Review. Use of psychrometers in field measurements of plant material: accuracy and handling difficulties

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Abstract

The determination of leaf water potential is useful in the establishment of irrigation guidelines for agricultural crop management practices and requires the use of various methods, among which thermocouple psychrometers (TCP). TCP have been widely used for this purpose. However, the psychrometric technique is complex and difficult to understand and the instrumentation required is difficult to handle. For this reason, a profound knowledge of the different aspects involved in the technique—which have not been clearly explained in the literature—is required. This paper reviews a number of research areas of TCP and focuses on three very specific fields: a) main applications of psychrometry in field studies; b) determination of the measurement accuracy of psychrometric equipment, accuracy per se and accuracy tested against alternative methods; c) main errors and handling difficulties of TCP in the field. Research in these areas provides an updated overview of TCP as a method for determining water relations in plant material that will contribute criteria to select the most suitable technique according to the type of plant material and the purpose of the research and will highlight the types of instruments, accuracies and errors that have detrimental effects on measurements.

Additional key words: hygrometric technique; irrigation; isopiestic technique; pressure chamber; psychrometric technique.

Introduction

For years, one of the major concerns of agronomists has been the study of how variations in the crop environment can affect proper crop development. A variety of factors must be considered, including factors such as plant genetics, climatic conditions or soil, and factors directly dependent on man such as technology or labour. In the search for sustainable development, all these factors must coexist in perfect harmony in order...
to achieve a rational use of water that may guarantee
the existence and availability of this scarce and poorly
used resource in a near future.

Water management practices for agricultural crops
demand knowledge of plant water requirements and of
the yield response to water application, so that a rational
use of water during irrigation can be attained most effi-
ciently. Measurement of soil/plant water potential is pro-
bably the most effective indicator to assess crop water
availability (Merrill and Rawlins, 1972). Thermocouple
psychrometers (TCP) are a versatile and valuable tool to
study the soil-water-plant system (Zollinger et al., 1966).

Since the early 1950s, TCP have been used to measure
soil and plant water status (McAneney et al., 1979).
Water status can be determined at different locations
of the system by using the same measurement techni-
que (Hoffman and Splinter, 1968; Campbell, 1985).
As compared to other techniques, the flexibility of TCP
and the rapid and reliable response provided by these
instruments have enabled the study and characteriza-
tion of the system, of crop resistance to water stress,
and of the different water potential gradients and their
effects on crop development. These aspects have been
studied by conducting continuous or discontinuous
measurements of water potential in roots, stems/trunks,
leaves, seeds or soils. In this case, water potential is
considered as the sum of matric potential and osmotic
potential (Hillel, 1998). Therefore, changes in TCP
readings may evidence changes in any or both of the
components. Moreover, changes in one of the compo-
nents could be masked by changes in the opposite
direction in the other component (Campbell and
Gardner, 1971). Therefore, a number of precautions must
be taken to ensure proper TCP performance, among
which are the following: knowledge of the technique,
types of TCP and suitability of each type of TCP for
different uses, and correct instrument calibration and
cleaning. Different authors have discussed the theore-
tical aspects of this issue (Barrs, 1968; Brown and Van
Haveren, 1972; Savage and Cass, 1984b; Mullins, 2001;
Andraski and Scanlon, 2002).

Given the many possible research areas within the
field of psychrometry, this review paper has focused
on three specific areas related to the use of psychro-
metry on plant material: a) main applications of psy-
chrometry in field studies; b) determination of the
measurement accuracy of the equipment: accuracy per
se and accuracy tested against alternative methods;
c) main errors and handling difficulties of TCP in the
field.

What type of TCP must be used? How accurate are
TCP readings? How must the device be handled
according to measurement conditions? Or, more impor-
tantly, are TCP really useful in the determination of
water potential in plant material in the 21st century,
considering the development of advanced technology?
Below, the results of research studies conducted in the
field of psychrometry are presented and some answers
to the above questions are provided.

**Types of psychrometers**

TCP are instruments used to determine the energy
state of water. The first types of TCP appeared during
the 1950s, with the earliest research on these techni-
ces. The use of TCP comprises readings based on
psychrometric techniques (wet-bulb temperature de-
pression), hygrometric techniques (dew-point tempera-
ture depression) (Turner, 1981) and isopiestic tech-
niques for water potential measurement. Yet, psy-
chrometry has been the most widely used technique
(Andraski and Scanlon, 2002). Some theoretical aspects
of these measurement techniques were discussed by
Campbell et al. (1966), Rawlins (1966), Peck (1968,

At first, psychrometry was restricted to laboratory
use because accurate temperature control was required.
However, with the development of improved designs
and electronic instrumentation that provided rapid and,
sometimes, non-destructive in situ measurements, the
uses of psychrometry have been extended (Xanthopoulos,
2002). Currently, there are three types of TCP available:
nonequilibrium, dewpoint and isopiestic TCP (Mullins,
2001; Kirkham, 2005).

**Nonequilibrium**

The first TCP considered as such were nonequi-
librium TCP, which included the wet-loop TCP
described by Richards and Ogata (1958), known as «Rich-
ards type» TCP, and the Peltier TCP described by
Spanner (1951), known as «Spanner type» TCP. Such
thermocouples had good sensitivity, but showed
difficulties in calibration and sample preservation
(Richards and Ogata, 1958). Both types of TCP consist
in a couple of thermocouple junctions placed in a
closed chamber. The main difference between them is
the method used to apply water to the measuring junc-
The measuring junction of Richards-type TCP is wetted by mechanically placing a drop of distilled water (Bingham et al., 1972) on a ceramic bead or on a small silver ring that is welded to the junction. Spanner-type TCP are wetted by cooling the measuring junction below the dew-point temperature before each measurement, according to the thermoelectric principle based on the Peltier effect.

