

AN APPARATUS FOR MEASURING WATER POTENTIALS IN THE XYLEM OF INTACT PLANTS

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[Manuscript received October 15, 1964]

Summary

A new method and apparatus for estimating water potentials in the xylem of intact plants are described. The apparatus is based on the thermocouple psychrometer method of measuring water potentials, but measurements are made on leaves which remain attached to plants. Illustrative experiments are described, in which continuous records of the estimated water potential in the xylem of cotton and pepper plants are given for periods of up to 10 hr.

I. INTRODUCTION

If a leaf which is still attached to a plant were brought to vapour pressure equilibrium with a small enclosed space at constant temperature, the energy of the water vapour in the space would be the same as that in the leaf. Since at equilibrium there would be no water flow along the petiole of the leaf, there would be no energy gradient along the petiole. Therefore, the energy of the water in the leaf would be the same as that in the plant stem. Thus, the energy of the water in the vapour phase in equilibrium with the attached leaf gives a measure of the energy of the water in the conducting elements of the stem xylem. We have recently found that Rawlins (1963) has also proposed similar arguments.

This paper describes a new method and apparatus for estimating the water potentials in the xylem of a plant, based upon the above reasoning.

Notable assumptions are that equilibrium is attained, that negligible transpiration occurs from the petiole, and that the measuring technique does not disrupt the normal leaf physiology. The validity of these assumptions is not easily verified. For this reason the results have been called ψ_{ξ} values, indicating that they are an approach to the true water potential in the xylem (ψ_x).

The apparatus is a development from the thermoelectric method of measuring vapour pressure introduced by Hill (1930) and improved by subsequent workers, notably Baldes (1934), Spanner (1951), and Richards and Ogata (1958). A critical comparison has recently been made (Barrs 1965) of the methods of Spanner and of Richards and Ogata when applied to measuring water potentials of isolated plant tissue. The present method differs from its predecessors in two ways: first, the leaf remains attached to the plant, and secondly the enclosed space containing tissue is not immersed in a water-bath controlled at about ± 0.001 degC.

Constant temperature of the leaf-vapour system is a critical requirement for the technique, but with leaves attached to plants it is plainly inconvenient to use a constant-temperature water-bath. The basis for the ± 0.001 degC temperature

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control normally used appears to be that an error of 0.001 degC in the wet-bulb depression corresponds to an error of about 12 J.kg⁻¹ in the water potential, which is the precision normally sought when the thermoelectric technique is used. It appeared that rapid fluctuations of millidegrees must be avoided; however, a slow drift of temperature may possibly be tolerated. The apparatus provides temperature control of this type.

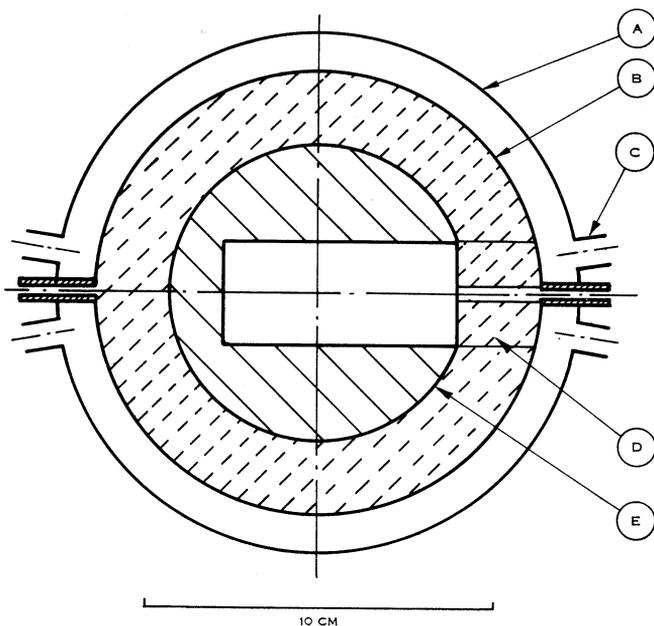


Fig. 1.—Temperature controller (cross-section): *A*, water jacket; *B*, insulation; *C*, water conduits; *D*, plug of insulation; *E*, copper sphere.

II. DESCRIPTION OF APPARATUS

The apparatus consists of two main parts, a temperature controlling device (Fig. 1), and a cell for enclosing samples and measuring wet-bulb depressions (Fig. 2). The cell fits inside the temperature controller.

