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In Situ Measurement of Plant Water Potentials by Equilibration with Microdroplets of Polyethylene Glycol 8000[1]

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ABSTRACT

Microdroplets (3–5 nanoliters) of polyethylene glycol 8000 solution were allowed to equilibrate with plant water potential by placing the microdroplet on an abraded surface and covering it with mineral oil to prevent evaporation. Osmolality was followed by cryoscopic measurements, accurate to about ±0.1 bar, on subnanoliter samples.

Under constant environmental conditions, apparent equilibrium between microdroplet and plant water potentials was attained in about 1 to 2 hours. Microdroplet osmolality responded promptly to treatments (illumination, excision, osmotica) which changed plant water status. The values obtained for plant water potentials appeared to be physiologically reasonable. However, comparison with values obtained by other means (dewpoint hygrometry, treatment of tissue with polyethylene glycol solutions, calculation from turgor and osmotic pressures) suggest that they might be somewhat more negative than the actual tissue water potential.

Aside from the advantage of providing *in situ* measurements of plant water status, the method is not temperature sensitive and requires only about 10 square millimeters of surface area, which allows its use on even small structures with little interference by shading or with gas exchange.

Thermocouple hygrometry is presently the method most generally preferred for making *in situ* measurements of plant water potential. However, its dependence on vapor phase water transport makes it very sensitive to temperature gradients. As a result, the psychrometer chamber and necessary insulation comprise a moderately bulky package which must be attached to the leaf at the point of measurement, and considerable technical care is required in its use (*e.g.* 9).

In the course of making osmotic pressure measurements on the endosperm cavity sap of wheat grains, it appeared that these values should reflect closely the endosperm water potential, since there was no evidence of pressure in the cavity. This suggested the possibility that water potential measurements might be similarly obtained on other plant parts if small solution volumes could be exposed to apoplastic water without evaporation. Several approaches to water potential measurement are based on identifying the solution concentration at which no net water movement occurs between tissue and solution (10). However, those most usually employed are dependent on the use of replicate tissue samples exposed to a graded series of solutions of relatively large volumes. In the present approach, a very small solution volume is used. This allows equilibrium conditions to be reached with movement of only a nanoliter or so of water between the solution and plant, with negligible effect on plant water status. Of published accounts, the procedure most closely resembles that of Stocking (12), who injected sucrose solutions (1–2 ml) into the hollow petioles of squash plants and followed concentration changes by refractometry.

MATERIALS AND METHODS

Plant Material. All measurements were obtained using wheat plants (*Triticum aestivum* L. cv Sun 9E) grown in a glasshouse (21°C d, 16°C night) in the CSIRO Phytotron. Seeds were sown in perlite-vermiculite and watered twice a day, once with Hoagland solution and later with distilled H_2O. For convenience, most measurements were made on plants after they had reached anthesis, but some measurements (not reported) were also obtained with equal facility from much younger plants.

For several experiments where more negative water potentials were desired, plants were transferred to a growth cabinet (14 h, 21°C d; 10 h, 16°C night) and water was withheld for 4 to 6 d.

Basic Procedure. To prevent evaporation, the solution microdroplet must be covered with mineral oil. This was accomplished by sealing a small well constructed of a 1- to 2-mm segment of plastic tubing, 2 to 3 mm i.d., to the plant surface with Dow-Corning RTV silicone rubber polymerized with the fast-setting catalyst "4". This and subsequent operations were carried out under a dissecting microscope. After the adhesive set, the well was filled with distilled H_2O. The broken end of a fine-tipped pipet was used to scrape the surface layer from several hundred micrometers of sclerenchyma overlying a vein. The water was sucked from the well, taking care that the scraped area did not dry. A small droplet (3–10 nl) of PEG 8000 solution (Sigma Chemical Co.) was ejected onto the scraped area and the well was filled promptly with mineral oil. If desired (*e.g.* to reduce the time to reach equilibrium), the droplet volume could be adjusted at this time or brought closer to a desired initial concentration by changing most of the volume several times with the desired PEG concentration. The latter procedure was necessary to achieve a particular starting concentration, since the initial droplet was sufficiently small that appreciable evaporation occurred before it was fully covered with mineral oil. Droplet volume was adjusted to about 2 to 6 nl. Adjustments in the droplet were made without allowing mineral oil to displace any of the droplet from the plant surface, which would result in less effective exchange between the plant tissue and the droplet.

Droplet osmolality was followed by taking small aliquots (several tenths of a nl) at appropriate intervals for measurement of freezing point depression using a nanoliter osmometer (Clifton Technical Physics). Sampling pipets were prepared from disposable 100 μl pipets by pulling a fine tip over a small flame, flaming the tip shut, and silanizing the outer surface of the pipet. Just before use, the tip was broken off so that the opening was about 5 to 15 μm in diameter. After the sample was pulled into the

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pipet, it was followed by a short length of mineral oil to prevent evaporation.