«Richards-type» sensors can be used only with the psychrometric measurement technique, while «Spanner-type» sensors allow for the performance of both psychrometric and hygrometric measurements (Andraski and Scanlon, 2002). Only Spanner-type TCP can be used in the field, while Richards-type sensors are restricted to laboratory use.

Dewpoint

Dewpoint TCP are based on the hygrometric technique introduced by Neumann and Thurtell (1972). Dewpoint TCP include two subtypes: i) «hygrometers», which are structurally similar to other TCP and are named hygrometers because of their mode of operation, and ii) «water activity meters» (WAM), whose operation and components differ from TCP in general.

Hygrometers detect dewpoint temperature depression of water vapour inside the sample chamber and, therefore, detect title —the fraction of saturated vapour in a unit mass of liquid and saturated vapour—; as opposed to nonequilibrium TCP, which respond to wet-bulb temperature.

Gee et al. (1992), Scanlon et al. (2002) and Cancela et al. (2006), among other authors, described and used a recent development in dewpoint instrumentation applied to water potential measurement, WAM, termed also «dewpoint potentiometers». WAM are based on the chilled mirror dewpoint technique, which equilibrates the moist plant sample with water vapour in the air within the sealed chamber. When equilibrium is reached, a photodetector attached to a chilled mirror, whose temperature is controlled by Peltier cooling, detects when water starts to condense on the mirror by means of a change in the reflection of the mirror. This technique assumes that the dissolved salts in the plant are negligible as compared to matric forces, so that the potential can be calculated from the Kelvin equation. Because of the special mode of operation of WAM (Martínez and Cancela, 2011), WAM have not always been classified as a subtype of dewpoint TCP, and some classifications consider this type of sensor as an instrument independent from the early TCP (Villar and Ferrer, 2005). Both types of TCP can be used to perform in situ measurements, but the accuracy of the determinations performed with WAM under non-controlled conditions must be verified. In this case, measurements are destructive.

Isopiestic

Isopiestic TCP are similar to dewpoint sensors (Baughn and Tanner, 1976a). Isopiestic sensors are a modification of Richards-type TCP (Boyer, 1972b,c), whose application has not been much extended (Boyer, 1995). Isopiestic TCP are the only type of TCP that can be used only in plant material (Boyer, 1966; Knipling and Kramer, 1967; Boyer and Poter, 1973; Gollan et al., 1985). In isopiestic TCP, a solution of known potential is placed on a ring containing a thermocouple junction that is enclosed in a thermally insulated container above the sample. If the temperature of the junction is different from the temperature at which the metals are joined to other metals, such as the terminals of a voltmeter, a small voltage is generated that may be related to temperature difference. Any situation in which water evaporates or condenses is interpreted by the TCP as a change in temperature. By performing measurements with solutions of known potentials that are close to the potential of the sample, we can determine the potential of a solution that would produce the same reading as a dry thermocouple, and the potential obtained would show the same value as the water potential of the sample (Mullins, 2001).

Isopiestic TCP is a destructive method recommended for laboratory use under controlled conditions.

Commercial equipments

Despite the initial trend for the use of own manufactured equipment (Spanner, 1951; Ehlig, 1962; Campbell and Campbell, 1974; Dixon and Tyree, 1984), current TCP comprise mainly Dewpoint TCP, wetbulb method TCP (Richards TCP) and hygrometers commercialized by Wescor (Logan, UT, USA) or J.R.D. Merrill Specialty Equipment (Logan, UT, USA), isopiestic TCP manufactured by Isopiestic Co. (Lewes, DE, USA) or, alternatively, WAM developed by Decagon Device, Inc.
Main applications of TCP on plant material

Soil and atmosphere moisture conditions are important because they affect plant transpiration rate and absorption rate. The result of the interactions of these factors, which involve a better soil-plant relationship, is plant water balance. The most interesting aspect of these relationships may be represented by water deficit in plant tissues insofar as water deficit in plant tissues directly affects the physiological and biochemical processes that control plant growth (Gounot and Monteny, 1967). In this sense, stomatal behaviour and photosynthesis —among other processes— may be affected by the decrease in leaf water potential caused by water stress at any developmental stage of the plant (Rodrigues et al., 2003). Therefore, water potential in leaves and other plant tissues is usually measured with TCP. Measurements have been conducted more frequently in leaves, but water potential has also been measured in roots, trunks, branches, fruits or the whole plant (Jobling et al., 1997). The material used as a sample may be collected from the plant. However, non-destructive methods can be used, and in situ measurements can be performed using TCP that have been designed for that purpose (Ehret et al., 2001).

Measurement of leaf water potential

The first study that addressed measurements of leaf water potential was carried out by Ehlig (1962), who measured osmotic potential and diffusion pressure deficit in individual leaves by using a slightly modified Richards-type TCP in greenhouses. Ehlig (1962) presented TCP as a method that allowed for the determination of osmotic potential and diffusion pressure deficit with a measurement range 0.33-1.13 MPa requiring a single leaf and short preparation time. At that time, it was impossible to determine water potential in growing parts of the plant. Lambert and Schilfgaarde (1965) suggested a measurement method based on a psychrometer probe and a sample chamber that enabled measurements of intact plant material. Their study was further improved and applied to tobacco (Nicotiana tabacum) plants by Hoffman and Splintern (1968), who used a psychrometer probe similar to the instrument used by Rawlins and Dalton (1967). The results of their study evidenced the strong influence of factors such as the location of measurements in the leaf, relative air humidity and diurnal variations in the relationship...
between soil and water potential. Such a relationship was found to be non-linear, and it was observed that a gradient was present in leaves from the base to the periphery. Campbell et al. (1966) refined this conclusion and affirmed that leaf water potential was maintained at the periphery and decreased towards the midrib and the petiole.

