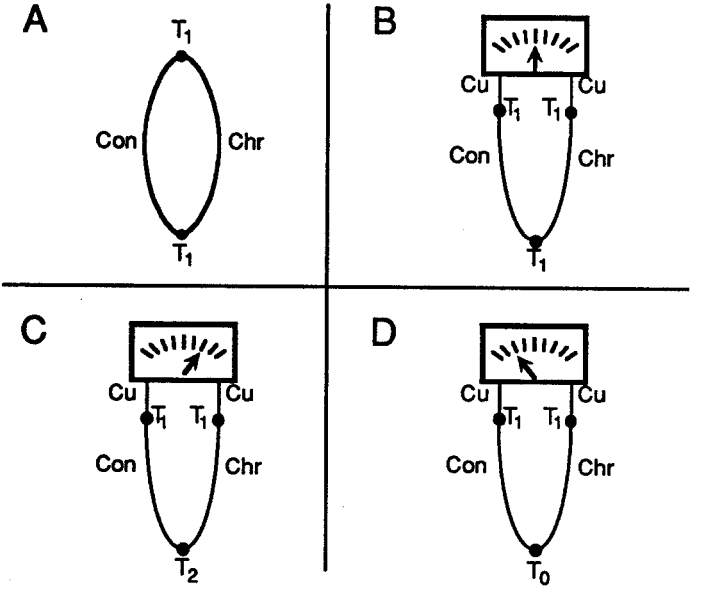

Figure 3.4. Thermocouple circuits. A) Basic thermocouple constructed of two dissimilar metals (Chr, chromel P; Con, constantan). Under isothermal conditions (T_1 at both junctions), the voltage is the same but opposing at the two junctions and no current flows. B) Same thermocouple as in A but with a voltmeter inserted at one junction. The voltmeter and wires are made of copper (Cu). In isothermal conditions (T_1), the voltmeter does not affect the circuit voltage and shows zero volts. C) Increasing the temperature of the lower junction (T_2) raises its voltage, and the voltmeter displays a positive voltage difference between the junctions at T_1 and T_2 (Seebeck thermoelectric effect). D) Decreasing the temperature of the lower junction (T_0) decreases its voltage, and the voltmeter displays a negative voltage difference between the junctions at T_1 and T_0.

effect is used to cool the measurement junction (and release heat at the reference junction) in some thermocouple psychrometers.

Types of Thermocouple Psychrometers

All psychrometers depend on the temperature of a thermocouple junction in contact with water or an aqueous solution

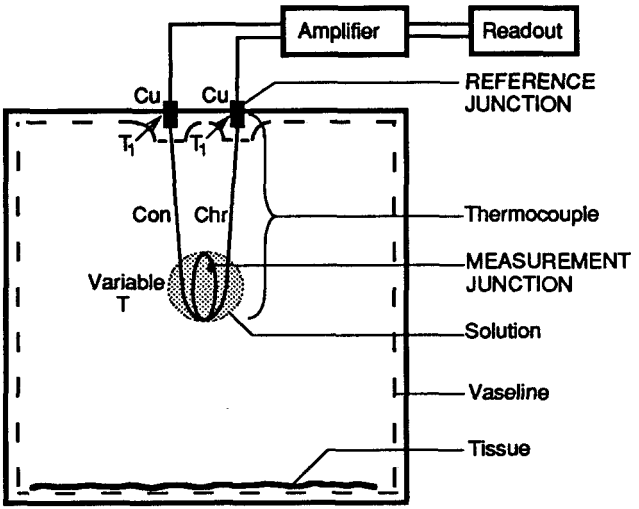

Figure 3.5. Typical thermocouple psychrometer circuit (Cu, copper; Con, constantan; Chr, chromel P). Reference junctions are at the copper posts, and the measurement junction is at the black dot. The voltage is measured between the posts at T_1 and the junction at variable T. Since both posts at T_1 have the same temperature, the voltage associated with copper cancels and only the voltage difference between T_1 and variable T is displayed by the amplifier and readout. Vaseline coats the chamber walls to minimize sorption of water vapor. The tissue sample is on the bottom.

suspended in the atmosphere above the sample (Fig. 3.2). According to the rate of evaporation, the thermocouple gives an electrical voltage. Because the output is small, it must be amplified (Fig. 3.5) before it can be sent to the voltmeter (readout device).

The amplifier introduces other junctions and switches into the circuit. These parts can act as thermocouples themselves and, to avoid interference with the measurements, they are kept isothermal. If there is doubt about a junction, a simple test is to warm it in your fingers and observe the effect in the readout of the instrument. If the junction is active there will be a change in the readout and extra steps should be taken to prevent temperature fluctuations around the junction.

It is essential that the walls of the psychrometer chamber are as nonsorptive as possible to facilitate vapor equilibrium. The most nonsorptive surface found so far is petrolatum (Vaseline) after it has

been melted and resolidified to coat the chamber walls (Fig. 3.5). The melting and resolidification apparently hide impurities so that the surface becomes highly hydrophobic (Boyer, 1967a). Although some manufacturers do not include this coating in their procedures, it decreases interaction of the thermocouple with the chamber walls and has other beneficial uses (described later).

The detailed design of a psychrometer depends on whether it is used with samples removed from their surroundings, i.e., plant parts or soil samples, or *in situ*, e.g., attached to leaves or undisturbed parts of the soil profile. The sizes and shapes of the instruments can vary accordingly. However, the principles of the measurements fall into three categories: the isopiestic method, the dew point method, and the Peltier method.

ISOPIESTIC PSYCHROMETERS

Isopiestic means equal pressure and isopiestic psychrometers determine the vapor pressure of a known solution that equals the vapor pressure of the unknown (Boyer and Knipling, 1965). The thermocouple wire is bent to make a spiral loop that holds a droplet of solution (Fig. 3.6A) and contains the measurement junction. The water or solution is placed in the spiral, the thermocouple is inserted into the vapor chamber, and the output is observed. When the output is steady, the thermocouple is removed, the solution is cleaned off, a new solution is placed in the same spiral, and the thermocouple is reinserted into the chamber. The new steady output indicates the voltage change for the potential change caused by the new solution on the thermocouple. The potential of the solution can then be calculated that gives the output of a dry thermocouple, which is neither cooled nor warmed by evaporation or condensation. This solution is isopiestic and does not exchange water vapor with its surroundings. The water potential of the solution is known and is the same as in the sample, so no calibration is required. Diffusive characteristics of the sample do not affect the measurement, and the temperature is corrected simply by using the solution for the measurement temperature given in Appendix 3.2.

Any vapor pressure can be measured as long as a solution exists with a similar vapor pressure. The method has been tested for its accuracy (Boyer, 1966) by using plant tissue of known water potential and measuring the potential with the isopiestic technique (Fig. 3.7). The absolute accuracy was ±0.01 MPa; that is, the isopiestic value was within 0.01 MPa of the true water potential of the tissue and remained so for several hours. This is the only technique for which such a high accuracy has been demonstrated.

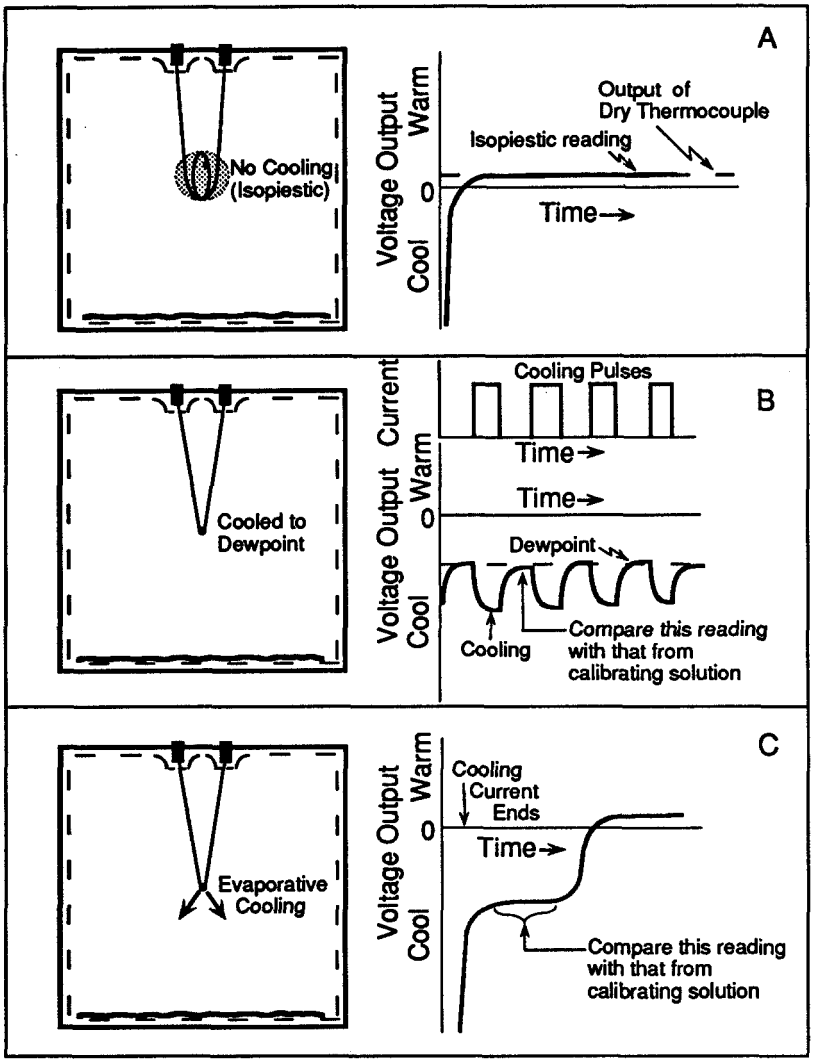

Figure 3.6. Three types of thermocouple psychrometers. A) Isopiestic psychrometer with a thermocouple that holds a droplet of solution of known vapor pressure (water potential). The thermocouple can be removed and the solution can be replaced with another having a different vapor pressure. The solution causing an output identical to that of the dry thermocouple is neither losing nor gaining water from the atmosphere and is thus isopiestic with the

DEW POINT HYGROMETERS

This method uses a Peltier current to cool the thermocouple just enough to keep the junction at the dew point (Neumann and Thurtell, 1972) where the condensed water has a vapor pressure that is the same as in the sample. The dew point is detected by determining the increase in Peltier cooling needed to cool the increased thermal mass of the measurement junction when it becomes wet. The dew point can be approximated by cooling in pulses and observing between pulses (Fig. 3.6B). This principle, described by Campbell *et al.* (1973), is used in a commercial unit (Appendix 3.1) that has an electrical circuit for automatically cooling and making the measurements (Fig. 3.6B).

Dew point instruments need to be calibrated with a range of solutions of known water potential (Appendix 3.1). The unknown dew point is compared with the dew points on the calibration curve to find the water potential of the unknown. The calibration is sensitive to ambient temperature.

Reproducibility is usually ±0.05 MPa (Campbell and Campbell, 1974; Nelsen *et al.*, 1978). With the commercial unit (Appendix 3.1), the true dew point is only approximated and there can be significant diffusion of water vapor between the sample and the junction, causing diffusive error (Shackel, 1984). At the true dew point, the diffusive error is minimized.

PELTIER PSYCHROMETERS

Figure 3.6C shows that in the typical configuration for a Peltier psychrometer (Spanner, 1951), Peltier cooling is used to condense water from the vapor atmosphere onto the thermocouple. The cooling is brought about by inserting a battery into the thermocouple circuit and disconnecting the amplifier and readout device. The battery moves an electrical current through the thermocouple in a direction that causes the junction to cool (the Peltier effect). The temperature drops below

atmosphere and tissue. No calibration is required. B) Dew point psychrometer using pulsed current from a voltage source to cool the junction to the dew point (Peltier effect). The pulses are varied in size to hold the junction at the dew point temperature. The output of the thermocouple is measured between pulses and compared to outputs previously measured with calibrating solutions. C) Peltier psychrometer using current imposed by a voltage source to cool the junction below the dew point (Peltier effect). Turning off the cooling current allows condensate to evaporate from the junction. The output decreases to a semi-stable value that is compared to outputs previously measured with calibrating solutions.

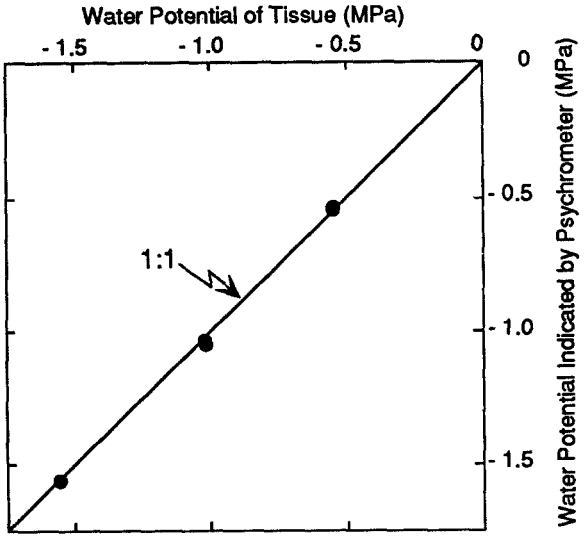

Figure 3.7. Test of the absolute accuracy of an isopiestic psychrometer. Water potential was measured with the psychrometer and compared with the known water potential for the same tissue. The known water potential was achieved by equilibrating the sunflower leaf tissue with an atmosphere of known water potential. Some replicate data points lie too close to one another to be resolved in this plot. From Boyer (1966).

the dew point and water condenses on the thermocouple junction. When the current is switched off, the condensate evaporates to the atmosphere (Fig. 3.6C). The rate of evaporation is greater when the atmosphere contains less vapor. By quickly switching the amplifier and readout into the circuit, the rate of evaporation can be determined and calibrated with solutions of known vapor pressure (water potential, see Appendix 3.1). Comparing the rate on the calibration curve with that of an unknown allows the water potential of the unknown to be determined (Fig. 3.6C).

A variant of this method places a droplet of water on the thermocouple (Richards and Ogata, 1958). The water is held in place around the junction by a porous ceramic bead or a ring. The output of the thermocouple becomes steady as water evaporates. The output is calibrated in the same way as for a Peltier psychrometer.