Figure 1 shows a solid copper sphere (*E*) with a bored cylindrical hole. The sphere is surrounded by insulation of foamed polyurethane* moulded *in situ* (*B*) and a plug of loose insulation (*D*). The whole is enclosed in a water jacket (*A*) made in two halves to allow access to the centre of the sphere, and with tubes (*C*) for water circulation. The jacket is made from ball floats.

The sample cell shown in Figure 2 is a stainless steel tube (*G*) with a specially designed plug (*K*). The plug is split along the centre line of the hole (*F*, through which the leaf petiole passes), and a slot (*H*) is milled transversely across the split.

* The polyurethane was made from Daltolac 41, Suprasec D, and Arceton 11, supplied by Imperial Chemical Industries Limited.

A thermocouple assembly (*N*) is inserted into the plug. The assembly combines a shortened Richards and Ogata (1958) thermocouple psychrometer (*M*) with 0.25 mm diameter copper leads to decrease the thermal conductivity along the lead to the outside air, with a chromel–alumel thermocouple junction (*L*) of 0.25 mm diameter wires for measuring the temperature within the cell. The wires are sealed into the assembly with Araldite adhesive.

Seals between the two parts of the plug (*K*) and the leaf petiole are made with Apiezon Q compound.* O-rings (*J* and *P*) seal the remaining metal faces.

Water is circulated through the water jacket (*A*) from a Colara water-bath (KT40S) with a temperature differential of ± 0.02 degC.

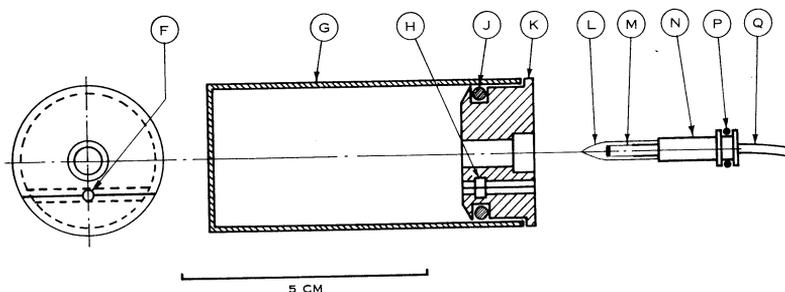


Fig. 2.—Sample cell (cross-section): *F*, hole for petiole; *G*, stainless steel shell; *H*, milled slot for seal; *J* and *P*, O-rings; *K*, plug; *L*, chromel–alumel thermocouple; *M*, modified Richards and Ogata psychrometer; *N*, thermocouple assembly; *Q*, leads.

The output from the psychrometer, which is in the range 0–40 μ V, is measured either with a microvolt potentiometer (Teale and Schumann 1939), or with a microvolt amplifier (Keithley Model 149) connected to a potentiometric recorder. Both measuring systems are calibrated frequently with a microvoltage source consisting of a standard cell in series with a voltage divider, which has a low temperature coefficient of resistance, and negligible thermal e.m.f. in the microvoltage output.

The temperature in the sample cell is measured with the chromel–alumel thermocouple (*L*) together with a stirred-ice junction and a Leeds and Northrup Type K3½ potentiometer in the circuit. This system gives discrimination of 0.01 degC.

III. CALIBRATION OF APPARATUS

(a) Methods

(i) *Calibration with Salt Solutions.*—Because of variations between different psychrometers, it was necessary to calibrate each couple in atmospheres of known water vapour pressure (Klute and Richards 1962).

The inner walls and bottom of the sample cell were lined with filter paper, and about 0.6 ml of sodium chloride solution of known molality added so as to saturate the filter paper and leave a small pool at the bottom of the cell. The plug (*K*) of the cell

* Apiezon Q is a high-vacuum preparation, like plasticine, made by A.E.I. (Manchester) Ltd., England.

was inserted, the thermocouple assembly (*N*) loaded with a drop of water and fitted into the plug, the cell inserted into the bored hole of the copper sphere, and the remainder of the apparatus assembled.

The temperature of the water circulating in the water-jacket (*A*) was adjusted so that the temperature within the copper sphere (*E*) was as constant as practicable (a drift of 0.01 degC.hr⁻¹ was normally attained), usually without attempting to control the temperature of the sphere at a predetermined value. Consequently, it was necessary to calibrate the psychrometers over a temperature range.