To obtain accurate measurements that are not affected by various factors, Boyer (1966) suggested the isopiestic technique, and applied this technique to sunflower (Helianthus annuus) leaves using tissue under equilibrium and non-equilibrium conditions. He demonstrated that the technique provided highly repeatable results under variable experimental conditions (Boyer, 1972c).

Barrs and Kramer (1969) conducted measurements in leaves of different plant species grown in greenhouses and observed that the potential measured in sliced tissue yielded a value that was higher than the potential measured in unsliced tissue. They explained that the cause for such results was the effect of sap and of the vacuolar cytoplasm released by cut cells, which was accumulated by the surrounding intact cells. Such an accumulation caused an increase in volume and in pressure potential that exceeded the decrease in osmotic potential caused by solute uptake. However, they concluded that this effect could be incorrect if too small tissues were used.

Millar et al. (1970) studied the importance of leaf excision as compared to in situ measurements in onions (Allium cepa). In situ measurements differed 0.05 to 0.1 MPa from measurements performed in leaves, and erratic measurements were obtained in the morning and in the evening because of rapid heating in early morning and rapid cooling in late evening. In order to avoid temperature changes between the sample and the TCP chamber, the sensors were introduced in the sample in both cases. Because this process could not be conducted in samples formed by thin plant material such as leaves, Hoffman and Rawlins (1972) suggested a silver-foil TCP that demonstrated that the temperature difference between the sensor and the sample was low enough to allow for in situ leaf water potential measurements. They conducted experiments in tomato (Solanum lycopersicum) and sunflower plants, and observed the response of the sensor to diurnal temperature changes and to changes in water potential, which increased under irrigation.

Other authors such as Brown and McDonough (1977) performed in situ measurements in leaves using TCP to observe the effects of water availability. Machado and Paulsen (2001) measured water potential in leaves of wheat (Triticum aestivum) and sorghum (Sorghum bicolor) to study the effects of drought and high temperatures as yield limiting factors. Auge et al. (1998) observed the foliar dehydration tolerance and Liu et al. (2003) measured, among other factors, stomatal conductance and leaf water potential in sand rice (Atriphyllum squarrosum) using a WAM, and observed that the increase in soil water content significantly affected the physiological traits analyzed, while the change in the photosynthesis was delayed as compared to changes in water potential. Starting the current trend for the use of WAM, Niu et al. (2004) studied other aspects such as gas exchange and chlorophyll fluorescence response to simulated rainfall in plants under water stress by measuring leaf water potential. Other authors such as YuBing et al. (2007), Xu and Zhou (2008), and Li et al. (2010) used WAM in their research, but the suitability of the use of this type of TCP in the field has not been verified.

Measurements of water potential in stems, fruits, roots and seeds

Measurements of stem water potential enable the analysis of crop growth. The studies conducted by Michel (1977) and Dixon and Tyree (1984) using stem hygrometers with temperature correction, and the study carried out by Dixon et al. (1984) using a hygrometer are some examples of water potential measurement in stems. Vogt (2001) measured seasonal differences in stem water potential in two shrub species using a stem psychrometer, and Nonami and Boyer (1993) verified the occurrence of a water potential gradient in growth regions of soybean stems, and concluded that a radial gradient was present in the growth region that formed a water potential field in three dimensions around the xylem.

In addition, measurements can be performed in the fruits of the various species. Bower (1985) observed the effect of long-term irrigation on avocados (Persea americana) and the consequences of that effect on fruit ripening and physiology. Johnson et al. (1992) combined water potential measurements in stems and fruits of tomato plants during several days using automated TCP. They observed the presence of a strong correlation between the stem-fruit water potential gradient and the changes in diameter. They concluded that low
Some researchers have performed water potential measurements in plant roots. Such measurements are useful in the analysis of water transport processes in the soil-plant system. The determination of water potential in roots and in the adjacent soil reflects the ability of plants to extract water from soil and provides information about the limits of such an ability, and about root resistance to water flow. Different authors have been concerned with this issue (Martre, 1999; Zou et al., 2000). Ficus (1972) determined water potential in corn (Zea mays) roots and in the adjacent soil, and observed good correlations between both potentials, obtaining more negative values for roots than for soil. By applying irrigation, water potential became less negative in both cases, and leaf resistance decreased. Oosterhuis (1987) evaluated the changes in root water potential for cotton (Gossypium hirsutum). He observed that osmotic adjustment occurred as a response to water tension, which allowed for continuous growth of the plant through cell turgor maintenance, even under certain drought conditions.

Measurement of water potential in seeds is not frequent, but Daws et al. (2004) studied the effect of heat during seed development on the physical, physiological and biochemical traits of Horse-chestnut (Aesculus hippocastanum) seeds. Cavalieri and Boyer (1982) measured water potential in intact dark-grown soybean (Glycine max) hypocotyls, and suggested the presence of a longitudinal water potential gradient. Daws et al. (2006) focused on the study of WAM.

Continuous measurement of water potential

The capability to monitor water potential in plants that undergo dynamic changes in water status is particularly interesting. However, such a capability has seldom been described in reports because recording frequent measurements at inconvenient times is constrained by manpower resources. Yet, monitoring plant water potential is necessary to better understand the causes of the differences in yield between plants that grow in different soils, and to improve irrigation requirements (McBurney and Costigan, 1988). Brunini and Thurtell (1982) demonstrated that the response of a hygrometer to monitor changes in water potential in a soil-plant system under dynamic conditions was very good. These authors showed that the difference found in water potential between leaf and soil after a dark period appears to be related to plant growth or to unequal distribution of moisture in soil.