Neither of these methods operates at thermodynamic equilibrium because they measure the rate of evaporation rather than

the vapor pressure that prevents evaporation. As a consequence, calibration is required and is highly sensitive to a number of factors including temperature. Cool temperatures give less thermocouple output than warm temperatures. For best results, the calibration should be repeated immediately before and after the measurement of a sample. Peltier and Richards/Ogata psychrometers have the advantage of simplicity, but the transient nature of the Peltier measurement causes a significant amount of variation in repeated measurements, and Richards/Ogata psychrometers also are variable. With plant tissue and soils, one can expect these psychrometers to have a reproducibility of about ±0.15 MPa (Savage and Cass, 1984; Savage *et al.*, 1983). In both psychrometers, the diffusion characteristics of the vapor path between the thermocouple and the sample affect the measurements (see Diffusion Error), causing systematic error that is usually 5 to 10% of the reading (Boyer and Knipling, 1965) but can be as high as 30 to 50%.

How to Make Measurements

In the following section we briefly discuss the principles for making psychrometer measurements but distinguish between calibrated and noncalibrated instruments. You will need a set of solutions having various water potentials (osmotic potentials), and Appendix 3.2 gives the water potential (osmotic potential) for sucrose solutions. KCl or NaCl solutions also can be used but should be left in contact with the instrument only briefly to prevent corrosion.

PRELIMINARY CHECKS

Electrical Performance. Before using the instrument for the first time, check its electrical performance. Make sure that each thermocouple communicates with the readout (temperature change at thermocouple causes change at readout). Then check that each thermocouple gives a stable reading over long periods of time. It is best to use a recorder to conduct these tests. If there is instability, determine whether it is caused by a loose connection (usually rapid noise) or thermally active junctions (slow drifts in output). Wiggle suspected junctions to check for good contact and warm suspicious parts of the circuit to test for thermal activity. The output of the thermocouples is about 5.0 μV per MPa. Judge the stability in light of the variation you can accept in your final measurements.

For calibrated instruments, check that the thermocouple cooling works properly. Place a solution in the vapor chamber, pass a Peltier current through the thermocouple, and observe thermocouple output immediately after the current is switched off. You should see an output

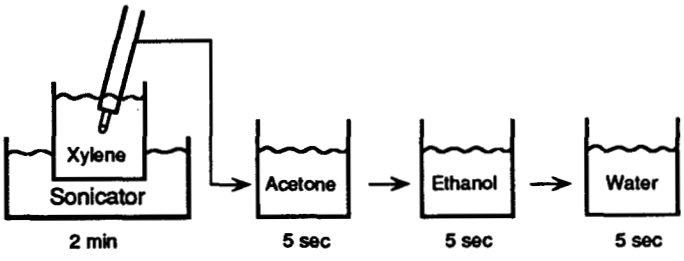

Figure 3.8. Cleaning a thermocouple from an isopiestic psychrometer.

indicating that the thermocouple is cool but the thermocouple should quickly return to zero output.

For isopiestic instruments, check that the thermocouple output is zero when water is on the thermocouple and on the bottom of the psychrometer chamber ($\Psi_w = 0$). This test is important not only to check whether the thermocouple works isopiestically, but also whether the thermocouple is clean, the electrical circuit is noise-free, and the chamber has been properly loaded with sample. If your thermocouple passes this test, it should be ready for use. Careful handling of the thermocouple usually will preserve this condition for many months, with only occasional testing necessary.

Cleaning the Thermocouples. If the thermocouple is contaminated with foreign material (e.g., Vaseline), the output will drift. Cleaning usually can be done by rinsing in water and blowing dry, but if this does not cure the problem, remove excess foreign material using a small spatula. Submerge the entire thermocouple, including the copper wires at the base, into xylene and sonicate for 1 to 2 min (Fig. 3.8). Follow this with a 5-sec rinse in acetone, then ethanol and finally water. Be careful not to bend the thermocouples during this operation. Reapply fresh Vaseline to the surfaces around the thermocouple as shown in Fig. 3.5, taking care not to cover the thermocouple itself.

ROUTINE PROCEDURES

The vapor pressure of water in samples is so high that surfaces can interact with the vapor and affect the measurements. To avoid this effect, keep chamber surfaces clean and make them as inactive as possible by coating with petrolatum (Vaseline). The following procedures work well for making measurements:

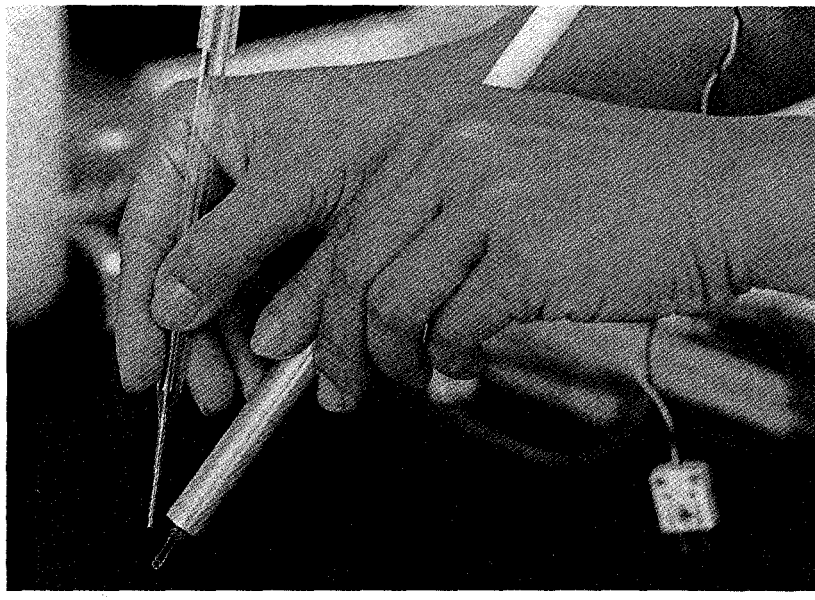

Figure 3.9. Drying the thermocouple from an isopiestic psychrometer in an air stream after rinsing the junction with water. Thermocouple and air stream should be directed downward to move water droplets away from the thermocouple.

1) Clean the thermocouple with a water rinse. Dry in an air stream (Fig. 3.9). Check that all seals are in good condition so that the vapor chamber can be made airtight.
2) Coat the vapor chamber with Vaseline by warming the cup to melt the Vaseline, then covering the bottom and walls and inverting to cool (Fig. 3.10). Use a spatula to spread a thin coating of Vaseline on as much of the chamber top as possible without coating the thermocouple (Fig. 3.5). For isopiestic psychrometers, coat the hole through which the thermocouple enters the chamber by wiping Vaseline into the hole, then removing the excess with a spatula that leaves only a thin coating.
3) For calibrated instruments, calibrate by placing thin tissue paper, i.e., Kimwipe or Kleenex, on the bottom of the cup, and cover the paper with calibrating solution. Assemble the apparatus and note the output of the thermocouple when vapor conditions become stable. Stability usually is achieved within 10 to 20 min. A calibration curve should be

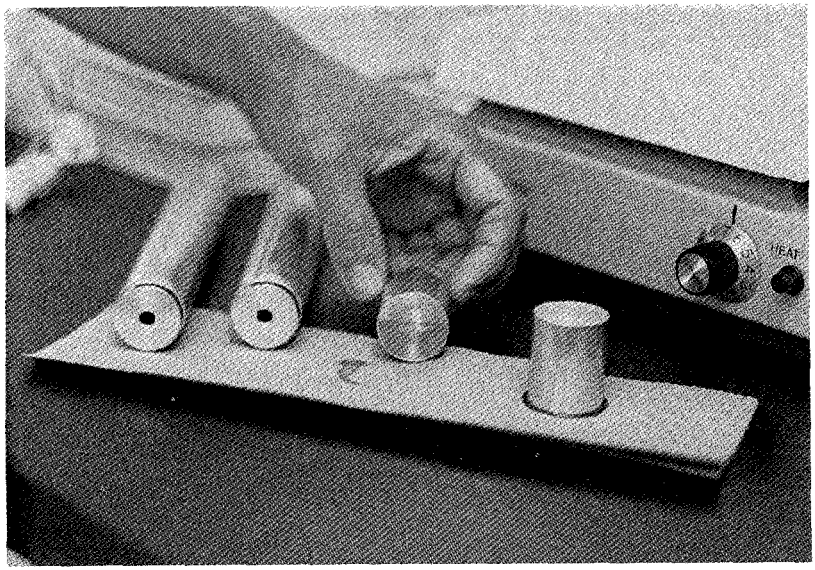

Figure 3.10. Coating the walls of a psychrometer cup with melted and resolidified Vaseline. The cup is cooled completely before reassembling the psychrometer. The chamber top, the walls of the entrance hole for the thermocouple, and the reference junctions of the thermocouple also are coated using unmelted Vaseline as in Fig. 3.5.

constructed to give the output of the system as a function of the water potential (osmotic potential) of the solution as in Fig. 3.11. In general, calibrate before and after measuring each unknown at the temperature being used for the unknown. For isopiestic instruments, this step is omitted.

4) Prepare the tissue or soil to be sampled. The aboveground parts of plants usually have foreign material on the surface. Depending on the growth environment, this may range from dust and salt to spray residues and deposits from watering. Rinse the tissue with water (Fig. 3.12) by wiping with saturated tissue paper, e.g., Kimwipe. Be sure that all surfaces have been thoroughly wetted. Dry by blotting the tissue to remove adhering water. Restore water potential gradients to normal by allowing the tissue to remain in the growth environment for several hours after washing and blotting dry.

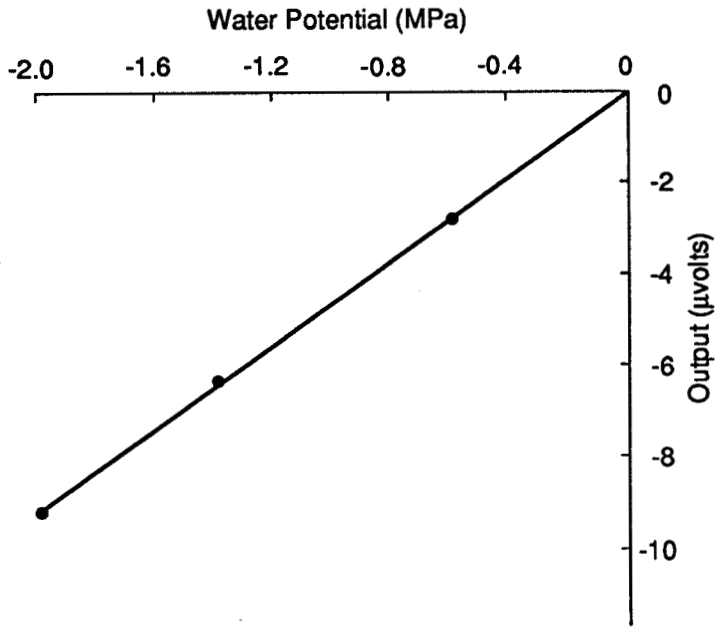

Figure 3.11. Typical calibration curve for Peltier or dew point psychrometer.

Some investigators abrade the surface of plant tissue with carborundum or remove the cuticle with xylene (e.g., Savage *et al.*, 1984). These practices should be avoided because they wound the tissue and disrupt the metabolic and growth activity. Cells lose turgor when they are wounded and they stop growing. Large changes in metabolism can be observed with these treatments. The best measurements are obtainable with clean but otherwise unaltered tissue. Isopiestic psychrometers overcome most of the problems of the cuticle without abrading (see Diffusion Error).

5) Rapidly load the sample into the cleaned and Vaseline-coated cup (see Working with Plant Tissue or Working with Soils for sample placement and handling techniques). Press the tissue into the Vaseline layer at two or three places to ensure good thermal drainage of metabolic heat to the chamber walls. Loading and sealing the cup should take no longer than 10 sec to avoid dehydrating the tissue. For

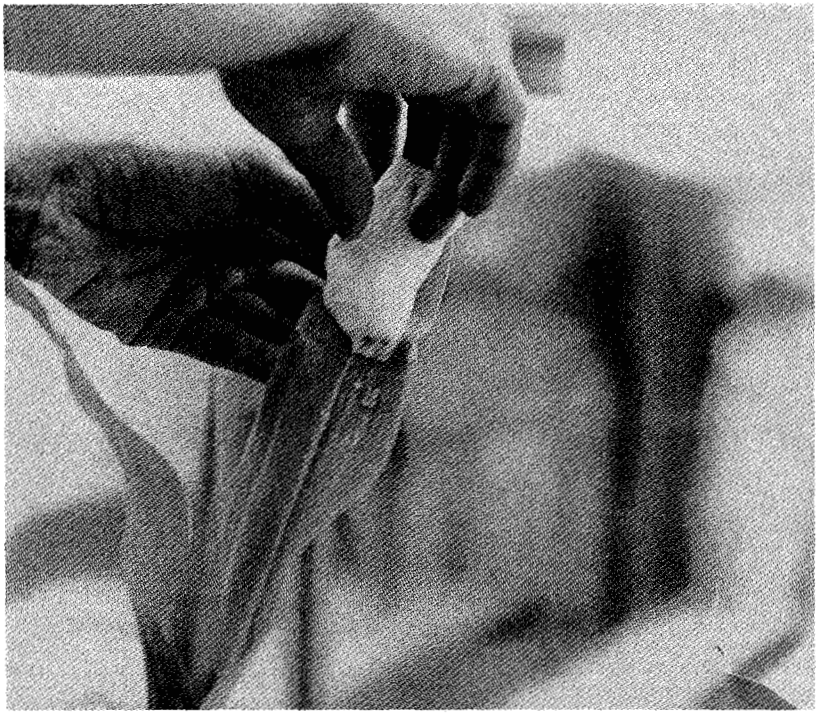

Figure 3.12. Washing plant tissue. After washing, blot the tissue dry with tissue paper. Allow the tissue to reestablish water potential gradients for a few hours before sampling.

leaves, a punch can help because it loads the cup when the disk is cut (Fig. 3.13). If longer sampling times are required, work in a saturated atmosphere. High humidity can be achieved by constructing a simple glove box (Fig. 3.14). Load the cup with one layer of tissue only. Additional layers cause problems because they cannot lose metabolic heat rapidly enough, and liquid condenses between the warm inner layers and cool outer layers causing erroneous readings.

