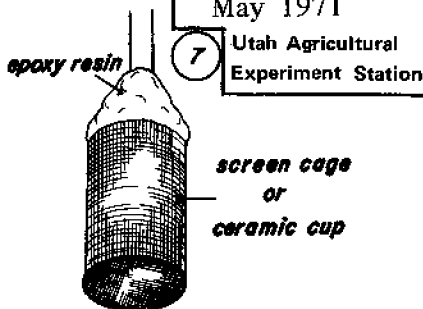
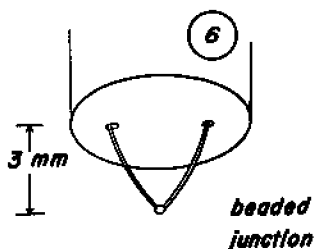
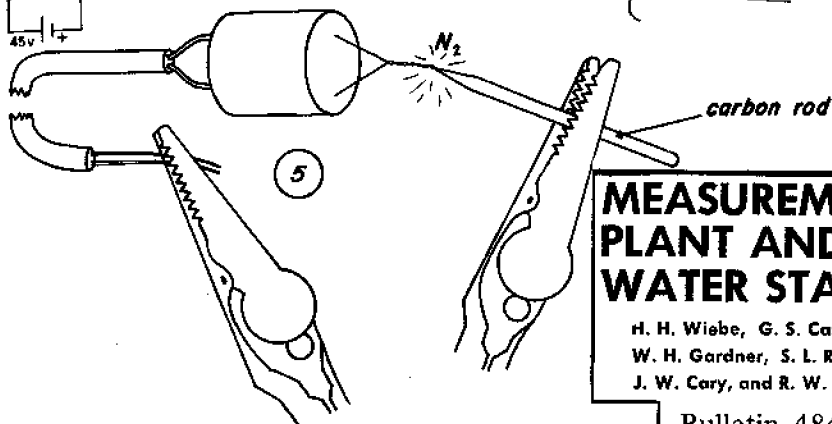
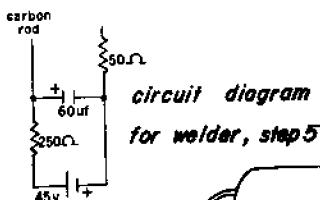
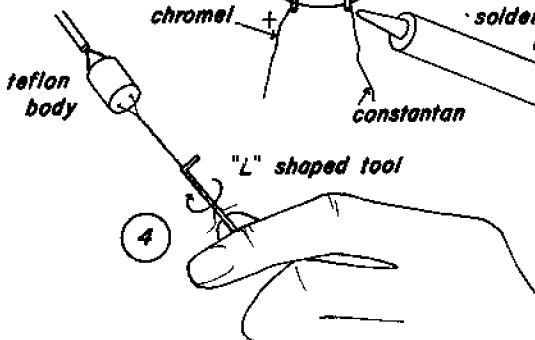
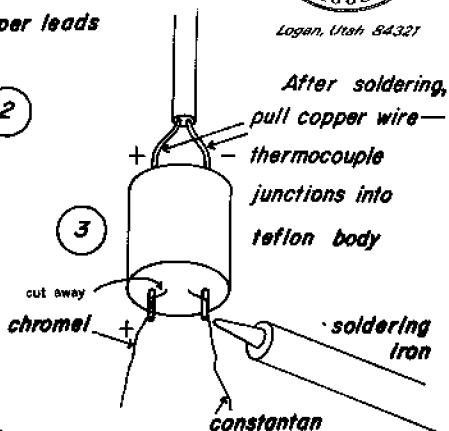
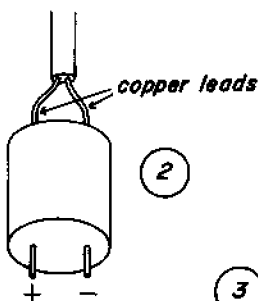
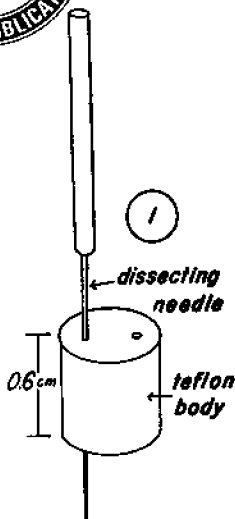




Western States Agricultural Experiment Stations
 in cooperation with
 The United States Department of Agriculture



Logan, Utah 84321



MEASUREMENT OF PLANT AND SOIL WATER STATUS

H. H. Wiebe, G. S. Campbell
 W. H. Gardner, S. L. Rawlins
 J. W. Cory, and R. W. Brown

Bulletin 484
 May 1971

Utah Agricultural Experiment Station

MEASUREMENT OF PLANT AND SOIL WATER STATUS

A bulletin based on research conducted under the sponsorship of Western Regional Research project W-67 with the 12 Western States and Hawaii in cooperation with the United States Department of Agriculture, Agricultural Research Service.

Herman H. Wiebe, Professor of Botany, Utah State University,
Logan, Utah 84321

Gaylon S. Campbell, Assistant Professor of Soils, Washington
State University, Pullman, Washington

Walter H. Gardner, Professor of Soils, Washington State
University, Pullman, Washington 99163

Stephen L. Rawlins, Research Soil Scientist, USDA-ARS-SWC
US Salinity Laboratory, Riverside, California 92502

John W. Cary, Research Soil Scientist, USDA-ARS-SWC
Snake River Conservation Research Center, Kimberly, Idaho
83341

Ray W. Brown, Plant Physiologist, U.S. Forest Service,
Logan, Utah 84321

Edited by
Herman H. Wiebe

Utah Agricultural Experiment Station, Utah State University,
in cooperation with the Experiment Stations of the 12 Western
States and the United States Department of Agriculture, Agricul-
tural Research Service.

Under procedure for cooperative publications, this regional
report becomes in effect an identical publication of each of the
cooperating agencies, and is mailed under the frank and indicia
of each.

CONTENTS

ACKNOWLEDGEMENTS	iv
I. INTRODUCTION	1
II. THEORY	2
III. SPANNER (Peltier) PSYCHROMETERS	7
A. Apparatus	7
1. Thermocouples	7
a. Simplified construction procedure	8
2. Sample chambers and changers	11
3. Soil psychrometers	12
a. Ceramic cups vs screen cages	13
4. Measuring equipment	14
a. Amplifiers	14
b. Switchbox	14
c. Characteristics and measurements of cooling current	16
5. Temperature control for laboratory psychrometers	19
B. Calibration	22
1. Calibration of soil psychrometers	25
2. Effect of temperature on calibration	26
C. Use of psychrometers in the laboratory	26
1. Sample preparation	26
2. Soil measurement in the laboratory	28
3. Leaf measurements	30
4. Measuring extremely low potentials	31
5. Summary of measurement procedure for Peltier thermocouple psychrometers	31
D. Spanner psychrometers in field studies	32
1. Soil measurement	32
2. Tree stems	33
3. Attached leaves	35
a. Construction of compensating psychrometers	37
b. Calibration of compensating psychrometers	39
c. Discussion of compensating psychrometers	42
IV. THERMISTOR PSYCHROMETER	42
A. Thermistor psychrometer apparatus	43
B. Measurement procedure	43
V. GRAVIMETRIC VAPOR EXCHANGE	45
A. Apparatus	45
B. Procedure	46
VI. PRESSURE CHAMBER OR BOMB	47
VII. DYE METHOD	48

VIII. FREEZING POINT METHOD 49

A. Theory 49

B. Construction 51

C. Calibration 54

D. Use with plant samples 55

E. General precautions 55

IX. OSMOTIC POTENTIAL DETERMINATIONS 56

A. Extraction of sap 57

B. Osmotic potential determinations of extracted sap 58

 1. Vapor exchange methods 58

 2. Cryoscopic methods 59

C. Visual melting point method 59

X. MATRIC POTENTIAL DETERMINATIONS ON PLANTS 59

XI. LITERATURE CITED 61

XII. APPENDIX A 67

XIII. APPENDIX B 71

ACKNOWLEDGEMENTS

Soil and plant scientists from the Western States and Hawaii have cooperated for many years in studying soil-plant relations. These studies have been aided by Western Regional Research Project W-67, and earlier by projects W-29 and W-9. As a result of their cooperative work, this group has contributed substantially to the development of measurement techniques. This publication gives primary emphasis to methods they have found most useful. It is intended as a guide, based primarily on the experience, and perhaps whims, of the authors. Stephen Rawlins wrote the theory section. Gaylon Campbell wrote most of the section about Spanner psychrometers. Walter H. Gardner wrote the section about the compensated psychrometer, while John Cary wrote about the freezing point meter.

I. INTRODUCTION

Research in soil-plant water relations during the last two decades has successfully addressed itself to the problems of expressing water status in soils and plant tissues in terms of free energy. The initial impetus was provided by Spanner (1951) in his classical work on the psychrometer. During the ensuing years the science of water relations was provided with a new theoretical approach based on thermodynamic principles and terminology. This was complemented by a substantial amount of research on basic techniques. The result has been a rather sophisticated technology which provides a means of describing the free energy status of water in natural systems in quantitative terms consistent with modern thermodynamic theory. This marks a significant turning point, because now we are capable of expressing the flow of water in the soil-plant continuum in meaningful terms of energy status. The recent comprehensive review by Barrs (1968) cites over 50 references on the use of the Spanner psychrometer and related vapor exchange methods, and a great many other useful references on various techniques for evaluating other aspects of plant water status. However, with this and other reviews (Boyer, 1969; Brown, 1970; Peck 1968, 1969; Rawlins, 1966) there still appears to be a need for a practical guide on the use of thermocouple psychrometers and other methods of measuring water potential and its components.

Water in the soil-plant continuum is dynamic, and as such is rarely, if ever, in equilibrium with water in adjacent locations. The driving forces responsible for water transfer are gradients in the free energy status of water, or the water potential, which result from transpiration, evaporation, temperature gradients, and other factors. Water moves along these gradients in the soil-plant continuum from regions of higher to regions of lower potential. As such, plant responses to water stress are closely related to the energy required to remove a unit of water from the soil. Thermocouple psychrometers are particularly well

suited for measuring water potential, since they are capable of distinguishing small changes in energy levels based on vapor exchange.

II. THEORY

Scientists realized for many years that water potential can be inferred accurately from measurements of relative humidity. At any given temperature, T , the theoretical relation between water potential and relative humidity is

$$\psi = \frac{RT}{V} \ln \frac{e}{e_0} \quad [1]$$

where ψ is the water potential, R is the ideal gas constant, T is the absolute temperature, V is the volume of a mole of liquid water, and e/e_0 is the relative humidity. The only assumption involved in this relation is that water vapor behaves as an ideal gas, which introduces negligible error for the pressure normally encountered.

Two kinds of thermocouple psychrometers have been developed for measurement of relative humidity in soil and plant samples. These operate on the same principle as ordinary psychrometers for measurement of atmospheric relative humidity; that is, they measure the temperature depression of a wet surface. The two kinds of thermocouple psychrometers differ mainly in the mode in which water is applied to the wet bulb. The psychrometer developed by Spanner (1951) makes use of the Peltier effect to condense water from the atmosphere in the psychrometer chamber onto the measuring junction. The psychrometer developed by Richards and Ogata (1958) has a small silver ring supported by 0.0025-cm diameter chromel and constantan wires to hold a droplet of water.

Several questions have arisen concerning the validity of the relative humidity measurements made with these thermocouple psychrometers. For one thing, they are used in static air, while normally psychrometer wet-bulbs are aspirated to achieve accurate readings. It also has been found that the wet-bulb depression of the psychrometers is not a unique function of the

relative humidity of the chamber in which they are placed. Sensitivity increases with increasing temperature and decreases with increasing pressure. To answer these questions, Rawlins (1966) derived a theoretical equation relating the voltage output of the thermocouple psychrometer to psychrometer dimensions and to chamber temperature and pressure. The equation is based on the energy balance of the wet junction and assumes that convection within the equilibration chamber is negligible. The wet junction gains heat by radiation from the surrounding chamber walls, conduction down the thermocouple wires, and conduction through the chamber air. At steady state, the heat gain to the wet junction by these three modes is balanced by evaporative cooling, which is the product of the evaporation rate and the latent heat of vaporization. For a given psychrometer, equations were written for each of these heat transfer processes by using well known laws of heat and vapor transport. By equating the heat gain and heat loss from the wet junction, the condition at steady state, the temperature depression of the wet junction, Θ was given by

$$\Theta = \frac{[(4 r_j r_c) / (r_c - r_j)] D L c_{T^s} [1 - \exp(\psi \Psi / R T)]}{16 \sigma r_j^2 T^3 + r_w^2 k_w \gamma + [(4 r_j r_c) / (r_c - r_j)] (k_a + D \beta L)} \quad [2]$$

where r_j , r_c , and r_w are the respective radii of the wet junction (assumed to be a spherical water drop), the equilibration chamber, and the thermocouple wire; k_w and k_a are the thermal conductivities of the wire and the air; D is the diffusivity of water vapor in air; L is the latent heat of vaporization of water; c_{T^s} is the saturation specific humidity; β is the slope of the curve of c_{T^s} with temperature; σ is the Stefan-Boltzmann constant; $\exp(\Psi \Psi / R T)$ is e/e_0 , substituted from equation [1]; and γ is a combination of these parameters given by

$$\gamma = 2 \left[\frac{(2 \pi k_a) / [\ln(r_c/r_w)] + 8 \pi r_w \sigma T^3}{\pi r_w^2 k_w} \right]^{1/2}$$

In equation [2] the dependence of Θ on psychrometer geometry is included explicitly in the r_w , r_c , and r_j factors and implicitly in γ . Temperature appears in the equation explicitly in T as well

thermocouple wires is small and the radiation term of the equation is negligible, the equation reduces to

$$\Theta = c_r S (1 - p/p_0 / \beta + k_a/LD) \quad [3]$$

If κ is substituted for k_a , where κ is the thermal diffusivity of air and c_p is the specific heat of the air at constant pressure, and κ is set equal to D for convection dominated transfer, equation [3] becomes identical to equation 3.23 of Slatyer and McIroy (1961) — the equation for a fully ventilated psychrometer. Thus, the theory of operation of psychrometers used for water potential measurement differs from that for aspirated psychrometers only by the absence of the convection term. The important requirement in use of either psychrometer is to insure that heat transfer through the air is either entirely by convection or entirely by conduction. In either case the calibration of the psychrometer will be predictable.

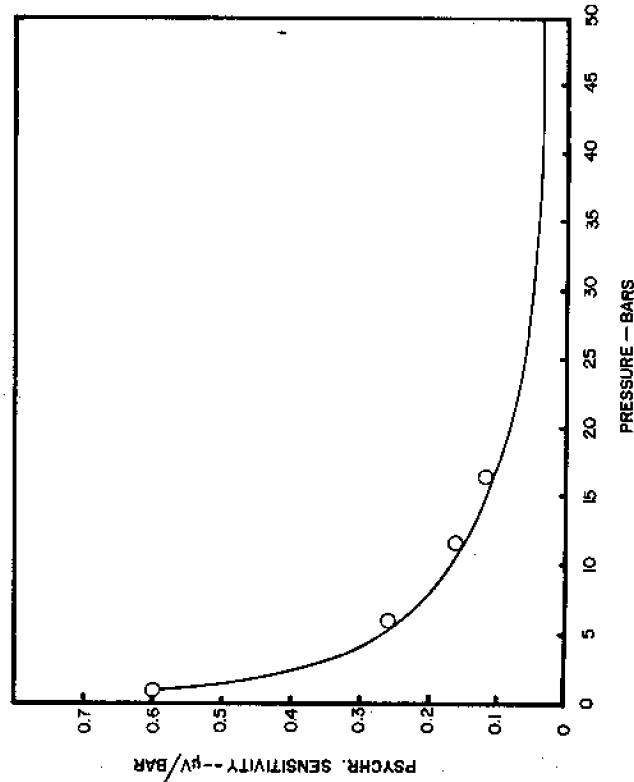


Figure 3. Theoretical psychrometer sensitivity versus pressure from equation (2) at $t = 25$ C for a Richards psychrometer. The data points are psychrometer sensitivity in $\mu\text{V bar}^{-1}$ at 1, 6, 11 and 16 bars pressure determined by Richards et al. (1964) (from Rawlins, 1966).

Assuming on the basis of this evidence that thermocouple psychrometers measure relative humidity within the equilibration chamber, any errors in measurements of water potential must stem from lack of either vapor pressure or temperature equilibrium between the sample and the air in the equilibration chamber. This can occur in two obvious ways: (1) sinks of sources for water vapor within the chamber can lower or raise the vapor pressure measured by the psychrometer from that within the sample; (2) heat production within the sample, as for example by respiration, can raise its temperature above that of the chamber.

III. SPANNER (Peltier) PSYCHROMETERS

A. Apparatus

The Peltier-type thermocouple psychrometer has found many applications because it is convenient to use in field psychrometers (Rawlins and Dalton, 1967) where it would be difficult to remove a wet-loop psychrometer to add a water droplet, and because it is relatively free from measurement errors arising from the addition or removal of water from the system (Zollinger et al., 1966). The bulk of this discussion will apply to the Peltier psychrometer. Information on construction and measurements using the wet loop psychrometer were given by Richards and Ogata (1958). In this section we will present information on construction, application, and measurements using the Peltier psychrometer.

The construction of thermocouples and sample holders for Peltier psychrometers has been described in several publications (Spanner, 1951; Monteith and Owen, 1958; Campbell et al., 1966; Rawlins and Dalton, 1967; Campbell et al., 1968; Merrill et al., 1968; Barrs, 1968; and Brown, 1970).