The first authors who monitored oscillations of water potential in plant stems were McBurney and Costigan (1984). Because plant transpiration is induced by changes in plant water potential, these authors aimed at finding a relationship between such oscillations and plant transpiration. The study was conducted in Brussels sprouts (Brassica oleracea) subjected to controlled temperature and to a range of evaporative demands, high vapour pressure deficit and low light intensity. With the reduction in light intensity, stem water potential immediately increased (became less negative) and transpiration rate decreased. These authors further developed their studies by applying a water-jacketed stem-attached psychrometer to the same plant species (McBurney and Costigan, 1987). Turner et al. (1984a, 1985) and Gollan et al. (1985) focused their attention on this approach. Turner et al. (1984a) used an in situ TCP to study the response induced in photosynthesis, transpiration and leaf conductance by changes in vapour pressure deficit. First, the study was conducted for both herbaceous and woody species and, later, the study was repeated for herbaceous species (Turner et al., 1985). Gollan et al. (1985) went further in these studies and observed the relationships between vapour pressure deficit, leaf water potential, soil water content and leaf gas exchange in a woody species.

In addition, continuous monitoring of water potential is required to better understand the causes of differences in yield between plants that grow in different soils and to improve irrigation requirements in crops, as suggested by Schaefer et al. (1986).

To perform continuous measurements of water potential, adapted TCP must be used. New measurement technologies such as WAM are extractive, destructive methods that do not allow for continuous monitoring. WAM measurements can be monitored at specific moments throughout the season, but cannot be performed in the same plant material.

Determination of accuracy: comparison with other types of equipment

The convenience of choosing a particular type of TCP from among all the models available depends —among other aspects— on the measurement range of the instrument. The upper and lower measurement limits are dependent on the design of the sensor, the
measurement protocol, and the resolution of the voltmeter used. The upper limit is set at –0.03 to –0.2 MPa (Andraski and Scanlon, 2002), while the lower limit varies for each type of sensor.

The lower limit of water potential measurements is –300 MPa for Richards-type TCP and –8 MPa for Spanner-type TCP. Mullins (2001) reported a measurement range of 0 to –300 MPa (5-10%) for the Richards type and 0 to –7 MPa (5-10%) for the Spanner type, but such limits are rather vague and some authors disagree with these measurement ranges. Gee et al. (1992) limited the measurement range at 0 to –200 MPa for Richard-type sensors and –0.2 to –8 MPa for Spanner-type sensors.

The measurement range of hygrometers is 0 to –40 MPa (100 kPa) (Mullins, 2001), while Scanlon et al. (1997) established the measurement range of WAM at 0 to –312 MPa. However, the possibility of obtaining values near 0 MPa is limited by the accuracy of the device and by the relationship between temperature and relative humidity.

The measurement range of isopiestic TCP is 0 to –40 MPa (10 kPa) (Mullins, 2001).

The values reported above are theoretical and guidance values that may vary according to operating conditions, which questions the reliability of water potential measurements. Hence the need for studies that verify the variability of measurements using a different type of TCP or a different technique as a reference.

### Comparison of psychrometric methodologies per se

Usually, different methodologies are compared in order to verify that the instrumentation used in the research work is the most suitable instrumentation. Within TCP, different types of sensors have been compared, as shown in Table 2.

For nonequilibrium psychrometers, Barrs (1964) compared the results obtained in leaves using both a

<table>
<thead>
<tr>
<th>TCP type</th>
<th>Species used</th>
<th>Method</th>
<th>Comparative method</th>
<th>Empirical relationship</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sp</td>
<td>Pelargonium zonale</td>
<td>P</td>
<td>R</td>
<td>( \Psi(\text{R}) &lt; \Psi(\text{Sp}) )</td>
<td>Barrs, 1964</td>
</tr>
<tr>
<td>Sp</td>
<td>Helianthus annuus</td>
<td>P</td>
<td>R</td>
<td>( \Psi(\text{R}) = \Psi(\text{Sp}) )</td>
<td>Zollinger et al., 1966</td>
</tr>
<tr>
<td>I</td>
<td>Taxus cuspidate</td>
<td>I</td>
<td>PC</td>
<td>( \Psi(\text{PC}) \pm 2 \text{ bar} = \Psi(\text{I}) ) (1 and 2)</td>
<td>Boyer, 1967</td>
</tr>
<tr>
<td>I</td>
<td>Helianthus annuus</td>
<td>(1)</td>
<td></td>
<td>( \Psi(\text{I}) - 4 &lt; \Psi(\text{PC}) &lt; \Psi(\text{I}) + 2.5 ) (2)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Rhododendron roseum</td>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Liriodendron tulipifera</td>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Cornus florida</td>
<td>I</td>
<td>DM</td>
<td>( \Psi(\text{R}) = 1 - 5 \Psi(\text{DM}) ) (0-30 bar)</td>
<td>Knipping and Kramer, 1967</td>
</tr>
<tr>
<td>I</td>
<td>Oxycodrum arboreum</td>
<td>I</td>
<td></td>
<td>( \Psi(\text{D}) = \Psi(\text{PC}) )</td>
<td>Wiebe et al., 1970</td>
</tr>
<tr>
<td>D</td>
<td>Liriodendron tulipifera</td>
<td>H</td>
<td>PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Ulmus americana</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Ulmus pumila</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Elaeagnus angustifolia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Hacer glabrum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt</td>
<td>Solanum lycopersicum</td>
<td>P</td>
<td>PC</td>
<td>( \Psi(\text{PC}) = -0.456 )</td>
<td>Duniway, 1971</td>
</tr>
<tr>
<td>Pt</td>
<td>Diseased plants:</td>
<td></td>
<td></td>
<td>( \Psi(\text{PC}) = -0.51 \pm 0.20 )</td>
<td></td>
</tr>
<tr>
<td>Pt</td>
<td>Healthy plants:</td>
<td></td>
<td></td>
<td>( \Psi(\text{PC}) = -0.57 \pm 0.23 )</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Solanum lycopersicum</td>
<td>I</td>
<td>R and Sp</td>
<td>( \Psi(\text{Sp}) ) and ( \Psi(\text{R}) &lt; \Psi(\text{I}) ) (1)</td>
<td>Boyer, 1972a</td>
</tr>
<tr>
<td>I</td>
<td>Magnolia grandiflora</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Phiodendron hastatum</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values reported above are theoretical and guidance values that may vary according to operating conditions, which questions the reliability of water potential measurements. Hence the need for studies that verify the variability of measurements using a different type of TCP or a different technique as a reference.
Table 2 (cont.). Comparison of the application of different methodologies in plant material