PEG 8000 solutions of known water potentials were prepared from the equation published by Michel and Kaufmann (7), and their osmolalities were determined experimentally. Sodium chloride standards were used to calibrate the osmometer.

Several other solutes were also tried. These included mannitol, sorbitol, and dextran (nominal mol wt = 10,000). All were obtained from Sigma Chemical Co.

Confirmation of PEG 8000 and Dextran Osmotic Properties. Owing to the unusual properties of PEG solutions (including a pronounced negative temperature coefficient for water potential [7]) and the possibility that they might differ for the particular batch in use, measurements were made to confirm its anticipated behavior. Water potential measurements were made using a Wescor thermocouple hygrometer operated in the dewpoint mode, and vapor pressure measurements were made on the same solutions with a Hewlett-Packard vapor pressure osmometer. Each measurement was made in duplicate on PEG concentrations of 0.2, 0.3, and 0.4 g PEG/g H_2O.

Similar measurements were made for dextran solutions (0.2, 0.4, and 0.6 g/g H_2O) to confirm the anticipated relationships between freezing point depression and water potential.

Estimation of Measurement Accuracy. Three approaches were taken to evaluate the accuracy of water potential measurements made by the microdroplet method. These were (a) comparison with conventional thermocouple hygrometer measurements, (b) measurements taken from tissues whose water potential was 'controlled' by PEG solutions of known concentration, and (c) comparison with water potentials calculated from measurements of sieve tube turgor pressures and osmotic potentials.

For comparison with thermocouple hygrometer measurements, a water potential measurement was first obtained from a PEG microdroplet placed on the midrib of a leaf blade. After a steady value was reached, punches were taken from the blade above and below the microdroplet position and quickly placed in hygrometer chambers. Measurements were made in the dewpoint mode 3 to 4 h later.

In an attempt to control tissue water potential by exposure to solutions of konwn concentrations, standard PEG 8000 solutions were passed through excised wheat peduncles. The peduncles were wrapped in Saran wrap except for an opening which exposed the mineral oil bath with its microdroplet. In addition, the peduncle was shaded with aluminum foil. When changes were made between perfusing PEG concentrations, the new concentration was passed through at about 5 to 7 ml/min for several minutes, after which the rate was slowed to a few drops/min. Occasional samples were taken to check effluent osmolality; no significant differences from the entering osmolality were noted.

Calculated water potentials were obtained from micromanometric measurements of sieve tube turgor and aphid stylet exudate osmotic pressure (13). Stylets of *Rhopalosiphum padi* (L.) feeding on the peduncle were severed by radio-frequency microcautery (6).

RESULTS

With the initial concentrations illustrated here, microdroplet osmolality reached a steady state value within 1 to 2 h (Figs. 1–5). While the time course was not truly first-order, it may nevertheless be characterized usefully as having a half-time of about 20 to 30 min. Low mol-wt compounds were not satisfactory osmotica for the microdroplets (Fig. 1), since the droplets did not attain a steady osmolality and eventually disappeared altogether. Both problems presumably arose from the ability of smaller-sized solutes to enter the cell walls (4). On plants under constant environmental conditions, PEG (and dextran) droplets reached fairly constant osmolalities (Figs. 1–5), and the microdroplets usually remained for a day or more. However, PEG droplets would also disappear if they contracted damaged chlorenchyma, which then provided a pathway for infiltration of the solution into intercellular spaces.

The steady state concentration attained was independent of

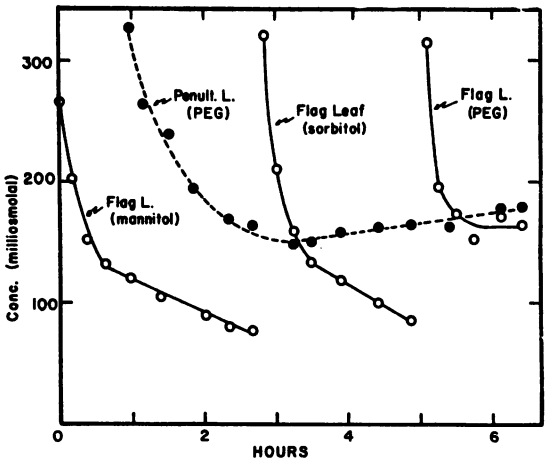

FIG. 1. Comparison of different solutes as microdroplet osmotica. Microdroplets of mannitol, sorbitol; and PEG 8000 were applied successively to the same abraded spot on a flag leaf. A single PEG droplet was present continuously on the penultimate leaf.