1. Thermocouples

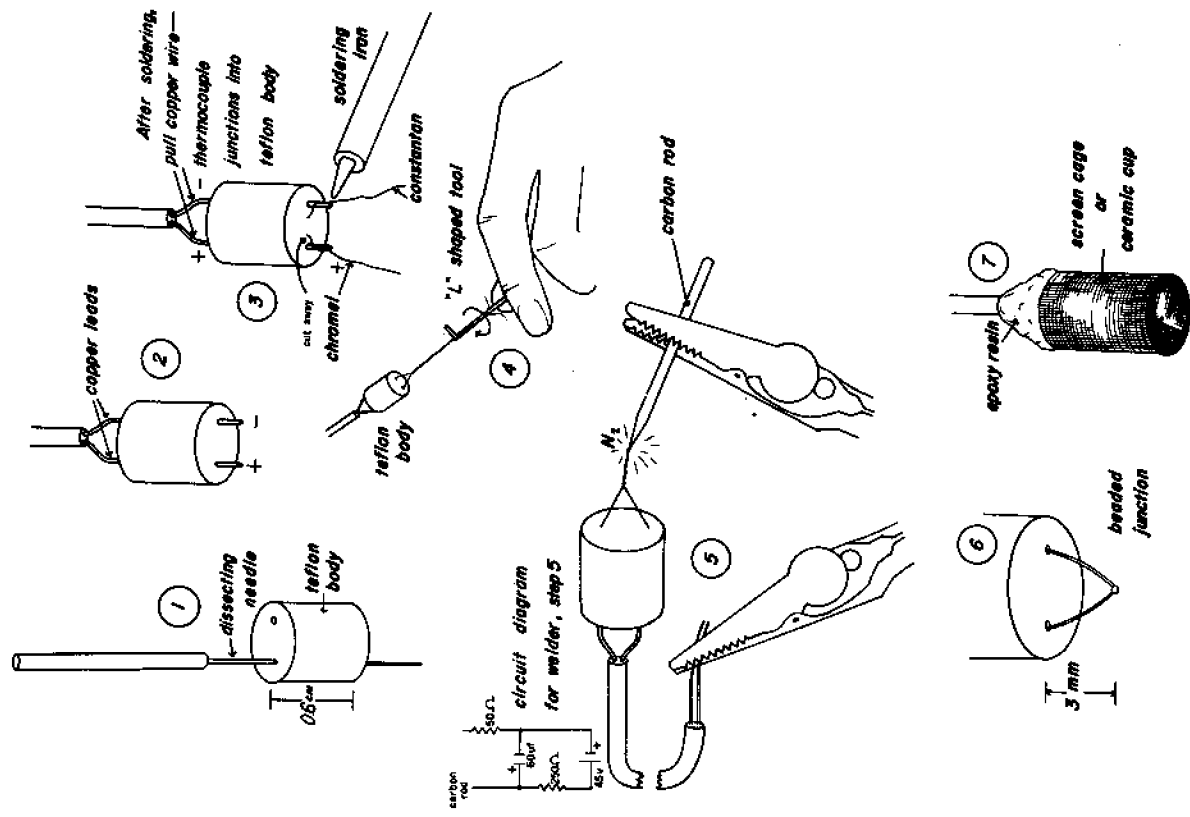
The thermocouples now in general use are constructed from chromel and constantan wire. These materials are chosen because they are readily available, easy to work with, and have a high sensitivity and Peltier coefficient. It is desirable to cause as little thermal and vapor disturbance in the sample chamber

as possible when readings are made with the psychrometer, and the psychrometer is most easily constructed and used if the reference junctions are small. The smaller the thermocouple wire used, the less the chamber will be disturbed and smaller heat sinks will be required. Thermocouple wire with a diameter of 0.0025 centimeter (0.001 inch) is commercially available (Omega Engineering Inc.)*, and is generally used.

Wire with a diameter of 0.0013 centimeter (0.0005 inch) is also available and has been used with good results. Construction of thermocouples with this smaller wire is more difficult, however, and the readings were the same as those obtained by using larger wire. Welded thermocouples made from 0.0025 centimeter chromel and constantan are available commercially (Omega Engineering Inc.) and have been used for thermocouple psychrometers, but thermocouples welded according to a technique described below are superior for this application because the measuring junction can be made larger.

Details on construction of the thermocouple unit are available (Merrill et al., 1968; Campbell et al., 1968; Brown, 1970; and Lopushinsky and Klock, 1971). The Merrill technique provides heat sinks for the reference junction of the size prescribed by Dalton and Rawlins (1968). The Campbell and Brown techniques are much simpler, but provide only the lead wires and reference junction surroundings for dissipation of the heat generated at the reference junction by the Peltier effect. This is apparently adequate when 0.0025-centimeter diameter or smaller wire is used in the thermocouples because no reference junction heating has been detected with psychrometers constructed in this manner. For larger thermocouple wire, reference junction heating becomes more of a problem and it is more important to provide adequate heat sinks for the reference junction.

a. *Simplified construction procedure.* The essential parts of the Peltier psychrometer consist of a Teflon body within which the copper lead wires are attached to the thermocouple (figure 4). After cutting the Teflon body to the proper length (0.48 centimeter diameter rod cut to a length of 0.65 centimeter), two fine holes approximately 0.025 centimeter in diameter are made



* Addresses of commercial sources of supply are given in Appendix B.

Figure 4. Psychrometer construction steps.

through the long axis of the Teflon with a fine dissecting needle. The chromel and constantan thermocouple wires (0.0025-centimeter diameter) are then inserted through the holes from the bottom side of the Teflon body with about 4 centimeters of each wire allowed to protrude. About 0.5 centimeter of the ends of the copper lead wires (Beldon #8640, 26 gauge) are scraped carefully and inserted into the holes from the top side of the Teflon body. The positive copper lead is tightly wedged into the hole with the chromel wire, and the negative lead with the constantan wire. This provides a tight contact between the copper and thermocouple wires. An alternative method (illustrated as steps 2 and 3, figure 4) is to insert the copper lead wires all the way through the Teflon insert, allowing each copper wire to extend about 3 millimeters beyond the Teflon. Then wrap the chromel and constantan wires around each appropriate copper lead a few times, and solder each junction with high grade silver solder. Then pull the copper-thermocouple wire junctions back up into the Teflon body.

The chromel and constantan wires protruding from the bottom of the Teflon body are then twisted together with an "L"-shaped piece of wire (26 gauge, 3 centimeters long with an "L" bend about 3 millimeters). The "L"-shaped tool is held between the thumb and fore-finger, and is twirled slowly around the two thermocouple wires, twisting the two wires tightly together. Twisting is continued until the junction is about 2 millimeters away from the Teflon; then the "L"-shaped tool is inserted between the chromel and constantan wires and the junction pulled out to 3 millimeters from the Teflon body. This tightens the junction and provides a uniform length to the thermocouple assembly. The excess twisted wire can be cut away with scissors, leaving about 3 millimeter of twisted wire below the junction. The thermocouple is now ready to be welded or soldered. Welding is preferred because soldered junctions tend to age more quickly and do not have a linear response throughout the range of vapor pressures of interest (Campbell et al., 1968).

Welding can be accomplished by means of several different techniques, but the method discussed here has been found to be most satisfactory (see also Lopushinsky and Klock, 1971). Circuit diagrams of arc welders are given in figure 4, step 5a. The negative lead from the welder is attached to both copper leads,

and the positive lead is attached to a sharpened graphite rod (4-H graphite lead). The welding operation is accomplished by touching the graphite rod to the end of the twisted thermocouple wires. Welding should be done slowly at first, building up a head of fused metal. The head should be enlarged until it is about three times the diameter of the thermocouple wire. Welding can be terminated when about one twist remains behind the beaded junction. The largest junction diameter possible with this technique appears to be about five times the wire diameter. Best results are obtained if the welding operation is carried out under low weight oil in a petri dish, under a binocular scope.* The oil prevents oxidation deposits from building up on the junction, and it permits greater control over the rate of welding. The thermocouple can be cleaned subsequently by rinsing in acetone. Some workers have had success by welding in a nitrogen atmosphere; a chamber flooded with nitrogen gas is sufficient, although some recommend a special air-tight chamber built to house the thermocouple and the graphite rod assembly.

2. Sample chambers and changers

The thermocouple assembly can be used with a variety of enclosures for measurement of water potentials of samples or *in situ* measurements. The primary requirement of the sample chamber is that there be no vapor sources or sinks present (see Theory section). A suitable choice of materials for construction of the sample chamber and proper cleaning procedures are important to assure that vapor sources and sinks are not present in the chamber. A smooth Teflon surface apparently is best. Stainless steel also works well, and brass has been used with generally satisfactory results. Glass, rubber, and acrylic surfaces apparently absorb water and have not worked well for sample chambers.

Chamber shape and size are not critical for measurements in soil and can be adapted to the specific research problem. For plant tissues, where heat of respiration can be a problem, the chamber should be shaped to completely surround the thermocouple by tissue sample. A cylindrical shape works well. Measurements have been made in chambers with volumes of only a few cubic millimeters, with no apparent effect of volume on the reading, so the lower limit in chamber size seems to be one of con-

*We thank Eric Campbell for this suggestion.

struction difficulties rather than disturbance of the chamber environment by taking readings.

It is important that the chamber be sealed to prevent vapor exchange with the surroundings, but care must be taken to maintain the pressure in the chamber at atmospheric pressure or to know the pressure and correct the psychrometer readings. The error, calculated from figure 3 in the Theory section, introduced by pressure changes is about 1 percent for every 10 millibar pressure change near atmospheric pressure. If the chamber is constructed so that pressure builds up when the chamber is assembled, a small vent tube may be provided to maintain the chamber at atmospheric pressure. In a sealed chamber, an error of about 1 percent will occur for every 1 percent change in volume. Error introduced by changes in atmospheric pressure will usually be negligible, but calibrator curves can differ significantly at different elevations.

Two types of sample holders are currently in wide use, the *single chamber* type and the *sample changer*. Different types of sample changers can be obtained from Technical Services, Utah State University, or from Wescor Engineering. The sample changer is similar in principle to the single sample chamber, but speeds up calibration and allows more precise comparisons of samples (Campbell et al., 1966).

3. Soil psychrometers

For *in situ* measurements of soil water potential, the thermocouple is protected by a cup-shaped device that maintains a void in the soil. The thermocouple shield should permit rapid vapor pressure equilibrium between the soil and the chamber surrounding the thermocouple. Ceramic cups and bulbs have been used (Rawlins and Dalton, 1967; Wiebe et al., 1970; and Lopushinsky and Klock, 1971), as well as fine-mesh screen cages (Lang, 1968; Brown, 1970). The cup geometry is not particularly critical and can be modified according to the specific research problem. Cups varying in size and geometry from spheres with diameters of about 2 centimeters to cylinders with inside diameters of about 1 millimeter have been used successfully. The shape of the cup acts only to maintain a fixed dimension within the soil, and to provide protection for the thermocouple. Commercial units are available from Wescor.

a. *Ceramic cups vs screen cages*. A legitimate question can be raised concerning the influence of ceramic on the maintenance of water potential equilibrium, particularly when liquid contact between the soil and ceramic cup is broken. Brown (1970) compares equilibrium times for bare, ceramic cup shielded and 200 mesh stainless steel screen shielded psychrometers. Figure 5 illustrates the shorter time required to achieve vapor equilibrium for bare and shielded psychrometers.

Because the thermocouple psychrometer calibration is temperature sensitive (see Theory and Calibration sections), a thermocouple thermometer should be added to psychrometer units used under field conditions. The temperature sensing instrument should be small enough to allow its junction to be embedded in the epoxy cement immediately above the teflon body. Usually a copper-constantan thermocouple constructed of 24 gauge or smaller wire works very well, and an appropriate circuit can be added to facilitate temperature measurements. Temperature measurement to the nearest degree C is usually sufficient.

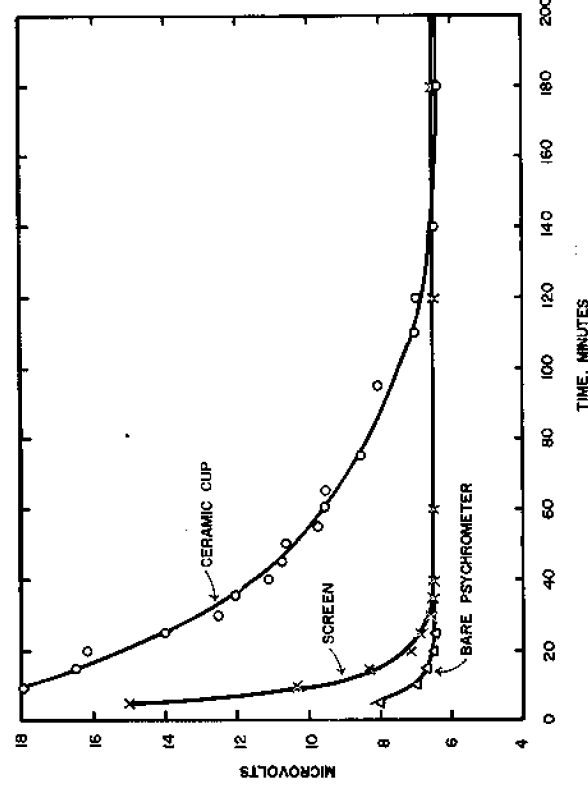


Figure 5. The lag in response of thermocouple psychrometer to vapor equilibrium over a 0.3-molar KCl-solution at 21°C.

4. Measuring equipment

Readings are made with Peltier psychrometers by first measuring the voltage with *both* junctions dry. Current is then passed through the thermocouple to cool the measuring junction and condense water on it. The change in voltage caused by cooling produced from the evaporation of the water is then measured. In this section we discuss amplifier requirements, switching circuits, cooling current magnitude and duration, output interpretation, and temperature corrections.

a. Amplifiers. The output of the thermocouple psychrometer generally ranges from 0 to about $30\mu\text{v}$. Most amplifiers capable of accurate measurements in this range are suitable for use with psychrometers although full scale sensitivities of $3\mu\text{v}$ are required for accurate indication of high water potentials. A number of amplifiers are presently available with internal battery power supplies and can be used for both laboratory and field measurements. The Keithley models 148, 150b, and 155 and the Hewlett Packard Model 419A have been used successfully for these measurements. Wescor sells a modified Keithley 155 unit with the switching unit incorporated. The Leeds and Northrup model 2437 null detector can also be used, but it does not have fixed ranges and requires an external test signal to set the sensitivity to the desired range. Other amplifiers of comparable quality are, no doubt, also available, and we can expect new ones to become available in the near future because of the rapid advances now being made in the area of microvolt measurements.

Readings are generally taken directly from the meter of the amplifier. Amplifiers provided with an output connector can be used to record if one wishes to do so.

b. Switchbox. The switching circuit provides the cooling current to the thermocouple and connects the thermocouple to the amplifier. A separate position may also be provided for a heating current to quickly dry residual water from the thermocouple after a reading is taken. This may also be accomplished by reversing the thermocouple leads and using the cooling current. A zeroing voltage may also be applied to the thermocouple circuit from the switchbox. The switchbox is constructed from standard components. Soldered connections, however, must be made with a solder having a low thermal emf with respect to copper (available from

Leeds and Northrup) and the amplifier and thermocouple binding posts should be gold plated to minimize contact potentials. The circuit is constructed in an aluminum box which provides electrical shielding and protection of components from rapid temperature fluctuations. The circuit diagram is shown in figure 6. The components enclosed in the dashed lines are optional. Component "a" is a zero adjustment. When measurements are made in the presence of a large zero offset and this offset is compensated by the zero suppress of the amplifier, the amplifier goes off scale while current is being passed through the thermocouple and often does not return to the proper position on the meter scale rapidly enough to indicate the proper reading. The circuit shown in 6a allows the zero suppression to be applied directly to the thermocouple so the meter remains at zero while the thermocouple is being cooled. Figure 6b is a shunting resistor which is used with the Hewlett Packard 419A to eliminate the meter deflection caused by switching transients. This type resistor is not required with the Keithley Amplifier.

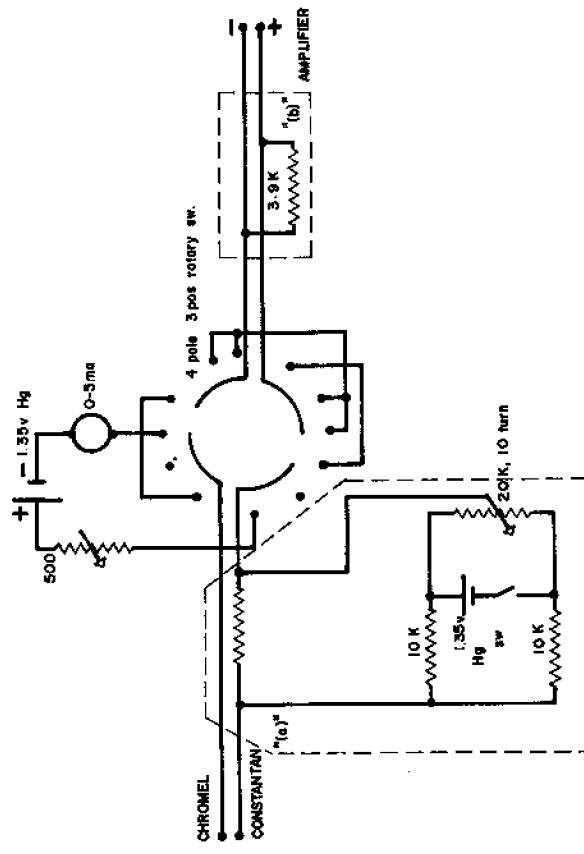


Figure 6. A switching circuit for two-wire psychrometers. Optional features are (c) a zero suppress circuit and (b) a shunting resistor for use with the Hewlett Packard 419A to suppress the switching transient. All resistors are 1-percent tolerance.

The switch is wired so that the amplifier input is shorted at the off (center) and current positions. This keeps the amplifier from drifting off scale while the thermocouple is being cooled.