<table>
<thead>
<tr>
<th>TCP type</th>
<th>Species used</th>
<th>Method</th>
<th>Comparative method</th>
<th>Empirical relationship</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>Zea mays</td>
<td>H</td>
<td>BG</td>
<td>(\Psi (D) = \Psi (BG))</td>
<td>Neumann and Thurtell, 1972</td>
</tr>
<tr>
<td>Sp</td>
<td>Prosopis glandulosa</td>
<td>—</td>
<td>PB or DM</td>
<td>Better Sp</td>
<td>Easter and Sosebee, 1974</td>
</tr>
<tr>
<td>D</td>
<td>Solanum tuberosum, Helianthus annuus, Capsicum annuum, Glycine max, Avena sativa «Lodi»</td>
<td>H</td>
<td>PC</td>
<td>—</td>
<td>Baughn and Tanner, 1976a</td>
</tr>
<tr>
<td>D</td>
<td>Glycine max, Triticum aestivum, Hordeum vulgare</td>
<td>H</td>
<td>I</td>
<td>(\Psi (D) &gt; \Psi (I)) for different plants; (\Psi (D) = \Psi (I)) for the same plant</td>
<td>Nelsen et al., 1978</td>
</tr>
<tr>
<td>L</td>
<td>Citrus jambhiri</td>
<td>—</td>
<td>PC</td>
<td>(\Psi (LP) = \Psi (PC)) (light abrasion); (\Psi (LP) &gt; \Psi (PC)) (coarse abrasion)</td>
<td>Savage et al., 1983</td>
</tr>
<tr>
<td>IP</td>
<td>Glycine max, Thuja occidentalis</td>
<td>P</td>
<td>PC and Sc</td>
<td>(\Psi (IP) &gt; \Psi (PC)) and (\Psi (Sc))</td>
<td>Oosterhuis et al., 1983</td>
</tr>
<tr>
<td>D</td>
<td>Medicago sativa</td>
<td>H</td>
<td>PB</td>
<td>(\Psi (D) &lt; \Psi (PB)) (without temperature correction)</td>
<td>Dixon and Tyree, 1984</td>
</tr>
<tr>
<td>LH/LP</td>
<td>Helianthus annuus, Triticum aestivum, Vigna unguiculata, Nerium oleander, Pistacia vera, Corylus avellana</td>
<td>H/P</td>
<td>PC</td>
<td>(\Psi (LH/LP) = \Psi (PC))</td>
<td>Turner et al., 1984b</td>
</tr>
<tr>
<td>Sp</td>
<td>Medicago sativa, Glycine max, Zea mays</td>
<td>—</td>
<td>PC</td>
<td>(\Psi (LP) &lt; \Psi (PC)); (\Psi (LP) &gt; \Psi (PC))</td>
<td>Bennett et al., 1986</td>
</tr>
<tr>
<td>D</td>
<td>Brassica oleracea</td>
<td>H</td>
<td>PC</td>
<td>Psychrometer without jacket: (\Psi (D) &lt; \Psi (PC)); Psychrometer with jacket: (\Psi (D) = \Psi (PC))</td>
<td>McBurney and Costigan, 1987</td>
</tr>
<tr>
<td>IP</td>
<td>Tradescantia virginiana</td>
<td>—</td>
<td>PRP</td>
<td>(\Psi (IP) = \Psi (PRP))</td>
<td>Shackel, 1987</td>
</tr>
<tr>
<td>D</td>
<td>Cicer arietinum</td>
<td>H</td>
<td>PC</td>
<td>(L = 0.45P^3 + 0.75P^2 + 0.67P - 0.34); (r^2 = 0.68) (A); (L = 1.02P + 0.11); (r^2 = 0.84) (B); (L = 1.19P + 0.23); (r^2 = 0.9) (C); (L = 0.23P^3 - 0.50P^2 - 0.89P - 0.54); (r^2 = 0.90) (D)</td>
<td>Turner et al., 2000</td>
</tr>
<tr>
<td>EW</td>
<td>Chlorophytum comosum, Zea mays subsp. mays, Gossypium hirsutum</td>
<td>P</td>
<td>VPO</td>
<td>(\Psi (EW) = 1.052 \Psi (VPO)); (\Psi (EW) = 1.030 \Psi (VPO)); (\Psi (EW) = 0.842 \Psi (VPO))</td>
<td>Ball and Oosterhuis, 2005</td>
</tr>
</tbody>
</table>

1 D: dewpoint; R: Richards; Sp: Spanner; I: isopiestic; Pt: Peltier; IP: in situ psychrometers; LP: leaf psychrometers; LH: leaf hygrometers; EW: end-window psychrometer. 2 Measurement method: P: psychrometric; H: hygrometric; I: isopiestic. 3 PC: pressure chamber; DM: dye method; BG: beta gauge; PB: pressure bomb; PRP: pressure probe; Sc: screen-caged psychrometer; VPO: vapour pressure osmometer. 4 (A) the cutter of the psychrometer; (B) a new pair of razors; (C) a disposable biopsy punch; (D) a biopsy punch, where the leaf was subsequently damaged with a nail punch.
Richards-type sensor and a Spanner-type sensor. He observed that the Richards-type sensor was affected by temperature changes in the chamber, with estimated variations of 0.18 MPa as compared to the Spanner-type sensor, and concluded that the Spanner-type sensor was not affected by slight temperature changes in the chamber. Conversely, Zollinger et al. (1966) used both types of TCP but did not find significant variations in sunflower leaves.