Two psychrometers were calibrated in the apparatus at irregular intervals of temperature between 5 and 30°C with 0.1002, 0.2010, 0.4040, 0.6089, and 0.8158 molal* sodium chloride solutions. Two other psychrometers were calibrated in the apparatus at a single temperature (25°C) with 0.2, 0.4, 0.6, and 0.8 molal sodium chloride solution. Readings of the output of a psychrometer were made at intervals, or recorded continuously in later experiments, until constancy was achieved.

Water potentials were calculated from the equation:

$$\psi = RT \ln a_w, \quad (1)$$

where

- ψ = the water potential (J.kg⁻¹),
- R = the gas constant (J.kg⁻¹.degC⁻¹),
- T = absolute temperature (°K), and
- a_w = the activity of water.

Water potential, as used in this paper, is analogous in symbol, magnitude, and sign to the total water potential of soil water defined by the International Society of Soil Science (Aslyng 1963). Activities at 25°C were taken for the various molal solutions of sodium chloride from the composite table of Robinson and Stokes (1959) which probably contains the most reliable values available. For temperatures other than 25°C, an adjustment to ψ was made for the change in absolute temperature; a_w was taken as independent of temperature. (Changes in the activity of water with temperature were calculated from the data of Robinson and Harned (1941), Robinson and Stokes (1959), and interrelations given by Robinson and Stokes, using graphical integration. The equivalent error in ψ did not exceed 2% for the extreme temperature and concentration.)

(ii) *Temperature Drift of Sphere.*—The rate of change (drift) of the temperature of the copper sphere depends upon the difference in temperature between the jacket and sphere. This interrelation was determined with a relatively large difference of 3–7 degC, and measuring the changing temperature of the sphere with the thermocouple in the cell. Measurements were made either with the cell dry, or containing salt solutions at temperatures ranging from 4.2 to 30°C, or containing cotton leaves.

(iii) *Temperature Drift and Measured Output of Psychrometer.*—These experiments were usually done in conjunction with those of the previous paragraph. After equilibration as described above, the jacket temperature was changed, and the new

* A molal solution contains 1 mole of solute per 1000 g of solvent.

value of the psychrometer output, together with the rate of change of temperature within the cell, was determined.

(b) Results

(i) Calibration with Salt Solutions.—To allow construction of a family of calibration curves at intervals of 5 degC from the results at irregular temperatures, it was first necessary to calculate the regression of microvolts against degrees Celsius for

TABLE 1
CALIBRATION OF PSYCHROMETERS FROM 5 TO 30°C

Regression coefficients for $\hat{Y} = a + bX$, where \hat{Y} is the calculated output of the psychrometer (μV) and X is the temperature ($^{\circ}C$). The sample standard deviation from the regression equation is given by $s_{y \cdot x}$ (Snedecor 1956)

Psychrometer No.	Regression Coefficient	Molality				
		0.1002	0.2010	0.4040	0.6089	0.8158
1	a	1.938	2.684	3.767	5.889	8.873
	b	0.0573	0.1003	0.2322	0.3173	0.430
	$s_{y \cdot x}$	0.29	0.04	0.36	0.74	0.57
2	a	1.762	2.773	4.987	6.709	8.921
	b	0.0422	0.1011	0.2035	0.3308	0.4431
	$s_{y \cdot x}$	0.04	0.38	0.14	0.21	0.04

each molality. Table 1 gives the regression coefficients and deviations for microvolts against temperature. The points shown in Figure 3 are the solutions of the regression equation for microvolts at the temperatures 5, 10, 15°C, etc. Snedecor's (1956) statistical

TABLE 2
CALIBRATION OF TWO PSYCHROMETERS AT 25°C

Regression coefficients for $\hat{Y} = c + d\psi$, where Y is calculated output of psychrometer (μV) ψ is water potential ($J.kg^{-1}$). The sample deviation from the regression equation is $s_{y \cdot \psi}$ and the deviation of the regression coefficient, d , is s_d (Snedecor 1956)

Psychrometer No.	c	d	$s_{y \cdot \psi}$	s_d
3	0.334	-4.99×10^{-3}	0.14	4.8×10^{-5}
4	0.859	-4.76×10^{-3}	0.27	11.2×10^{-5}

nomenclature and methods are used in this paper. Table 2 lists the regression coefficients and deviations for microvoltage output against water potential for the two psychrometers calibrated at 25°C only.