6) Assemble the apparatus. If storage is necessary during transportation from the field, place the apparatus in a Styrofoam box away from direct sunlight, generally for no longer than 15 to 30 min.

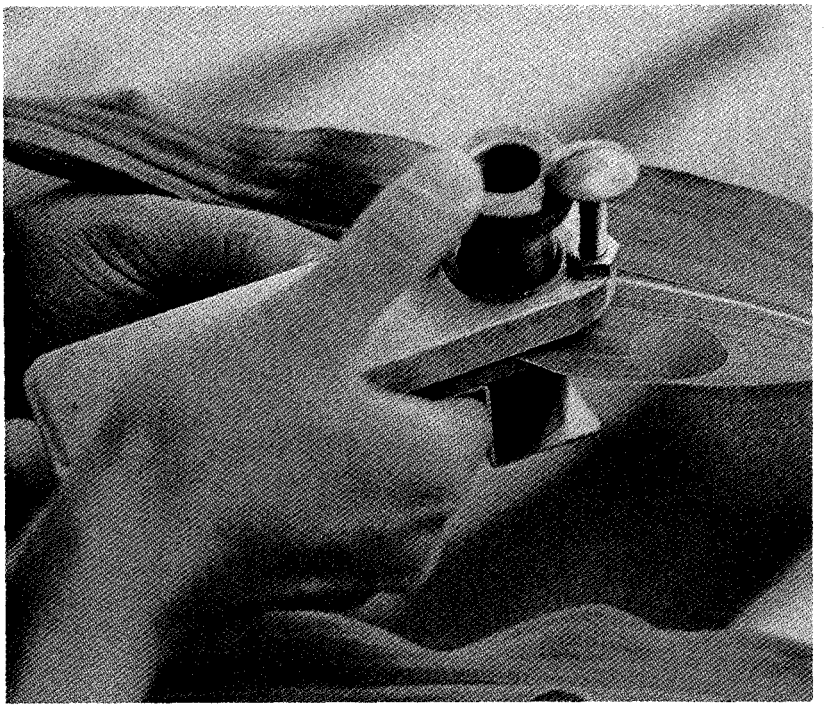

Figure 3.13. Cutting a tissue sample with a leaf punch and loading the psychrometer cup. Work swiftly to avoid sample dehydration.

7) For isopiestic instruments, load water or a solution on the thermocouple. Allow the vapor atmosphere to become stable (0.02 MPa/hr or less). This usually takes 1 to 3 hr, but always test stability by measuring thermocouple output. If times longer than 6 to 8 hr are required, there is usually a problem with the sample. The tissue surface may be dirty (sometimes the tissue may need to be soaked in water for several hours in order to clean the surface, see Step 4 above) or the tissue may degrade during the measurement (rapidly metabolizing tissue in small vapor chambers may run out of oxygen and you will need to use a larger chamber, e.g., 5-10 cm^3, see section on Volume of Vapor Chamber). Some tissues may exude cell liquids onto the surface at high humidities (in this

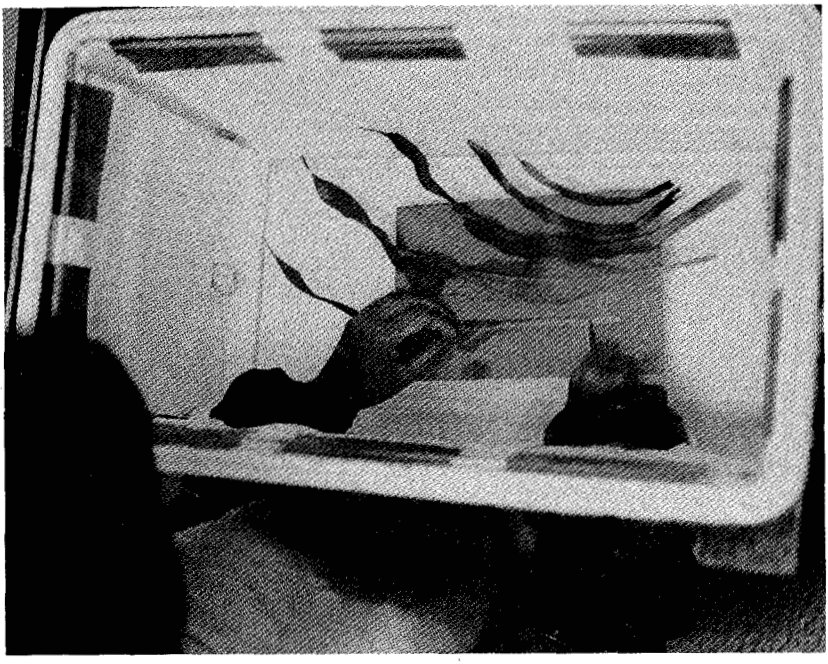

Figure 3.14. Working in an atmosphere saturated with water vapor in a glove box.

case, measurements may not be possible). Occasionally, too little tissue is loaded to adequately communicate with the thermocouple (use more tissue, see section on Working with Plant Tissue).

8) For calibrated instruments, measure the difference between the dry reading before cooling and the stable reading after cooling. Compare these with those during calibration (done with a series of solutions of differing potential prior to the measurements with the tissue) and obtain the water potential of the sample. For the best measurements, it is often necessary to repeat the calibration after the sample measurements have been completed. Calibrations must be at the same temperature as the sample measurements or must be corrected for temperature because these are not equilibrium measurements.

For isopiestic instruments, the calibrations are omitted and the measurements are continued as follows. Remove the thermocouple and replace it with a plunger to seal the psychrometer chamber. Wash the

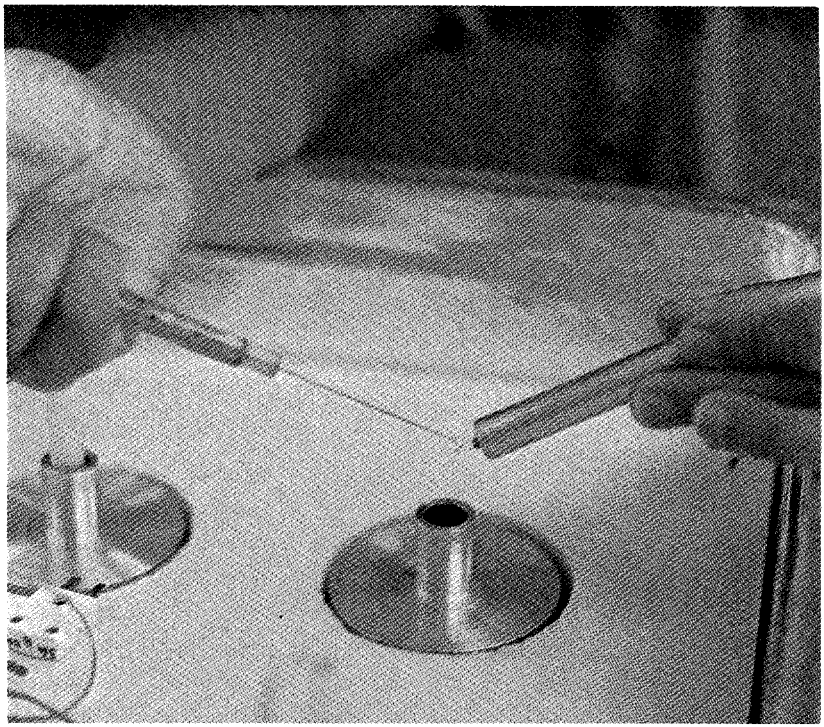

Figure 3.15. Replacing the solution on an isopiestic thermocouple. The droplet size has little effect on the thermocouple output (see Fig. 3.29).

thermocouple in distilled water and dry it with an air jet. Remove the plunger seal, load the spiral with a new solution having a different water potential (Fig. 3.15), and rapidly insert the thermocouple into the psychrometer chamber (Fig. 3.16). Note the new steady output (usually after 0.5 hr, Fig. 3.17), remove the thermocouple, wash and dry it, and insert the dry thermocouple into the chamber. Note the steady output of the dry thermocouple. Because the dry thermocouple neither evaporates nor condenses water from the atmosphere, it identifies the output for the isopiestic solution and corrects for any small thermal activity in the tissue (see section on Isothermal Conditions). Calculate the water potential as shown below. Temperature corrections are unnecessary as long as the potentials chosen from Appendix 3.2 are at the measurement temperature.

Figure 3.16. Inserting a thermocouple with new solution into a psychrometer system. Work swiftly to avoid dehydration of the droplet.

9) After these steps are completed, remove the sample, clean the vapor chamber and thermocouple junction with distilled water, and dry them with an air jet. Reassemble the unit to protect from dust and damage during storage. This completes the measurements.

CALCULATING WATER POTENTIALS

With Peltier and dew point psychrometers, the water potential is read from the previously prepared calibration curve (Fig. 3.11) by comparing the voltage produced by the unknown with that of the calibrating solutions. For isopiestic psychrometers, the water potential must be calculated by extrapolating to the solution that causes an output that is the same as for the dry thermocouple. The extrapolation is linear because the steady output of the thermocouple is proportional to the potential difference between the thermocouple solution and the tissue (see Fig. 3.18 and also Eq. 3.10). Thus, if a potential difference of 0.5 MPa gives an output of 2 μV, a 1.0 MPa difference will give an output of 4

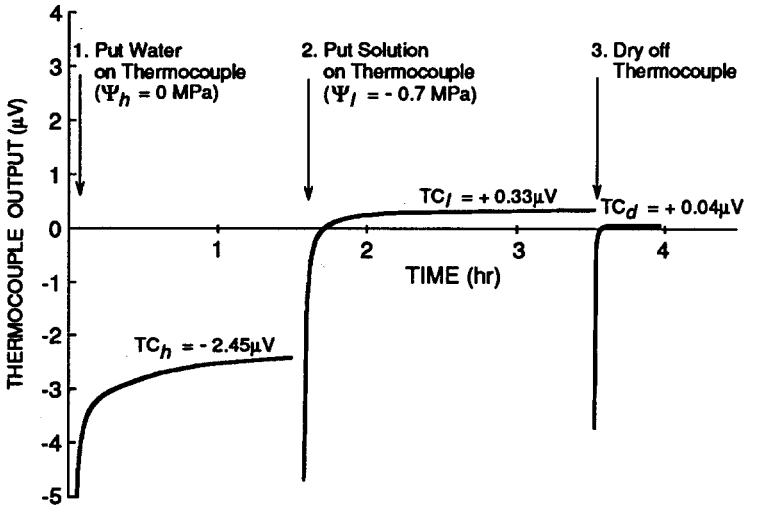

Figure 3.17. Recorder tracing of an isopiestic determination for a sunflower leaf. Measurement 1 gives a rough estimate of the water potential. Measurement 2 gives a more accurate estimate. The isopiestic value is the most accurate estimate and was -0.63 MPa, calculated to give the output of TC_d (Fig. 3.18 and Eq. 3.2).

μV. The isopiestic point is obtained by extrapolating to any fraction of this difference.

The calculation can be formalized with the following equation. Let TC_h be the steady voltage displayed by the thermocouple with the known solution at the higher potential (Ψ_h, closest to 0), TC_l be the steady voltage displayed by the thermocouple with the solution at the lower potential (Ψ_l), and TC_d be the steady voltage of the dry thermocouple (Fig. 3.17). The solution that gives the output of the dry thermocouple is the isopiestic value (Fig. 3.18) and is calculated from:

$$\frac{TC_h - TC_d}{TC_h - TC_l} \cdot (\Psi_l - \Psi_h) + \Psi_h = \text{isopiestic value.} \qquad (3.2)$$

Care should be taken to follow algebraic sign conventions when making this calculation. Thus, a TC_h of -2.45 units (i.e., measurement junction cool) and TC_l of +0.33 units (i.e., measurement junction warm) for solutions

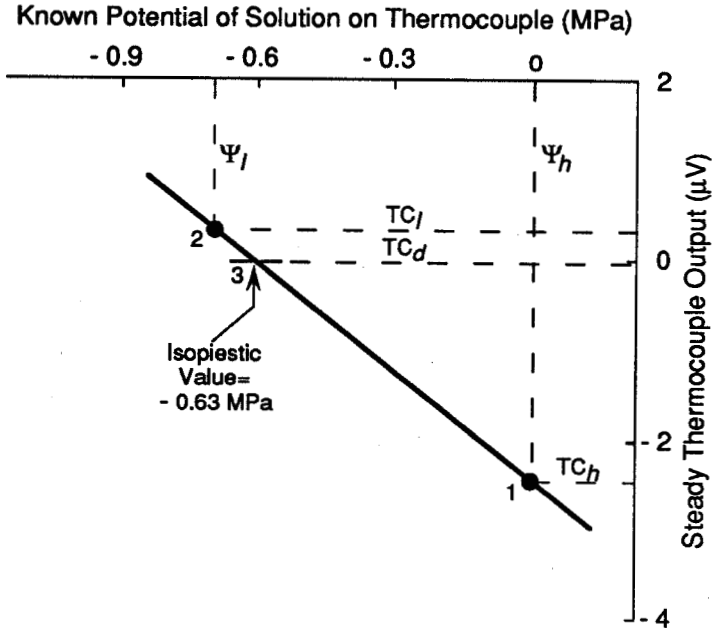

Figure 3.18. Plot of the steady readings in Fig. 3.17. In the first one, water was used (point #1). In the second, a solution was used (point #2). In the third, the thermocouple was dry (point #3). The steady output of the thermocouple was linearly proportional to the difference in potential between the thermocouple and the sample. Therefore, the output could be extrapolated short distances to the isopiestic value as shown (from point #2 to point #3). The extrapolation identifies the solution that would not exchange vapor with the surroundings (that is, the solution causing the same output as the dry thermocouple).

having Ψ of 0 and -0.7 MPa, respectively, with a TC_d of +0.04 units will give an isopiestic value of:

$$\frac{-2.45 - [+0.04]}{-2.45 - [+0.33]} \cdot (-0.7 - 0.0) + 0.0 = -0.63 \text{ MPa.} \qquad (3.3)$$

For bars, multiply this result by 10.

The extrapolation can be checked by placing a solution having the calculated potential on the thermocouple. The reading should be identical to that of the same thermocouple when dry.