Variations found within the same type of TCP can be due to the number of terminals considered in TCP design, as reported by Millar et al. (1970), who compared the results obtained using a four-terminal TCP and a two-terminal TCP, and observed a change in the output of 0.5–1 µV °C⁻¹ for the four-terminal TCP, as compared to 13 µV °C⁻¹ obtained with the two-terminal TCP.

When new measurement techniques are developed, the tuning and verification of such techniques is usually carried out against other techniques. When isopiestic techniques were first developed, Boyer (1972a) compared isopiestic TCP with Richards-type and Spanner-type TCP by applying the instruments to different plant species. He verified that the isopiestic technique was not affected by the frequent problem of resistance to vapour diffusion in tissues, which produced errors of 2.5–7.5% for Spanner-type TCP and 4.5–12% for Richards-type TCP. Nelsen et al. (1978) continued these studies and performed measurements in leaves of soybean and cereals using a dewpoint hygrometer and an isopiestic TCP. The results obtained with both sensors were similar for soybean, but too high potentials were obtained for cereals with the hygrometer, with poor agreement between the two techniques, in the range –0.5 to –1.5 MPa. Because the sampling techniques were different, the authors attributed such a poor agreement to sample size, and recommended using a large sample in order to avoid anomalous results caused by the effect of damaged cells. These conclusions corroborated the results reported by Barrs and Kramer (1969). The studies presented above reveal the need for the use of the same type of sample under the same conditions. Yet, the accuracy of the determinations is dependent on the design characteristics of the device and on the characteristics of the measurement technique used.

Comparison of psychrometry with other methodologies

The analysis of results can be carried out by comparing the results obtained using sensors based on different methodologies (Table 2), such as pressure chamber, pressure probe, dye method, beta gauge or vapour pressure osmometer.

Using a pressure chamber, Boyer (1967) determined water potential in leaves of yew (Taxus cuspidata), rhododendron (Rhododendron roseum) and sunflower. As compared to isopiestic TCP, values of ±0.2 MPa were obtained for sunflower and yew. The results obtained for rhododendron using the pressure chamber ranged from 0.25 MPa (less negative) to 0.4 MPa (more negative). Boyer attributed such a lack of agreement to the effect of refilling tissues different from the xylem with xylem sap during measurements. Conversely, Wiebe et al. (1970) compared the results obtained using an in situ pressure chamber and a laboratory dewpoint TCP, and obtained good agreement between in situ and laboratory results.

Using a Peltier TCP, Duniway (1971) determined the relationship between the measurements performed with a pressure chamber and a TCP in plants of tomato. According to his observations, the results obtained with the TCP might be misleading because the value of the water potential measured showed dependent on the method used to sample the plant tissue (how the plant was cut) and on possible water movement. For all these reasons, he concluded that pressure chamber might be more suitable than TCP for these types of measurements.

Baughn and Tanner (1976a) compared hygrometric measurements with measurements obtained using a TCP and a pressure chamber in potato (Solanum tuberosum), pepper (Capsicum annum), soybean, sunflower and oats (Avena sativa). For high (wet) ranges of water potential, the values obtained with the pressure chamber were lower (drier) than the values obtained with the hygrometer (from 0 to –1.5 MPa). For drier ranges of water potential, the highest values were obtained using the pressure chamber (from –0.5 to –1.5 MPa). They suggested that hygrometers are more reliable than TCP and recommended the use of hygrometers as a method for chamber calibration. Brown and Tanner (1981) applied both sensors to alfalfa (Medicago sativa) leaves for the first time, and obtained higher values with the hygrometer than with the pressure chamber in different alfalfa plants in a range –0.15 to –1.52 MPa. However, the results were similar for measurements performed in the same plant. These results contradicted the results obtained for Brussel sprouts by McBurney and Costigan (1987), who used a water-jacketed stem-attached psychrometer and obtained good agreement between both methodologies.
Sometimes, the lack of agreement stems from the protocol used to remove the leaf cuticle before the measurement is made. Savage et al. (1983) compared the results obtained with an in situ TCP and a pressure chamber in young plants of Citrus jambhiri. Leaves were abraded using two techniques: light abrasion and coarse abrasion. The results of light abrasion were statistically equal to the results obtained with the chamber, but coarse abrasion caused wetter potentials when TCP were used.

The sampling protocol must be appropriate. Wright et al. (1988) found that the pressure chamber over-estimated leaf water potential by an average of 0.4 MPa over the range −0.5 to −5.0 MPa. They suggested that the pressure chamber technique might be appropriate in comparative studies of peanut water stress. However, where absolute measurements are required, as in the calculation of leaf turgor potential, a correction factor should be applied or, preferably, the TCP technique should be used. These results were verified by Turner et al. (2000), who assumed that discrepancies occurred because the TCP damaged the leaves and substantially modified leaf tissue (as reported by Barrs and Kramer in 1969). Therefore, Turner et al. (2000) recommended collecting the sample using a new razor blade. In addition, they suggested that the chamber that contained the sample be humidified in order to avoid loss of water. The samples were individually inserted into plastic bags to avoid any gradient associated to transpiration, as suggested by Shackel (1987).

