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In Situ Field Measurement of Leaf Water Potential Using Thermocouple Psychrometers[1]

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ABSTRACT

Thermocouple psychrometers are the only instruments which can measure the *in situ* water potential of intact leaves, and which can possibly be used to monitor leaf water potential. Unfortunately, their usefulness is limited by a number of difficulties, among them fluctuating temperatures and temperature gradients within the psychrometer, sealing of the psychrometer chamber to the leaf, shading of the leaf by the psychrometer, and resistance to water vapor diffusion by the cuticle when the stomates are closed. Using *Citrus jambhiri*, we have tested several psychrometer design and operational modifications and showed that *in situ* psychrometric measurements compared favorably with simultaneous Scholander pressure chamber measurements on neighboring leaves when the latter were corrected for the osmotic potential.

Some workers regard the psychrometric technique as the absolute technique, but few research workers have reported *in situ* field measurements of leaf water potential (1, 2) and xylem water potential comparisons. Yet we know from personal communications that many have tried, but with only limited success. A major problem is fluctuating thermal gradients causing temperature gradients between the measuring thermojunction and the leaf surface which preclude an acceptable level of precision in the determination. In addition, the diffusion resistance of the cuticle-stomate system may delay equilibrium especially when the resistance is high.

The objective of this investigation was to improve nondestructive techniques for measuring leaf water potential in the field using *in situ* thermocouple psychrometers and to compare these measurements with Scholander pressure chamber measurements.

MATERIALS AND METHODS

Eleven leaf psychrometers (3), calibrated according to previous methods (4–6), and juvenile plants (*Citrus jambhiri*) grown in 5- to 15-L pots were used in this investigation. However, in this study, psychrometers were field calibrated and measurement errors in water potential determined (7).

In order to use leaf psychrometers for *in situ* field measurements of leaf water potential, it was necessary to reduce the large temperature gradients associated with exposure of the aluminum block to direct solar radiation and wind. This was done by covering all surfaces of the aluminum housing of a commercial psychrometer (Wescor Inc., Logan, UT) with thermal insulation material (3). Polystyrene covered with aluminum foil tape (Y434 by 3M, Industrial Tape Division) proved less satisfactory than Scotch mount double coated foam tape (4009 by 3M) (Fig. 1a). About 12-mm-thick insulation material was applied to the top and bottom of the housing and about 6 mm to all sides except the non-slit side (Fig. 1a, side A), which was 9 mm thick. This arrangement minimized leaf shading. The insulation material was covered with highly reflective aluminum foil tape ensuring that radiation of all wavelengths was reflected. A cylindrical, narrow bore plastic tube was glued to the piston top in order to extend its length, accommodating the increased depth resulting from the insulation material around the psychrometer (Fig. 1a).

Cleaning of the leaf psychrometer chamber is important. A toothpick was used to carefully remove any sealant that had entered the psychrometer chamber. The lower piston was then soaked in boiling distilled H_2O, immediately washed in acetone followed by 4 mol/kg NH_4OH, cleaned with a jet of steam and finally rinsed in distilled H_2O. Merrill (Merrill Speciality Co., Logan, UT) psychrometers were more difficult to clean if sealant entered the chamber, due to the protective wire screen covering the sensing junction.

The effect of abrasion on the measured leaf water potential was investigated using no abrasion, light particle abrasion, and coarse abrasion treatments. The abrasion technique was similar to that of Brown and Tanner (1) except that 400-grit (particle diameter of about 60 μm) carborundum powder was used for a light abrasion treatment and calcined aluminum oxide (wet sieved through a 75-μm sieve) for a coarse abrasion treatment. The leaf was first cleaned with distilled H_2O. The area to be abraded was marked by a piece of Parafilm wax paper folded around the edge of the leaf and having a punched hole with a diameter slightly larger than that of the psychrometer chamber. The leaf area exposed by the punched hole was marked with the abrasive slurry, and after removal of the wax paper, the marked leaf area was carefully abraded (circular motion) using the index finger covered with thin cotton cloth using the index finger of the other hand as a support. The abrasive slurry was formulated from non-ionic detergent, water, and abrasive powder, as suggested by Brown and Tanner (1). After abrasion, the leaf was again cleaned with distilled H_2O and gently blotted dry with paper towel.

