Comparison of Water Potentials Measured by In Situ Psychrometry and Pressure Chamber in Morphologically Different Species

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ABSTRACT

Leaf water potentials measured by in situ psychrometry were compared with leaf water potentials measured by the pressure chamber technique at various values of water potential in Helianthus annuus, Helianthus nuttallii, Vigna unguiculata, Nerium oleander, Pistacia vera, and Corylus avellana. In V. unguiculata, the leaf water potentials measured by the in situ psychrometer oscillated at the same periodicity as, and proportional to, the leaf conductance. In all species, potentials measured by in situ psychrometers operating in the psychrometric mode were linearly correlated with potentials measured with the pressure chamber. However, the in situ psychrometers underestimated the leaf water potential in the two Helianthus species at low water potentials and overestimated the water potential in P. vera, N. oleander, and C. avellana. The underestimation in the two Helianthus species at low water potentials resulted from differences in water potential across the leaf. The overestimation in P. vera, N. oleander, and C. avellana was considered to arise from local conductances in these species even after abrasion of the cuticle. Pressure-volume studies with Lycopersicon esculentum showed that less water was expressed from distal than proximal leaflets when the whole leaf was slowly pressurized. The implication of this for water relations characteristics obtained by pressure-volume techniques is discussed. We conclude that in situ psychrometers are suitable for following dynamic changes in leaf water potential, but should be used with caution on leaves with low epidermal conductances.

In situ psychrometers have been commercially available for some years, but have had limited use because of difficulties with sealing, calibration, and temperature and water potential equilibration with the leaf. Recently, Brown and Tanner (5) described procedures and modifications to the commercially available psychrometers that gave reliable observations on Medicago sativa L. under field conditions.

Comparison of water potentials measured by in situ psychrometry with those measured by the pressure chamber have shown a 1:1 relationship in a range of species both in the growth room (6) and in the field (5, 9). Comparisons, however, were usually made using psychrometers in the dew point mode. In the present study, water potentials measured by in situ psychrometers in the psychrometric mode were compared with the water potentials measured by the pressure chamber in a range of mesophytic and xerophytic, herbaceous and woody plants. The influence of resistances to water flow within the leaf, and between the leaf and atmosphere, was studied. Additionally, the ability of the psychrometers to follow the dynamic changes in leaf water potential induced by stomatal oscillation was followed.

MATERIALS AND METHODS

The studies were performed on plants of cultivated sunflower (Helianthus annuus L. cv Suncross 150), wild sunflower (Helianthus nuttallii Torrey and Gray ssp. nuttallii), cowpea (Vigna unguiculata L.), oleander (Nerium oleander L.), pistachio (Pistacia vera L.), hazel (Corylus avellana L.), and tomato (Lycopersicon esculentum) grown in a well fertilized potting soil in a greenhouse. Prior to measurement, the plants were transferred to one of two controlled environment chambers, each with a day/night temperature of 30/25°C, a water vapor pressure deficit of 1 kPa, a quantum flux density of between 600 and 1000 μmol m⁻² s⁻¹, and a 14-h photo- and thermo-period. Unless indicated otherwise, one of six modified L-51 Wescor (Wescor Inc., Logan, UT) leaf hygrometers/psychrometers was attached to the adaxial surface of a leaf and connected to a Wescor PS-625 psychrometer scanner set to provide an 8 mamp cooling current for 10 to 15 s. In some studies, the PS-625 psychrometer scanner was replaced with a Wescor HR-33T microvoltmeter, so that the in situ psychrometers could be read in both the dew point and psychrometric modes and the cooling time and reading time varied. Each L-51 hygrometer/psychrometer was modified by replacing the metal thermal block of rectangular cross section with a cylindrical block of similar material and size which could be more easily insulated as recommended by Brown and Tanner (5). Prior to use, each psychrometer was calibrated against NaCl solutions by vapor equilibration. To reduce equilibration times, the leaf cuticle was abraded by carefully rubbing the epidermis exposed to the chamber with 500 grit carborundum. In H. annuus and N. oleander, the epidermal conductance before and after abrasion was measured with a LI-1600 Li-Cor (Li-Cor, Inc., Lincoln, NE) steady state porometer. The leaf was then washed with distilled H₂O and dried before the psychrometer was attached and sealed with a 50:50 mixture of paraffin wax and anhydrous lanolin.

Several experiments were conducted. (i) A V. unguiculata leaf was enclosed in a leaf cuvette (10) within the controlled environment chamber, and the leaf conductance and transpiration were induced to oscillate by quickly changing the vapor pressure deficit around the leaf. The rate of transpiration and leaf conductance were measured each minute and the leaf water potential of the
same leaf was measured at 5-min intervals with a leaf psychrometer enclosed in the cuvette.