Working with Plant Tissue

Because thermocouple psychrometers detect the vapor pressure of the surface water, the vapor must proceed from the outer surface of the cells through the intercellular spaces to the atmosphere to be detected by the thermocouple. However, at equilibrium in excised tissue, the water potential of the cell interior is in equilibrium with the water potential at the cell surface that in turn is in equilibrium with the atmosphere in the psychrometer chamber. There is no net water or vapor movement and the entire system is at the same Ψ_w.

In intact plants, *in situ* measurements allow gradients in water potential to exist over distances of several cells because the tissue remains attached to its water supply and water movement can occur. However, water vapor diffuses rapidly and the vapor usually represents an average for the gradient. Boyer *et al.* (1985) showed that psychrometers give average values for the sample whether water is entering or not and thus whether gradients are present or not. This simplifies the interpretation of psychrometer measurements.

It is worth considering what kind of average the psychrometer provides. As discussed in Chap. 2, excising plant tissue removes its water supply, and preventing evaporation allows internal gradients in water potential to equilibrate, giving an average for the tissue at the time of excision. Tyree and Hammel (1972) and Tyree and Jarvis (1982) point out that at equilibrium the potential for a tissue should be a volume average that accounts for the fact that the volume of water having a particular potential determines the amount of work that can be done by that water, and larger volumes will do more work and thus contribute more to the average than small volumes will. Accordingly, the volume-averaged Ψ_w is

$$\textit{Average } \Psi_w = \frac{\Sigma\, V^i \cdot \Psi_w^i}{V}, \tag{3.4}$$

where V^i is the water volume in the protoplasm of cell i, Ψ_w^i is the water potential of cell i, and V is the total water volume in the protoplasm of all the cells (the symplasm). The symbol Σ sums the effects of each cell V^i and Ψ_w^i in the tissue, and the $V^i \cdot \Psi_w^i$ weights the potential of each cell by the volume of water in that cell. This volume-averaging concept applies to the components of Ψ as well as to any other cell parameters that depend on tissue measurements of Ψ.

Figure 3.19 shows the effect of volume averaging on the Ψ_w measured in a leaf. Before excision, there is a gradient in Ψ_w extending

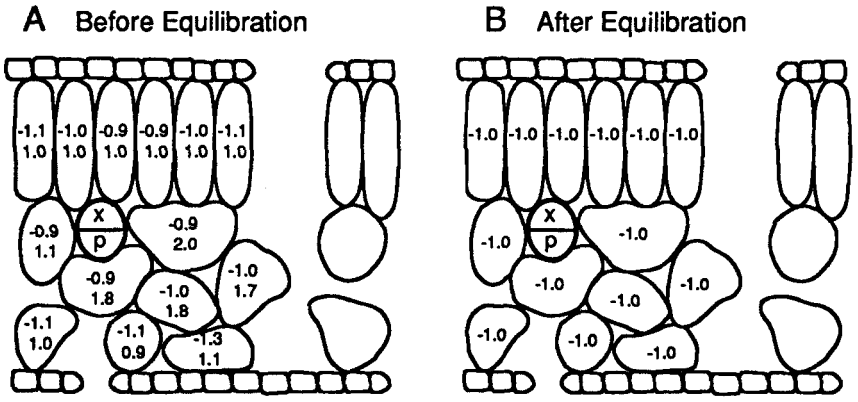

Figure 3.19. Water potential gradients in a hypothetical leaf before and after equilibration in a vapor chamber. A) Before equilibration, a gradient exists in the intact, freely transpiring leaf. The Ψ_w is highest (-0.9 MPa) next to the vein (x/p) and lowest (-1.3 MPa) far from the vein (see negative values in each cell). The volume of water in each cell is also shown (positive values). B) After the sample is excised and equilibrated internally, the Ψ_w represents a volume average for the gradient. The volumes change slightly in each cell as water is exchanged between the cells during the approach to uniform potentials. Regardless of whether a gradient exists as in A or equilibration has occurred as in B, vapor pressure measurements with psychrometers are volume averages because the vapor mixes rapidly in the intercellular spaces,

from the veins to the leaf surface. The Ψ_w are higher close to the veins (-0.9 MPa) than far from the veins (-1.3 MPa), and the volume of water varies from cell to cell (e.g., see Nonami *et al.*, 1991). After excision, the Ψ_w equilibrate according to Eq. 3.4, and the Ψ_w is -1.0 MPa in all the cells.

A similar principle holds for the vapor above the gradient. The vapor pressure above cell i reflects Ψ_w^i (Eq. 3.4) and, because the vapor in the intercellular spaces is very mobile compared to the liquid, the vapor mixes in a volume-averaged fashion. Thus, the psychrometer gives a volume-averaged Ψ_w whether a gradient is present or not (Boyer *et al.*, 1985).

From the foregoing, it is clear that two approaches may be taken to ensure that the sample represents the potential in the intact plant. In the first, one may remove the sample from the plant and rapidly seal it in the vapor chamber so that internal equilibrium occurs and the measurement represents a volume average of the potentials present in

the intact plant before excision. Alternately, one may enclose part of the intact plant in a vapor chamber and measure the volume average of any gradients that are present. Although gradients may persist, the volume average clearly applies to the intact plant. However, by enclosing the tissue, transpiration is prevented and the potentials in the intact plant may not be the same as before enclosure. The excised tissue has the advantage that the volume average applies to the Ψ_w before excision but the intact plant has the advantage that no excision artifacts are possible. Depending on the experiment, one approach may be more desirable than the other.

EXCISED TISSUE

Because the major value of excised tissue is that it indicates the average water status at the instant of excision in the freely transpiring plant, it is particularly useful for routine measurements with a wide range of tissues. As long as the ratio of cut surface to intact surface is small (generally less than 10%), the cut surface does not affect the psychrometer significantly and can be neglected. If the cut surface is more extensive, it should be hidden by coating with Vaseline (see section on Sorption Effects).

For relatively dry plant tissue or soil where the water content is small, it is important to avoid dehydrating or rehydrating the tissue with the water on the thermocouple. Isopiestic psychrometers use potentials close to those of the tissue (thermocouple output is kept small), and hydration changes can be kept minimal. Peltier and dew point systems use water from the tissue or soil to humidify the air and coat the thermocouple, so a certain amount of dehydration is inevitable.

Leaves. After cleaning the leaf surfaces with water (Fig. 3.12), blotting dry, and allowing the leaves to reequilibrate with their surroundings for several hours, excise a sample using a leaf punch (Fig. 3.13). Place the disk on the bottom (Fig. 3.2) of the Vaseline-coated psychrometer cup and seal it within 10 sec of removal from the plant. If no leaf punch is available, place an oversized leaf fragment in a glove box having a saturated atmosphere (Fig. 3.14) and trim a sample to size. For needles or small fragments of leaves, work in the glove box and place the tissue fragments on the walls and the bottom of the cup until an area equivalent to the bottom is covered. Press the tissue into the Vaseline on the cup surfaces to hold it in position and to drain away metabolic heat. Use only one layer of tissue and do not allow any overlap between the fragments.

Leaf disks with diameters of 2.0 to 2.5 cm usually are large enough to have negligible cut surfaces. Because it is desirable to avoid the effects

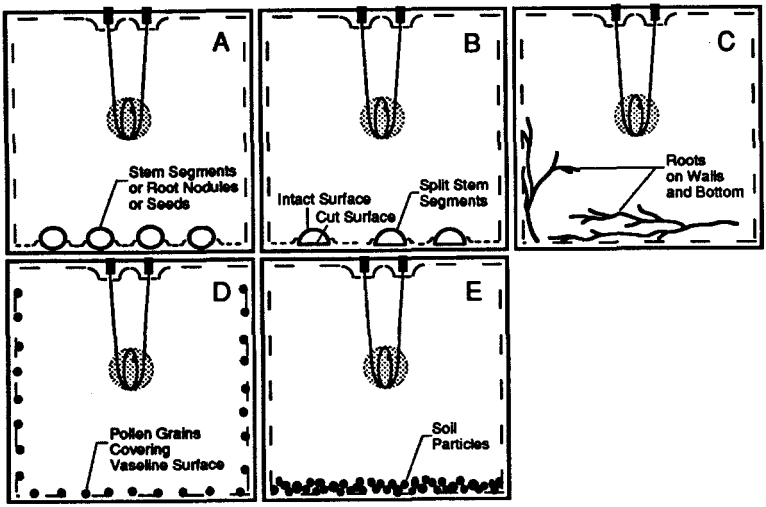

Figure 3.20. Placement of various plant parts or soil in the vapor chamber. A) Whole stem segments, root nodules, or seeds. B) Split segments of large stems. C) Roots. D) Pollen grains. E) Soil. Leaf placement is shown in Fig. 3.2.

of cut surfaces as much as possible (Nelsen *et al.*, 1978), leaf samples should be this size or larger. If thick leaves are being sampled, large amounts of cut surface may be inevitable. In this case, coat the edge with Vaseline before loading.

Stems. Place the stems into a saturated glove box (Fig. 3.14) for sampling. Small diameter stems (to 3 mm) can be cut into lengths of 2.0 to 2.5 cm without exposing too much cut surface. Place four to six segments in the cup so they do not touch each other (Fig. 3.20A). For larger stems, cut short lengths and split the stems. With the split surface down, submerge the cut surfaces in the Vaseline on the bottom (Fig. 3.20B). The Vaseline layer should be thick enough to completely bury the cut surface, hiding it from the vapor atmosphere. Of the surface exposed to the thermocouple, at least 90% should consist of intact cells.

Roots. Place the soil/root complex into a saturated glove box (Fig. 3.14) and dissect the root segment to be measured. Flick away adhering soil particles. Use caution because even light brushing can cause the cells to collapse. For short segments of small diameter (to 3 mm), place four to six segments on the cup bottom and the walls (Fig. 3.20C). The cut surface generally can be neglected. Avoid root-root contact between the samples. For long roots, wrap the root around your index finger and

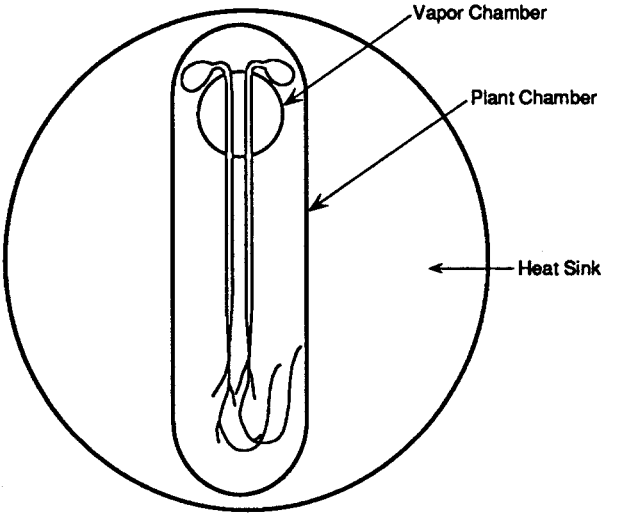

Figure 3.21. Example of a thermocouple psychrometer for measuring the water potential in intact plants. Etiolated seedlings are held in a plant chamber in a large aluminum heat sink. The vapor chamber for the psychrometer encloses the part of the plant to be measured. The vapor chamber is sealed around the stems with Vaseline.

insert it into the cup, releasing it so the root can uncoil against the wall. Check to be certain that no roots extend into the region to be occupied by the thermocouple. For fleshy roots, cut a section but coat the cut surfaces with Vaseline and expose the remaining uncoated surface to the thermocouple. At least 90% of the exposed surface should be intact.

Root Nodules. Place six to eight small nodules or three to four large nodules into the cup and press into the Vaseline on the bottom (Fig. 3.20A). The Vaseline layer should be thick enough to cover the lower half of the nodules and to drain away metabolic heat. Cut surface is usually negligible.

Seeds. Same as with nodules (Fig. 3.20A).

Pollen. Collect pollen in a glassine bag. Rapidly insert the bag into a saturated glove box (Fig. 3.14) and cut away the lower corner of the bag. Pour the pollen from the open corner into the cup. Tilt the cup to coat the Vaseline-covered surface with pollen (Fig. 3.20D). Place the cup on the thermocouple unit.

INTACT TISSUE

The psychrometer is the only method of measuring plant water status in completely intact plants. One advantage of using intact tissue is that it can be observed for long times while growth occurs or the tissue hydrates (Boyer *et al.*, 1985). This is because the attachment to the rest of the plant gives a path along which oxygen, water, and nutrients can move and keep the tissue vigorous.

Measurements of intact tissue follow the same principles as for excised tissue and are made by sealing the tissue into an airtight, temperature-controlled vapor chamber. The measurements require specialized instruments, an example of which is shown in Fig. 3.21. As in any other psychrometer measurement, it is essential to use clean tissue, coat chamber surfaces with melted and resolidified Vaseline, and maintain good contact between the tissue and the Vaseline to drain away metabolic heat.

Working with Soils

SOIL SAMPLES

The water potential of soils can be measured with samples placed in the psychrometer cup. Put an oversized soil sample into a saturated glove box (Fig. 3.14). Subsample the soil sufficiently to cover the bottom of the cup with a layer 2 or 3 mm thick (Fig. 3.20E). An approach that has worked well is to obtain a soil core using a tube with a sharpened edge, then push out the core and catch a sample from mid-core in the psychrometer cup. Be careful to avoid regions of soil that have been subject to air drying because they usually do not represent the bulk environment around the roots. When placing the cup on the thermocouple unit, keep it upright to prevent soil from making contact with the thermocouple. For relatively dry soils where water films are small, avoid large potential differences between the soil and the thermocouple. Large potential differences allow too much vapor transfer between the soil and the thermocouple, which can hydrate or dehydrate the soil and prevent a steady output from being achieved. To keep potential differences small with an isopiestic psychrometer, place a solution on the thermocouple that has a potential close to that of the soil. With extremely dehydrated soil, it may be necessary to operate the psychrometer in an atmosphere of high humidity to avoid dehydrating the sample when changing solutions during a determination. For Peltier and dew point instruments, some dehydration of the sample is inevitable because of the condensation of water from the sample onto the thermocouple surface.