A beeswax-lanolin mixture was used to seal the psychrometer piston against the leaf. The relative amounts of each wax determines the rheological properties of the mixture.[3] The softening

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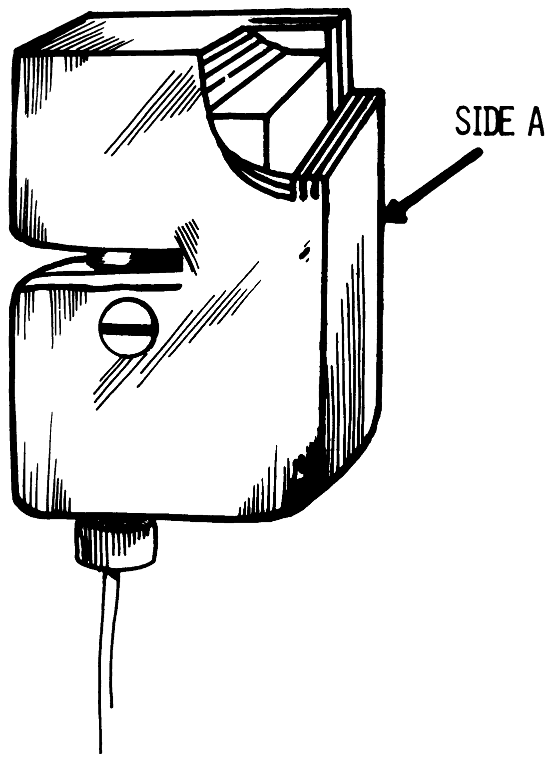
[3] If T_s is the softening temperature and R the volume ratio of lanolin to beeswax, then $R = 20.76 - 0.43\ T_s$ for the waxes used (with total degrees of freedom 7, $r = 0.987$ and $S_{y.x} = \pm\ 0.52$).

a



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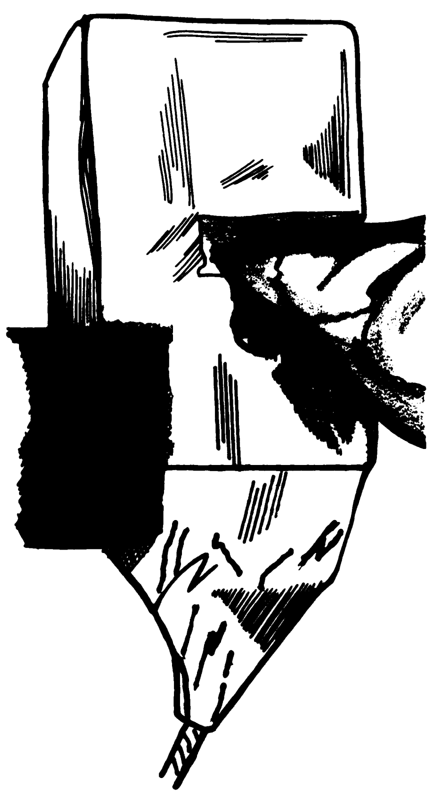

FIG. 1. Diagrammatic representation of an *in situ* leaf psychrometer covered with thermal insulation and reflective aluminum foil. Four layers of insulation material (each 3 mm thick) at the base of the psychrometer were used with two layers on all sides to minimize leaf shading (except the non-slit side which had three layers). Total shaded area of the leaf was at least 8% of the total leaf area. a, Cut-away view showing the narrow bore plastic tube and brass securing screw; b, aluminum foil cover over the piston top and lead outlet.