Automated switching units have been used by some researchers (Barrs, 1969; Hoffman et al., 1969; and Walter H. Gardner and C. Calissendorff, 1968; personal communication). The reader is referred to these authors for details on the construction and use of these units.

c. *Characteristics and measurements of cooling current.* Readings obtained from the psychrometer will depend on the *magnitude and duration of the cooling current applied*. Dalton and Rawlins (1968) present equations and curves showing that the optimum cooling current for 0.0025 centimeter diameter wire is 3 to 4 milliamperes, depending on the length of the thermocouple wires. The expression for measuring junction temperature is a quadratic in i (current) and has a fairly broad range near its maximum where cooling is quite independent of current. Therefore, it is not necessary to adjust the current to exactly 3 milliamperes each time the cooling current is applied. If the 500-ohm potentiometer (figure 6) is set to give 3.5 to 4 milliamperes with the binding posts shorted by a piece of copper wire, no further adjustment of the current is necessary during the use of the circuit. The primary function of the meter is to indicate whether or not current is flowing through the thermocouple. This aids trouble shooting because it may indicate an open circuit (broken thermocouple) or a low battery.

The proper *duration of cooling current* is more difficult to specify because it depends on the water potential of the sample. A cooling time adequate for low water potentials is much longer than necessary for high potentials. Keeping cooling time to a minimum reduces zero drift during cooling, allows the junction to dry sooner, and reduces the heat produced at reference junctions. Since the cooling time influences the reading (particularly at low water potentials) and since one does not know the water potential of a sample prior to measurement, a compromise must be reached. A cooling time of 15 seconds is adequate for most plant and soil samples if the thermocouples are constructed as previously outlined. If samples are likely to be drier than about -30 bar, a second calibration curve should be constructed by using a longer cooling time of 30 seconds or 1 minute. When

water potentials are near zero, cooling times of 2 or 5 seconds may be used with appropriate calibration curves based on these times. At these high water potentials, up to 5 minutes may be required to evaporate all water from the thermocouple. Replotted readings may give erroneously high ψ values if the thermocouple remains wet. The thermocouple may be dried with a heating switch, built into the switching box, or by reversing the thermocouple leads and using the cooling current switch. Some workers caution that heating may disturb the equilibrium for several minutes, however.

Figure 7 shows the *effect of cooling time on the reading* for a solution with a water potential of -30 bar. The curves were made by using a double thermocouple arrangement in which the measuring junctions of the two thermocouples were welded together. One thermocouple was used to cool the junction while measurements of cooling during both the condensing and evaporating stages of the reading were made with the other thermocouple. The dotted line shows the reading which would have been seen with various cooling times using a conventional psychrometer. Cooling curves obtained with the double thermocouple are not identical to ones which would be found for single thermocouples at the same water potential because more heat is conducted to the junction through the extra wires. However, the curve shapes are typical. If one understands what is happening during the condensing phase of the reading and correlates this with the shape of the curve obtained during the evaporating phase, it is easier to determine the proper cooling time. The initial increase in cooling with cooling time at low potentials probably is the result of nonuniform temperature distribution on the surface of the measuring junction. With longer cooling, more of the surface becomes covered with water. This also is reflected in the rapid decrease in evaporative cooling over time for short cooling periods. The water film on the measuring junction is apparently changing area as water evaporates which results in the rapid decay of the curves. With longer cooling times, the area covered by the water film apparently stabilizes and the thickness of the water layer increases with cooling time. This results in the leveling off of the condensation curve with longer cooling times. (Peck, 1968, 1969). The evaporation curves now begin to have a plateau or period of more gradual slope where the area of the water film remains fairly constant during evaporation.

When this constant water film stage is reached, cooling current duration has little effect on psychrometer output. This would be the ideal situation, but it is often compromised at low water po-

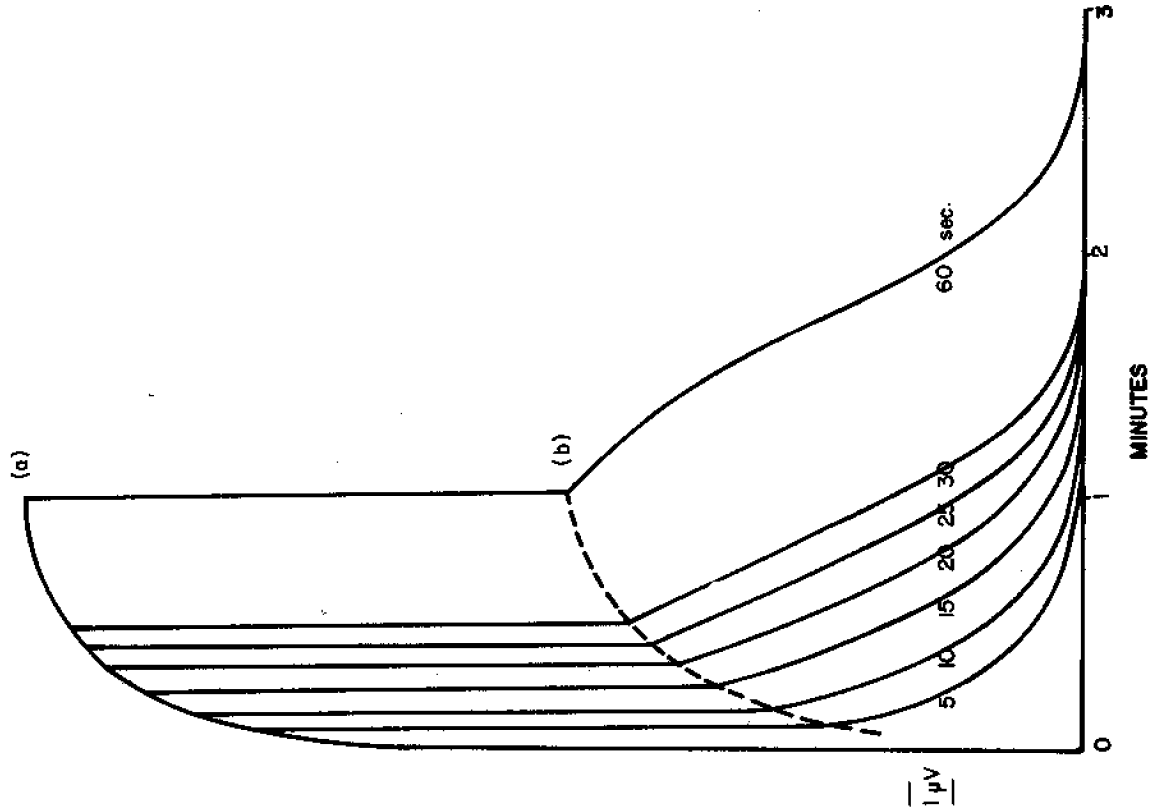


Figure 7. Effect of the cooling time on temperature of the measuring junction during (a) cooling and (b) evaporation.

tential by using a cooling time more suitable to higher potentials. As mentioned earlier, 15 seconds seems to work well for samples which are wetter than -30 or -40 bars, and 2 to 5 seconds for samples wetter than about -2 or -5 bars.

Once the thermocouple has been cooled, a *measurement reading* is made by switching the thermocouple to the amplifier and noting the maximum meter deflection or chart recorder reading (excluding deflection caused by switching transients). Condensation and evaporation curves were obtained at several water potentials by using the double thermocouple mentioned earlier to show typical psychrometer readings and how they change with water potential. These are shown in figure 8. Again, the curves are not the same as would be obtained with a conventional thermocouple because of the additional wires in the junction, but the curve shapes and changes in shape with water potential are typical. Figure 9 shows curves obtained with a conventional thermocouple.

5. Temperature control for laboratory psychrometers

Presentation of psychrometer theory (Rawlins, 1966) and work with the field psychrometer (Rawlins and Dalton, 1967) have led to a reassessment of constant temperature bath requirements for laboratory psychrometers. It has now been recognized that short term temperature drift must be small for accurate measurements, but long term drift of several degrees per day can be tolerated with no adverse effects as long as corrections are made for temperature sensitivity of the psychrometer. Two factors determine the amount of short term drift which can be tolerated: (1) changes in reference junction temperature during the time the thermocouple is being cooled, and (2) temperature and vapor equilibrium within the chamber. The relative magnitudes of these factors depend on: (1) sample chamber geometry and volume, (2) diffusion resistance of the sample surface, (3) heat conductivity of the chamber and sample, and (4) the coupling between ambient temperature fluctuations and reference junction temperature. Changes in reference junction temperature can be minimized by using a temperature compensated thermocouple (Section III D 3). The problem, then, becomes one of maintaining temperature and vapor equilibrium. For most sample chambers and samples, this is accomplished by

8 to 12 liters, filled with water, in a room with a diurnal temperature fluctuation of 5 C or less. Drill holes in the styrofoam box lid to allow insertion of the psychrometers. Readings taken in temperature baths constructed in this manner can be as precise as those taken in our best laboratory temperature controlled baths.

If it is not feasible to operate at ambient temperature, very precise temperature control is required. This may be accomplished by constructing a *constant temperature bath* (Richards, 1965; and Rawlins et al., 1968) and by using a Hallikainen Thermotrol temperature controller. Cold tap water has been satisfactorily used in some installations for cooling. If the bath is operated at 5 C or more above ambient temperature, cooling by the surroundings is adequate for precise control. Ambient temperature should stay relatively constant for good temperature control. Diurnal changes should be less than 5 C. The best control is attained when the cooling and heating in the bath are minimal. The bath should give adequate control when the continuous cooling is just balanced by a thermo-regulator activated 40-watt incandescent lamp. If light sources are used for heating, it is absolutely essential that the samples are in darkness to prevent radiant heating. Glass or transparent plastic sample holders may be covered with foil to exclude light.

Remember to keep short term temperature fluctuations at a minimum. Some long term drift can be tolerated. Short term fluctuations should be less than ± 0.0005 C for proper precision.

B. Calibration

In the Theory section of this paper, an expression was obtained for thermocouple output as a function of water potential by assuming the measuring junction was fully wet. Calibration curves for the Spanner psychrometer have this general shape at high potentials, but as the water potential decreases, the calibration curve deviates from the ideal. Figure 10 shows a typical calibration curve. The curve drops off because the Peltier cooling is no longer able to condense the water on the wet junction necessary to make the reading. The water potential at which the curve begins to fall off depends on properties of the thermocouple junction and varies considerably from thermocouple to thermocouple. For welded thermocouples of the type described

by Merrill et al. (1968), and Campbell et al. (1968), the peak may be below -50 bars.

The curve in figure 10 shows that a given thermocouple reading may correspond to two different water potentials. There is usually no problem in determining which of the two readings is the correct one, but if there is a question, it usually can be resolved by noting the width of the thermocouple output curve. The curve becomes very narrow at low water potentials and eventually appears as just a spike (figure 9).

Even though psychrometer theory is well understood, there exists enough variability in thermocouple characteristics to require individual calibration of psychrometers for accurate measurements. Psychrometers are calibrated by using solutions having known water potentials. Potassium chloride and sodium chloride have been used. Water potentials and preparation procedures for calibrating solutions are given in the appendix (tables 1, 2, and 3).

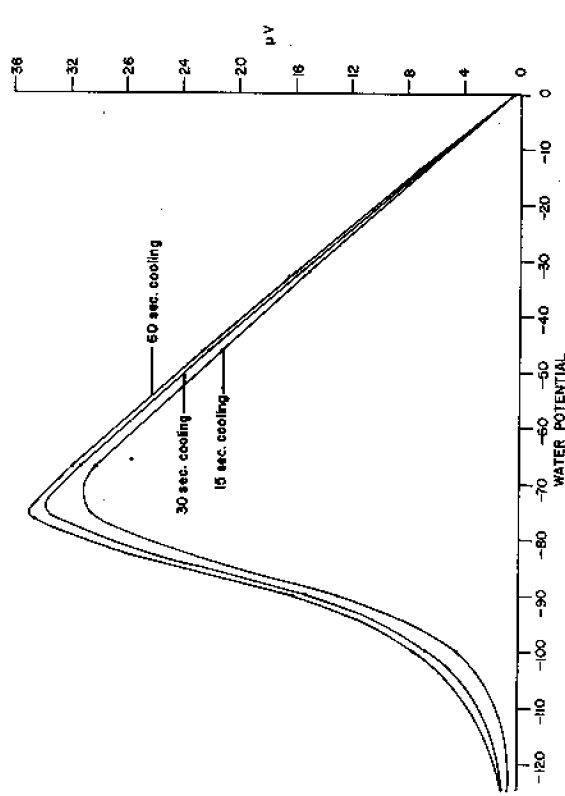


Figure 10. Calibration curve for Spanner psychrometer.

Equation 2 (Theory) shows that the psychrometer output depends on the size of the sample chamber. Therefore, the chamber should have a geometry during calibration similar to that which will be used with samples. We also know from the Theory section that exposed chamber surfaces may act as vapor sources or sinks. It is therefore desirable to cover as much of the chamber surface with sample as possible. Peck (1969) gives a thorough discussion of sample configuration.

Filter paper is usually used to obtain the proper calibrating geometry. Three calibrating geometries will be presented here. Two have been used for leaf samples and one for soil samples. Leaf sample configurations are: (1) a leaf disk in the bottom of the chamber leaving sides and top of the chamber exposed; and (2) a leaf disk in the bottom of the chamber, a strip of leaf tissue formed into a cylinder to cover the cylinder walls, and a disk for the top of the chamber with a small hole for the thermocouple. Soil samples cover the chamber walls and have a conical depression for the psychrometer. More details on these will be given later. To calibrate the psychrometer for plant samples, filter paper is cut to give a chamber similar to either configuration 1 or 2 and treated with sufficient calibrating solution to wet the filter paper. For soil, a strip of filter paper is formed into a cylinder to cover the walls of the chamber and several drops of calibrating solution are placed in the bottom of the chamber. This method does not give the exact chamber geometry obtained with soil, but it is close enough to give accurate calibrations. Experience has shown that best results are obtained if the filter paper cylinder is about 1 millimeter shorter than the chamber. If the filter paper extends to the top of the chamber in the sample changer, the solution may move up the filter paper, across the chamber cover, and down into the next hole.

Generally, five or six calibration points are adequate to establish a calibration curve. Solutions of KCl at 0.1, 0.3, 0.5, 0.8, and 1.0-molar concentrations work well (see appendix). The order in which the solutions are run is not critical, although calibrations in the sample changer are accomplished more rapidly when the more concentrated are read first because vapor equilibrium is fastest at low water potential.

Once the calibration points are obtained, they can be plotted as a function of water potential to get a calibration curve. A curve

similar to figure 10 should result. Departures of individual points from the curve should be less than ± 0.1 bar. When a constant temperature bath without temperature control is used, readings should be corrected to 25 C to plot the curve. The calibration curve may indicate that at 0 bar the thermocouple still has a positive output. This happens frequently with laboratory psychrometers, and may be the result of vapor absorption on exposed chamber walls or from heating of the reference junctions. This is apparently not serious if similar equilibration times are used with samples and standards. Clean Teflon surfaces should minimize the absorption problems.

1. Calibration of soil psychrometers

Soil psychrometers enclosed in ceramic cups can be immersed directly into a flask containing a standard solution for calibration. The flask containing the solution and the psychrometers are in turn immersed in a constant temperature bath, and the psychrometer outputs are recorded after temperature equilibrium is reached. The bath temperature can then be changed or the flask transferred to another bath for calibration at other temperatures. After calibration, the psychrometers must be washed for several hours in several changes of distilled water to remove all traces of solute. Outputs deviating from zero in wet soil may be caused by traces of solute.

Screen-cage soil psychrometers are calibrated by mounting the complete psychrometer in a small test tube lined with filter paper that has been moistened with a KCl or NaCl solution. The psychrometer is lowered into the test tube until the screen cup is below the filter lining, and is then sealed with a rubber stopper through which the copper lead wires extend. The seal can be insured by using vacuum grease. The test tube and lead wires should be immersed in a constant temperature bath to avoid heat conduction down the wires and temperature gradients within the psychrometers.

The required frequency of calibration depends somewhat on the accuracy required. If the psychrometer is in constant use, it should probably be calibrated every month or so. As a general practice, when a sample changer is used, one or two calibration points should be determined each time the samples are being run. Some operators routinely use two positions in a sample

changer for standard solutions which bracket the expected unknown values. Any deviation in readings from the standard alerts the operator to irregularities. If a single chamber is used, a calibration point should be established before and after each sample to make sure the calibration is accurate. With experience, the operator should be able to determine an adequate frequency of calibration to maintain the required accuracy.

2. Effect of temperature on calibration

As was shown in the Theory section, thermocouple sensitivity is a function of the temperature of the psychrometer. Readings taken at any temperature may be converted to some standard temperature of, say, 25 C by use of a temperature correction factor. A temperature correction factor is obtained from psychrometer calibrations made at several temperatures. A theoretical temperature correction based on Peck's (1968) equation for Peltier psychrometers is presented in figure 11 along with data obtained from several experiments. To obtain the factor from figure 11, draw a vertical line at the psychrometer temperature. A horizontal line drawn to the left indicates the correction factor to be multiplied by the psychrometer reading to obtain the reading at 25 C. This value is then used with the calibration curve to obtain water potential.

A note of caution should be added here about the use of figure 11 for temperature corrections. Although the theoretical curve provides a good *average fit* to the data points, individual psychrometer corrections may differ considerably from those given in the figure. It would therefore seem advisable to calibrate psychrometers individually at several temperatures in the range of interest if precise measurements are required or if psychrometer temperatures deviate considerably from 25 C.