Leaves are not the only plant material for which measuring instruments were compared. Dixon and Tyree (1984) applied a Scholander pressure chamber and a hygrometer in stems. Hygrometric measurements differed from pressure chamber measurements between 0.2 and 1 MPa. Turner et al. (1984b) compared TCP measurements using the psychrometric technique with pressure chamber measurements. Results were variable for morphologically different species. In some cases, the correlation between both techniques was strong, but higher or lower values were obtained in other cases. They attributed these results to the influence of low epidermal conductance (leaf cuticle was abraded to increase conductance) or to the occurrence of large water potential gradients within the same sample (leaves, in this case). They concluded that in situ TCP were suitable for monitoring changes in leaf water potential, but recommended caution in case of low epidermal conductance. Oosterhuis et al. (1983) used two different types of TCP (leaf psychrometer and screen-caged psychrometer) and pressure chamber for in situ measurement of soybean water potential; Bennett et al. (1986) used a Spanner-type TCP to measure xylem water potential and osmotic potential; and Hardegree (1989) applied TCP to Pinus ponderosa. More recently, Rodrigues et al. (2003) compared leaf water potential measurements with pressure chamber and TCP at different developmental stages of wheat.

Another method used was the beta gauge, which was compared to the hygrometric technique by Neumann and Thurtell (1972), who obtained similar results for both methods.

At the beginning of psychrometric studies, the dye method was used by Knipling and Kramer (1967) and compared to TCP by applying both techniques to leaf water potential measurements. Contrary to common practice, psychrometry was not compared to another reliable methodology, and the TCP was taken as reference method. Knipling and Kramer (1967) used a modification of the Richards-type TCP with the isopiestic technique (Boy er, 1966), and observed that both methods differed 0.1-0.5 MPa for a range of water potentials from 0 to −3 MPa. At extreme potentials (very negative or close to zero), the dye method yielded too low values, while at intermediate potentials, the dye method yielded very high (less negative) values. The cause for such a discrepancy was based on the presence of contaminants in the sample, which avoided appropriate osmotic exchange with the thin leaf tissue. Easter and Sosebee (1974) identified an advantage of Spanner-type TCP as compared to pressure chamber or the dye method: TCP allow for measurements in plant roots, trunks, or branches of different sizes, while the use of the other methods is limited and dependent on the size of the branch or leaf.

A different methodology was used by Shackel (1987), who compared the performance of a TCP with the performance of a pressure probe on Tradescantia virginiana and determined that the error that was most difficult to control during measurements was the lack of thermal equilibrium in TCP. The test conducted by Shackel (1987) was the first direct comparison between in situ measurements in tall plants and methods for measuring potential in cells and tissues.

The methods for the determination of osmotic potential have seldom been compared. Ball and Oosterhuis (2005) determined osmotic potential using an end-window psychrometer and a vapour-pressure osmometer in roots and leaves of various species.

Recently, Busso (2008) has highlighted the greater accuracy of TCP as compared to Scholander pressure
chamber, the most common technique to measure water potential in plant material, and defined TCP as «a convenient, accurate and reliable method in the determination of water potential if good sampling techniques are used and appropriate precautions are taken». The studies reviewed in this paper support this statement. The accuracy of the device is questioned by poor handling, by the performance of measurements under adverse environmental conditions or by the lack of consistency or diversity of the protocol considered. If the appropriate precautions are taken and comparisons are performed under the same conditions, conclusive results are obtained for the verification of the accuracy of the device. In addition, the selection of the most suitable equipment is determined by the type of material measured, the testing conditions (in situ or laboratory measurements) and the accuracy required, which is inherent to the device.

Handling difficulties: main errors

TCP are extremely useful for measuring water potential in different types of samples if the appropriate precautions are taken. Each TCP is unique and, therefore, requires individual calibration. The reliability of the data obtained depends on correct calibration. The conditions under which actual measurements are performed must be reproduced as accurately as possible during the calibration process, and the protocol used must be consistent with the protocol used for actual measurements. Savage and Cass (1984b) suggested that calibration should be conducted ensuring that there are no thermal gradients within the instrument.

In addition, equilibration time must be considered in the determination of water potential in plant material. It is essential that the time for vapour equilibration between the sample and the air around the sample is adequate because allowing for adequate equilibration time is critical in order to obtain reliable results. When equilibration time is not adequate, apparent water potential is too dry. In plant material, the time required to achieve vapour pressure equilibrium between the plant material and the chamber environment varies by controlling tissue hydration. Equilibration time decreases with decrease in water deficit (Gounot and Monteny, 1967) and is dependent on resistance to water vapour diffusion, which is a limitation in leaf samples with waxy surfaces (Joly, 1985). Too long equilibration times are not desirable in leaf samples because such long times could allow for changes in the analyzed tissue (Barrs, 1965; Peck, 1969). Lambert and van Schilfgaarde (1965) suggested reducing equilibration times by illuminating the sample with low fluorescent light in order to maintain stomata open and accelerate equilibration. Cuticle removal by light abrasion facilitates water vapour exchange in plant tissue and accelerates equilibration time (Campbell and McInnes, 1999), which helps minimize the changes that occur inside the reading chamber (for laboratory TCP). Manoharan et al. (2010) rate the average equilibration time required at approximately 60 minutes.

Any modification of the ideal design or the ideal environmental conditions may cause significant changes in TCP sensitivity. However, such changes do not necessarily lead to systematic errors in measurements. Many authors have tried to detect, assess and solve the errors that may arise during sensor operation when used with plant samples. Some of the studies were carried out in the 1970s and 1980s (Hisieh and Hungate, 1970; Baughn and Tanner, 1976b; Savage and Cass, 1984a; Shackel, 1984; Wullschleger et al., 1988), but this issue has been studied from the beginnings of psychrometry (Waister, 1965; Boyer, 1969).