(ii) In all species except L. esculentum, comparisons between the psychrometers and pressure chamber were made at a range of leaf water potentials induced by allowing the soil to dry slowly. At intervals during the drying cycle, the leaf psychrometers were read and removed. Immediately afterwards, the leaf was enclosed in a plastic bag to reduce errors arising from water loss (13) and the leaf water potential was measured by the pressure chamber technique using a pressurization rate of 0.005 M Pa s\(^{-1}\).

(iii) A 20 cm\(^2\) area of H. annuus and a C. avellana leaf was covered in the afternoon with aluminum foil and the foil was sealed to the leaf with transparent tape to prevent transpiration. The following morning, three discs from the covered portion and three discs from an adjacent uncovered portion were punched from the leaf, placed in two cross-calibrated Wescor C-52 chambers, equilibrated for 3 h, and the water potentials were measured.

(iv) Two sensors were attached to the adaxial surface of a H. annuus leaf, one at the edge and the second near the midrib of the same leaf. After equilibration at various leaf water potentials and on leaves of various sizes, the leaf water potential of the two portions of the leaf was measured simultaneously with the psychrometers and immediately thereafter with the pressure chamber.

(v) Pressure-volume curves of L. esculentum leaves were established using the pressure chamber technique (7, 13, 15). The leaves were cut under distilled H\(_2\)O, rehydrated overnight and the volume of sap expressed was determined after the leaves were held at pressures of 0.35, 0.60, 0.75, 0.90, 1.15, 1.35, 1.60, and 2.00 M Pa for 40 min. The balancing pressure after each period of pressurization was measured and used in the construction of a pressure-volume curve from which the apoplastic water content was determined by extrapolation. The fully turgid weight, the weight at the conclusion of pressurization to 2.00 M Pa, and the oven-dry weight of individual leaflets were also measured. The volumetric modulus of elasticity of individual L. esculentum leaflets was also established from the pressure-volume relationships at relative water contents above 95\% (12, 13).

RESULTS AND DISCUSSION

When calibrated against NaCl solutions of known potential, all six psychrometers gave similar outputs of 4.6 m N \(\text{m}^2\) M Pa\(^{-1}\). The responsiveness of the \textit{in situ} psychrometers to changes in leaf conductance is shown in Figure 1. Leaf conductance and leaf water potential measured on the same leaf of \textit{V. unguiculata}. Leaf conductance was measured by gas exchange techniques and leaf water potential with an \textit{in situ} psychrometer.

![FIG. 1. Changes with time in the leaf conductance and leaf water potential measured on the same leaf of \textit{V. unguiculata}.](image)

Leaf conductance (m mol m\(^{-2}\) s\(^{-1}\))

Time (min)

Leaf conductance (m mol m\(^{-2}\) s\(^{-1}\))

Leaf water potential (M Pa)

![FIG. 2. Relationship between leaf water potential (\(\psi\)) and leaf conductance (\(g_l\)) in leaves of \textit{V. unguiculata} in which the stomata were oscillating (\(\bullet\)) or nonoscillating (\(O\)). In the oscillating leaves, the leaf water potential was plotted against the conductance measured 5 min earlier. In nonoscillating leaves the differences in leaf water potential were obtained by exposing the plants to different vapor pressure deficits. The line is the fitted linear regression to the data from oscillating leaves: \(\psi = -0.001 g_l - 0.50 (r^2 = 0.81)\).](image)

![FIG. 3. Relationship between water potential measured by the \textit{in situ} psychrometer and that measured by the pressure chamber for six plant species. For clarity, points are plotted for the two \textit{Helianthus} species (\(C\)) and \textit{Corylus avellana} (\(\bullet\)) only. The dashed line gives the 1:1 relationship and the solid lines give the fitted linear regressions, which had coefficients of determination \((r^2)\) of 0.81, 0.88, 0.90, 0.76, and 0.74 for \textit{Corylus avellana}, \textit{Nerium oleander}, \textit{Pistacia vera}, \textit{Vigna unguiculata}, and the two \textit{Helianthus} species, respectively.](image)

