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Effects of Salt Secretion on Psychrometric Determinations of Water Potential of Cotton Leaves

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Abstract. **Thermocouple psychrometers gave lower estimates of water potential of cotton leaves than did a pressure chamber. This difference was considerable for turgid leaves, but progressively decreased for leaves with lower water potentials and fell to zero at water potentials below about −10 bars. The conductivity of washings from cotton leaves removed from the psychrometric equilibration chambers was related to the magnitude of this discrepancy in water potential, indicating that the discrepancy is due to salts on the leaf surface which make the psychrometric estimates too low. This error, which may be as great as 400 to 500 %, cannot be eliminated by washing the leaves because salts may be secreted during the equilibration period. Therefore, a thermocouple psychrometer is not suitable for measuring the water potential of cotton leaves when it is above about −10 bars.**

The water potential of cotton leaves may be surprisingly low when measured psychrometrically. For leaves of well-watered plants, Lang and Barrs (8) found values of −11.4 bars for cotton but −4.6 bars for pepper. Box (3), also using a thermocouple psychrometer, obtained values of −6 to −13 bars for cotton leaf tissue previously floated on water. Other psychrometric data (6) indicate that the water potential of cotton leaves is considerably lower than that of pepper, trefoil, and sunflower. The present report shows that psychrometric determinations of water potential of cotton leaves are spuriously low as a result of salts which are secreted onto the leaf surface.

Materials and Methods

Leaves for this study were taken from plants of cotton (*Gossypium barbadense* L. var. Pima S-2) and sunflower (*Helianthus annuus* L. var. Large Grey) grown in Hoagland's solution in a greenhouse. Generally, leaves were washed with distilled water on the plant an hour or 2 before sampling. However, whenever excised leaves were left with the cut petiole in water in a humid chamber overnight to become turgid, they were washed immediately prior to use. Two estimates of water potential were made on the same leaf, first with a pressure chamber (4, 10) and then with a Peltier-cooled thermocouple psychrometer (2). Thermocouple outputs were measured with an automatic scanning and recording system (Trickett and Barrs, in preparation). When the leaf tissue was removed from the psychrometric equilibration chambers, it was washed with 5 ml distilled water and the electrical conductivity of this water was determined.

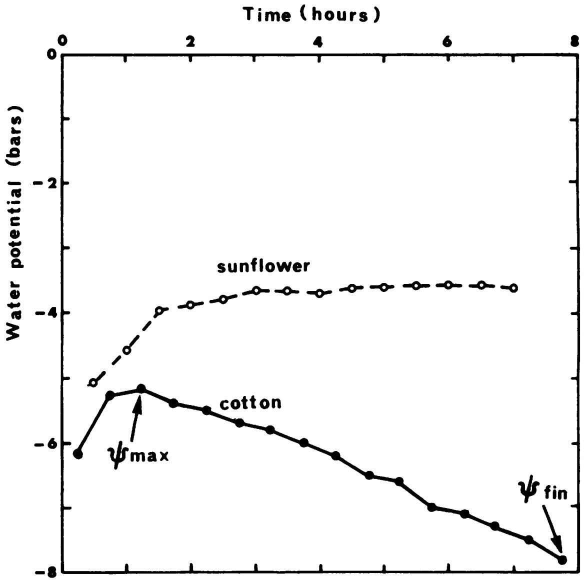

FIG. 1. The time courses of psychrometric readings of water potential for sunflower and for cotton leaves enclosed in equilibrium chambers. ψ_{max} and ψ_{fin} are described in the text.

Results and Discussion

Figure 1 shows the time course of a typical psychrometric determination of water potential for a cotton leaf and a sunflower leaf. For cotton, a maximum water potential (ψ_{max}) was soon reached and thereafter water potential fell continually; the value at the end of the experiment is designated

ψ_{fin}. For the sunflower leaf, the estimate of water potential did not change once ψ_{max} had been attained. Clearly cotton leaf tissue, unlike that of sunflower, does not reach a stable maximal water potential. Such behavior is unusual and has not previously been reported. The attainment of equilibrium illustrated by the curve for sunflower is typical of a wide range of species, *e.g.*, tobacco, geranium, pepper, and rock-melon (2), and pea, and *Tradescantia* (9). The form of the curve for cotton indicates that some factor operates while the tissue is in the chamber to prevent the attainment of a stable equilibrium.