C. Use of Psychrometers in the Laboratory

1. Sample preparation

Accurate measurements of soil and plant water potentials require that serious attention be given to *sample preparation and loading*. Water loss from samples during loading can result in rather large measurement errors. For this reason it is highly

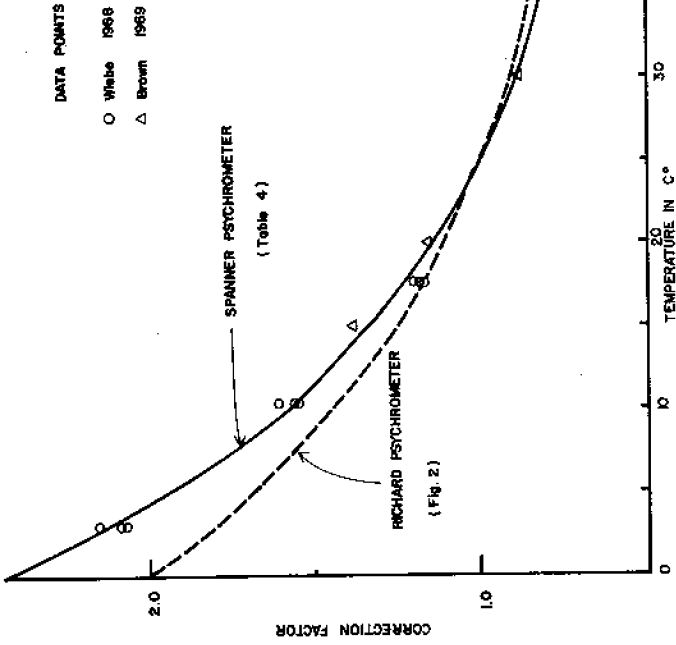


Figure 11. Temperature correction factors for Richards and Spanner psychrometers.

desirable to transfer samples to psychrometers in a *humid transfer box* or glove box. The box should be lined with wet blotter paper, have a viewing window at the front or top, and have access holes for the hands. If the blotter paper is kept wet, the humidity in the box will remain near 100 percent. Since the samples are close to 100 percent relative humidity, the moisture exchange of samples with the surroundings inside the box is reduced. Low level, indirect illumination of the box is also desirable to minimize radiation heating and drying of the samples.

Water loss is generally more serious for dry soil samples, which show a greater drop in potential for a given quantity of evaporation than wetter samples. Even with a small amount of evaporation, turgid leaf samples may show a large drop in potential.

If the *sample chamber and sample are warmer than the constant temperature bath*, condensation of water may occur in

the chamber when the psychrometer is placed in the bath. Condensation problems may be avoided if the chamber is covered and cooled on ice for a few minutes before the psychrometer is assembled and placed in the bath. As long as the sample remains cooler than the chamber surfaces, no condensation will occur on the chamber walls.

One of the most important factors in making a good psychrometer reading is *clean chamber surfaces and thermocouples*. Good results have been obtained by rinsing all surfaces that are exposed inside the chamber during readings with boiling distilled water after each use and removing the excess water by shaking. Steel wool has been used on brass surfaces to remove corrosion caused by calibration solutions. The thermocouple also should be cleaned frequently in boiling distilled water. Acetone rinses also are useful, especially if grease is deposited on the thermocouple. It is essential to clean the thermocouple thoroughly if it should accidentally be touched by the operator's finger.

2. Soil measurement in the laboratory

Measurement of *soil water potential* is accomplished by placing a given volume of soil into the psychrometer chamber, packing it with a conical packer, and measuring the equilibrium reading obtained over the sample. Samples are conveniently loaded in the sample changer by using the device shown in figure 12. The apparatus is fastened to the sample changer cylinder with a screw and the bottom disc is rotated until it is aligned with the first chamber. The hole in the top disc is filled with soil from the sample and then rotated until the soil drops through the bottom hole into the chamber. The packer is then inserted to form the conical depression. The soil loader may be constructed from various materials, but Teflon works well. A 0.9-centimeter deep hole in the top plate provides about the right volume of soil.

Assuming that appropriate temperature calibration has been carried out for a particular thermocouple psychrometer (i.e. calibrated against osmotic solutions at different temperatures using appendix tables 1 and 2 and a calibration curve constructed as in figure 11, there remains a question as to how much, if any, the temperature of the sample may affect the water potential of

the sample itself. Experimental values of the water potential of four different soil types at constant water content as a function of temperature have been obtained by Campbell and Gardner (1971). These indicate only small changes in water potential with temperature except in fairly dry soils. Even then, they do not occur in all of the soil types (see Campbell and Gardner figure 2). Thus, measurements of the water potential of field samples under laboratory rather than field temperatures often will introduce negligible error, but where precise values are required, this should be checked.

Bulk density changes in soil samples result in alteration of pore space, which in turn may affect water potential, particularly

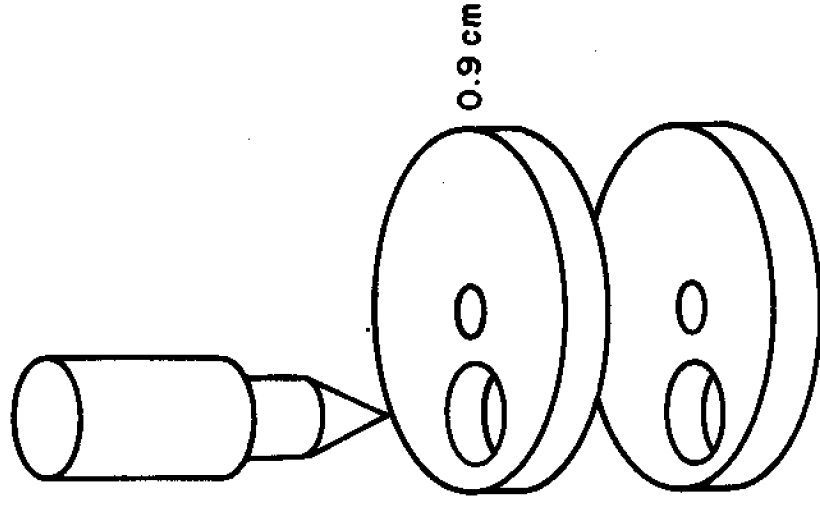


Figure 12. Soil loader for sample changers.

in the wet range. Some disturbance of sample bulk density is inevitable during the sampling and sample loading process so that laboratory measurements of water potential might differ some from those under field conditions. Campbell and Gardner (1971) measured the effect of imposed changes in bulk density on samples of three soils. Changes with bulk density were relatively small except for the swelling and shrinking subsoil for which care must be taken to reproduce field bulk density as may be observed in the curves of Campbell and Gardner, figure 4.

3. Leaf measurements

The two configurations used for *leaf water potential measurements* have been previously mentioned. The leaf disc method is more convenient because samples may be placed directly into the psychrometer chamber by using a leaf punch (Campbell et al., 1966; Brown, 1969). This method, however, leaves much of the chamber interior exposed and may be subject to errors because of leaf respiration (see Theory). Campbell et al. (1966) suggested using a shallow sample changer to reduce the exposed chamber surface. The same effect can also be accomplished by machining brass or Teflon plugs to fill the holes of the deep changer to a depth of 4 to 5 millimeters or by machining Teflon cup liners to fit into the holes. Thus, the same changer may be used for both soil and plant samples. The respiration problem is reduced by inserting small rings made from stainless steel wire into the holes to hold the leaf tissue against the bottom of the chamber.

The problem of sources and sinks on the chamber walls and the respiration problem are reduced by surrounding the thermocouple as much as possible with leaf tissue. Equilibrium should also be more rapid because more leaf tissue is available to contribute water vapor. This technique is preferred over the disc method if sufficient leaf tissue is available. The discs and strip are cut from the leaf tissue using appropriately sized punches and templates. This operation and the loading of the chambers should be done in the humid transfers box to minimize water loss from the tissue. There are reports (Barrs and Kramer, 1969) that leaf water potential will rise within a few minutes after excision because xylem tension is released. It may, therefore, be best to punch samples directly from the attached leaves into the psychrometer, only one sample being taken from each leaf.

Any solutes, dust, or exudations on the leaf surface will give an excessively low water potential reading by psychrometric methods. Such surface accumulations can be removed generally by washing the leaf on the day before sampling with distilled water and allowing it to dry. Certain species, cotton for example, may, however, continue to excrete salt while in the psychrometer, again leading to depressed ψ values (Klepper & Barrs, 1968). In these cases, the pressure bomb may be the preferable method.

According to Barrs and Kramer (1969), any unnecessary slicing or injury of leaf tissues should be avoided on loading psychrometers, as this generally results in higher values. They propose that the remaining intact cells actively accumulate solutes released from the cells on slicing. The diluted sap then diffuses into the intact cells, raising their turgor pressure and water potential.

4. Measuring extremely low potentials

Psychrometers in sample changers have been used to measure water potentials as low as -800 bars (Wilson and Harris, 1968) by condensing water on the thermocouple over a -10 -bar KCl solution for 10 minutes and then quickly rotating the cylinder to the appropriate chamber and making a reading as the water evaporates. Wiebe (unpublished) used a variation in which water was condensed over a 0.05 molal solution in one chamber for 4 hours. The cylinder was then rotated to read samples in the other chambers, allowing 5 to 10 minutes for equilibration in each chamber. In this procedure, the Peltier psychrometer is used like a Richards and Ogata wet loop psychrometer, and is subject to the same limitations (Zollinger, et al., 1966; Barrs, 1968) such as diffusion resistance of the sample. Accuracy may be comparable to the Peltier psychrometer at a water potential of about -50 bars, with reduced accuracy at lower potentials. Lithium chloride solutions are used for calibrating the psychrometer at these water potentials.

5. Summary of measurement procedure for Peltier thermocouple psychrometers

1. Load sample holders with material to be measured, using care to avoid desiccation during the cutting and loading procedure. Place them in the constant temperature bath and allow to equilibrate for several hours.

2. Attach psychrometer leads to the switchbox, and this in turn to a voltmeter or an amplifier and recorder. Set the switchbox to Measure (or Read) and set the voltmeter to read "0." Output should be zero with both junctions dry and at the same temperature.

3. Apply the cooling current.
4. Return the switch to the read position and note the maximum deflection of the voltmeter. It should return to "0," quickly for dry samples, more slowly for moist ones.
5. Steps 2, 3 and 4 should be repeated until reproducible results are obtained.
6. When a sample changer is used, several minutes should be allowed for equilibration after changing chambers; then repeat steps 2, 3, and 4.
7. The calibration procedure is identical except that filter paper wetted with standard solutions is used for samples.

Readings should be made at 10-to 15-minute intervals until duplicate readings are obtained. The initial equilibrium will usually take less than 3 hours. If the sample changer is used, the cylinder is rotated to the next sample after repeat readings are obtained and successive readings are again taken until equilibrium is reached. This usually requires less than 30 minutes per chamber. After some experience has been obtained, one can predict fairly well how much time will be required for equilibrium without taking readings every 10 to 15 minutes.

D. Spanner Psychrometers in Field Studies

1. Soil measurement

For use in soil, the ceramic or screen psychrometers are buried at the desired depth in the soil and left for extended periods. The hole can be made with a shovel, and soil replaced over the psychrometer, or a hole can be made with a soil auger, the psychrometer placed in the hole, and the hole refilled with the soil which was removed from it. The psychrometer should be in good contact with the soil to facilitate moisture exchange. It is best to place the probe in a horizontal position, so that the two

thermocouple junctions are more likely to be at the same temperature. If in a vertical position, they may be at different temperatures due to vertical temperature gradients often existing in soil. These gradients are generally steeper near the surface. Equilibrium time is mainly dependent on the soil reaching temperature and moisture equilibrium after installation. Moisture equilibrium with the soil is achieved most rapidly if the cup is wet before installation.

Soil temperature must be known at the time readings are made so that the microvolt readings can be corrected to a water potential at a known temperature (Section III B 2). The psychrometers with built-in temperature measuring thermocouples provide for the most convenient soil temperature measurement. Some workers find that the calibration of psychrometers left in the soil changes markedly within a month, thus reducing their usefulness in longer studies. Perhaps this change is caused by corrosion of the psychrometer wire. Others have left them in soil for 3 months and found that the calibration was within 1 percent of the original. It is recommended that after soil studies, psychrometers be removed and recalibrated to alert workers to any changes.

2. Tree stems

Psychrometers have been installed in tree trunks to monitor water potentials for periods in excess of 2 months (Wiebe, et al, 1970). The psychrometers were covered with a ceramic cap measuring about 1.2 centimeters long and 0.8 centimeters in diameter, the same psychrometers as those sold for soil use (Wescor). These were installed in holes drilled into the cambium and outer sapwood of tree trunks and of branches as little as 2 centimeters in diameter. It is best to orient the psychrometer parallel to the branch to avoid the radial temperature gradient which occurs in tree stems. The implanted psychrometer must be completely covered and sealed with some water proofing compound to prevent desiccation (Asphalt compounds used in tree surgery were used). Thermal insulation against wind and sunlight is provided by covering the area with a 2-inch layer of plastic foam spray, or with foam sheets held in place with tape. It is also desirable to shade the entire implant region from direct or intermittent sunlight.

Since tree temperatures fluctuate with the environment and the psychrometer output changes with temperature, it is necessary to calibrate the psychrometers at different temperatures (see section III B 2).

When readings are taken while the temperature is changing, temperature gradients may exist within the psychrometer (Lang and Barrs, 1965). With such gradients, the two junctions of the thermocouple may be at different temperatures, even though both are dry. The ceramic cup temperature may also differ from that of the thermocouple, resulting in false water potential values. To test the influence of temperature gradients, psychrometers were immersed in a 0.5-molal-NaCl calibrating solution (Wiebe et al., 1970). Their outputs were then measured when the temperature was constant, and while it was raised or lowered at the rate of 4 degrees per hour. The readings averaged 0.3 bar high during the cooling cycle, and 0.3 bar low during warming (figure 13). These deviations were much less than those reported for a similar experiment (Lang and Barrs, 1965), but the much smaller psychrometers used by Wiebe et al. apparently resulted in smaller temperature gradients, and in turn, less pronounced deviations in water potential measurements.

The zero or base line will change with changing temperatures. Slow change can be partly corrected with a zero offset control. Examination of recorder traces (figure 14) indicates that the base line may continue to change during the Peltier cooling, but that the base can be interpolated from visual inspection. Three-wire, temperature-compensated psychrometers should be considered in future tree trunk studies. Temperature changes are also more gradual during daily temperature maxima and especially during the minima. Fluctuations in wind and cloud cover may also cause fluctuations. During nighttime, clouds are no problem and wind also is generally reduced.

In the use of psychrometers in massive plant organs, care must be taken to see that exudation into holes as a result of wounding does not negate their usefulness. This can be detected by water potential readings near zero. This happened regularly after less than a week with honey locust trees, but did not appear to be a problem with rocky mountain juniper, or a number of the hardwoods studied.

It should be possible to install the thermocouples directly

without the protective ceramic or screen cap. This would reduce equilibrium and response time, but the thermocouple would be more easily damaged by exudations during installation, or it might touch wood fibers which would interfere with its operation.

Although the psychrometers were used in tree trunks and branches, it should also be possible to use them in other massive plant structures such as storage roots, tubers, fruits, and perhaps some inflorescences and buds. Further miniaturization also should be possible. They also might be used inside hollow structures like onion leaves or grass stem internodes.

3. Attached leaves

Thermocouple psychrometers have also been used to measure

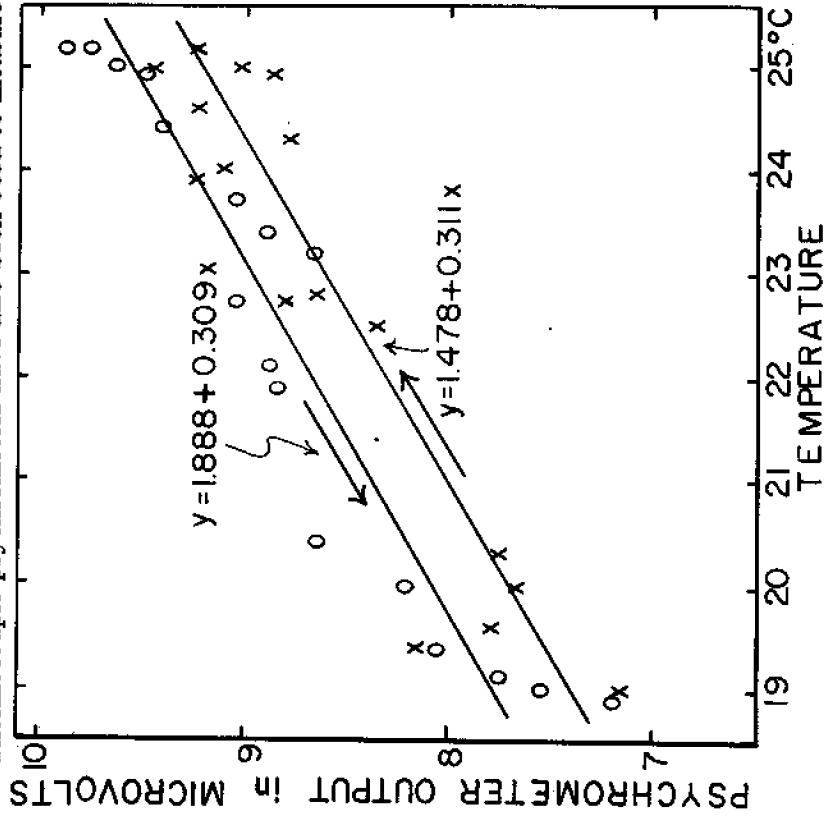


Figure 13. Psychrometers output during changing temperature. Rate of change was 4 C per hour with a 1-molal sucrose calibrating solution.

the water potential of attached leaves. Lambert and van Schilf-gaarde (1965), Lang and Barrs (1965) and Manohar (1966, a, b and c) enclosed the leaf with a psychrometer chamber. This interferes with normal transpiration, and therefore probably changes the water potential of the leaf being measured. Hoffman and Splinter (1968) and Hoffman and Herkelrath (1969) have successfully attached miniature psychrometers to plant leaves

and made water potential measurements within closely controlled temperature chambers. Close temperature control was required because zero drift of psychrometers constructed in the usual way is otherwise too great. A compensating technique was suggested by Hsieh and Hungate (1970) which reduced this zero drift. Three lead wires are brought to a Teflon plug (figure 15) and attached to two thermocouples which produce opposing voltages. Cooling current is applied between A and B, and the reading is made between A and C. The output is proportional to the temperature difference between a and b. Since they are both in the same thermal environment and have about the same heat capacity, b compensates for rapid temperature changes which occur during the reading.