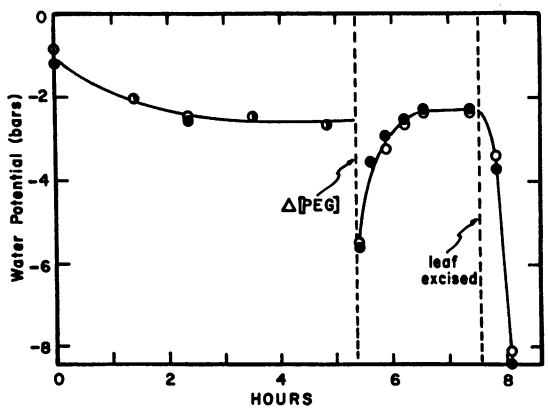

FIG. 2. Approach to equilibrium from different directions, and the response of microdroplet water potential to excision. Measurements were taken from two droplets established about 3 cm apart on the same vein of the flag leaf.

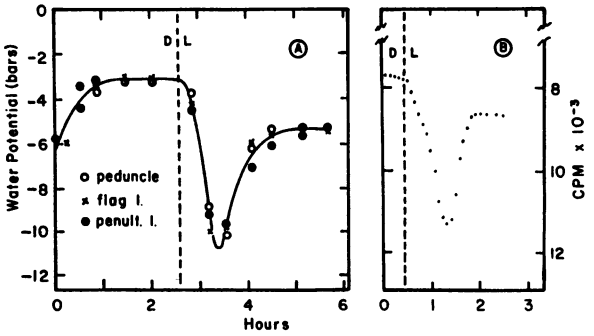

FIG. 3. Response of microdroplet water potential (A) and leaf water content (B) to illumination. Microdroplets were established on the peduncle, flag leaf, and penultimate leaf of the same plant (A). Flag leaf water content (B) was monitored in a similar experiment with a beta gauge 2 d later (B).

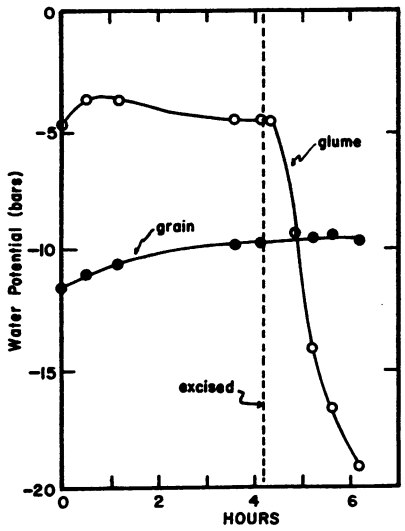

FIG. 4. Response of grain and glume water potentials to excision below the flag leaf node. PEG microdroplets were sited on adjacent spikelets.

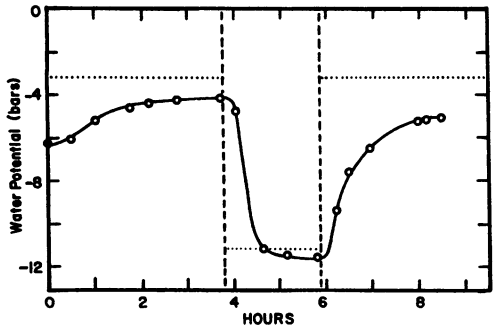

FIG. 5. Responses of microdroplet water potential during attempts to achieve known tissue water potentials by perfusing known concentrations of PEG solutions (· · · · ·) through a length of peduncle.

Table I. *Measurement of Water Potentials by Microdroplet Equilibration using Different Solutes*

Comparison of values obtained with PEG 8000 and dextran (nominal mol wt = 10,000). Microdroplets were sited about 2 cm apart on the same vein of the peduncle.

PEG	Dextran
bars	
−6.6	−6.4
−4.0	−4.2
−4.4	−4.0
−17.9	−17.7

the direction from which equilibrium was approached (Fig. 2), indicating that water exchange was freely reversible. Replicate droplets on the same plant agreed closely with one another whether they were on the same leaf (Fig. 2) or were separated by substantial distances (Fig. 3). Droplet osmolality responded promptly to changes in plant water status initiated by excision (Figs. 2, 4), illumination (Fig. 3), or exposure of peduncle tissue to differing PEG concentrations (Fig. 5). As expected from the reported independence of grain water relations from the rest of the wheat plant (1, 2), grain water potential was insensitive to excision of the peduncle while a nearby glume showed an immediate decrease (Fig. 4).

Response to illumination was particularly interesting (Fig. 3).