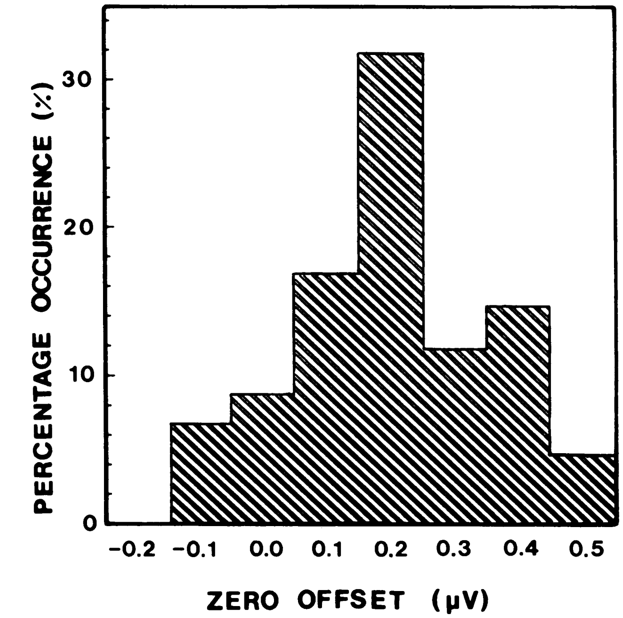

FIG. 2. Histogram plot of the percentage occurrence of the various psychrometer temperature gradients occurring in the field situation where block temperature varied between 12 and 30°C. Zero offset measurements are as important as the measured voltages corresponding to the wet bulb temperature. During this experiment, a total of 243 zero offset measurements were collected. These measurements were obtained using the '0 to 100' 'μv range on the HR-33T microvoltmeter and the output recorded on a chart recorder. This enabled voltages to be measured to within ±0.025 μV (±25 nv). Generally, negative zero offset values were measured during the night. Most measurements were obtained during hot and dry environmental conditions.

temperature is defined as the temperature at which no beeswax-lanolin mixture flowed off a thermometer dipped into the heated mixture. Generally, the mixture that was used had a softening temperature 2°C greater than the expected 1400 h block temperature. No infiltration of this mixture into the leaf was noted even after a week with temperatures exceeding 35°C.

The insulated aluminum psychrometer housing supported by metal retort stands and laboratory clamps were positioned so that the abraded areas of the respective leaves fit without stress into the psychrometer slit. Sealant was applied to the edge of the psychrometer piston which was then pushed firmly against the leaf surface and secured with a brass set screw (Fig. 1a). A screw driver with a long shaft was used to tighten this screw ensuring minimal disturbance to the plant (Fig. 1a). Psychrometers were usually attached in the late afternoon some 10 min after abrasion, but not if dew was noted on the leaf surface.

For the crops used in this study, psychrometers were always sealed on abaxial leaf surfaces. The leaf angle of citrus changes with water stress and it was necessary to occasionally alter the psychrometer position if measurements continued for extended time periods. When the abaxial leaf surface faced upwards, the plastic rod and lead wire were exposed to incoming solar radiation. In such cases, aluminum foil was taped over the plastic rod (Fig. 1b) to reduce heat conduction to the piston. The tape was not used as an umbrella, thereby casting minimal shade on the leaf. The psychrometer slit was positioned towards north (or