Calissendorff and Gardner (Calissendorff, 1970) used a small compensated psychrometer attached directly to the leaf for similar measurements and have, in addition, measured the temperature differences between the leaf and the region of the psychrometer junctions. They have measured temperature differences of as much as several tenths of 1 degree C in the psychrometer cup on leaves subjected to sunlight in nature. In theory, an 8 bar difference in water potential may result from a 0.1 C temperature difference. Probably because of positioning of the differential thermocouples, measured differences in water potentials were only about 7 bars per 0.1 C. Measurements made on calibrating solutions in filter paper used in place of a leaf indicate precisions of about ± 2 bars to be possible under the kind of temperature differentials and rapid temperature fluctuations observed in nature.

a. Construction of the compensating leaf psychrometer with thermocouples for measuring temperature differential between leaf surface and psychrometer. The compensated psychrometer, with three lead wires, is the left unit in figure 15, while the differential thermocouple for measuring temperature differences is the right hand unit. The two sets of thermocouples are placed in a 6-millimeter diameter and 2-millimeter deep cavity in the Teflon body as shown in figure 15. The psychrometer thermocouples are arranged approximately in the middle of the cavity with the junctions about 1 millimeter apart. The differential temperature thermocouples are arranged with one, q, so it will touch the leaf surface, and the other, p, near the psychrometer.

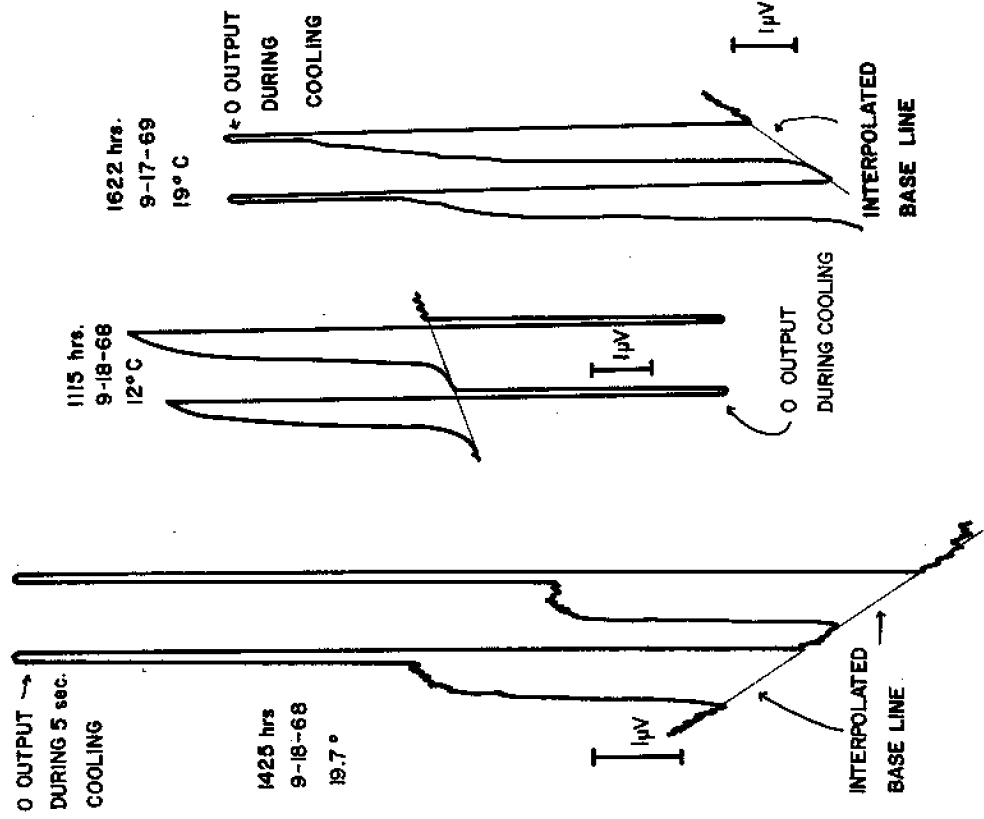


Figure 14. Selected recorder tracings of Peltier cooled psychrometers installed in tree trunks.

Two sets of chromel-constantan thermocouple junctions made from .0025-millimeter diameter wire are press-fit against heat sinks made of 6-millimeter lengths of 0.64 millimeter diameter copper wire in holes punched through a Teflon body forming the psychrometer cup as shown in figure 15. Lead wires of small diameter to reduce heat flow are soldered to the 6-millimeter long

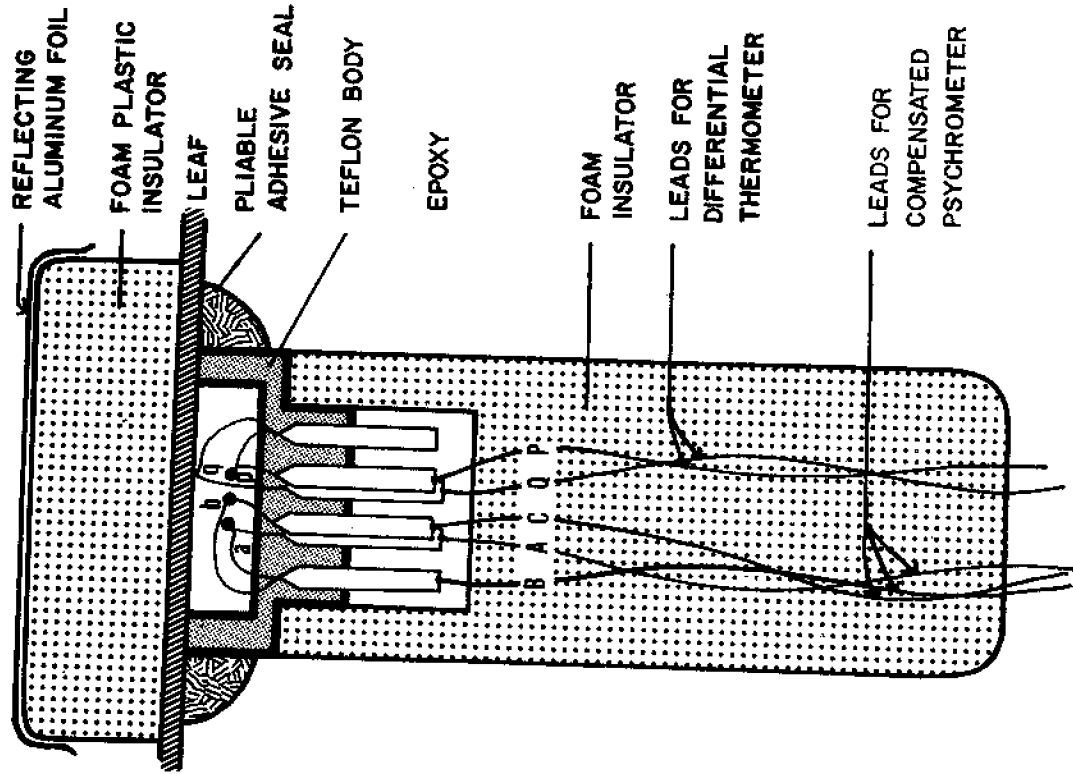


Figure 15. Diagram of double psychrometer.

wires; these are covered with a "poured in place" rigid polyurethane foam. This unit is fastened to a leaf surface by using a water-proof contact adhesive made from adhesive scraped from a commercial sticky tape (C-505 Adhesive Tape, Arno Adhesive Tapes), and mixed with a small amount of petroleum jelly to increase pliability. The portion of the leaf opposite the psychrometer unit is insulated with soft foam plastic and an outside covering of aluminum foil to reduce temperature fluctuations from radiation. The unit is held in place with a spring wire clip.

Cooling and reading circuitry are shown in figure 16.* As in conventional psychrometry, a current is passed through junction b to cool it and condense water on the surface. To avoid heating, the current is not passed through the adjacent reference junction, a. The reading is made by connecting leads A and C to the meter. During cooling, the differential thermometer is connected to the meter to read the difference in temperature between the leaf and the psychrometer junctions.

b. *Calibration of compensating psychrometers.* Calibration with no temperature differential is done using a piece of filter paper saturated with an osmotic solution. The filter paper is covered so as to be vapor tight with a piece of glass (slide cover) and inserted in place of the leaf. The unit then is placed in a constant temperature bath at the desired calibrating temperatures and psychrometer readings are taken (also see section on calibrations).

Calibration for temperature differential within the psychrometer cavity is accomplished by placing a Peltier heat pump against the glass over the psychrometer with osmotic solution-soaked filter paper in place of the leaf as before. The temperature of the Peltier element is changed incrementally to provide a series of temperature differentials between the filter paper and the region of the psychrometer wet bulb. The differential temperatures are measured along with the water potential. Parallel curves like those shown in figure 17 are obtained. Care should

*A circuit for automatic reading of the psychrometer temperature differentials and the ambient temperature is available from the soil physics laboratory, Department of Agronomy and Soils, Washington State University, Pullman, Washington 99163.

be taken that the thermocouple junctions used to measure the temperature differential are in the same position each time the psychrometer is calibrated or attached to a leaf, and that these junctions are vertically aligned. Since this is not easily achieved, horizontal temperature gradients should be avoided during calibration and use on a leaf insofar as is possible. Since precise arrangement and spacing of the junctions is difficult to control, each psychrometer must be calibrated individually. In addition to the slope of the psychrometer output-temperature differential curves obtained in the manner described (figure 17), a conventional calibration curve of psychrometer output against water potential for zero temperature differential must be obtained.

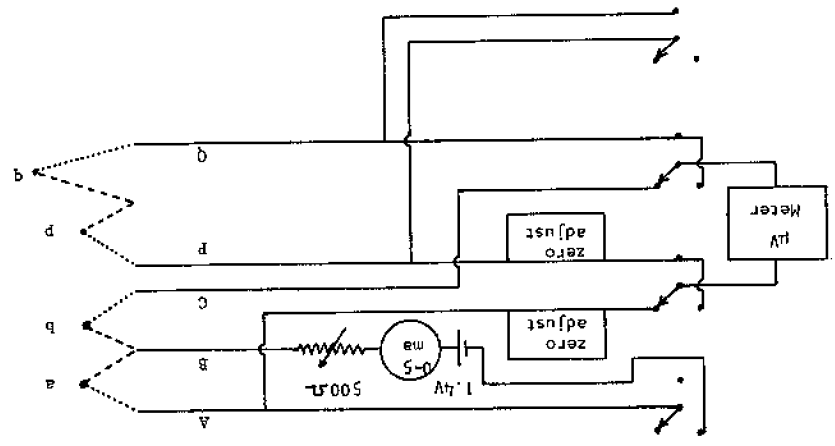


Figure 16 Switching circuit for use in cooling the psychrometer wet bulb and taking the psychrometer and differential temperature readings. The dotted line represents chromel and the dashed line constantan thermocouple wire.

From such calibration data, it is possible to construct a family of curves like those given in figure 17 at regular water potential intervals for use in converting psychrometer and temperature differential readings to water potential. If desired, curves for water potential as a function of psychrometer output may be constructed using the same data with temperature differential appearing as the parameter.

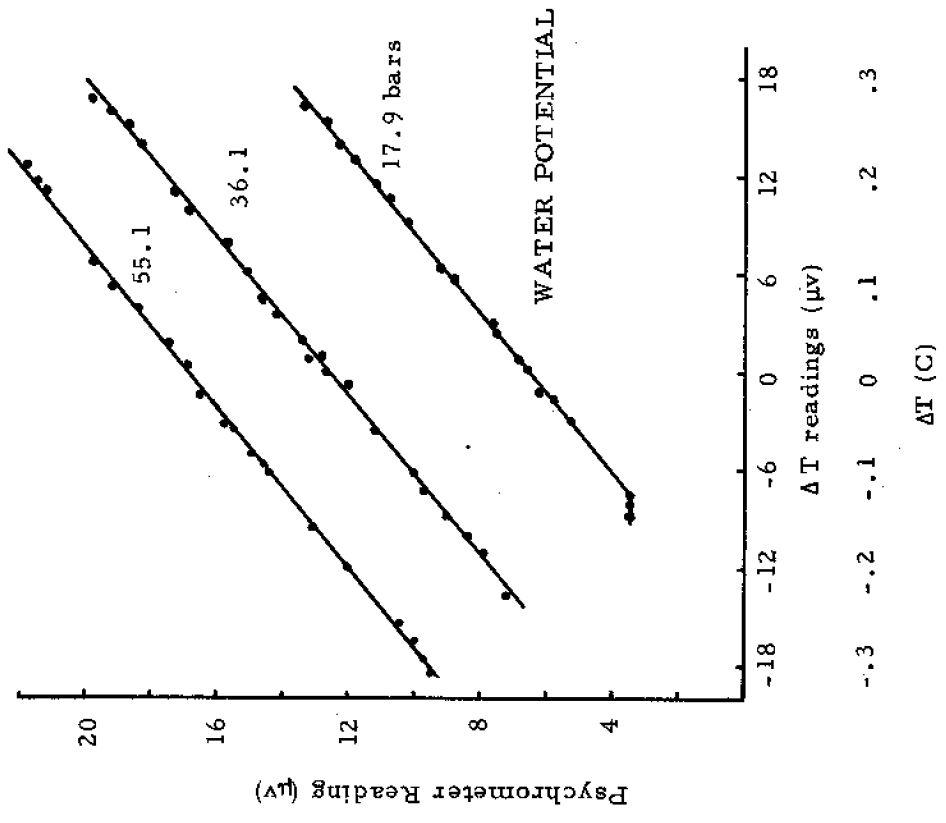


Figure 17. Psychrometer EMF is a function of the temperature differential ΔT between the leaf surface and the psychrometer junctions. A position ΔT means that the cavity is warmer than the leaf. The Parameter is the water potential of the calibrating solution.

c. *Discussion of compensating leaf psychrometers.* It is evident that water potentials in the psychrometer cavity can be measured with reasonable precision, even under conditions where temperature differentials exist between the calibrating filter paper surface and the junctions. However, results obtained thus far using the psychrometer indicate some uncertainty as to the meaning of water potentials measured with respect to the leaf.

Three general requirements exist for obtaining accurate leaf water potentials: (1) the EMF measured must be due solely to evaporative cooling; (2) the water potential between leaf and the psychrometer junctions must be in equilibrium or a correction must be available for non-equilibrium conditions; and (3) the psychrometer must not upset the water potential of the leaf. Success with measurements made on osmotic solutions simulating a leaf indicates that the first requirement is under reasonable control, although some additional precision may ultimately be achieved. The second requirement is met in part where corrections for temperature differentials between leaf and junctions are quickly reflected in the psychrometer cup. It is observed that the psychrometer responds rapidly to changes in environmental stress. However, further testing is necessary to establish this definitely. The final requirement is difficult to evaluate because of the lack of any standard of comparison for *in situ* leaf water potentials. Several independent measures are available, such as the pressure bomb and psychrometer measurements made upon excised leaf samples. No technique yet exists, however, for making measurements directly upon a leaf without some disturbance. It appears that the compensating psychrometer described here comes closer than any known instrument in permitting measurement without disturbance. However, considerable further effort is required before firm conclusions can be drawn.

IV. THERMISTOR PSYCHROMETER

Temperature sensitive resistance units, or thermistors also have been used in psychrometers (Richards, 1965; Kreeb, 1965; Barrs, 1968). A modified Kreeb unit has been used by one of the present authors (H. H. Wiebe) and is described in detail below. It could be modified for use with either of the sample changers. It lacks the versatility of the Peltier psychrometers in that the

water droplet must be applied to the thermistor bead, as in the Richards and Ogata psychrometer.

A. Thermistor Psychrometer Apparatus

A commercial thermistor with about 1.5 K ohm resistance at 25 C was imbedded in a glass tube 1.2 millimeters in diameter and about 30 centimeters long (figure 18). Insulated wire leads were soldered to the thermistor leads, and the solder joints protected with insulating spaghetti. The thermistor was then cemented into the end of a 4-millimeter diameter glass tube 10 centimeters long. The thermistor tip extended about 1 centimeter from the glass tube, and the wire leads extended through the tube. A small stopper was fashioned from Tygon tubing by using a pencil sharpener, then slipped over the psychrometer and cemented in place. The entire assembly was mounted in a rubber stopper. A cup was constructed from copper tubing and copper rod. The tubing diameter was chosen so that the cup formed a tight seal with the Tygon stopper. A second hole, just the right diameter to permit the handle of the cup to slide easily was drilled through the large rubber stopper and the cup handle was inserted through this hole and bent. Since this apparatus is immersed in a water bath, it is essential that the handle fit so there is no leakage. Silicone stopcock grease helped. A vial to hold the sample completed the apparatus.

The measuring circuit consisted of a Wheatstone bridge type circuit, (Kreeb, 1965), for plant and soil materials. The loading procedure is similar to that used with other psychrometers.

B. Procedure

A drop of water is placed on the thermistor bulb, which is then covered by the cup, assembled on the vial containing the sample, and the assembly equilibrated in the water bath for several hours. Since speed is essential in loading the apparatus to reduce evaporation from the samples, it is well to moisten the thermistors in advance.

The following steps are followed during measurement:

1. Cover the thermistor with the cup to prevent evaporation;

then adjust the measuring circuit so there is no current flow through the thermistor. This gives the "dry bulb" temperature.