(ii) *Temperature Drift of Sphere.*—The temperature drift of the copper sphere was interpreted by the equation:

$$dT_s/dt = k(T_j - T_s), \quad (2)$$

where

T_s = temperature of sphere ($^{\circ}\text{C}$),

T_j = temperature of jacket ($^{\circ}\text{C}$),

t = time (hr), and

k = a heat conductance coefficient ($\text{degC}\cdot\text{hr}^{-1}$).

The term k had a value of 0.23 (sample standard deviation = ± 0.030).

(iii) *Temperature Drift and Measured Output of Psychrometer.*—Table 3 summarizes the results.

TABLE 3
TEMPERATURE DRIFT AND MEASURED OUTPUT OF PSYCHROMETER

Expt. No.	Sample	Psychrometer No.	Nominal Temperature ($^{\circ}\text{C}$)	Rate of Change of Sphere Temperature with Time, \dot{T} ($\text{degC}\cdot\text{hr}^{-1}$)	Change in Psychrometer Output per Unit of \dot{T} ($\mu\text{V}\cdot\text{degC}^{-1}\cdot\text{hr}$)
1	Dry	1	22	0.98	5.6
				-1.21	5.8
2	0.2m NaCl	1	21	0.80	7.3
				-1.18	5.8
3	0.6m NaCl	1	21	0.93	6.3
				-1.16	4.9
4	0.4m NaCl	2	30	-0.99	6.9
5	0.2m NaCl	2	5	0.92	5.6
				-0.98	5.3
6	0.8m NaCl	2	5	1.33	5.0
				-0.80	6.8
7	0.2m NaCl	2	30	0.76	6.5
				-0.53	5.4
8	0.8m NaCl	2	30	-0.86	6.6
				-0.45	8.8
9	Cotton	1	26	0.84	6.7
				-1.51	4.6
10	Cotton	1	26	0.95	5.4
				-1.39	4.9
11	Cotton	2	25	-1.05	4.4
				-1.11	4.4
12	Cotton	2	26	1.01	4.7
				-1.27	4.3

(c) *Discussion*

(i) *Calibration with Salt Solutions.*—The results shown in Figure 3 may be compared with those of Klute and Richards by determining the variations of slopes of the calibration curves with temperature whence:

$$K'_t = -1.07 \times 10^{-4}t - 2.44 \times 10^{-3}, \quad (3)$$

where K'_i is the slope of the calibration curve ($\mu\text{V}\cdot\text{J}^{-1}\cdot\text{kg}$) and t is degrees Celsius. When allowance is made for the different units, the values of Klute and Richards are -1.09×10^{-4} for the constant of proportionality and -2.75×10^{-3} for the intercept. The agreement is satisfactory considering the differences in couples and measuring systems.

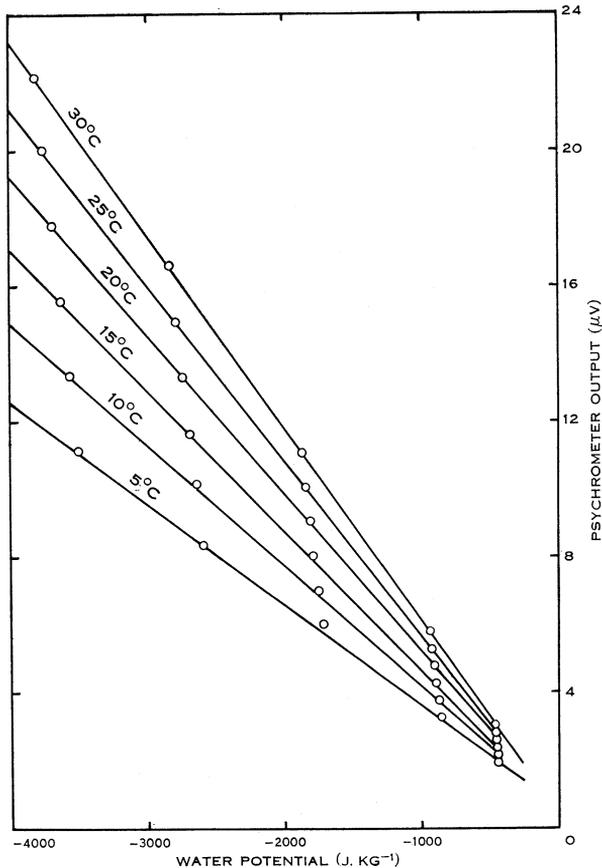


Fig. 3.—Calibration curve for psychrometer No. 2. Points were calculated from regression equations given in Table 1.