Table II. *Comparison of Water Potential Values Obtained by Microdroplet Equilibration with Values Obtained by Dewpoint Hygrometry*

Microdroplet Equilibration	Dewpoint Hygrometry
bars	
−4.6	−3.1
−5.1	−2.2
−8.3	−5.3
−23.4	−23.7
−24.0	−21.7
−46.7	−31.5

Table III. *Comparison of Water Potential Values Obtained by Microdroplet Equilibration with Values Calculated from Measurement of Sieve Tube Turgor Pressure and Osmotic Pressure*

P	π	ψ_{calc}[a]	ψ_{meas}
	bars		
9.9	29.8	−19.9	−22.5
10.6	15.4	−4.8	−5.2

[a] $\psi_{calc} = P - \pi$.

Readings taken from three widely removed sites (penultimate leaf, flag leaf, and peduncle) were in close agreement at all times during a dark/light transition. On illumination, a rapid drop in water potential to at least −11 bars was followed by recovery to a more positive water potential (−5.4 bars) which in turn was more negative than the preillumination value (−3.2 bars). These changes, due presumably to overshoot during stomatal opening (3), were reflected in changes in leaf water content as measured with a beta gauge in a similar plant 2 d later (Fig. 3).

Possible difficulties arising from the use of PEG as an osmoticum were not realized. While dextran and PEG solutions had quite different effects on freezing point depression on a bar-for-bar basis (approximately 50 *versus* 41 milliosmolal units per bar at 22°C for PEG and dextran, respectively), the two solutes gave closely similar water potential values for the same plant (Table I). Hygrometric measurements of standard PEG solutions agreed to within 3% of published values for PEG 8000 (7), as did vapor measurements (7) (data not shown).

Attempts to achieve defined peduncle water potential values by flowing standard PEG solutions through the peduncle often yielded microdroplet water potential values which were somewhat more negative than the perfusing solution (Fig. 5; one of three experiments with similar results), although some were within 0.5 bar. Similarly, microdroplet water potential values were often more negative than hygrometric measurements on the same leaf (Table II). Only two comparisons of measured and calculated (from sieve tube sap osmolality and turgor pressure) water potential values were reliable (Table III), owing to difficulties in achieving a pressure seal to the heavily cutinized peduncle. In one case, the difference between calculated and measured water potential values fell within the experimental error (±0.4 bar) (13) for the measurements. In the second case, the microdroplet value was about 2.6 bars (12%) more negative than the calculated value.

DISCUSSION

The possibility of measuring plant potential by the use of microdroplet equilibration appears to offer a feasible alternative for following plant water status. The 'theory' of the measurement is straightforward and familiar, the accuracy of cryoscopic measurements is good (±0.1 bar), the entire range of water status can be covered, and the small size of the microdroplet and well allows

measurements on even small structures with minimal interference by shading or with gas exchange.

The method has some disadvantages which, aside from the question of accuracy (discussed later), are generally minor. Infiltration of intercellular spaces, by either the microdroplet or mineral oil, is probably of most concern. The microdroplet can be kept from wetting intercellular spaces by abrading a tissue without intercellular spaces, the approach taken here, or it should be possible to apply a gentler method of abrasion to the outer walls of epidermal cells (5, 11). Infiltration of mineral oil, however, occurred to some extent wherever stomates were present. This was of little concern in the present measurements, since stomates either were not present (grain) or the abraded sclerenchyma had good hydraulic access to the xylem (veins). Other, more minor shortcomings include microdroplet volume reduction by sampling or by decreased water potential (correctable by replenishing with an appropriate PEG concentration) and the fact that it is inherently an *in situ* method not suitable for use with excised tissue. Finally, due to the negative temperature coefficent for the water potential of PEG solutions (7) and solute-PEG interactions (7). apoplastic solutes, if present at significant concentrations, may be expected to alter the relationship between osmolality and water potential observed for solutions containing PEG alone. This may be remedied by using dextran instead of PEG.

While the water potential values obtained by microdroplet equilibration appear physiologically reasonable, it is difficult to judge their accuracy. Based on comparisons made so far, they may be more negative than the actual values. However, values obtained by dewpoint hygrometry may be spuriously high, especially for wheat (8), due apparently to the reabsorption of solutes released from damaged cells. The more negative values obtained from peduncles perfused with standard PEG solutions are more puzzling, since equilibrium conditions were apparently attained in the experiments and the solute in the solutions on either side of the peduncular tissue (a distance of about 1 mm) was identical. Probably the best criterion of accuracy would be agreement between measured and calculated values (from sieve tube turgor and osmotic pressure) of water potential. Unfortunately, technical problems with the turgor measurements precluded a thorough comparison on this basis. In general, however, the results appear sufficiently accurate and reproducible to warrant further use of the technique in assessing plant water status.

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