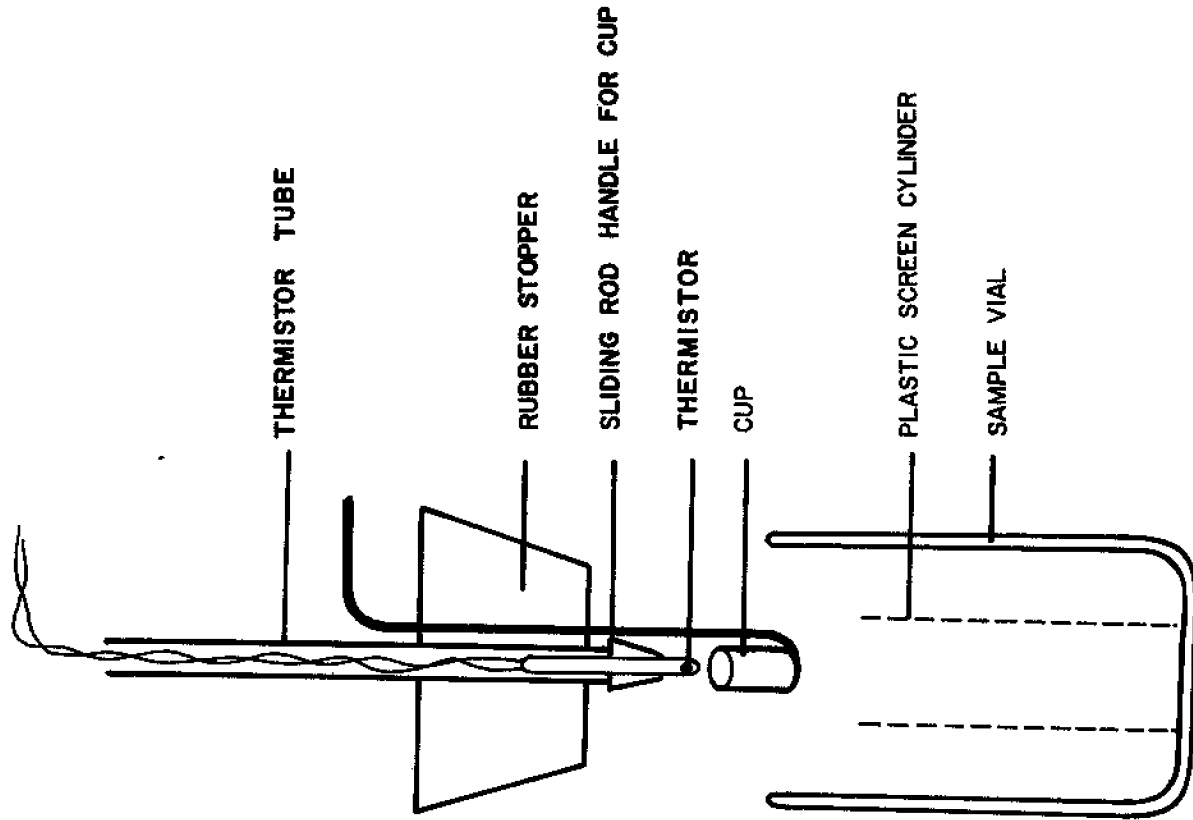


Figure 18. Diagram of a thermistor psychrometer with movable cup.

2. Lower the cup. The galvanometer will register a deflection, which is generally maximum within 1 minute. Note reading (alternately, the variable resistance is changed to return the deflection to "0", and the change in resistance is noted).

3. Cover the thermistor again with the cup and repeat steps 1 and 2.
4. Calibrate the instrument against standard osmotic solutions on filter paper.

V. GRAVIMETRIC VAPOR EXCHANGE

The gravimetric vapor exchange method of water potential determination is a modification of the liquid exchange method which is found in many elementary plant physiology laboratory manuals. It has been reviewed by Barrs (1968).

Replicate leaf discs are weighed, then placed in separate vapor equilibration jars containing a graded series of osmotic solutions for several hours to a day. The discs are then weighed again. The water potential of the leaf is equivalent to the osmotic pressure of the solution which causes no change in weight. Its advantages are: (1) the equipment needed is generally available, (2) experience with electronics is not required, and (3) an elaborate water bath is not essential. The method is, however, more cumbersome and requires more plant material. More time is required for a measurement, during which the water potential may change.

A. Apparatus

Any water bath with 0.1 C or less temperature fluctuation is adequate. The water bath must be large enough to hold about ten jars. Pint size wide mouth fruit jars with lids are convenient and can readily serve as vapor equilibration jars. Fill these jars to within 3 centimeters of the top of the glass beads. Then pour in the respective osmotic solution until it just covers the beads. A weight may be added to submerge the jar in the bath. The beads serve to reduce splashing of the solution, and provide support for the screen. Attach a plastic window screen with epoxy cement to a plastic cylinder about 5 millimeters high and wide

enough to slip easily into the jar. This little platform rests on the glass beads, and the screen serves as a support for the leaf samples. It is essential that the screen be dry at all times and not come in contact with the osmotic solution.

Tared vials may be small chemical-type glass containers. Low form, wide mouth vials are most convenient. Any *analytical balance* that weighs to 1 milligram or less can be used.

Osmotic solutions can be prepared according to the appendix. If the water potential is not approximately known, or is expected to vary widely among samples, solutions increasing in steps of 4 to 5 bars between 0 and 40 bars may be chosen. If the approximate water potential is known, solutions increasing in 1 or 2 bar steps over a limited range bracketing the expected values are preferable, and give more accurate results.

B. Procedure

1. Collect discs from leaves; place directly in tared vials and promptly stop. It is important to collect representative samples from the same regions of leaves of similar age and exposure. Salts should have been washed off previously, as discussed previously.
2. The vials are *weighed*.
3. Place one vial in each of the *equilibration* jars. Remove the vial cover and quickly seal the jar.
 - 3a. An *alternate method* is to remove the leaf discs from the vial and place them either directly on the screen, or on comb-like holders fashioned by cutting slits in rigid plastic. This method permits more rapid vapor exchange, but incurs the risk of excessive evaporation during transfers. These transfers are best made in a humid chamber.
4. Submerge the jars in the water bath for 2 to 24 hours (weights in the jar will help submerge and stabilize them in the bath). After some experience, a submersion time can be selected which gives a weight change sufficient to measure.

5. Remove the jars from the bath, stopper the vials, and weigh again on the analytical balance.
6. Typically, leaf sections held over distilled water and dilute solutions will gain weight, while those over more concentrated solutions will lose weight. Some solutions will cause no weight change, or it may be necessary to estimate the osmotic potential which causes no weight change by interpolation. The water potential of the leaves is equal to the osmotic potential of the solution which causes no weight change, or which is initially in equilibrium with the tissue.
7. It is possible, by this method, to run a number of determinations simultaneously. The limits are the number of vials that can be fitted into each jar, and operator efficiency.

VI. PRESSURE CHAMBER

Although attempted by Dixon (1914), the pressure chamber was first used extensively by Scholander et al. (1965) and by Waring and Cleary (1967). Commercial units, some of them portable if small gas cylinders are used, are available from PMS Instrument Company.

Detailed instructions are given in the above citations and by Barrs, 1968. A twig is peeled to the cambium and inserted in a rubber stopper insert which is then inserted in the pressure chamber lid and attached to the chamber (figure 19). When assembled, the end of the twig protrudes from the lid, so that it can be observed for sap exudation. The pressure is increased until the sap just begins to exude from the twig. The water potential of the twig is numerically equivalent to the minimum pressure required to cause exudation. The twig should be cut one time only during removal from the tree, preferably with a sharp knife or razor blade. All temptations to make a second, smooth cut should be resisted, as these invariably lead to excessively high water potential values. The method has been used with individual leaves as well as twigs. In comparative studies with psychrometers, the two procedures gave comparable result for many species, but not for porous hardwoods like oak and hickory. Determinations require only 1 to 2 minutes and can be readily repeated. It is perhaps the most convenient field method and has been used extensively with trees. Because of the pressures

results. It deserves serious consideration for field studies and as an independent check on other methods. Procedures and precautions are outlined in the above citations.

VIII. FREEZING POINT METHOD

A. Theory

The chemical potential of water in a solution may be calculated from the equilibrium vapor pressure, the boiling point, or the freezing point. The effect of chemical potential on freezing point is relatively large. A decrease of 12 bars potential causes a 1 C decrease in the freezing point. The freezing point method has been used for many years to measure osmotic pressure. A review of freezing point methodology was published by Abele in 1963. In 1969, Cary and Fisher suggested using freezing point measurements to measure total plant water potential. The instrument they developed is shown in figure 20. The freezing unit is powered by a 12-volt battery and can be used in the field. Measurement of plant water potential takes about 4 minutes. The instrument is easy to operate and its components cost less than \$200.

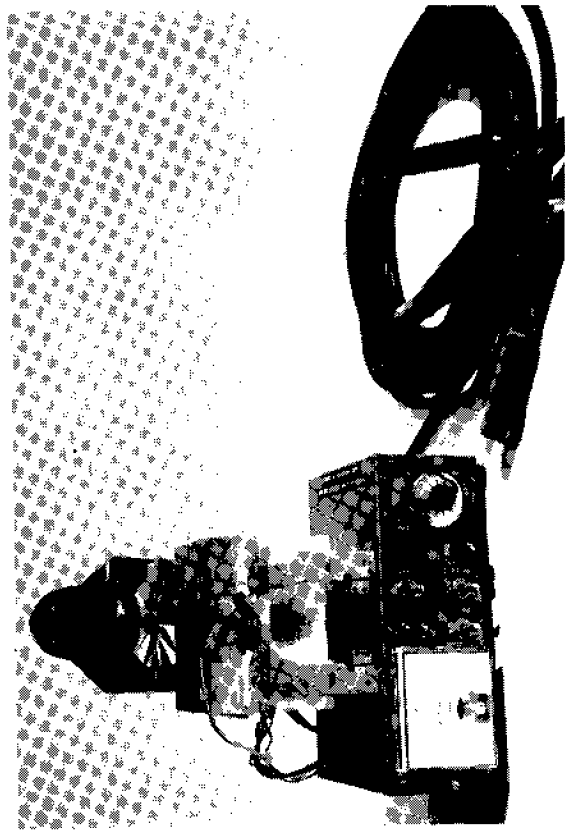


Figure 20. A photograph of the freezing point meter.

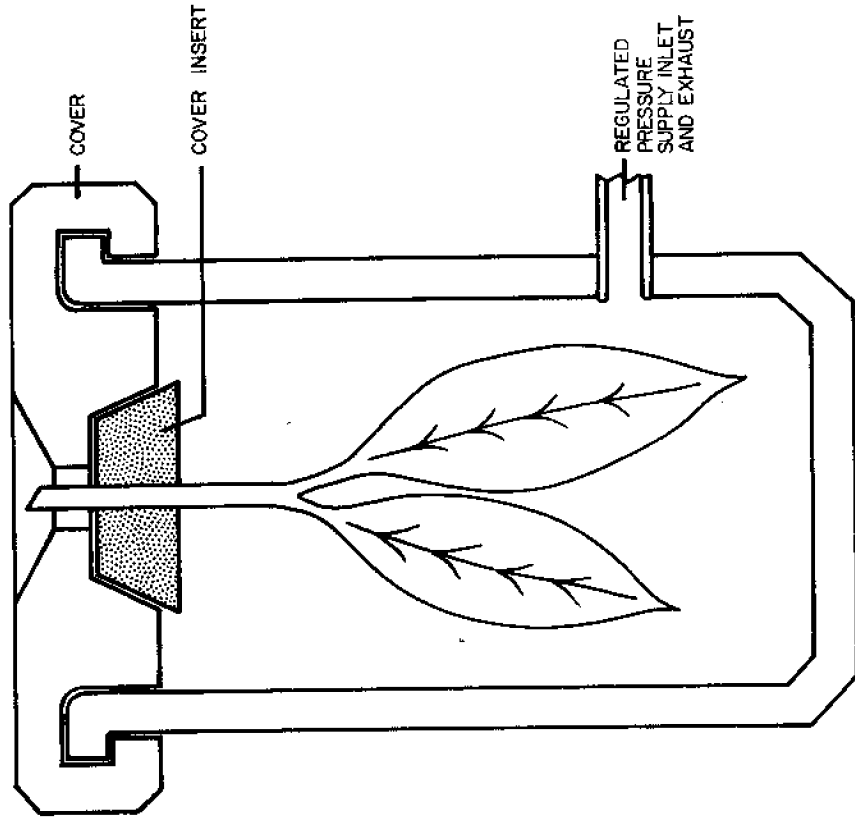


Figure 19. Diagram of a pressure chamber (courtesy B. Cleary).

involved, it is wise to use the bomb with due caution. According to at least three reports, the apparatus, when improperly used, becomes a bomb in the literal sense and has caused serious injury. With proper use, properly constructed chambers have been extensively used without accident.

VII. THE DYE METHOD

The dye method, generally attributed to Shardakov, has been described and reviewed recently by Barrs (1968), Knippling and Kramer (1967), and by Knippling (1967). The method requires no expensive equipment, and gives reasonably accurate

The freezing point of plant material can be measured, but whether this freezing point may be interpreted as a measure of the total plant water potential rather than a measure of the osmotic component of the total water potential may be questioned. The freezing plant water may rupture cell membranes and walls and cause a loss of cell turgor pressure. Under these conditions, the freezing point would not be a measure of the total plant water stress before freezing started. On the other hand, the first ice to form in plant material is often in the extracellular spaces. As freezing continues, the water moves from inside the cells out to the growing ice crystals in the extracellular spaces, and, depending upon the freezing rate and other plant factors, ice may also begin to form inside the plant cells (Mayland and Cary, 1970). In any case, water with the highest freezing point will first turn to ice once nucleation starts. Consequently, the first freezing point temperature should represent the potential of the water in the extracellular spaces which is near equilibrium with the intracellular solutions. Thus, it does seem possible that the total water potential might be measured by the freezing point method if the measurements are carried out correctly.

The instrument can be calibrated with an osmotic solution, but to measure the total plant water potential in a freezing point apparatus calibrated with osmotic solutions, three major requirements must be met: (1) the freezing point must be measured in the first few seconds after freezing starts before significant cell rupture and mixing of osmotic contents with the extracellular water; (2) the temperature dependence of the plant water potential must be reasonably close to the temperature dependence of the potential of the calibration solution; and (3) the rate of freezing and the heat transfer characteristics of the calibrating solution and the plant sample must be similar.

One way to test whether or not these requirements have been met is to compare the results of the freezing-point method with the results from other independent measurements. One such example is shown in figure 21. Four samples were taken simultaneously from single plants grown in the greenhouse. Two samples were run in the psychrometer, and two in the freezing point meter. The average difference between duplicates was about 1 bar from both the vapor pressure and freezing point methods, while the average difference was 2.6 bars between the two methods. Most measurements made by freezing point were within

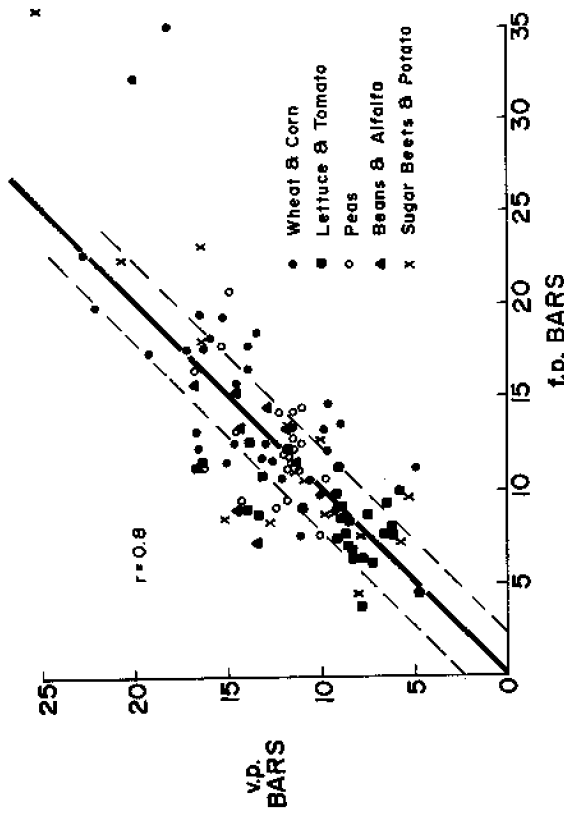


Figure 21. Comparison of freezing point and psychrometric water potential determinations.

2 or 3 bars of measurements made with the vapor pressure psychrometer. The limited data presently available, however, suggest that the difference may be greater at potentials less than -30 bars. The greater number of measurements that can be made by the freezing point method tends to compensate for the loss of accuracy.

The discrepancy between measurements made with the freezing point meter and the pressure bomb shows a different pattern than that in figure 21. For such plants as beans, alfalfa, potatoes, and sunflowers, the pressure bomb gives potential measurements several bars higher than those shown by the freezing point meter.*

B. Construction

A diagram of the cross section of the freezing chamber is shown in figure 22. The sample chamber is made by drilling a hole 2 centimeters in diameter and 2 centimeters deep in an

*Cary and Fisher, unpublished data.

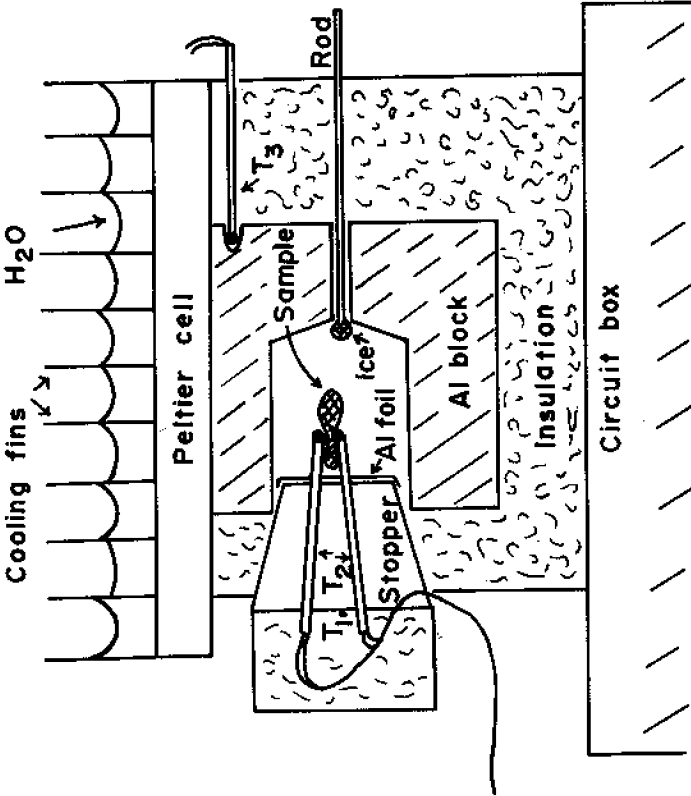


Figure 22. Diagram of a freezing point meter.

aluminum block. Thermal grease is used between the aluminum block and the cold side of the Peltier cell. The thermistors are of the glass rod type, about 2 centimeters long and 1.5 millimeters in diameter. They are set in the stopper at an angle so that the sample fits firmly between the tips, making a good point for heat exchange. The tips of the thermistor should be about 1 millimeter apart.