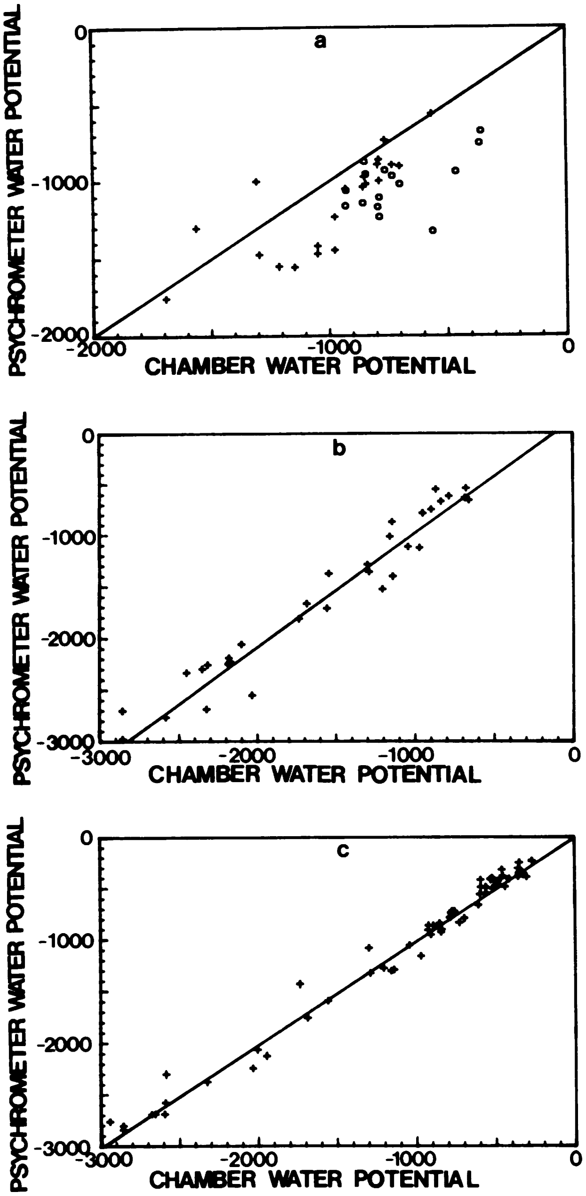

FIG. 3. Comparative relationship between psychrometer and pressure chamber measurements of citrus leaf water potential for various treatments. Each point represents an average of two to three pressure chamber measurements on adjacent leaves: a, no abrasion ([+], wide aperture psychrometer measurements; [O], narrow); b, coarse abrasion; c, light abrasion.

south in the Northern Hemisphere) to minimize leaf shading.

It was possible to check whether the psychrometers were successfully sealed against the leaf within 5 min after sealing. A 30-s cool followed by switching to read indicated that the chamber was approaching equilibrium with the substomatal cavity if the output voltage was less than about 15 μv. If not, the brass screw was released and pressure applied between the psychrometer piston top and the leaf with the piston rotated slightly to improve the vapor seal. The screw was then retightened. One hour was generally sufficient for vapor equilibration between the substomatal cavity and the psychrometer chamber for the abrasion treatment used. Measurements were performed 4 h after sealing.

When all psychrometers were sealed, the corresponding stem or branch was tied to the metal support rod and neighboring

Table I. *Associated Statistical Parameters for the Linear Regression Curves*

The curves are shown in Figure 3, b and c (psychrometric *versus* pressure chamber or xylem potential) and Figure 4 (narrow aperture psychrometer measurements *versus* wide aperture).

Statistical Parameter	Figure No.		
	3b	3c	4
Slope	1.106	1.004	0.966
SE slope	0.0431	0.0159	0.0284
Intercept (kPa)	126	−18	−15
SE intercept (kPa)	76.7	1.6	40.8
r	0.976	0.984	0.982
$S_{y\cdot x}$ (kPa)	179.8	100.0	148.3
No. of points	35	66	44
t value for unit slope[a]	2.451	0.235[b]	−1.194[c]
t value for zero intercept	−1.659[c]	0.862[b]	0.374[b]

[a] Null hypothesis is that slope is unity and intercept is zero.
[b] Statistical significance at 99% level of significance.
[c] Statistical significance at 95% level of significance.

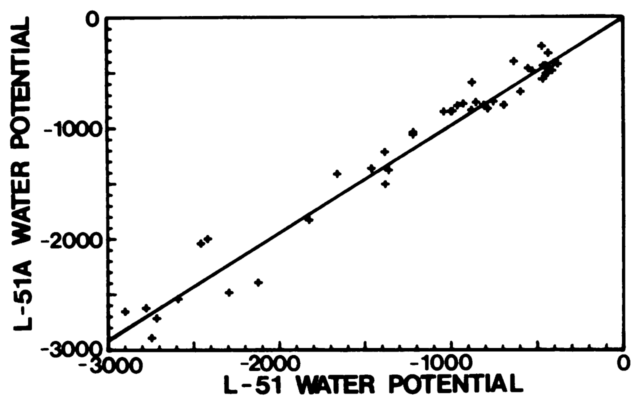

FIG. 4. Comparison between psychrometer measurements (intensive light abrasion) using the wide aperture unit (L-51) and those using narrow (L-51A) aperture unit. Individual measurements were performed using the same leaf.

stems or branches to reduce the possibility of wind-induced stresses breaking the seal. The support rod was also pushed into the soil of potted plants when feasible.