Leaf water potential may fall in species other than cotton after the tissue has been enclosed in psychrometric chambers for long periods (1, 2, 11). However, this only occurs some time after a stable equilibrium at ψ_{max} has been reached and is accompanied by a reduction in respiration and obvious deterioration of the tissue. The effect is brought about by the reduction of oxygen concentration below some critical level with consequent loss of tissue semi-permeability and leakage of cell solutes. This is unlikely to explain the decline in water potential in cotton leaves since the decline occurs very soon after the tissue is put into the chamber. Also, there is no corresponding sudden drop in the heat of respiration and the tissue appears green and healthy when removed from the chamber. However, visual examination of such tissue shows minute droplets on its surface. Since Ehlig (5) reported that the accumulation of salt on the surface of cotton leaves caused spurious depressions in his psychrometric determinations of water potential, salt secretion could be responsible for the failure to attain a stable equilibrium in our experiments with this species.

This possibility was tested by measuring the conductivity of 5 ml washings from parallel subsamples of leaves of cotton and sunflower taken after they had been in the psychrometer equilibration chambers for various periods of time. The leaves had been washed immediately before they were placed in the chambers. If salt secretion were responsible for the decline in the estimates of cotton leaf water potential, the conductivity of washings should increase the longer the samples were held in the chambers. Figure 2 shows that this happens for cotton but not for sunflower, as would be expected since sunflower tissue reaches a stable equilibrium water potential (fig 1). Therefore, valid psychrometric estimates of cotton leaf water potential cannot be made by washing the leaf the day before sampling, as Ehlig (5) has suggested, since significant salt secretion occurs while a cotton leaf is in the equilibration chamber and water potential estimates fall even though the leaf is washed immediately before sampling (see fig 1).

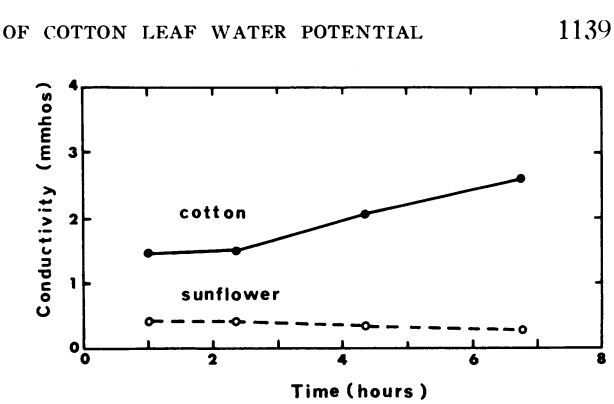

FIG. 2. Conductivities of washings from sunflower and cotton leaves at different times after enclosure in psychrometric chambers. Tissue was washed immediately prior to enclosure.

The presence of salts on the surface of cotton leaves was confirmed by chemical analysis. Excised leaves were thoroughly washed and were put overnight with their petioles in water in a humid chamber. The next morning the leaf surfaces had become obviously wet. The inorganic ions in this superficial liquid were determined for a solution made by washing each leaf in 5 ml distilled water. The solution contained largely potassium and magnesium bicarbonate (table I); the concentration of potassium was especially high. Similarly very high concentrations of potassium and relatively high concentrations of magnesium occur in guttation fluid (7). However, the significant amounts of calcium found in guttation fluid (7) were not found in the fluid on the surface of cotton leaves. Sugars, which also occur in guttation fluid, could have been present in the cotton leaf secretion, but we did not test for them. Of course, guttation fluid results from xylem sap being pushed out of leaves by root pressure whereas the fluid on cotton leaves presumably results from the leakage of cell contents so that differences in composition would be expected.

To assess the magnitude of the error caused by salt secretion, estimates made with the psychrometers (ψ_{max}) were compared with those made on the same leaf with a pressure chamber (ψ_{ch}), where this error

Table I. *The Major Inorganic Ions in the Liquid on the Surface of Cotton Leaves*

Leaves were each washed with 5 ml of distilled water. The values given are concentrations of ions in the washings.

Sample no.	Concentration								
	K^+	Mg^{2+}	Ca^{2+}	Na^+	NH_4^+	HCO_3^-	NO_3^-	Cl^-	HPO_4^{2-}
					meq/l ..				
1	3.3	1.0	. . .	0.07	0.12	4.1	0.23	0.06	0.002
2	2.8	1.1	0.3	0.04	. . .	5.4	. . .	. . .	. . .