In Situ SOIL MEASUREMENTS

Some psychrometers are built to be buried in soil where they can provide frequent measurements at constant positions in the soil profile (Brown and Collins, 1980). The thermocouples should be calibrated before being placed in the soil. The procedure is to expose the thermocouples to the atmosphere above salt solutions of known water potential in a temperature-controlled water bath. After calibration, bury the thermocouples and the surrounding protective housing, such as a porous ceramic cup or screen cage, well before measurements are taken so that the soil has a chance to settle (Brown and van Haveren, 1972). Generally, calibrations should be repeated immediately after the psychrometer is removed from the soil at the end of the experiments. This can detect any shift in calibration that occurred while the thermocouples were in the soil. In theory, isopiestic measurements could be made *in situ* and would avoid the need for calibration, but they have not been attempted.

Measuring the Components of the Water Potential

So far this chapter has dealt with measuring the water potential, but it is often just as important to determine the components of the water potential (Eq. 2.1). Because psychrometry indicates the vapor pressure of the surface solution in the walls of the cells, the potentials in other parts of the cell are measured by appropriate mixing with the surface solution. As pointed out earlier, the surface solution in the apoplast consists of the components

$$\Psi_{w(a)} = \Psi_{s(a)} + \Psi_{m(a)}, \tag{3.5}$$

and in the protoplasts there are different components

$$\Psi_{w(p)} = \Psi_{s(p)} + \Psi_{p(p)}. \tag{3.6}$$

Each equation contains three variables and measuring any two allows the third to be calculated. A simplification is the equilibrium between the wall solution and the protoplast solution that allows $\Psi_{w(p)}$ to be considered identical to $\Psi_{w(a)}$. Measuring the osmotic potential in both compartments then allows the other component ($\Psi_{m(a)}$ or $\Psi_{p(p)}$) to be calculated from Eqs. 3.5 and 3.6.

OSMOTIC POTENTIAL

Solutions sometimes behave nonideally because of dissociation of the solute (NaCl for example) or binding of water to the solute (some sugars and macromolecules) or relatively independent motions of various parts of polymers. These effects alter the Ψ_s of the solution from that

expected from the concentration of solute alone. Thermocouple psychrometers measure water activities in the vapor phase and thus in the liquid phase. Any nonideal effects that alter liquid water activity are detected and the true Ψ_s is automatically measured.

Apoplast Osmotic Potential. The $\Psi_{s(a)}$ can be determined as described in Chap. 2 by using a pressure chamber to obtain a sample of apoplast solution (Scholander *et al.*, 1964, 1965; Boyer, 1967a; Nonami and Boyer, 1987; Jachetta *et al.*, 1986).

1) Excise a leaf, branch, or root system and place it in the pressure chamber so that the cut end extends through the top to the outside of the chamber.
2) Place water on the cut surface and wipe it away several times to remove solutes released from the cut.
3) Pressurize the tissue until apoplast solution appears on the cut surface and wipe away the first 2-5 µl to remove any solute that may have been released during the pressurization.
4) Collect the next 5 µl of exudate in a microliter syringe.
5) Place a droplet of exudate on the spiral thermocouple of an isopiestic psychrometer above a known solution on the bottom of the cup. After the output of the thermocouple becomes steady, move the thermocouple to a second vapor chamber containing a different known solution (Fig. 3.22) and obtain a steady reading. The isopiestic point is found by extrapolation. The calculation is similar to that for measuring Ψ_w with living tissue (Eq. 3.2) except that TC_d is zero (no metabolic heat) and the definition of the other TC terms is *reversed* because the unknown is on the thermocouple rather than in the cup. Accordingly, let TC_h be the steady voltage displayed by the thermocouple with the *cup solution* having the higher potential (Ψ_h, which gives the higher thermocouple voltage), and TC_l be the steady voltage displayed by the thermocouple with the *cup solution* having the lower potential (Ψ_l, which gives the lower thermocouple voltage), and TC_d be zero. Thus, a TC_h of +3.0 units above a solution with Ψ_h of -0.4 MPa and a TC_l of -7.2 units above a solution with Ψ_l of -0.7 MPa will give an osmotic potential (Fig. 3.22) of

$$\frac{+3.0 - 0}{+3.0 - [-7.2]} \cdot (-0.7 - [-0.4]) + (-0.4) = -0.49 \text{ MPa.} \quad (3.7)$$

This procedure works well for determining $\Psi_{s(a)}$ in samples of 3-5 µl and, by bending the thermocouple spiral to a smaller size, isopiestic psychrometers can measure samples as small as 0.1 µl. It is also possible to presample a leaf, branch, or root system to measure the water potential in a psychrometer and then pressurize the remaining plant part in a

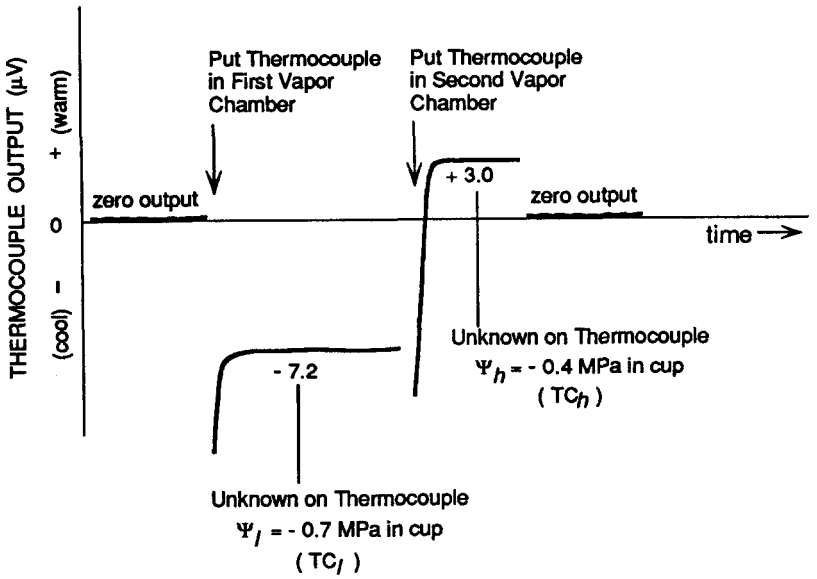

Figure 3.22. Recorder tracing of an isopiestic osmometer measurement. The isopiestic value is -0.49 MPa, which is the osmotic potential needed in the cup to give a thermocouple reading of zero. See Eq. 3.7 for the calculation.

pressure chamber to remove the xylem solution for measuring $\Psi_{s(a)}$. With these presampled tissues, it may be necessary to coat cut surfaces with Vaseline in order to prevent excessive leakage of gas in the pressure chamber.

Occasionally it is possible to obtain apoplast solution from completely uncut tissue by pressurizing the roots of intact plants, collecting exudate from the surfaces of the shoot tissue, and measuring $\Psi_{s(a)}$ of the exudate. This method has proven successful for obtaining apoplast solution from stem elongating tissues (Nonami and Boyer, 1987) and from leaf hydathodes, which exude xylem exudate (Klepper and Kaufmann, 1966). It has been possible to use a microcapillary to sample the region below developing seeds (Maness and McBee, 1986).

Protoplast Osmotic Potential. The $\Psi_{s(p)}$ is obtained by breaking the protoplast membranes, extracting the mixed apoplast/protoplast solution, and measuring the osmotic potential of the solution (Ehlig, 1962).

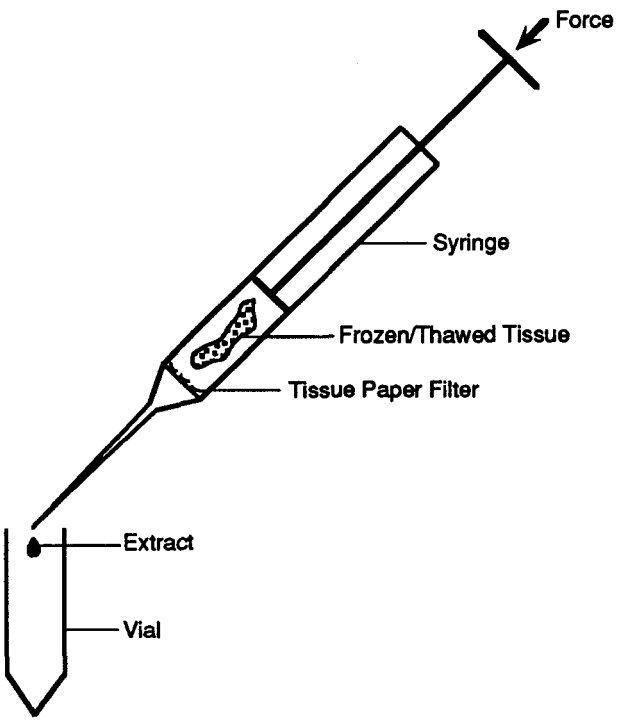

Figure 3.23. Extraction procedure after freezing and thawing plant tissue in the barrel of a syringe. Note tissue paper filter to prevent plant tissue from entering the syringe needle.

1) Place a tissue sample into the barrel of a 1-ml syringe with a half millimeter wad of tissue paper as a filter at the bottom (Fig. 3.23). Insert the plunger above the tissue, insert the needle tip into a rubber stopper to act as a seal, and place the syringe/stopper into a freezer.
2) After at least 20 min in the freezer, remove the syringe and thaw. Immediately after thawing, unseal the needle tip, press firmly on the syringe plunger, and catch the cell extract in a vial as it exudes from the syringe needle. Seal the vial and mix the extract.
3) Place a droplet of the extract on the thermocouple spiral of an isopiestic psychrometer (usually 3-5 µl volume) and measure $\Psi_{s(p)}$ as described earlier for measuring the apoplast osmotic potential. If you have a large volume of extract (usually more than 50 µl), it is possible to use a Peltier

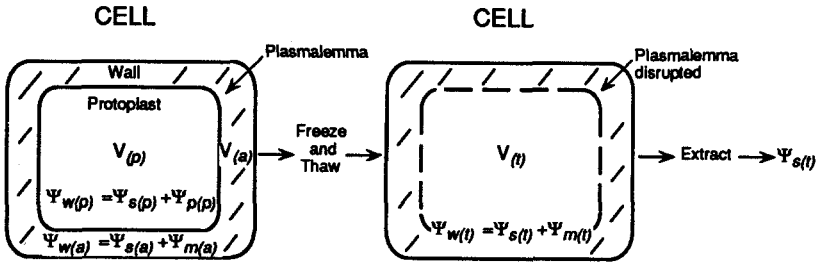

Figure 3.24. Compartmentation of components of the water potential before and after freezing and thawing a tissue sample. Initially, the protoplast solution in the cells is separated from the wall by the plasmalemma. After freezing and thawing, the compartments $V_{(a)}$ and $V_{(p)}$ mix to give a single solution $V_{(t)}$. The $\Psi_{w(t)}$ of the frozen/thawed solution indicates $\Psi_{s(t)}$, which has been diluted by the water in the walls, and $\Psi_{m(t)}$, which has been flooded by solution from the protoplast. The $\Psi_{s(p)}$ can be determined by correcting $\Psi_{s(t)}$ for dilution after extracting the cell solution (see Eq. 3.9). The volume $V_{(t)} = V_{(a)} + V_{(p)}$.

or dew point psychrometer to measure the osmotic potential. Place the extract into the vapor chamber and determine the osmotic potential from a calibration curve made with solutions of known osmotic potential (see Appendix 3.2).

Sometimes it is not possible to make an extract for the measurement of $\Psi_{s(p)}$. An alternate method is to measure the osmotic potential *in situ* in the frozen and thawed tissue. To do this, freeze the tissue in the psychrometer cup after sealing the top with plastic film. Freeze as rapidly as possible, preferably in liquid nitrogen. The faster the freezing rate, the more uniform the ice crystal distribution and the shorter is the time necessary to establish steady state conditions after thawing. Thaw the walls and top of the chamber first to drive any condensed water off these surfaces and onto the tissue. Immediately place the cup onto the thermocouple assembly and measure the water potential of the thawed tissue ($\Psi_{w(t)}$), then measure the matric potential ($\Psi_{m(t)}$) as indicated in the section on Matric Potential (the subscript *t* refers to frozen/thawed tissue). Calculate $\Psi_{s(p)}$ from $\Psi_{w(t)} - \Psi_{m(t)}$.

Compartment Mixing. As long as the wall volume is small, there is only a small error caused by mixing the apoplast and protoplast solutions during thawing. If the wall volume is significant (5-10% of cell volume or greater), mixing causes significant error because the apoplast solution

is dilute but the protoplast solution is concentrated (Boyer and Potter, 1973). Consider the volume of water in the protoplasm ($V_{(p)}$) and apoplast ($V_{(a)}$) to be mixed in the total volume after freezing and thawing ($V_{(t)}$; Fig. 3.24). To correct for mixing, assume the apoplast solution is pure water (an oversimplification, but the solution is usually so dilute that the assumption is safe). The protoplast solution mixed with the apoplast solution after freezing and thawing is $\Psi_{s(t)}$. The relationship of $\Psi_{s(p)}$ to $\Psi_{s(t)}$ is then

$$\Psi_{s(p)} \cdot V_{(p)} = \Psi_{s(t)} \cdot V_{(t)} \tag{3.8}$$

and

$$\Psi_{s(p)} = \Psi_{s(t)} \frac{V_{(t)}}{V_{(p)}}. \tag{3.9}$$

For example, $\Psi_{s(t)}$ of -1.0 MPa measured with extract from frozen/thawed tissue having $V_{(t)}/V_{(p)}$ of 1.2 will have a $\Psi_{s(p)}$ of -1.2 MPa. The volume $V_{(t)}$ and $V_{(p)}$ can be measured microscopically in fresh tissue sections (also see Chap. 2 for another method of measuring $V_{(t)}$ and $V_{(p)}$).

TURGOR

The $\Psi_{p(p)}$ is measured by first determining the water potential of the living tissue ($\Psi_{w(a)} = \Psi_{w(p)}$) followed by measuring the $\Psi_{s(p)}$ as above. Calculate the $\Psi_{p(p)}$ by difference according to Eq. 3.6.

MATRIC POTENTIAL

Because matric potentials arise from surface effects of porous solids in contact with water or solutions (Fig. 3.25), the $\Psi_{m(a)}$ of the surface solution can be measured with a psychrometer in living tissue by first determining $\Psi_{w(a)}$ and $\Psi_{s(a)}$ as described earlier, then calculating $\Psi_{m(a)}$ by difference from Eq. 3.5.