The microvoltmeter lid was covered with aluminum foil tape and the inside of the lid filled with polystyrene to reduce sudden temperature changes and spurious voltage (zero offset) fluctuations in the electronic circuit. In particular, the lid of the microvoltmeter was never fully opened whenever connecting wires or operating the function switch, thus preventing radiation from directly entering the unit. Heating of the psychrometer lead wires was prevented by mounting a polystyrene block covered with aluminum foil tape to the side of the microvoltmeter. This reduced heat energy flow to the sensor connecting copper posts of the microvoltmeter, and eliminated the apparent temperature gradients and fluctuating zero offsets (1).

A rechargeable power supply was substituted for that supplied by the manufacturers. This consisted of six rechargeable batteries (Yuasa, Japan) each 6 v and 6 amps·h housed in a plastic box covered with aluminum foil tape to reduce radiant heating of the batteries in the field. The capacity of the battery pack was sufficient for the microvoltmeter to be kept on continuous power throughout the investigation (after 1 week, trickle recharging was necessary). The 10- to 15-min drift, on turning the meter on (1), was prevented by this procedure.

A mains chart recorder was used to record thermocouple psychrometer output with a Keithley multimeter connected to the Wescor microvoltmeter as a cross-check.

On a few occasions, the aluminum covering and the thermal insulation resulted in static charge accumulation. This resulted in large apparent zero offset voltages, normally associated with large leaf and sensing junction temperature differences. The accumulation of charge was dissipated by connecting the psychrometer earth lead wire to the chart recorder earth connection. Generally, psychrometer measurements only commenced at about 900 h (SAST). Winter dew frequently occurred and predawn measurements were often not possible in windy conditions as droplets of water entered or moved out of the slit area. This water movement often caused large leaf (cavity) and sensing junction temperature differences and also zero drift errors during measurement of the wet bulb temperature. Early morning post-dawn measurements were also often not possible due to the water movement from the slit area, in particular from the piston. This resulted in large temperature differences (zero offsets) particularly in windy conditions or when the leaf was partially exposed to solar radiation.

Xylem water potentials were obtained using pressure chamber measurements made on adjacent leaves whenever psychrometer measurements were made. Prior to excision, the leaf was covered with a slightly moistened rectangular piece of cotton cloth folded over the leaf and then covered with adhering plastic wrap. Some leaf tissue was trimmed away from the petiole as needed, to lengthen it and facilitate insertion into the chamber rubber stopper. Pressure was increased at a rate of about 10 kPa/s, to improve endpoint accuracy.

RESULTS AND DISCUSSION

In the field experiment, measured block temperatures ranged between 12 and 30°C, and the zero offsets associated with temperature gradients between the reference and sensing junctions were never greater than 0.6 μv (Fig. 2). This maximum value was measured when direct solar radiation entered the slit area, striking the psychrometer piston. Previous comparisons of measurements obtained from Merrill leaf psychrometers indicated that the Wescor L-51 (wide aperture) and L-51A (narrow aperture) leaf psychrometers exhibited smaller temperature gradients under similar conditions of high energy load than did Merrill psychrometers. Consequently, the latter were not used in the field.

Zero offsets measured during the investigation ranged between −0.1 and 0.6 μv (Fig. 2). The zero defects accepted by Brown and Tanner (1) were less than 0.3 μv but their psychrometer shaded more of the leaf than ours (9 cm^2 compared to our 6 cm^2). Some workers have accepted less than 1 μv, where 1 μv ≃ 250 kPa (at 25°C). Complete shorting of the binding posts using a short piece of copper wire indicate that meter zero offsets range between 0.1 and 0.2 μv (GS Campbell, personal communication, 1983). The zero offsets shown in Figure 2 should therefore be reduced by this amount.