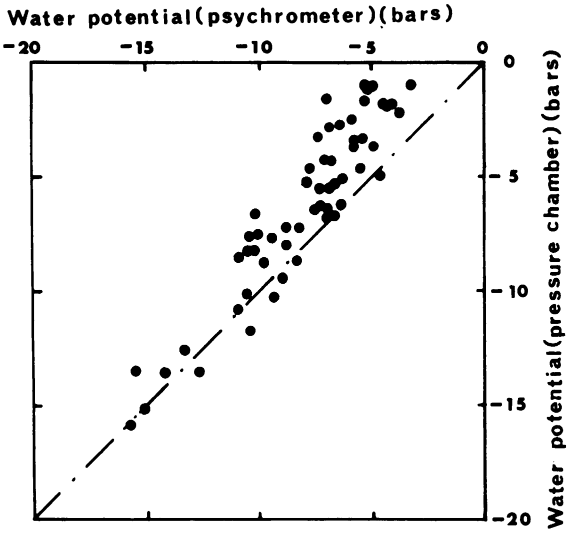

FIG. 3. Comparison of cotton leaf water potential as measured psychrometrically (ψ_{max}) and with pressure chamber (ψ_{ch}).

would be absent. ψ_{max} was used since this gives the smallest psychrometric error. Figure 3 shows reasonable agreement between ψ_{max} and ψ_{ch} at leaf water potentials below about −10 bars, but ψ_{max} falls progressively below ψ_{ch} at higher leaf water potentials. A maximum difference of 4 to 5 bars occurs for nearly turgid leaves having ψ_{ch} values of about −1 bar. Thus valid estimates of cotton leaf water potentials may be obtained with a thermocouple psychrometer only at water potentials below about −10 bars. As full turgor is approached, psychrometric values are increasingly depressed, the maximum error being about −5 bars at full turgor.

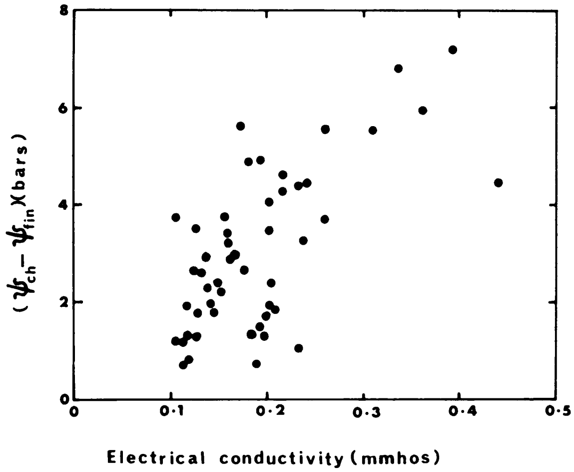

FIG. 4. Relationship of the difference between cotton leaf water potential measured after some hours in a thermocouple psychrometer chamber (ψ_{fin}) and the potential measured with a pressure chamber (ψ_{ch}) to the conductivity of washings obtained subsequently from the leaves.

If salt secretion decreases psychrometric estimates of cotton leaf water potential, the magnitude of the error should be related to the amount of salt secreted. Figure 4 shows that large depressions of water potential (ψ_{ch} − ψ_{fin}) are indeed associated with high conductivity readings of washings from the leaves (measured after ψ_{fin} had been determined) and that small depressions are associated with low conductivity readings.

Acknowledgments

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Literature Cited

1. BARRS, H. D. 1964. Heat of respiration as a possible source of error in the estimation by psychrometric methods of water potential in plant tissue. Nature 203: 1136–37.
2. BARRS, H. D. 1965. Comparison of water potentials in leaves as measured by two types of thermocouple psychrometer. Australian J. Biol. Sci. 18: 36–52.
3. BOX, J. E., JR. 1965. Measurement of water stress in cotton plant leaf discs with a thermocouple psychrometer. Agron. J. 57: 367–70.
4. BOYER, J. S. 1967. Leaf water potentials measured with a pressure chamber. Plant Physiol. 42: 133–37.
5. EHLIG, C. F. 1962. Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiol. 37: 288–90.
6. EHLIG, C. F. AND W. R. GARDNER. 1964. Relationship between transpiration and the internal water relations of plants. Agron. J. 56: 127–30.
7. GOATLEY, J. L. AND R. W. LEWIS. 1966. Comparison of guttation fluid from rye, wheat and barley seedlings. Plant Physiol. 41: 373–75.
8. LANG, A. R. G. AND H. D. BARRS. 1965. An apparatus for measuring water potentials in the xylem of intact plants. Australian J. Biol. Sci. 18: 487–97.
9. MANOHAR, M. S. 1966. Effect of the excision of leaf tissues on the measurement of their water potential with thermocouple psychrometer. Experientia 22: 386–88.
10. SCHOLANDER, P. F., H. T. HAMMEL, E. D. BRADSTREET, AND E. A. HEMMINGSEN. 1965. Sap pressure in vascular plants. Science 148: 339–46.
11. WAISTER, P. D. 1964. An improved thermocouple for assessing leaf water potential by vapour pressure measurement. Israel J. Botany 12: 192–96.