As discussed for measuring the osmotic potential of tissues *in situ* after freezing and thawing, it sometimes is desirable to measure the matric potential in frozen/thawed tissue ($\Psi_{m(t)}$). The matric potential is no longer that of the intact tissue (Boyer, 1967b) because the wall has been flooded with solution from the protoplasm, and the matric potential becomes less negative. If you wish to measure $\Psi_{m(t)}$, use an isopiestic psychrometer, and place a solution extracted from frozen/thawed tissue on the thermocouple and a frozen/thawed sample of the same tissue in the cup (Fig. 3.26). The difference in potential between the solution on the thermocouple, which is affected only by the osmotic component, and the frozen/thawed tissue in the cup, which is affected by the osmotic

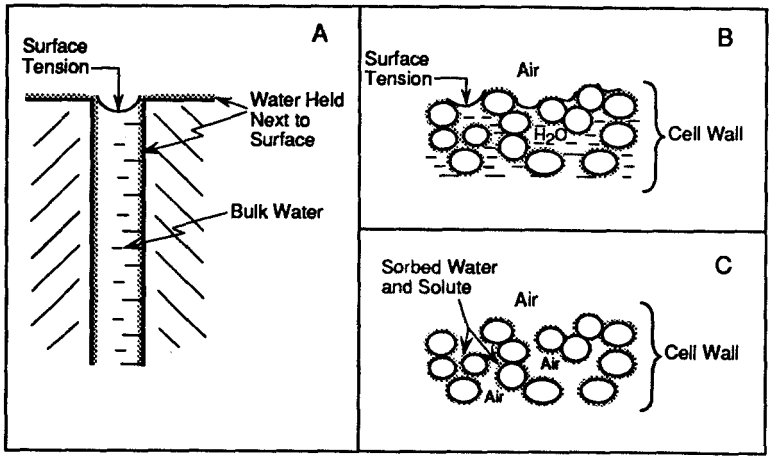

Figure 3.25. Origin of matric potential. A) In a single capillary with completely wettable walls, molecules of water are held next to the wall mostly by hydrogen bonds and electric charges. Electrolytes are attracted by charges on the wall, and the high solute concentrations also attract water. Water tends to fill the pore because hydrogen bonds, electrostatic attractions between the weak water dipoles, and other intermolecular forces hold the liquid water together. There is a meniscus at the air-water interface maintained by the intermolecular forces, which create surface tension. B) In a cell wall, there are many small pores (about 5 to 8 nm diameter) that remain water-filled against large tensions. The round structures in this view are cross sections of the microfibrils and matrix polymers forming the solid structure of the wall. Water fills the spaces and is continuous with the protoplasts and the vascular system, forming a hydraulically connected system. C) In a cell wall that has been desiccated sufficiently to drain the pores, hydraulic contact is lost between the pores and the protoplasts and vascular system. Air fills most of the pores and pressures cannot be measured easily. However, water remains adsorbed to microfibrils and its vapor pressure can be measured with a thermocouple psychrometer.

and matric components, gives the matric component of the frozen/thawed tissue.

GRAVITATIONAL POTENTIAL

Although we often ignore gravitational potentials, they can be substantial. For every 10 m of depth, gravity causes an increased potential of 0.1 MPa. The effects are observed mostly in tall trees (Scholander *et* al., 1965), marine environments, and deep soils. In a column of free water, the gravitational potential is expressed as pressure that increases at

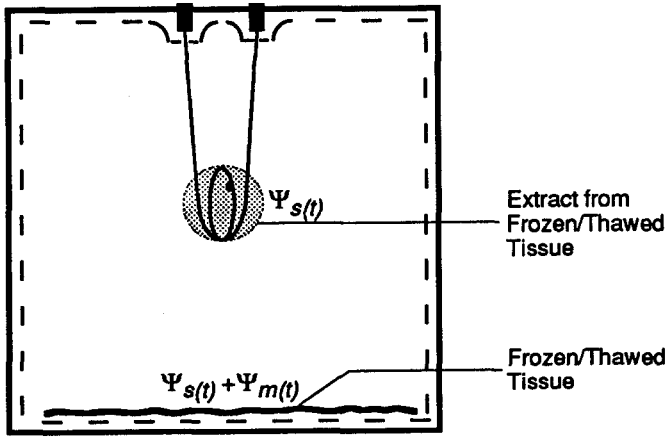

Figure 3.26. Measurement of matric potential in frozen/thawed tissue *in situ* ($\Psi_{m(t)}$). The difference in potential between the frozen/thawed tissue ($\Psi_{s(t)}$ + $\Psi_{m(t)}$) and the osmotic potential ($\Psi_{s(t)}$) extracted from a parallel sample is the $\Psi_{m(t)}$ in the frozen/thawed tissue.

increasing depths. If the column is in a capillary and the weight of water is allowed to put a tension on the meniscus in the capillary, as in a tree, the pressure will be negative immediately under the meniscus and will increase (become less negative) at increasing depths.

The difference in potential at two heights indicates the effect of gravity only if the water column is stationary and the composition of the solution is uniform. In tall trees and in soils, the water often is moving and the solution may not be uniform in composition, so care must be taken to measure all other potentials. Psychrometers can be mounted at various positions and gravitational effects can be monitored *in vivo*. For excised tissue, excision and psychrometer loading should take place at the sampling height in the tree to allow rapid sampling (within 10 sec).

Precautions

DIFFUSION ERROR

Diffusion determines the interaction between the thermocouple and the sample and involves vapor and heat transfer (Boyer, 1969b; Boyer and Knipling, 1965; Rawlins, 1964). The physical arrangement can be idealized to two exchange surfaces, one at the thermocouple and one inside the tissue. For calibrated psychrometers, measurements are not made

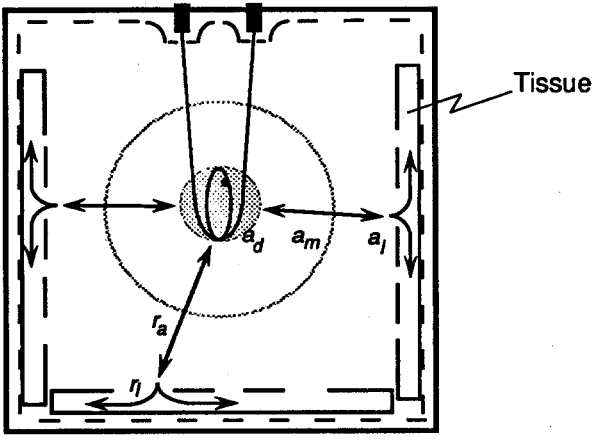

Figure 3.27. Diffusion properties of a thermocouple psychrometer. In order to simplify the mathematics, the tissue lines the sides and bottom of the vapor chamber and is shown with a porous surface that restricts diffusion due to stomata. The diffusive resistance of the air between the thermocouple droplet and tissue (r_a) is in series with the diffusive resistance of the porous tissue (r_l). The area governing diffusion is the geometric mean area (a_m, shown as a shaded sphere around the thermocouple) that accounts for the differing areas of the droplet (a_d) and tissue (a_l).

at equilibrium and water vapor diffuses between the two surfaces governed by the geometry, surface area, and diffusive resistance of each part of the diffusion path. There is always a possibility of diffusion error in these instruments (Boyer and Knipling, 1965; Shackel, 1984). On the other hand, there is no net vapor movement at the isopiestic point in isopiestic psychrometers and thus there is no diffusion error. This difference can have an impact on measurements with psychrometers.

Because of the opportunity for diffusion errors in calibrated instruments, it is useful to analyze vapor transfer and how the errors originate. Figure 3.27 shows the diffusion properties of a psychrometer with a water droplet on the thermocouple and plant tissue lining the walls. There are two paths in series for vapor transfer when tissue is present and each has its own diffusive resistance (Fig. 3.27). One is between the droplet and the sample surface (diffusive resistance r_a, sec·m^{-1}) and the other is between the sample surface and the interior water surface (diffusive resistance r_l). The diffusion surfaces are of area a_d (m^2) for the water drop and a_l for the sample that can be simplified to a single area a_m which

is the geometric mean of the droplet and sample areas (defined as $4\pi R_d R_c$ where R_d and R_c are the radii of the droplet and sample, respectively, idealized as surfaces of spheres). According to Boyer and Knipling (1965), the vapor transfer is given by

$$\frac{dm}{dt} = -\frac{La_m a_l}{La_m r_l + a_l r_a} \cdot \frac{c_o \bar{V}_w}{RT} (\Psi_o - \Psi_w), \tag{3.10}$$

where dm/dt is the rate of vapor transfer from the droplet ($g \cdot sec^{-1}$) and $(\Psi_o - \Psi_w)$ is the driving force (MPa) with Ψ_o the water potential of the droplet and Ψ_w the water potential of the tissue. The term $c_o \bar{V}_w / RT$ is a constant at a particular temperature and serves to convert water potentials into the equivalent concentrations of water vapor (c_o is the saturation vapor concentration at the temperature of the chamber in units of $g \cdot m^{-3}$, R is the gas constant of 8.3143×10^{-6} $MPa \cdot m^{-3} \cdot mol^{-1} \cdot K^{-1}$, $\bar{V}_w$ is the partial molal volume in units of $m^3 \cdot mol^{-1}$, and T is the Kelvin temperature), and L is a constant that corrects droplet vapor concentration for the droplet temperature (has a value of 0.32 and indicates that water on the thermocouple is cool and thus at lower vapor pressure than it would be at chamber temperature).

When tissue is present, Eq. 3.10 indicates that the rate of transfer from the droplet to the tissue is determined by $La_m a_l / (La_m r_l + a_l r_a)$ but when a calibrating solution is present, r_l is zero and this relation becomes La_m / r_a. The lack of r_l causes diffusion to be faster for the calibrating solution than for the tissue even when the two have the same water potential. This difference in diffusion between the calibrating solution and the tissue is the source of the systematic diffusion error in calibrated psychrometers (Boyer and Knipling, 1965).

The size of the error is most easily estimated by noting that calibrated psychrometers determine the water potential by selecting the calibrating solution that gives the same thermocouple output as the sample being measured, that is by selecting for the same rate of vapor transfer for the calibrating solution and the sample, and reading the potential that gives this rate from the calibration curve. Accordingly, the water potential that gives this rate with tissue is Ψ_w and with calibrating solution is Ψ_a and these can be conveniently expressed as the ratio Ψ_w / Ψ_a. Because the rate of vapor transfer is equal for the calibrating solution and the tissue, Eq. 3.10 can indicate the equality as

$$\frac{La_m a_l}{La_m r_l + a_l r_a} (\Psi_w) = \frac{La_m}{r_a} (\Psi_a), \tag{3.11}$$

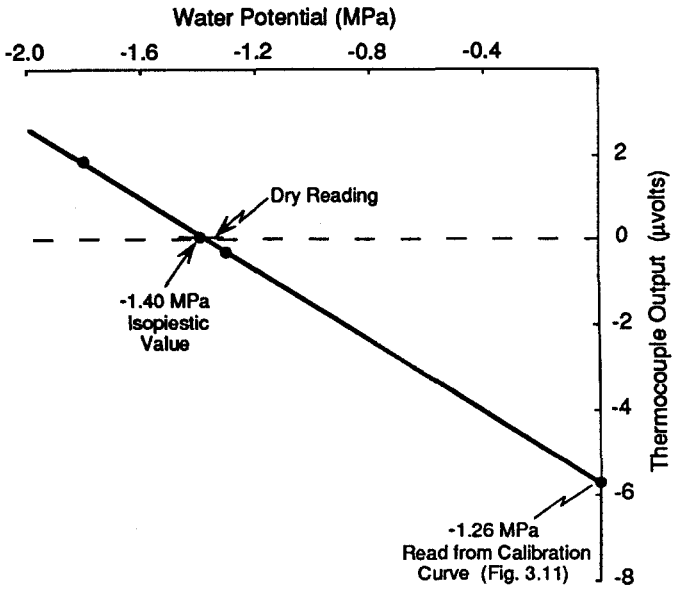

Figure 3.28. Diffusion error for a sample from a maize leaf. The true Ψ_w measured with the isopiestic psychrometer was -1.40 MPa while, with the same tissue sample, the same thermocouple measured only -1.26 MPa as a calibrated psychrometer (Richards and Ogata type) using the calibration curve in Fig. 3.11. Peltier psychrometers are subject to the same diffusion errors (Boyer and Knipling, 1965).

where the left side represents the tissue and the right side represents the calibrating solution, and $\Psi_0 = 0$ because the droplet is water. Thus, $\Psi_w/\Psi_a = 1 + La_m r_l/a_l r_a$ indicating that Ψ_w is numerically larger than Ψ_a (more negative than Ψ_a). In other words, for the same rate of vapor transfer (and electrical output of the thermocouple), the true Ψ_w for the tissue is more negative than that determined from the calibration curve.

How much lower can be demonstrated in a psychrometer arranged as in Fig. 3.27. For this psychrometer having a 2-cm-diameter chamber and a 0.2-cm-diameter droplet, Eq. 3.11 gives a calculated $\Psi_w/\Psi_a = 1 + 0.032 r_l/r_a$, and for r_a of about 400 sec·m^{-1} for the air and a frequently encountered r_l of 1000 to 2000 sec·m^{-1} for the tissue (Boyer and Knipling, 1965), the calculated Ψ_w/Ψ_a is 1.08 to 1.16. Figure 3.28 shows that the Ψ_w and Ψ_a in this psychrometer for a maize leaf sample were -1.40 MPa measured isopiestically (Ψ_w) and -1.26 MPa measured in the same sample

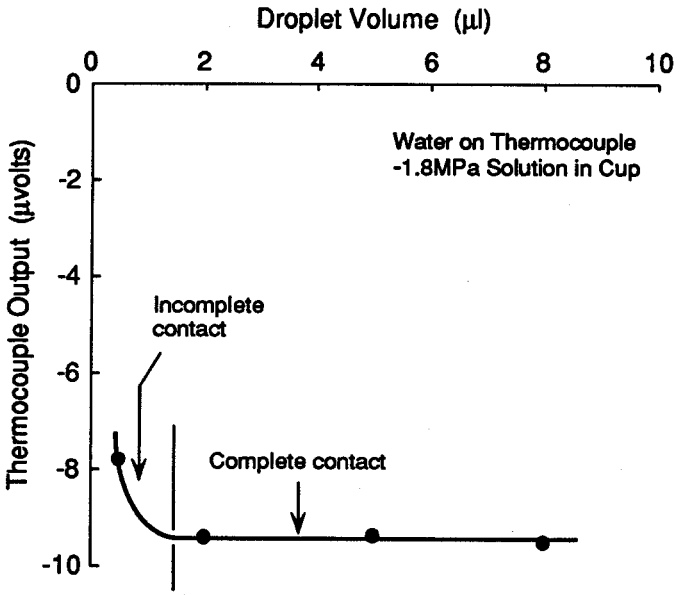

Figure 3.29. Effect of droplet size on thermocouple output. Measurements were made with an isopiestic thermocouple that completely contacted the droplet except when the droplet volume was decreased to 0.5 µl.

in the same psychrometer calibrated as a Richards/Ogata psychrometer (Ψ_a), that is, $\Psi_w/\Psi_a = 1.11$.