Agreement between psychrometer and pressure chamber measurements was poor unless the leaves were abraded prior to psychrometer measurements (Fig. 3, a–c). If leaves were not abraded prior to psychrometric measurement, the readings were excessively dry compared to pressure chamber values (Fig. 3a) and the 'plateaus' were poorly developed (3). The discrepancies became worse with lower (drier) leaf water potentials, ψ_w. In the case of the no abrasion treatment, the wide aperture psychrometer measurements compared more favorably with pressure chamber measurements on neighboring leaves, compared to the narrow aperture psychrometer measurements (Fig. 3a). Presumably, diffusion resistance limited the amount of substomatal water condensed on the thermojunction in the case of wide aperture measurements, but more so in the case of narrow aperture measurements (8).

The coarse abrasion treatment resulted in more variable leaf water potential measurements compared to light abrasion (Fig. 3c; Table I). The coarse abrasion treatment gave psychrometric water potential values greater than the pressure chamber measurements for $\psi_w > -1250$ kPa, but for drier values the reverse was true. The slope interval does not overlap with unity at a 95% confidence level (Table I). The variability of these data may be due to the severity of the abrasion treatment. We found that, with this abrasion treatment, damage to the epidermal tissue was more severe, compared to light abrasion, which may account for the lower (drier) psychrometric water potentials for $\psi_w < -2500$ kPa (8).

Psychrometric water potentials measured after a 60-s light abrasion treatment were not statistically different from measured pressure chamber values (Fig. 3c; Table I) for -2700 kPa $< \psi_w < -300$ kPa. Environmental conditions varied widely with measured block temperature ranging between 12 and 30°C. Brown and Tanner (1) measured leaf water potential on the same leaf using dewpoint and pressure chamber techniques and found no significant differences between the two. We used neighboring leaves for pressure chamber measurements and hence some of the scatter in our data could arise from spatial separation between psychrometer and pressure chamber leaves. The pressure chamber water (or xylem) potentials of Table I and Figure 3, a to c, were not corrected for the osmotic potential of the exuded sap. The mean osmotic potential of the exuded citrus sap from 28 measurements was −38.0 kPa with a standard error of ±3.6 kPa. If all pressure chamber potentials are corrected for sap potential, then the psychrometer water potential is closer to the total (xylem plus sap) water potential than to the pressure chamber (xylem) potential. Water potential values for the light abrasion treatment were greater than the corrected pressure chamber measurements by approximately 20 kPa on average. We conclude that the increase in leaf water potential caused by shading the leaf with the psychrometer was small in our case.

Further evidence for the success of the 60-s light abrasion treatment is indicated in Figure 4. Leaf water potential was measured using narrow and wide aperture leaf psychrometers on the same citrus leaf. The good agreement between these measurement values presumably would not be possible if resistance to water vapor diffusion was limiting the amount of substomatal water vapor condensed on the sensing thermojunction during cooling. There are, however, slight differences between these measurements. At low leaf water potentials, less than −2500 kPa as measured by the wide aperture psychrometer, the narrow aperture measurements were greater by 70 kPa (calculation based on data from Table I). At high leaf water potentials, the narrow aperture psychrometer measurements were lower than their wide aperture counterparts.

CONCLUSIONS

(a) *In situ* psychrometer leaf water potential measured on abraded citrus leaves agrees well with that measured on adjacent leaves using the Scholander pressure chamber (Fig. 3a; Table I).

(b) Light abrasion of the leaf resulted in lesser variability in measured water potential values than coarse abrasion. Furthermore, the coarse abrasion psychrometric values measured were greater (wetter) than the pressure chamber counterpart (uncorrected for exuded sap osmotic water potential) for $\psi_w > -1250$ kPa but smaller (drier) for $\psi_w < -1250$ kPa (Fig. 3a; Table I).

(c) The light abrasion treatment gave psychrometric water potential values that were statistically identical to the pressure chamber values (99% level of significance), uncorrected for the osmotic potential of exuded sap and the apparent effect of xylem tension relaxation following petiole excision (Fig. 3c; Table I). For this abrasion treatment, water potential measured using a wide aperture psychrometer were typically 70 kPa greater than those measured using a narrow aperture (at −2500 kPa), both instruments being sealed on the same leaf.

(d) If pressure chamber measurements are corrected for the osmotic potential of exuded sap, psychrometer potentials are greater than the corrected pressure chamber (xylem) water potentials by about 20 kPa. This effect may be due to local shading of the leaf by the psychrometer.

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