![FIG. 3. Relationship between water potential measured by the \textit{in situ} psychrometer and that measured by the pressure chamber for six plant species. For clarity, points are plotted for the two \textit{Helianthus} species (\(C\)) and \textit{Corylus avellana} (\(\bullet\)) only. The dashed line gives the 1:1 relationship and the solid lines give the fitted linear regressions, which had coefficients of determination \((r^2)\) of 0.81, 0.88, 0.90, 0.76, and 0.74 for \textit{Corylus avellana}, \textit{Nerium oleander}, \textit{Pistacia vera}, \textit{Vigna unguiculata}, and the two \textit{Helianthus} species, respectively.](image)

water potential in \textit{V. unguiculata} oscillated for over 1 h with a periodicity of 35 min; leaf water potential followed changes in leaf conductance with the same periodicity and amplitude, but with a phase shift of 5 min. This phase shift may simply reflect the time constants of the gas exchange and psychrometric systems, or may reflect the time constant of the plant hydraulics. Figure 2 shows that in oscillating leaves the relationship between leaf water potential and leaf conductance was linear when lagged
5 min. A similar relationship was observed in nonoscillating leaves. We conclude therefore that the in situ psychrometer can quickly equilibrate with the leaf and is a suitable instrument for following dynamic changes in leaf water potential.

However, the relationship between the leaf water potential measured by the psychrometers and that measured by the pressure chamber varied with species (Fig. 3). In V. unguiculata, the relationship was close to the 1:1 line, but in H. annuus and H. nuttallii values obtained by in situ psychrometry were lower at low water potentials, than those measured by the pressure chamber technique. Conversely, in P. vera, N. oleander, and C. avellana, the psychrometric values were higher than those observed with the pressure chamber. H. annuus and V. unguiculata are the only two of the six species in which the pressure chamber has previously been compared with the psychrometer (8). In both species, the relationship between the two methods was good, but at low water potentials in H. annuus the pressure chamber gave lower estimates of the leaf water potential than did the thermocouple psychrometer (2), the reverse of that observed in the present study.

One possible reason for the higher water potentials observed in C. avellana, N. oleander, and P. vera with the in situ psychrometers as compared to the pressure chamber is that there may be large resistances to flow within the leaf, resulting in higher water potentials in the shaded zone under the psychrometer than in the adjacent transpiring leaf (5). Resistances to flow within a leaf have been observed in H. annuus (1, 3, 14), but would need to be considerably greater in C. avellana, N. oleander, and P. vera than in H. annuus to account for the differences observed in Figure 3. The study with covered and uncovered portions of a leaf of H. annuus and C. avellana (Experiment iii) was designed to compare the magnitude of the resistance to flow within the leaf of these two species. When the water potential of the covered portion of leaf was −1.2 MPa in both species, the water potential of the adjacent uncovered area was −1.50 ± 0.18 MPa in C. avellana and −1.45 ± 0.06 MPa in H. annuus. The 0.30 and 0.25 MPa difference in water potential between the covered and uncovered portions of leaf in C. avellana and H. annuus, respectively, clearly shows that that water potential of the portion of leaf covered by the in situ psychrometer is likely to be higher than that in the remainder of the leaf, as observed previously in M. sativa (5). However, it also indicates that the resistances to flow within the leaf are similar in the two species and cannot account for the differences among species in water potential between psychrometer and pressure chamber observed in Figure 3. The overestimation of water potential under the psychrometer is likely to be less than the 0.25 to 0.30 MPa observed in this study since the area shaded by the foil was almost three times as great as that shaded by the psychrometer.

The development of differences in water potential across a leaf does, however, appear to account for the lower values of water potential measured by the in situ psychrometer compared to the pressure chamber at low water potentials in H. annuus and H. nuttallii. Measurements by two in situ psychrometers, one located near the edge of a H. annuus leaf and one located near to its midrib (Experiment iv), indicated that when the leaf water potential as measured by the pressure chamber was −1.65 ± 0.06 MPa, the leaf water potential by the psychrometer located near the edge of the leaf was 2.49 ± 0.05 MPa and the one near the midrib was −1.67 ± 0.13 MPa. No differences, however, were observed, in small leaves of H. annuus or in H. annuus leaves at high water potential. We conclude that considerable differences in water potential can develop across a sunflower leaf when stress becomes severe, and that the pressure chamber measures the water potential of cells near to the midrib and not an average for the leaf or the driest portion of a leaf.