Therefore, in this example, the measured diffusion error in the calibrated psychrometer is 11% and is about the size of the calculated error (8-16%), which is significant. As can be seen from Eq. 3.11, the error varies with the size of r_l, and larger r_l causes more error. In contrast, the r_l does not affect an isopiestic measurement because $dm/dt = 0$ and the coefficient $La_m a_l/(La_m r_l + a_l r_a)$ has no effect. The vapor pressure of the droplet is the same as in the tissue, and $\Psi_0 = \Psi_w$ at the isopiestic point.

It should be noted that the size of the droplet on the thermocouple has little influence on the outcome. Figure 3.27 shows that the area of the droplet is much smaller than the area of the sample. The droplet approaches the behavior of a point source, and the rate of vapor transfer is not much affected by small changes in droplet size. Figure 3.29 shows that a measurement of dm/dt at various droplet sizes has no measurable effect as long as there is good contact between the thermocouple junction and the droplet.

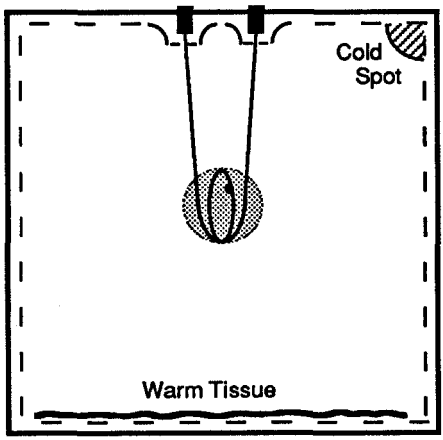

Figure 3.30. Effect of nonisothermal conditions (warm tissue or cold spots). External thermal gradients cause cold spots in the vapor chamber, resulting in condensation and disturbing the vapor conditions. Thermocouple readings cannot be corrected for these kinds of gradients. Internal gradients are caused by metabolic heat of the tissue. Measurements are corrected by noting the output of the thermocouple when dry and using the dry reading as the baseline for the output when wet.

ISOTHERMAL CONDITIONS

Uniform temperatures are important in psychrometry because the vapor pressure is temperature sensitive and the measurement circuit has its own thermoelectric activity. After uniformity is achieved, measurements can be made at different temperatures without a problem. Nonuniform temperatures can arise from two kinds of effects: temperature gradients imposed from the external environment and gradients generated internally, e.g., from the activity of cell metabolism inside the vapor chamber. Different techniques are used to correct the two types of gradients.

Externally imposed gradients cause one part of the vapor chamber to be colder than another as shown in Fig. 3.30 (cold spot). Water condenses from the chamber atmosphere, causing the water potential to appear very low to the thermocouple. A variation of ±0.1°C across the chamber results in a decrease in humidity equivalent to about 1.0 MPa. This cannot be corrected by measurements with the thermocouple because the cold area is remote and often small, and may alter the

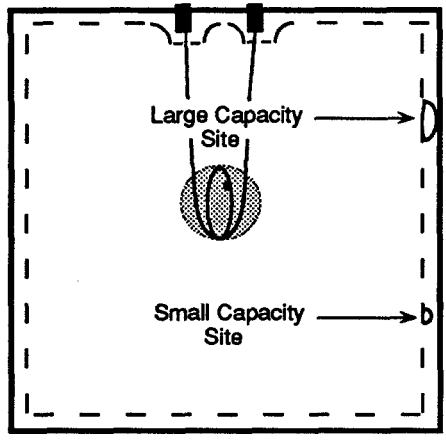

Figure 3.31. Sorption sites have large or small capacities in vapor chambers. Large sites take longer to equilibrate than small sites. Sites are present on walls and external tissue surfaces.

temperature of the thermocouple in unpredictable ways. Therefore, externally generated gradients must be avoided. To achieve an accuracy of ±0.01 MPa, temperatures must be uniform to ±0.001°C. This is usually achieved with a heat sink and insulation or with a water bath. Test for isothermal conditions in an isopiestic psychrometer by placing water on the spiral of an isopiestic thermocouple and on the bottom of the vapor chamber. If a steady reading of zero is obtained over long times, temperatures are uniform in the vapor chamber. In calibrated instruments, this test cannot be made.

Thermal gradients generated by metabolic heat are easier to deal with (Barrs, 1965). Metabolic heat affects the water potential readings by warming the thermocouple above the temperature it would normally have if the heat were absent (Fig. 3.30, warm tissue). As long as the heat does not raise thermocouple output more than 0.4 μV, measuring the output of the dry thermocouple can correct the output when the thermocouple is wet. In isopiestic measurements, the output of the dry thermocouple identifies the output at which a solution is isopiestic. Thus, isopiestic psychrometry automatically corrects for any heat produced by metabolism.

In addition to thermal effects in the vapor chamber, there also may be thermal effects in the measurement circuit. These can be detected by measuring the voltage produced by a dry thermocouple without any

tissue or water in the vapor chamber. If a steady zero output is obtained over long periods of time, isothermal conditions are sufficient for the measurement circuit.

SORPTION EFFECTS

In the high humidity of a psychrometer chamber, any sites that can adsorb water vapor will rapidly remove water from the air. As the sites become hydrated, their water potential rises and the rate of sorption slows down. Eventually their water potential equilibrates with that in the psychrometer and the humidity begins to reflect that of the solution in the sample, allowing the sample water potential to be measured.

It is clearly advantageous to keep sorption to a minimum. All solid materials adsorb water vapor, but some are more sorptive than others. Rubbers and plastics have a high capacity to hold water adsorbed from the air (Brown and van Haveren, 1972). Metals, particularly when highly polished, have an intermediate sorptive capacity. Oils and waxes generally have a low capacity. However, impurities from manufacture can significantly increase the sorptive capacity of oils and waxes. So far, melted and resolidified Vaseline provides the least sorptive surface known to the author. All chamber surfaces should be coated with it except for the thermocouple detector and the sample.

Both the water potential of the sorptive sites and their capacity to sorb water affect the time needed for a thermocouple to reach the steady state, and the capacity is the most important (Fig. 3.31). If a large amount of water must be sorbed to raise the water potential, equilibration will be slow. If the capacity is small, equilibration will be rapid.

Sorption occurs on chamber walls and on plant samples. Plants generally are coated with waxes that have dust or other foreign material on the surface, and these are highly sorptive. The foreign material needs to be removed by washing as described earlier (Fig. 3.12). For plants that have relatively old leaves, e.g., conifer needles, some success has been obtained by soaking the intact needles in water for several hours. Always avoid dirty tissues or tissues having exposed cut surfaces or wounds because the surface can be more sorptive in these areas. For underground portions of the plant, most soil can be shaken away but some can be left attached if sampling is done with care because the adhering soil is in near equilibrium with the roots.

Although sorption delays the approach to steady conditions, the psychrometer responds rapidly to changes after sorption is completed (Boyer, 1969a; Boyer *et al.*, 1985). Isopiestic psychrometers can respond to a step change in vapor pressure immediately and have a time constant of about 30 to 45 sec.

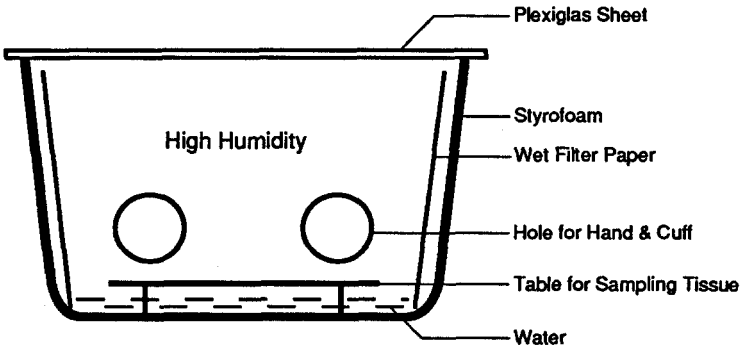

Figure 3.32. Glove box containing water-saturated air for sampling plant tissue and soils. The box has a clear top for viewing the sample on the table.

SAMPLING ERRORS

Evaporation after excision is probably the most frequent error in water potential measurements. To prevent it, tissue can be excised and loaded into the psychrometer rapidly (within 10 sec) or the tissue can be transferred immediately to a saturated atmosphere in a humid box where it can be loaded more slowly without significant evaporation.

A useful humid box can be constructed from a Styrofoam chest whose top has been replaced by a sheet of Plexiglas (Fig. 3.32). Cut two holes in the sides of the box for your hands. Attach plastic or rubber cuffs to the holes to seal around your wrists. Line the chest walls with wet filter paper and fill the bottom with water. Construct a small Plexiglas table to be placed inside. Place a dry paper towel on the Plexiglas table so that the sample is kept from contacting any wet surface. Insert your hands through the holes, sample the tissue on the table, load the Vaseline-coated psychrometer cup, and assemble the cup and psychrometer (Fig. 3.14).

Similar principles apply under field conditions. Carry the psychrometers to the field in a small Styrofoam chest and keep the chest out of direct sun. Complete the sampling rapidly or by using a glove box, as described earlier, then place the psychrometer units back into the Styrofoam chest. The sample should not be stored in the chest for longer than 15 to 30 min. In the laboratory, assemble the psychrometer system as soon as temperatures of the psychrometers approach laboratory temperatures.

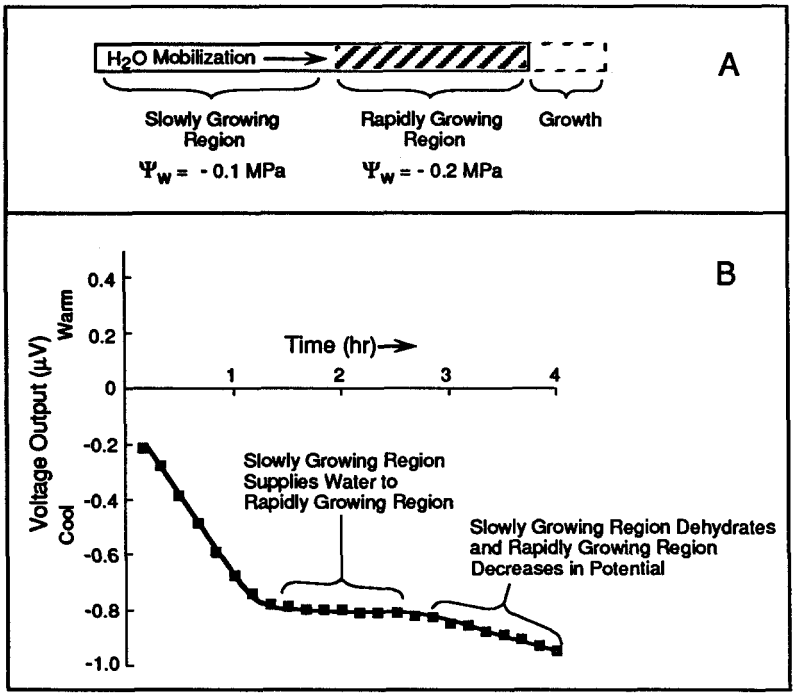

Figure 3.33. Growth can occur after plant tissue has been excised (A, dashed line). Water is mobilized from slowly growing to rapidly growing regions because the water potential is lower in the rapidly growing region. B) Psychrometer measurement in which rapidly growing and slowly growing soybean stem tissues were present in the vapor chamber. A stable output was obtained in the chamber between 1 and 3 hr after sampling, followed by a slow decline as water was withdrawn from the slowly growing region. The stable value gave the average water potential of the tissue and was the same as the potential in the intact plant. Provided readings were made between 1 and 3 hr, accurate measurements could be obtained. The slow decline that occurred after 3 hr did not relate to the intact plant and instead indicated changes occurring in the psychrometer chamber. Although the times vary, this is a typical behavior for all growing tissues.

GROWTH AFTER EXCISION

The water potential of tissue excised from growing regions may change slightly after excision (Boyer *et al.*, 1985). Excision disrupts the flow of water and solute into the enlarging tissue, but the cell walls continue

to extend. The walls relax without water uptake until the turgor decreases to the turgor threshold where further wall growth ceases (Boyer *et al.*, 1985; Cosgrove *et al.*, 1984). The net effect is that the water potential becomes slightly lower (about 0.1 MPa) than it would have been in the intact plant (Boyer *et al.*, 1985). Figure 5.4 shows examples of this effect.

If mature tissue and growing tissue are excised together, water in the mature tissue is moved to the enlarging tissue. Wall relaxation is delayed and slow growth occurs (Fig. 3.33A). The water potential of the enlarging tissue continues to reflect that in the intact plant before excision (Boyer *et al.*, 1985; Matyssek *et al.*, 1988). The potential of the mature tissue gradually declines over a period of 2 to 3 hr as water is withdrawn to the enlarging tissue (Fig. 3.33B). When measuring the water status of enlarging tissue, it is often wise to include some attached, slowly growing or mature tissue if possible to give yourself time to complete the measurement rapidly while the tissue has a water potential similar to that in the intact plant.

VOLUME OF VAPOR CHAMBER

Because psychrometer measurements generally require 1 to 3 hr, an oxygen supply must be available for the tissue. Oxygen is supplied by the air enclosed with the tissue. Since most plant tissues have a low rate of oxygen consumption, small chambers are adequate. On the other hand, growing tissues may consume oxygen 10 times faster than mature tissues and larger chambers may be required. For a sample that is growing rapidly and has about 10 mg of dry weight, the chamber volume should be about 5 cm^3.