A circuit diagram for temperature measurement is shown in figure 23. Two Wheatstone bridges are used, one to measure the temperature of the sample, and the other for monitoring the temperature of the cold chamber. The bridge on the left contains the two thermistors (T_1 and T_2) for measuring the sample temperature. These thermistors are placed in opposite arms of the bridge to provide increased sensitivity. The bridge is powered by two 1.35 volt mercury cells. Switch S_1 connects the ammeter to either the left or the right bridge. Switch S_2 turns the bridge on, while switch S_3 may be opened to decrease the sensitivity

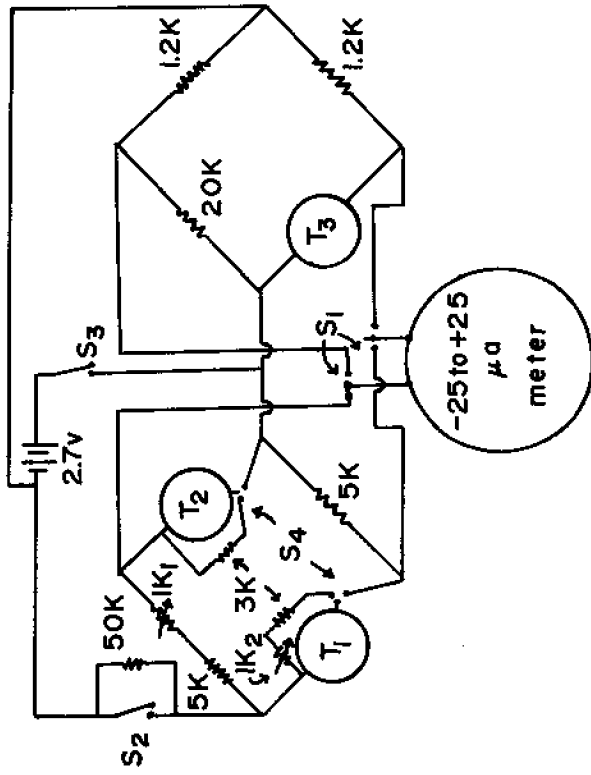


Figure 23. Circuit diagram for a freezing point meter.

of the temperature bridge during the sample loading period. Switch S_4 replaces the two thermistors with standard 3 K resistors. These are used to check the operation of the bridge.*

The bridge on the right is used to monitor the temperature of the freezing chamber. For best results, this temperature should be held constant within a few tenths of a degree. This may be done in either of two ways. The resistance between the Peltier cell and the car battery may be varied manually to give the

*The following component descriptions are listed for the convenience of the reader and in no way imply preferential endorsement of the manufacturer by the United States Department of Agriculture or Utah State University; T_1 , T_2 — VECO 31A13 thermistor 1 K Ω @ 25 C; T_3 — VECO 35A8 thermistor 5K Ω @ 25 C; 1K — Bourns 1 K Ω 10-turn potentiometer; S_1 — DPDT toggle switch; S_2 , S_3 — SPST toggle switches; battery — 2 Mallory Rn-4R 1.35-V mercury cells; meter — Knight 25-0-25 μ amp 3 $\frac{1}{2}$ inch panel meter; resistances are $\frac{1}{4}$ -watt, 10 percent tolerance, Cambion Peltier cell, 40-watt Model 803-8970-01 (Santa Monica-Bell Electronic Corp., Gardena, California). The fan motor on this cell can be replaced with a 12-volt automobile fan motor available at any auto supply store.

necessary amount of continuous cooling for the particular conditions under which the unit is operating. A resistance switch with an approximately 0.1-ohm step is required. These resistors should be rated for 8 to 10 amps. They can be purchased commercially, or made from short pieces of heavy nichrome wire. An alternate method for temperature control requires the use of enough cord between the battery and the Peltier cell to drop the voltage from 12 to the maximum amount recommended for the particular cell used. A temperature sensor and appropriate circuit may then be used to turn the power on and off, as is often done with the heater in laboratory temperature-controlled water baths. If the temperature control is to be achieved by automatic on-off switching, several systems are available.*

C. Calibration

After the instrument is assembled, it must be calibrated before plant water potential is measured. Fold a piece of filter paper or paper towel two or three times into a flat bundle approximately square and 1 to 2 millimeters thick. Slip this bundle between the thermistors as shown in figure 21 and wet to the dripping point with an osmotic solution of about 8 bars. Insert the stopper in the cooling chamber and adjust the Peltier battery so that the cooling rate of the sample is 1 μ amp every 1 or 2 seconds with switch S₂ closed. Note the microammeter reading of the T₃ thermistor at this point for future reference. The osmotic solution in the sample may be nucleated by tapping it with the ice crystal on the end of the sliding rod. As freezing begins, the sample temperature will rise, the ammeter will reverse its direction of movement, and then come to a halt at a constant value. The resistor, K₁, is then adjusted to set the microammeter reading to any convenient point on the scale; for example, + 7 μ amps. Switch S₁ should then be changed so that the 3K resistors are in the bridge rather than the thermistors T₁ and T₂. Resistor K₂ is then adjusted to zero, and this will serve as a standard reference setting for the bridge so long as the thermistors are not changed. Discard the osmotic sample, rinse the thermistors with distilled water, and put in place a new sample with

* A schematic for one type which operates from a 12-volt supply with thermistor for temperature sensing may be obtained upon request from Dale Fisher, Snake River Conservation Research Center, Route 1, Box 186, Kimberly, Idaho 83341.

a different osmotic pressure. Allow the sample to undercool 5 to 10 μ amps, initiate crystallization by tapping with the rod, and note the new steady point meter reading that will develop within 10 or 15 seconds. This process may be repeated with other osmotic solutions to form an appropriate calibration curve. The curve will be nearly linear, with the microammeter reading decreasing about 0.9 μ amp/bar decrease in water potential. The points will be reproducible within 1 μ amp.

D. Use With Plant Samples

After calibrating, the meter is ready for use with plant samples. Switch in the 3K reference resistors and check the microammeter for zero reading. If the reading is not zero, make the appropriate adjustment with the K₁ variable resistor. Check the T₃ thermistor to see that the sample chamber is at the correct temperature. A piece of plant leaf may then be cut off and folded to approximately the same size as the calibrating filter paper. Allow the sample to undercool several μ amps before setting the ice crystals, and then record the maximum reading which should follow within the next 30 seconds. The moisture potential of the plant sample is then read directly from the calibration curve.

The circuit is somewhat temperature sensitive, and the output will change during the day when used in the field under fluctuating temperatures. This is corrected for by adjusting the K₁ resistor to zero the meter with the 3K resistors in the circuit. It is also a good idea to carry some of the 8-bar calibrating solution and check the operation of the meter periodically each day, particularly when the temperature variations have been extreme, or when one suspects that any damage may have occurred to the thermistors or associated circuit.

E. General Precautions

1. Check the zero of the circuit as the ambient temperature changes, particularly under field conditions.
2. Watch for static charge buildup from dust on the meter face which will cause erratic behavior of the meter. Static charge may be eliminated by washing the meter face with a stream of distilled water from a wash bottle. The meter, of course,

must be sealed so that it is waterproof as well as dustproof.

3. Watch for cracks in the glass rod thermistors. If cracks develop, the calibrating solution may cause a partial short-circuiting.
4. Check the temperature of the freezing chamber during each sample measurement. If the temperature drifts appreciably, the calibration curve will be shifted. Colder temperatures cause a lower μ amp output for any given water potential.
5. Be sure that the sample is always in a firm position between the tips of the thermistors, but never allow the sample to touch any other part of the chamber.

IX. OSMOTIC POTENTIAL DETERMINATIONS

All methods for determining osmotic potential involve reduction of cell turgor to zero prior to the determination. In the plasmolytic method this is done with intact cells, but all other methods involve the disruption of cell membranes and osmotic potential measurement, usually after extraction of the cell sap. Accurate measurements of osmotic potential by either cryoscopic or vapor pressure methods present little difficulty; the greatest problem is that the extracted sap may not accurately represent the vacuolar sap. Unknown quantities of cytoplasm or cell wall water may be mixed with the vacuolar sap. Cell wall water, if present in any quantity, would probably dilute the sap. Also, ionic solutes in the vacuolar sap might be absorbed on cytoplasmic proteins or on cell walls after membranes rupture, again resulting in more dilute cell sap. Consequently, many sources of error must be considered when plant sap is extracted for any determination. In the last decade, great advances have been made in water potential determination on plant tissues, while little has changed in osmotic potential determination. It is true that we can measure osmotic potential of minute samples more accurately and conveniently, but we know little more about the osmotic potential of the vacuolar sap than earlier. It is also becoming apparent that it is the osmotic potential, or the solute environment, rather than the water potential or turgor pressure which is the important factor in metabolic or enzymatic function (Walter and Stadelman, 1969; Flowers and Hansen, 1969). Water movement into and through the plant is along water potential

gradients, while cell enlargement, stomate function and phloem translocation may depend on turgor pressure, but biochemical processes appear to be influenced primarily by the osmotic potential and the solute environment.

A. Extraction of Sap

Extraction from living tissues yields a sap which is more dilute than the cell sap, especially in the first samples extracted. When no shear forces are involved, as in the pressure bomb, almost pure water is recovered (Scholander, et al., 1965). When there is rupture of cells, especially at higher pressures in a press, successive fractions become more concentrated (Boyer, 1969; Bennett-Clark, 1959; Walter and Kreeb, 1969). In living cells the membranes thus act as ultrafilters, allowing the passage of water, but not solutes, at increased pressures. Such sap is not satisfactory for osmotic determinations, since it does not represent the vacuolar sap.

Various methods of rupturing membranes and thereby killing cells can be used. The most popular appears to be rapid freezing in a freezer, in solid carbon dioxide alcohol bath, or in liquid nitrogen. The tissue may also be heated in boiling water for 20 minutes. After disrupting the membranes, the sap is collected for osmotic determination. For moist tissues it can be simply squeezed out by hand. With very dry desert species it may be necessary to extract the sap with a pressure cylinder (Walter and Kreeb, 1970) or by centrifuge filtration. The two methods gave similar results in a comparative study (Wiebe and Walter, 1969, unpublished). It is essential to freeze or heat the tissue in sealed containers to prevent vapor exchange with the air, by condensation on frozen samples, or evaporation on heated ones. Water which has evaporated from the tissue will often condense on the walls of the container, and must be completely incorporated in the extracted sap. This can usually be done by wiping the sides and lid with the plant material.

Tissues should be killed quickly to prevent metabolic changes, especially hydrolysis of reserves. Some hydrolysis may also occur on boiling, although Walter did not find this a serious problem with most species (Walter and Kreeb, 1970).

In field work where it is not convenient to freeze or boil the

tissue, Kreeb (1963) has found that good results can be obtained by quickly drying the tissue, and subsequently reconstituting it to the original moisture content before expression of sap. Field facilities for fresh weight determination were essential. The soaking was done in a refrigerator to reduce metabolic changes.

B. Osmotic Potential Determination on Extracted Sap

1. Vapor exchange methods

Osmotic pressure of extracted sap can be measured by several vapor exchange methods. A commercial vapor pressure osmometer (Hewlett-Packard, Wescor) is probably the most convenient and can be used with samples of a fraction of 1 millimeter. Laboratories already having a psychrometer of any of the types discussed in the water potential measurement section could use them. The plant sap is merely placed on filter paper in the sample holder in technique identical to that used with calibrating solution. This method might be preferable in studies involving both water and osmotic potentials in that both are measured by the same method. The simplified method of Ehlig (1962) for measurement of both water and osmotic potentials on the same plant sample should also be considered in such studies. The water potential is measured in the psychrometers as discussed earlier. After this, the sample is frozen at -30° and after thawing and equilibration, its water potential is again measured to give the osmotic potential under zero turgor conditions. The sample may be frozen in the psychrometer sample holder or after transfer to another container. It is essential to seal the freezing container carefully to prevent any vapor exchange, which is especially critical with the small samples involved. It is also possible to immerse the tissue directly in liquid nitrogen for a few minutes, using a forceps to hold it, and then return it to the psychrometer chamber. Or liquid nitrogen can be poured directly into the chamber to freeze the sample, and allowed to evaporate. Condensation of water vapor from the air on the sample as dew or frost can be reduced by closing the sample container as it warms. Parallel before-and-after freezing determinations with standard solutions on filter paper are strongly recommended; any changes in water potential will indicate problems in technique. An osmotic potential value higher than water potential of the same sample should be carefully reinvestigated and preferably confirmed by independent determination with other techniques. An osmotic potential higher

than water potential would indicate a negative turgor pressure, which is unlikely except in the thick walled xylem cells (Wilson, 1967).

2. Cryoscopic methods

More osmotic determinations have been made by cryoscopy of the sap than by any other method, and it is still the most convenient and most frequently used method. The results appear to be generally valid, at least after correction of the osmotic potential to plant temperature. Colloids may interfere with the freezing point (Crafts et al., 1949). Presence of colloids may also increase undercooling and reduce the accuracy of the freezing point determination. Reduced solubility of salts also might be a problem at lower temperatures.

Detailed methods are given in many plant physiology laboratory manuals (Barrs, 1968). Micro methods are reviewed by Barrs. A number of commercial units are available.

C. Visual Melting Point Method of Measuring Osmotic Potential in Cells

Bearce and Kohl (1970) have reported a very promising cryoscopic method for measuring osmotic pressure of cells *in situ*. Thin slices of tissues (they used leaf epidermis sections) are rapidly frozen on a specially constructed microscope freezing slide. The sections are then observed under a microscope while being slowly thawed. The temperature at which the ice melts in each individual cell is recorded and used to calculate the osmotic potential of the cell sap. Observation of the ice is facilitated with a polarizing microscope.

X. MATRIC POTENTIAL DETERMINATIONS

Large molecules in cells, such as proteins in the cytoplasm and pectins, cellulose and other polysaccharides in the cell wall also lower the water potential. The contribution to water potential of colloidal surfaces, as opposed to solutes, is known as matric potential. The matric potential is probably qualitatively equivalent to bound water, although comparative studies have apparently not been made. Measurement of bound water is

reviewed by Gortner (1949), Kramer (1955), and Wilson (1967).

Wiebe (1966) has used the pressure membrane or ultra-filtration apparatus of Richards (1947) to study the matric pressure of several biocolloids and of plant tissues. Moisture release curves were obtained by equilibrating the material at various pressures.

A soil pressure membrane or ultra-filtration apparatus (a number of commercial units are available) is used with cellulose dialysis membrane, supported by nylon cloth. The apparatus can be modified for collection of the expressed liquid for osmotic analysis. Compressed cylinder nitrogen supplies the pressure. Plant material used to date includes slices of storage roots and ground leaf material. The leaf material could be ground in liquid nitrogen and used directly, or it could be dried, ground, and re-moistened before use. Leaf material which was merely macerated did not work because it became layered like shingles, and the sap did not move well. Sections of storage tissue must first be frozen to destroy the membranes because very little water can be extracted from living tissue.

The material is placed in the apparatus, and the gas pressure is applied for 48 hours. No additional sap is extracted after about 24-30 hours, but psychrometric water potential determinations of the residue gave values of only about 75 percent of the expected value, which is equal to the applied pressure, although of the opposite sign. Even after 10 days, complete equilibrium was not attained. After extraction, the moisture content as a fraction of the dry weight is determined gravimetrically. By equilibrating plant material at different pressures and determining the moisture content, a pressure release curve is obtained. This can then be used to estimate the matric potential of plant tissue of any given moisture content. The matric potential is well above -1 bar for the usual fresh weight water content of leaves and the storage organs studied. It would be lower in seeds meristems and zephyrites, although these have not been measured.

Several problems arise in interpretation of the matric potential, as determined by the above method. When the cells are killed, the vacuolar sap mixes with the cytoplasm and both permeate the cell wall. In the living state, the cell wall water is probably quite dilute. So gross matric potential is probably a composite of the individual potentials of the various colloids,

after mixing with the cell solutes. It does not necessarily represent the matric potential of the cell wall or of the cytoplasmic proteins. It is also possible that drastic treatments like freezing or drying alter the water holding capacity of the colloids. Frozen agar was found to hold less water at different pressures than fresh, unfrozen agar (Wiebe, 1966) and this could be true of proteins as well.

Boyer (1967, a and b) has used the pressure bomb together with frozen and thawed shoots to determine the matric potential of leaves. In killed tissue, the pressure just required to cause exudation is equal to the matric potential. For well watered shoots, Boyer found the matric potential to range from 0 to -0.8 bar, with values down to -10 bars in the wilting range. Cell walls accounted for most of the matric potential.

LITERATURE CITED

- Abele, J. E. 1963. The physical background to freezing point osmometry and its medical-biological applications. *Amer. J. Med. Electronics* 2:32-41.
- Barrs, H. D. 1968. Determination of water deficits in plant tissues in *Water Deficits and Plant Growth*. (T. T. Kozlowski, Ed.) Academic Press, New York. Vol. I, pp. 235-368.
- Barrs, H. D. 1969. Simple automatic scanning and recording system for measurement of water potential with droplet thermocouple psychrometers. *New Phytologist* 68:1041-1046.
- Barrs, H. D. and P. J. Kramer. 1969. Water potential increase in sliced leaf tissue as a cause of error in vapor phase determination of water potential. *Plant Physiol.* 44: 959-964.
- Bearece, B. C. and H. C. Kohl, Jr. 1970. Measuring osmotic pressure of sap within living cells by means of a visual melting point apparatus. *Plant Physiol.* 46:515-519.
- Bennett-Clark, T. A. 1959. Water relations in *Plant Physiology*. Academic Press, New York. (F. C. Steward, Ed.) Vol. II, pp. 105-192.
- Boyer, J. S. 1967. a. Leaf water potentials measured with a pressure chamber. *Plant Physiol.* 42:133-137.
- Boyer, J. S. 1967. b. Matric potentials of leaves. *Plant Physiol.* 42:213-217.