Some of the problems were caused by the heat produced during respiration (Barrs, 1964; Barrs and Kramer, 1969), the absorption of vapour on the walls of the chamber containing the sample (Lambert and Schilfgaarde, 1965), and leaf resistance to vapour transfer (Rawlins, 1964; Zanstra and Hagenzieker; 1977), which led to inaccuracies in the results obtained. In order to mitigate all these problems, Boyer (1966) described the isopiesometric technique. However, other problems were found, such as the effect of sample size (Walker et al., 1984; Bennett and Cortes, 1985), the position of in situ sensors (Easter and Sosebee, 1974) or the transportation of the sample from the field to the laboratory (Walker et al., 1983).

Assuming that TCP are the only non-destructive method available to measure water potential in the field, some general indications for TCP handling that guarantee the reliability of the determination are proposed.

For measurements made in the field, temperature fluctuations must be minimized using materials with high thermal conductivity (Campbell, 1985), such that leaf psychrometer watertightness is achieved by sealing the chamber with wax or petroleum jelly (Neumann and Thurtell, 1972). The effect of the temperature gradient was reported by Easter and Sosebee (1974), who suggested the convenience of installing the TCP inside
the trunk, sealing the hole, avoiding direct exposure to sunlight, and placing the sensor longitudinally and obliquely (Wiebe and Brown, 1979). Because of the interaction between water potential and temperature correction, a correction factor that changes linearly with temperature has been suggested. Errors can be corrected within a margin of ± 0.04 MPa by applying a correction equation proposed by Wullschleger et al. (1988) or Comstock (2000) across a range of 15-35°C.

The criteria for TCP selection affect measurement reliability and are a source of different types of errors. Selecting destructive or non-destructive measurements is a key element. Baughn and Tanner (1976b) analyzed the different causes of error in five herbaceous species. They observed that water potential in tissue can change due to different factors, such as sample dryness, xylem water tension, or variation of the inner tissue due to anoxia, cell expansion or other metabolic effects. These authors considered that errors occurred when tissue was extracted from the plant and analyzed, and that such errors might be considerable at wet potentials. Boyer et al. (1985) reported an error of 0.05-0.1 MPa as compared to in situ measurements. Such an error was caused by tissue growth after removal from the plant.

Because the importance of the sample was clear, further studies were carried out to observe whether the size of the sample was related to the measurements obtained. Walker et al. (1984) studied the ratio of cut surface area to sample volume in soybean leaves, and observed that the water potential measured decreased with the decrease in sample size, i.e. with the increase in the ratio. They suggested the use of larger leaf samples (with a lower ratio) to avoid the loss and dryness of the sample, which would produce measurements unrepresentative of the actual leaf water potentials. According to the suggestions made by these authors, the leaves should be transported in plastic bags inside a wet chamber in order to prevent transpiration. The mentioned study was further defined by Bennett and Cortes (1985). They suggested that, while the errors caused by water adsorption on the TCP chamber may be significant for small volumes of tissue, the same errors can be insignificant if a sufficient amount of tissue is used. The problems of water adsorption could have generated low values of water potential in previous studies. In the case of Walker et al. (1984), such problems were attributed to the ratio of cut surface area to sample volume. More recently, Brown and Oosterhuis (1992) recommended avoiding evaporative loss during sample collection, and detecting and assessing temperature gradients. According to Savage and Cass (1984a), the influence of temperature accounted for an error of 8-10% for temperatures below 20°C, while the error decreased to 4% for higher temperatures. Errors caused by evaporative loss during leaf collection can be solved by using a sampler that can be sealed (Brown, 1969).

Conclusions and future perspectives

The use of psychrometry in plant material has been widely described in the literature. TCP have been characterized by identifying and quantifying the main errors occurred during their application under controlled (in the laboratory or in greenhouses) or uncontrolled (in the field) conditions. Initially, such errors were solved by designing various devices, whose use was not extended in time. Yet, these devices were used as a basis for the development of the currently available commercial TCP. Today, the use of TCP is limited to a small number of commercial models. TCP measurements performed in the field and particularly in the laboratory under favourable and stable conditions are standardized. Yet, the errors found in the application of the earliest types of TCP are still detected because of the difficulty in the control of environmental parameters that strongly affect measurements, such as temperature, evaporation, or radiation, and because of the changes observed in the sample inside the reading chamber of some types of TCP. Because TCP measurements are reliable, current research focuses on the applicability of the psychrometric technique rather than on the verification of the goodness of the determinations.

The studies that have been performed in plant material and different plant tissues for decades support the usefulness of TCP. TCP are used mainly in leaves because leaves are easily transported to the laboratory. Psychrometry is a destructive technique but produces reliable water potential measurements that can be compared with measurements obtained with other techniques used for the same purpose. Yet, the applicability of TCP in the field is more questionable.

In the last decade, the increasing trend for the diversification of the types of TCP has caused a growing use of WAM against traditional TCP and against the Scholander pressure chamber, which was considered the reference method for the determination of water potential in leaves. WAM can be used in many types of samples, but these sensors are very sensitive to variations in moisture content and temperature. WAM
involve an extractive and destructive method, and their use is still limited. Some studies conducted with WAM in USA, Singapore, Germany, Portugal and Spain using soil samples suggest the advisability of controlling moisture content and temperature in plant material. Moreover, the use of WAM in field conditions has not been verified. For this reason, TCP will continue to be used in the near future to measure water potential in plant material, both in the field and in the laboratory. In addition, psychrometry is the only non-destructive method for the determination of water potential in plant material. Yet, future research should be aimed at verifying WAM measurements in plant material with a defined protocol and a control of the parameters that affect measurements because such factors can alter the determination of water potential, both in the field and in the laboratory. The verification of WAM measurements would result in a wider diffusion and use of WAM in the near future.

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