When tissue begins to run out of oxygen, the turgor decreases as membranes break down. The vapor pressure of cell water decreases and the tissue appears to become drier (not to be confused with the similar effects of wall relaxation in growing tissue). Eventually, the psychrometer indicates the osmotic potential for the protoplasts.

AVOIDING METABOLIC HEAT

The metabolic heat produced by plant tissue and by microorganisms in soil can be drained away by contact between the sample and the metal walls and bottom of the vapor chamber. Because these walls are coated with Vaseline, plant tissue can be pushed into the Vaseline to ensure good contact with the chamber surfaces (Fig. 3.20). Any residual heat is corrected by reading the output of the thermocouple when dry (Fig. 3.17). The correction is accurate only for small amounts of heat (dry reading to 0.4 μV). Never place more than one layer of tissue in the vapor chamber

because heat is not readily drained from the inner layer, and the overheating will cause erroneous readings.

POTENTIAL GRADIENTS

Gradients in potential exist over large distances in both soils and plants. The gradients exist because water moving through the material flows through various frictional resistances, causing some parts to be drier than others. Also, gravity may cause gradients over long vertical distances. A knowledge of the size and location of the gradients is necessary for interpreting the measurements. Nonami *et al.* (1991) have shown that Ψ_w gradients exist between tissues in a leaf. For excised tissue, the potential is a volume average for that part of the gradient in the tissue at the time of sampling. For intact tissue, the potential appears to be that of the vascular supply that provides water to the sample (except in growing tissue, see earlier).

Leaves that are farthest from the water supply are usually drier than leaves closer to the water supply. Leaves that are most apically situated also tend to be more brightly illuminated than those at the base of the canopy, which further lowers their water potential. The reproducibility between measurements is enhanced when gradients are carefully considered during sampling. A particular problem occurs when making leaf gas exchange measurements in cuvettes because leaves outside the cuvette can have a different water potential from those inside. In this situation, gradients not only are present along the shoot but they are imposed by the experimental apparatus. Therefore, water potentials should be measured only with tissue inside the same cuvette used for the other measurements.

Appendix 3.1-Psychrometer Manufacturers

Wescor, Inc.
459 South Main Street
Logan, Utah 84321
(801) 752-6011

J.R.D. Merrill Specialty Equipment
R.F.D. Box 140A
Logan, Utah 84321
(801) 752-8403

Decagon Devices, Inc.
P.O. Box 835
NW 115 State Street
Pullman, WA 99163
(509) 332-2756

Isopiestics Company
2 Harborview Road
Lewes, DE 19958
(302) 645-4014

Appendix 3.2-Water Potentials (Osmotic Potentials) of Sucrose Solutions

Osmotic potentials for sucrose solutions are given in Table 3.1, calculated from the equations below. The calculations are according to Michel (1972). The osmotic potential (in MPa) of a sucrose solution is determined by

$$\Psi_s = -\phi mDRT/1000, \tag{3.12}$$

where ϕ, the osmotic coefficient, is a function of the molal concentration m of the solution, m is in mol·(kg H_2O)$^{-1}$, D is the density of water (g·m^{-3}) as a function of temperature T, R is the gas constant (8.3143 x 10^{-6} MPa·m^3·mol^{-1}·K^{-1}), and T is the Kelvin temperature (K). The osmotic coefficient ϕ for sucrose is generated by the empirical equation

$$\phi = 0.998 + 0.089m. \tag{3.13}$$

Substituting ϕ from Eq. 3.13 and $k = DRT/1000$ into Eq. 3.12, the osmotic potential is a function of the molality and the temperature-dependent coefficient k

$$\Psi_s = -(0.089\ m^2 + 0.998m)k. \tag{3.14}$$

Solving Eq. 3.14 for m:

$$m = -5.6067 + \sqrt{31.4355 - 11.236\ \Psi_s\ k^{-1}}. \tag{3.15}$$

TABLE 3.1. Water Potentials (Osmotic Potentials) of Sucrose Solutions. Molalities are in moles per kg of water, osmotic potentials are in bars, temperatures (T) are in Celsius.

T Molality	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°	32°	34°	36°
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.05	-1.187	-1.195	-1.203	-1.211	-1.219	-1.227	-1.234	-1.242	-1.249	-1.257	-1.264	-1.271	-1.279
0.10	-2.385	-2.401	-2.417	-2.433	-2.448	-2.464	-2.479	-2.495	-2.510	-2.525	-2.540	-2.554	-2.569
0.15	-3.593	-3.617	-3.641	-3.665	-3.689	-3.712	-3.736	-3.759	-3.781	-3.804	-3.826	-3.848	-3.870
0.20	-4.812	-4.844	-4.876	-4.908	-4.940	-4.971	-5.003	-5.033	-5.064	-5.094	-5.124	-5.154	-5.183
0.25	-6.041	-6.082	-6.122	-6.162	-6.202	-6.242	-6.281	-6.319	-6.358	-6.396	-6.433	-6.470	-6.507
0.30	-7.281	-7.330	-7.379	-7.427	-7.475	-7.523	-7.570	-7.616	-7.662	-7.708	-7.753	-7.798	-7.842
0.35	-8.531	-8.589	-8.646	-8.703	-8.759	-8.814	-8.870	-8.924	-8.978	-9.032	-9.085	-9.137	-9.189
0.40	-9.792	-9.858	-9.924	-9.989	-10.05	-10.12	-10.18	-10.24	-10.31	-10.37	-10.43	-10.49	-10.55
0.45	-11.06	-11.14	-11.21	-11.29	-11.36	-11.43	-11.50	-11.57	-11.64	-11.71	-11.78	-11.85	-11.92
0.50	-12.35	-12.43	-12.51	-12.59	-12.68	-12.76	-12.84	-12.91	-12.99	-13.07	-13.15	-13.22	-13.30
0.55	-13.64	-13.73	-13.82	-13.91	-14.00	-14.09	-14.18	-14.27	-14.35	-14.44	-14.52	-14.61	-14.69
0.60	-14.94	-15.04	-15.14	-15.24	-15.34	-15.44	-15.53	-15.63	-15.72	-15.82	-15.91	-16.00	-16.09
0.65	-16.26	-16.36	-16.47	-16.58	-16.69	-16.79	-16.90	-17.00	-17.11	-17.21	-17.31	-17.41	-17.51
0.70	-17.58	-17.70	-17.82	-17.93	-18.05	-18.16	-18.28	-18.39	-18.50	-18.61	-18.72	-18.83	-18.93
0.75	-18.91	-19.04	-19.17	-19.29	-19.42	-19.54	-19.66	-19.79	-19.91	-20.02	-20.14	-20.26	-20.37
0.80	-20.26	-20.40	-20.53	-20.67	-20.80	-20.93	-21.06	-21.19	-21.32	-21.45	-21.57	-21.70	-21.82
0.85	-21.61	-21.76	-21.91	-22.05	-22.19	-22.33	-22.47	-22.61	-22.75	-22.88	-23.02	-23.15	-23.28
0.90	-22.98	-23.14	-23.29	-23.44	-23.59	-23.74	-23.89	-24.04	-24.19	-24.33	-24.47	-24.61	-24.75
0.95	-24.36	-24.52	-24.69	-24.85	-25.01	-25.17	-25.32	-25.48	-25.63	-25.79	-25.94	-26.09	-26.24

TABLE 3.1 (continued). Water Potentials (Osmotic Potentials) of Sucrose Solutions. Molalities are in moles per kg of water, osmotic potentials are in bars, temperatures (T) are in Celsius.

T Molality	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°	32°	34°	36°
1.00	-25.75	-25.92	-26.09	-26.26	-26.43	-26.60	-26.77	-26.93	-27.09	-27.26	-27.42	-27.57	-27.73
1.05	-27.14	-27.33	-27.51	-27.69	-27.87	-28.04	-28.22	-28.39	-28.57	-28.74	-28.90	-29.07	-29.24
1.10	-28.55	-28.74	-28.94	-29.13	-29.31	-29.50	-29.68	-29.87	-30.05	-30.23	-30.40	-30.58	-30.75
1.15	-29.97	-30.17	-30.37	-30.57	-30.77	-30.97	-31.16	-31.35	-31.54	-31.73	-31.92	-32.10	-32.28
1.20	-31.40	-31.61	-31.82	-32.03	-32.24	-32.44	-32.65	-32.85	-33.05	-33.24	-33.44	-33.63	-33.82
1.25	-32.84	-33.06	-33.28	-33.50	-33.72	-33.93	-34.14	-34.35	-34.56	-34.77	-34.97	-35.17	-35.37
1.30	-34.29	-34.52	-34.75	-34.98	-35.21	-35.43	-35.65	-35.87	-36.09	-36.30	-36.52	-36.73	-36.93
1.35	-35.75	-35.99	-36.23	-36.47	-36.71	-36.94	-37.17	-37.40	-37.63	-37.85	-38.07	-38.29	-38.51
1.40	-37.22	-37.48	-37.72	-37.97	-38.22	-38.46	-38.70	-38.94	-39.17	-39.41	-39.64	-39.87	-40.09
1.45	-38.71	-38.97	-39.23	-39.48	-39.74	-39.99	-40.24	-40.49	-40.73	-40.98	-41.22	-41.46	-41.69
1.50	-40.20	-40.47	-40.74	-41.01	-41.27	-41.53	-41.79	-42.05	-42.31	-42.56	-42.81	-43.05	-43.30
1.55	-41.70	-41.98	-42.26	-42.54	-42.81	-43.09	-43.36	-43.62	-43.89	-44.15	-44.41	-44.66	-44.92
1.60	-43.22	-43.51	-43.80	-44.08	-44.37	-44.65	-44.93	-45.21	-45.48	-45.75	-46.02	-46.29	-46.55
1.65	-44.74	-45.04	-45.34	-45.64	-45.93	-46.23	-46.52	-46.80	-47.09	-47.37	-47.64	-47.92	-48.19
1.70	-46.28	-46.59	-46.90	-47.21	-47.51	-47.81	-48.11	-48.41	-48.70	-48.99	-49.28	-49.56	-49.84
1.75	-47.82	-48.14	-48.46	-48.78	-49.10	-49.41	-49.72	-50.02	-50.33	-50.63	-50.92	-51.22	-51.51
1.80	-49.38	-49.71	-50.04	-50.37	-50.69	-51.02	-51.34	-51.65	-51.96	-52.27	-52.58	-52.88	-53.18
1.85	-50.94	-51.29	-51.63	-51.97	-52.30	-52.63	-52.96	-53.29	-53.61	-53.93	-54.25	-54.56	-54.87
1.90	-52.52	-52.88	-53.23	-53.58	-53.92	-54.26	-54.60	-54.94	-55.27	-55.60	-55.93	-56.25	-56.57
1.95	-54.11	-54.47	-54.84	-55.20	-55.55	-55.90	-56.25	-56.60	-56.94	-57.28	-57.62	-57.95	-58.28

TABLE 3.1 (continued). Water Potentials (Osmotic Potentials) of Sucrose Solutions. Molalities are in moles per kg of water, osmotic potentials are in bars, temperatures (T) are in Celsius.

T Molality	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°	32°	34°	36°
2.00	-55.71	-56.08	-56.46	-56.83	-57.19	-57.56	-57.92	-58.27	-58.63	-58.98	-59.32	-59.66	-60.00
2.20	-62.20	-62.62	-63.04	-63.45	-63.86	-64.27	-64.67	-65.07	-65.46	-65.86	-66.24	-66.62	-66.70
2.40	-68.87	-69.34	-69.80	-70.25	-70.71	-71.16	-71.60	-72.04	-72.48	-72.91	-73.34	-73.76	-74.18
2.60	-75.71	-76.22	-76.72	-77.23	-77.73	-78.22	-78.71	-79.19	-79.67	-80.15	-80.62	-81.08	-81.54
2.80	-82.71	-83.27	-83.82	-84.37	-84.92	-85.46	-85.99	-86.52	-87.05	-87.56	-88.08	-88.59	-89.09
3.00	-89.88	-90.49	-91.09	-91.69	-92.28	-92.87	-93.45	-94.02	-94.59	-95.16	-95.72	-96.27	-96.81
3.20	-97.22	-97.88	-98.53	-99.18	-99.82	-100.5	-101.1	-101.7	-102.3	-102.9	-103.5	-104.1	-104.7
3.40	-104.7	-105.4	-106.1	-106.8	-107.5	-108.2	-108.9	-109.6	-110.2	-110.9	-111.5	-112.2	-112.8
3.60	-112.4	-113.2	-113.9	-114.7	-115.4	-116.1	-116.9	-117.6	-118.3	-119.0	-119.7	-120.4	-121.1
3.80	-120.3	-121.1	-121.9	-122.7	-123.5	-124.3	-125.0	-125.8	-126.6	-127.3	-128.1	-128.8	-129.5
4.00	-128.3	-129.1	-130.0	-130.9	-131.7	-132.5	-133.4	-134.2	-135.0	-135.8	-136.6	-137.4	-138.2
4.20	-136.5	-137.4	-138.3	-139.2	-140.1	-141.0	-141.9	-142.7	-143.6	-144.5	-145.3	-146.2	-147.0
4.40	-144.8	-145.8	-146.8	-147.7	-148.7	-149.6	-150.6	-151.5	-152.4	-153.3	-154.2	-155.1	-156.0
4.60	-153.3	-154.4	-155.4	-156.4	-157.4	-158.4	-159.4	-160.4	-161.4	-162.3	-163.3	-164.2	-165.2
4.80	-162.0	-163.1	-164.2	-165.3	-166.3	-167.4	-168.5	-169.5	-170.5	-171.5	-172.5	-173.5	-174.5
5.00	-170.9	-172.0	-173.2	-174.3	-175.4	-176.6	-177.6	-178.8	-179.8	-180.9	-182.0	-183.0	-184.1
5.20	-179.9	-181.1	-182.3	-183.5	-184.7	-185.9	-187.0	-188.2	-189.3	-190.5	-191.6	-192.7	-193.8
5.40	-189.1	-190.4	-191.7	-192.9	-194.2	-195.4	-196.6	-197.8	-199.0	-200.2	-201.4	-202.5	-203.7