- Gortner, R. A. 1949. Outlines of Biochemistry, 3rd Edition. New York, J. Wiley & Sons.
- Hoffman, G. F. and W. N. Herkelrath. 1968. Design features of intact leaf thermocouple psychrometers for measuring water potential. Amer. Soc. Agr. Engr. Trans. 11:631-634.
- Hoffman, G. J. and W. E. Splinter. 1968. Water potential measurements of an intact plant-soil system. Agron. J. 60:408-413.
- Hoffman, G. J., W. N. Herkelrath and R. S. Austin. 1969. Simultaneous cycling of Peltier thermocouple psychrometers for rapid water potential measurements. Agron. J. 61:597-601.
- Hsieh, J. J. C. and F. P. Hungate. 1970. Temperature compensated Peltier psychrometer for measuring plant and soil water potential. Soil Sci. 110:253-257.
- Klepper, Betty and H. D. Barrs. 1968. Effects of salt secretion on psychromatic determinations of water potential of cotton leaves. Plant Physiol. 43:1138-1140.
- Knipling, E. B. 1967. Measurement of leaf water potential by the dye method. Ecology 48:1038-1041.
- Knipling, E. B. and P. J. Kramer. 1967. Comparison of the dye method with the thermocouple psychrometer for measuring leaf water potentials. Plant Physiol. 42:1315-1320.
- Kramer, P. J. 1955. Bound water in Encyclopedia of Plant Physiology. (H. Ruhland, Ed.) L:223-242. Springer-Verlag, Berlin.
- Kreeb, K. 1963. Untersuchungen zum Wasserhaushalt der Pflanzen unter extrem ariden Bedingungen. Planta 59:442.
- Kreeb, K. 1965. Untersuchungen zu den osmotischen Zustandsgrößen. II. Mitteilung: Eine elektronische Methode zur Messung der Saugspannung (NTC-Methode). Planta 66:156.
- Lambert, J. R. and J. van Schilfgaarde. 1965. A method of determining the water potential of intact plants. Soil Sci. 100: 1-9.
- Lang, A. R. G. 1967. Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40° C. Aust. J. Chem. 20:2017-2023.

- Boyer, J. S. 1969. Measurement of the water status of plants. Ann. Rev. Plant Physiol. 20:351-364.
- Brown, R. W. 1969. Leaf sampler for relative water content measurements: design and application. Agron. J. 61:644-647.
- Brown, R. W. 1970. Measurement of water potential with thermocouple psychrometers: construction and application. USDA Forest Service Res. Pap. INT-80.
- Calissendorff, C. 1970. An *in situ* leaf and soil water psychrometer having low temperature sensitivity. M. S. Thesis, Washington State University, Pullman, Washington.
- Campbell, G. S. and W. H. Gardner. 1971. Psychrometric measurement of soil water potential: temperature and bulk density effects. Soil Sci. Soc. Amer. Proc. 35:8-12.
- Campbell, G. S., J. W. Trull, and W. H. Gardner. 1968. A welding technique for Peltier thermocouple psychrometers. Soil Sci. Soc. Amer. Proc. 32:887-889.
- Campbell, G. S., W. D. Zollinger, and S. A. Taylor. 1966. Sample changer for thermocouple psychrometers: construction and some applications. Agron. J. 58:315-318.
- Cary, J. W. and H. D. Fisher. 1969. Plant moisture stress: a portable freezing-point meter compared with the psychrometer. Agron. J. 61:302-305.
- Crafts, A. S., H. B. Currier, and C. R. Stocking. 1949. Water in the Physiology of Plants. Chronica Botanica, Waltham, Mass.
- Dalton, F. N. and S. L. Rawlins. 1968. Design criteria for Peltier effect thermocouple psychrometers. Soil Sci. 105:12-17.
- Dixon, H. H. 1914. Transpiration and the Ascent of Sap in Plants. Macmillan, London.
- Ehlig, C. F. 1962. Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiol. 37:288-290.
- Flowers, T. V. and J. B. Hansen. 1969. The effect of reduced water potential on soybean mitochondria. Plant Physiol. 44: 939-945.

Lang, A. R. G. 1968. Psychrometric measurement of soil water potential *in situ* under cotton plants. *Soil Sci.* 106:460-464.

Lang, A. R. G. and H. D. Barrs. 1965. An apparatus for measuring water potentials in the xylem of intact plants. *Aust. J. Biol. Sci.* 18:487-497.

Lopushinsky, W. and G. Klock. 1970. Construction details of ceramic bulb psychrometers. Mimeo. USDA Forest Service, Forest Hydrology Lab., Wenatchee, Washington.

Manohar, M. S. 1966. a. Measurement of the water potential of intact plant tissues. I. Design of a microthermocouple psychrometer. *J. Exptl. Botany* 17:44-50.

Manohar, M. S. 1966. b. Measurement of the water potential of intact plant tissues. II. Factors affecting the precision of the thermocouple psychrometer technique. *J. Exptl. Botany* 17:51-56.

Manohar, M. S. 1966. c. Measurement of the water potential of intact plant tissues. III. The water potentials of germinating peas (*Pisum sativum* L.) *J. Exptl. Botany* 17:231-235.

Mayland, H. F. and J. W. Cary. 1970. Frost and chilling injury to growing plants. *Advances in Agron.* 22:203-234.

Merrill, S. D., F. N. Dalton, W. N. Herkelrath, G. J. Hoffman, R. D. Ingvalson, J. D. Oster and S. L. Rawlins. 1968. Details of construction of a multipurpose thermocouple psychrometer. Research Report No. 155, United States Salinity Laboratory ARS-USDA, Riverside, California.

Millar, A. A., A. R. G. Lang and W. R. Gardner. 1970. Four-terminal Peltier type thermocouple psychrometer for measuring water potential in nonisothermal systems. *Agron. J.* 62:705-708.

Monteith, J. L. and P. C. Owen. 1958. A thermocouple method for measuring relative humidity in the range 95-100 percent. *J. Sci. Instr.* 35:443-446.

Peck, A. J. 1968. Theory of the Spanner psychrometer. I. The thermocouple. *Agr. Meteorol.* 5:433-447.

Peck, A. J. 1969. Theory of the Spanner psychrometer. II. Sample effects and equilibration. *Agr. Meteorol.* 6:111-124.

Rawlins, S. L. 1966. Theory for thermocouple psychrometers used to measure water potential in soil and plant samples. *Agr. Meteorol.* 3:293-310.

Rawlins, S. L. and F. N. Dalton. 1967. Psychrometric measurement of soil water potential without precise temperature control. *Soil Sci. Soc. Amer. Proc.* 31:297-301.

Rawlins, S. L., W. R. Gardner and F. N. Dalton. 1968. *In situ* measurement of soil and leaf water potential. *Soil Sci. Soc. Amer. Proc.* 32:468-470.

Rawlins, S. L., G. J. Hoffman and H. T. Stumpf. 1968. A multiple temperature water bath. *Agricultural Eng.* 49:672-673.

Richards, B. G. 1965. Thermistor hygrometer for determining the free energy of moisture in unsaturated soils. *Nature* 208:608-609.

Richards, L. A. 1947. Pressure membrane apparatus—construction and use. *Agr. Engr.* 28:451-454.

Richards, L. A. 1965. Metallic conduction for cooling a thermocouple psychrometer bath. *Soil Sci.* 100:20-24.

Richards, L. A. and G. Ogata. 1958. Thermocouple for vapor pressure measurement in biological and soil systems at high humidity. *Science* 128:1089-1090.

Scholander, P. F., H. T. Hammel, E. A. Hemmingsen and E. D. Bradstreet. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. *Proc. Natl. Acad. Sci. U.S.A.* 52:119-125.

Slatyer, R. O. and I. C. McIlroy. 1961. *Practical Microclimatology*. UNESCO, Paris.

Spanner, D. C. 1951. The Peltier effect and its use in the measurement of suction pressure. *J. Exptl. Botany* 2:145-168.

Walter, H. and E. Stadelmann. 1968. The physiological pre-des Protoplasmas der Pflanzen und ihre oko-physiologische Bedeutung. *Protoplasmatologia II C 6*. Vienna, Springer-Verlag.

Walter H. and E. Stadelmann. 1968. The physiological prerequisites for the transition of autotrophic plants from water to terrestrial life. *BioScience* 18:694-701.

- Waring, R. H. and B. D. Cleary. 1967. Plant moisture stress: evaluation by pressure bomb. *Science* 155:1248-1254.
- Wiebe, H. H. 1966. Matric potential of several plant tissues and biocolloids. *Plant Physiol.* 41:1439-1442.
- Wiebe, H. H., R. W. Brown, T. W. Daniel and E. Campbell. 1970. Water potential measurements in trees. *BioScience* 20:225-226
- Wilson, A. M. and G. A. Harris. 1968. Phosphorylation in crested wheat grass seeds at low water potentials. *Plant Physiol.* 43:61-65.
- Wilson, J. W. 1967. The components of leaf water potential. *Aust. J. Biol. Sci.* 200:329-367.
- Zollinger, W. D., G. S. Campbell and S. A. Taylor. 1966. A comparison of water potential measurements made using two types of thermocouple psychrometers. *Soil Sci.* 102:231-239.

Table 1. Water Potentials of KCl Solutions at Temperatures Between 0 and 40 C (Campbell and Gardner, 1971).

Water Potential (bars or Joules/kg x 10⁻¹)

Molality	0°C	5°C**	10°C	15°C**	20°C	25°C	30°C**	35°C**	40°C
0.05	-2.14	-2.18	-2.22	-2.25	-2.29	-2.33	-2.37	-2.41	-2.45
0.1	-4.21	-4.29	-4.36	-4.44	-4.52	-4.59	-4.67	-4.74	-4.82
0.15	-6.25	-6.37	-6.48	-6.60	-6.71	-6.83	-6.94	-7.05	-7.16
0.2	-8.27	-8.43	-8.59	-8.74	-8.90	-9.05	-9.20	-9.35	-9.49
0.25	-10.29	-10.49	-10.68	-10.87	-11.07	-11.26	-11.45	-11.64	-11.82
0.3	-12.29	-12.53	-12.77	-13.00	-13.24	-13.47	-13.70	-13.92	-14.14
0.35	-14.29	-14.57	-14.85	-15.12	-15.40	-15.68	-15.94	-16.20	-16.46
0.4	-16.28	-16.61	-16.93	-17.24	-17.57	-17.88	-18.19	-18.49	-18.79
0.45	-18.26	-18.64	-19.01	-19.36	-19.73	-20.09	-20.43	-20.77	-21.11
0.5	-20.25	-20.67	-21.08	-21.48	-21.90	-22.30	-22.68	-23.06	-23.44
0.55	-22.22	-22.70	-23.16	-23.60	-24.06	-24.51	-24.93	-25.36	-25.78
0.6	-24.20	-24.73	-25.23	-25.72	-26.23	-26.72	-27.19	-27.65	-28.11
0.65	-26.17	-26.75	-27.31	-27.84	-28.45	-29.05	-29.65	-30.26	-30.86
0.7	-28.14	-28.78	-29.38	-29.96	-30.57	-31.16	-31.71	-32.26	-32.80
0.75	-30.11	-30.80	-31.46	-32.08	-32.74	-33.38	-33.98	-34.57	-35.16
0.8	-32.08	-32.83	-33.53	-34.21	-34.92	-35.61	-36.25	-36.88	-37.52
0.85	-34.05	-34.85	-35.61	-36.33	-37.10	-37.84	-38.52	-39.20	-39.88
0.9	-36.01	-36.87	-37.69	-38.46	-39.28	-40.07	-40.80	-41.53	-42.25
0.95	-37.97	-38.89	-39.77	-40.59	-41.47	-42.31	-43.09	-43.86	-44.63
1.0	-39.93	-40.92	-41.85	-42.72	-43.66	-44.55	-45.38	-46.20	-47.02

** Values given for water potential in bars are roughly the equivalent of those given in dyne-cm/cm²/10⁶.
 ** Interpolated values.

Table 3. Preparation of Calibrating Solutions

A 2 molal (m) stock solution is first prepared by dissolving 149.10 g KCl or 116.90 g NaCl in 1000 g distilled water. The salt should be reagent grade. This gives 7.7069 g. solution per gram KCl and 9.5543 g. solution per gram NaCl. The mass of KCl or NaCl required for any solution is the molality multiplied by the molecular weight. Multiplying the molality times the molecular weight times the mass of stock solution per unit mass of KCl or NaCl gives the mass of solution required. The mass of KCl or NaCl plus 1000 g gives the total mass of the solution, so the amount of water which must be added is obtained by subtraction of the stock solution mass from the total mass. The tables show the stock solution mass, mass of water required and total mass for KCl and NaCl solution.

Molality of KCl	Mass of 2-m stock solution	Mass of water	Total mass
0.1	57.45	950.00	1007.45
0.2	114.91	900.00	1014.91
0.3	172.36	850.00	1022.36
0.4	229.82	800.00	1029.82
0.5	287.27	750.00	1037.27
0.6	344.73	700.00	1044.73
0.7	402.18	650.00	1052.18
0.8	459.64	600.00	1959.64
0.9	517.09	550.00	1067.09
1.0	574.55	500.00	1974.55
1.5	861.82	250.00	1111.82
2.0	1149.10	0.00	1149.10

Table 2. Water Potentials of NaCl Solutions at Temperatures between 0 and 40 C (Lang, 1967)

Molality	0°C	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
2.0	-86.70	-89.20	-91.30	-93.60	-95.70	-97.80	-99.80	-101.60	-103.50
1.9	-81.90	-84.30	-86.30	-88.40	-90.40	-92.40	-94.30	-96.00	-97.80
1.8	-77.30	-79.40	-81.30	-83.30	-85.20	-87.00	-88.80	-89.40	-92.10
1.7	-72.60	-74.60	-76.40	-78.20	-80.00	-81.70	-83.30	-84.90	-86.50
1.6	-68.11	-69.96	-71.63	-73.30	-74.91	-76.52	-78.05	-79.50	-80.07
1.5	-63.59	-65.29	-66.84	-68.37	-69.86	-71.34	-72.76	-74.11	-75.48
1.4	-59.12	-60.68	-62.10	-63.50	-64.87	-66.23	-67.54	-68.80	-70.06
1.3	-54.70	-56.11	-57.42	-58.69	-59.94	-61.19	-62.39	-63.54	-64.71
1.2	-50.32	-51.60	-52.78	-53.94	-55.07	-56.20	-57.30	-58.35	-59.41
1.1	-45.99	-47.13	-48.20	-49.24	-50.26	-51.27	-52.26	-53.22	-54.18
1.0	-41.69	-42.70	-43.66	-44.59	-45.50	-46.40	-47.29	-48.15	-49.01
0.9	-37.43	-38.32	-39.17	-39.98	-40.79	-41.58	-42.37	-43.14	-43.90
0.8	-33.20	-33.98	-34.72	-35.43	-36.12	-36.82	-37.51	-38.18	-38.85
0.7	-29.01	-29.67	-30.30	-30.91	-31.51	-32.10	-32.70	-33.28	-33.85
0.6	-24.84	-25.39	-25.93	-26.44	-26.94	-27.44	-27.94	-28.43	-28.91
0.5	-20.70	-21.15	-21.58	-22.00	-22.41	-22.81	-23.22	-23.62	-24.02
0.4	-16.58	-16.93	-17.27	-17.59	-17.91	-18.23	-18.55	-18.86	-19.17
0.3	-12.47	-12.72	-12.97	-13.21	-13.44	-13.68	-13.91	-14.15	-14.37
0.2	-8.36	-8.68	-8.91	-9.15	-9.40	-9.62	-9.85	-10.07	-10.30
0.1	-4.23	-4.31	-4.39	-4.47	-4.54	-4.62	-4.70	-4.77	-4.85
0.05	-2.14	-2.18	-2.22	-2.26	-2.30	-2.34	-2.38	-2.42	-2.45

Table 3 (Continued)

Molality of NaCl	Mass of 2-m stock solution	Mass of water	Total mass
0.1	55.84	950.00	1005.84
0.2	111.69	900.00	1011.69
0.3	167.53	850.00	1017.53
0.4	223.38	800.00	1023.38
0.5	279.22	750.00	1029.22
0.6	335.07	700.00	1035.07
0.7	390.91	650.00	1040.91
0.8	446.76	600.00	1046.76
0.9	502.60	550.00	1052.60
1.0	558.45	500.00	1058.45
1.5	837.67	250.00	1087.67
2.0	1116.90	0.00	1116.90

Table 4. Temperature Correction Factors for Spanner Psychrometers, and Microvolts Per Bar for a Typical Psychrometer*

TC	Correction factor E25/Et	mv/bar
0	2.430	0.209
5	1.944	.261
10	1.573	.322
15	1.338	.379
20	1.134	.447
25	1.000	.507
30	.883	.575
35	.815	.623
40	.743	.684
45	.696	.729
50	.656	.774

*Calculated from Peck (1968) Equation 19, for welded thermocouple, 4 x 10⁻³ cm radius, and sample chamber radius of 2 cm. Other thermocouple or chamber sizes or geometries will deviate from their values.

APPENDIX B

Commercial sources of instruments and supplies mentioned in the text. These are listed for the convenience of the reader and do not imply preferential endorsement by the authors or their sponsoring institution.

Archer Daniels, Midland Co., 466 Faries Parkway, Decatur, Ill., 62525

Arno Adhesive Tapes, 4170 Ohio Street, Michigan City, Indiana 46360.

Belden Manufacturing Co., 415 T South Kilpatrick, Chicago, Illinois 60644.

Hallikainen Instruments, 750 National Court, Richmond, Calif. 94804.

Hewlett Packard, 1101 Embarcadero Road, Palo Alto, California 94303.

Keithley Instruments, 28775 Aurora Road, Cleveland, Ohio 44139.

Leeds and Northrup, North Wales, Pennsylvania 19454.

Omega Engineering Inc., 31 Knapp St., Connecticut 06907.

PMS Instrument Company, 405 Merrie Drive, Corvallis, Oregon 97330.

WESCOR Inc., 459 South Main, Logan, Utah 84311.

Reproduced by the U. S. Department of Agriculture
For Official Use