When we replaced the PS-625 scanner with the Wescor HR-33T microvoltmeter, we observed that the measured water potentials from the in situ psychrometers were different in the psychrometric mode than in the dewpoint mode in N. oleander. When the leaf water potential was −2.0 MPa as measured by the pressure chamber, the water potential measured in the psychrometric mode was −0.6 ± 0.03 MPa, whereas that in the dewpoint mode was −2.0 ± 0.04 MPa. The observations in the psychrometric mode were made after cooling for 10 to 15 s and measured 10 to 15 s after the cooling was terminated: lengthening the cooling time gradually increased the output so that with a cooling time of 50 s the measured water potential was −1.4 MPa. At all durations of cooling, increasing the delay time for reading reduced the measured output: no point could be detected when the rate of evaporation from the psychrometer was constant. These results are consistent with there being a low conductance to vapor exchange and hence slow equilibration between leaf and psychrometric chamber in N. oleander (18). Boyer and Knipping (4) showed that low conductance to vapor exchange between leaf and psychrometer resulted in values of water potential that were higher than those measured by their isopiestic technique. Presumably during cooling of the thermocouple, the vapor pressure in the chamber is lowered sufficiently to create a considerable vapor pressure gradient that withdraws water vapor from the leaf. When cooling is stopped, however, the low conductance at the leaf surface prevents rapid equilibration between chamber and leaf, resulting in a higher vapor pressure in the chamber than in the leaf and a spuriously high water potential. Although abrading the leaf markedly increased the epidermal conductance in both H. annuus and N. oleander, the conductance of the abraded N. oleander was still only 12% of that in the unabraded H. annuus and was in fact very similar to that in the unabraded H. annuus (Table I). We therefore conclude that the high water potentials observed in C. avellana, N. oleander, and P. vera arise from low epidermal conductances with our standardized abrasion technique. Nevertheless, the fact that the water potential measured by the in situ psychrometers read in the psychrometric mode was linearly related to the water potential measured by the pressure chamber does indicate that they can be used to measure absolute leaf water potentials provided they have been suitably calibrated.

The observation that measurable differences in water potential can exist within a leaf such as H. annuus and that the pressure chamber measured the water potential of the cells near to the midrib suggests that there are regions of the leaf in which the water is more readily removed under pressure and that in a large leaf such as H. annuus the water potential may not come to equilibrium throughout the leaf in the time taken to make a

### Table I. Epidermal Conductance (mmol m⁻² s⁻¹) of the Adaxial Surface of Abraded and Unabraded Leaves of Helianthus annuus and Nerium oleander

<table>
<thead>
<tr>
<th></th>
<th>Abraded</th>
<th>Unabraded</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. annuus</td>
<td>196 ± 33</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>N. oleander</td>
<td>24 ± 11</td>
<td>1 ± 0.6</td>
</tr>
</tbody>
</table>

### Table II. Water Content (% dry weight) of Various Leaflets of Tomato (Lycopersicon esculentum) That Had Been Slowly Pressurized to 2.0 MPa during the Determination of Pressure-Volume Curves and in Unpressurized Controls

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Middle</th>
<th>Outer</th>
<th>Terminal</th>
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<tbody>
<tr>
<td>Pressurized</td>
<td>381 ± 6</td>
<td>452 ± 20</td>
<td>519 ± 2</td>
<td>565 ± 37</td>
</tr>
<tr>
<td>Control</td>
<td>751 ± 18</td>
<td>709 ± 9</td>
<td>648 ± 15</td>
<td>646 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE of the mean of four or eight determinations.
measurement in the pressure chamber. This has implications for the pressure chamber technique for measuring both water potentials and pressure-volume curves. If equilibrium is slow, water potentials will be underestimated by the pressure chamber technique. The implications for the pressure-volume technique were demonstrated in L. esculentum which has a compound leaf with seven major leaflets. After pressurization to 2.0 MPa, 49% of the water was removed from the basal pair of leaflets, 36% from the middle pair, 20% from the distal pair, and 13% from the terminal leaflet when compared to the water in the unpressurized controls (Table II). Clearly, the cellular water was more readily available from the basal leaflets than from those farther from the cut petiole. Since the volumetric modulus of elasticity was similar at 11.3 MPa in all leaflets, the differences in water loss under pressure likely reflect differences in resistances to water flow within the leaf. One consequence of the differential removal of water from the leaf by the pressure chamber technique is the overestimation of leaf apoplastic water content. In the case of L. esculentum it was 54%, an unlikely value arising from the failure to remove water from the terminal leaflets. Tyree and Richter (16) analyzed the errors associated with estimating apoplastic water content from pressure-volume curves, but did not discuss the one observed here. We suggest that allowing the leaf to lose water by evaporation (11, 12, 17) may lead to smaller degrees of differential water loss and overestimation of the apoplastic water content than the widely employed overpressurization technique (7, 13, 15).

We conclude that in situ psychrometers can follow dynamic changes in leaf water potential. However, they should be used with caution for absolute measurements of leaf water potential in the psychrometric mode in species with low epidermal conductances. Abrasion of the cuticle may overcome the problems of low epidermal conductances, but before use we recommend that the psychrometers be carefully calibrated against a standard technique such as the pressure chamber or isopiestic psychrometer.

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