The reproducibility of results is indicated by the sample standard deviations for the regression equation ($s_{y,x}$ and $s_{y,\psi}$) given in Tables 1 and 2. The average was $\pm 0.27 \mu\text{V}$ (equivalent to $\pm 90 \text{ J}\cdot\text{kg}^{-1}$ at 5°C , and $\pm 50 \text{ J}\cdot\text{kg}^{-1}$ at 30°C) which may be compared with the value of $\pm 0.078 \mu\text{V}$ (or $\pm 15 \text{ J}\cdot\text{kg}^{-1}$, 25°C) for nine psychrometers calibrated in a water-bath with a control differential of 0.001 degC , using salt solutions with water potentials in the range $0-3760 \text{ J}\cdot\text{kg}^{-1}$.

The standard deviation of $\pm 0.009 \mu\text{V}$, recently quoted by Richards, Low, and Decker (1964), was calculated differently from the value given above, and was for the water potential range $0-900 \text{ J}\cdot\text{kg}^{-1}$. Recalculation using the two values supplied, and taking the origin as a third point for each regression of microvolts

against water potential gives an average *relative* standard deviation (C) of $\pm 0.017/450 = \pm 3.8 \times 10^{-5} \mu\text{V}\cdot\text{J}^{-1}\cdot\text{kg}$. As C is based upon only four independent values, and has the implicit assumption that the standard deviation depends upon the water potential, it may not be reliable; however, it agrees closely with $C = \pm 0.078/1800 = \pm 4.1 \times 10^{-5} \mu\text{V}\cdot\text{J}^{-1}\cdot\text{kg}$ for the water-bath results reported above. The deviation of ± 0.004 given by Richards and Ogata (1958) is presumably the sample standard deviation of the regression coefficient of microvolts against bars. This would be equivalent to $\pm 4 \times 10^{-5}$ in the units used in this paper, and should be compared with $\pm 4.8 \times 10^{-5}$ and $\pm 11.2 \times 10^{-5}$ of Table 2.

The standard deviation of calibration results from the apparatus is thus about three times that obtained when a water-bath is used.

(ii) *Temperature Drift of Sphere*.—As would be expected, the heat conduction coefficient, k [eqn. (2)], was unaffected by the sign of the temperature difference or the absolute temperature in the range 4.2 – 30°C . Also it was independent of whether the cell was empty or whether it contained salt solutions or plant leaves.

(iii) *Temperature Drift and Measured Output of Psychrometer*.—A drift of the sphere temperature of $1 \text{ degC}\cdot\text{hr}^{-1}$ caused a change in the output of the psychrometer of about $6 \mu\text{V}$ (equivalent to 0.1 degC). This change arises from a difference in the thermal paths to the tip and reference ends of the psychrometer; the tip is heated by the air in the cell, while the reference junctions are mainly heated by conduction along the copper leads. A drift in the sphere temperature of $0.01 \text{ degC}\cdot\text{hr}^{-1}$, which was normally attained, would cause an error of $0.06 \mu\text{V}$. This is equal to about one-quarter of the mean standard deviations of the values (Table 1 and 2) and undoubtedly causes part of the deviation.

It is clear that a satisfactory temperature stability is obtained with the apparatus for measurement of water potentials by the wet-bulb depression method, and that calibration results are similar to those obtained when the water-bath method is used.

IV. MEASUREMENTS WITH PLANTS

The experiments to be described illustrate the use of the apparatus in the estimation of water potential in the xylem of a plant.

(a) *Methods*

Cotton (*Gossypium hirsutum* L. cv. Empire) and pepper (*Capsicum frutescens* L. cv. Californian Wonder) plants grown in 5.5 -l plastic pots of soil in a glasshouse for about 3 months were used. The plants were severely wilted prior to testing, by withholding water for several days under hot summer conditions. At least 16 hr before enclosing a leaf it was trimmed to a rectangular shape 80 by 41 mm. With cotton plants this could be done without severing veins which supply the remaining part of the leaf; but this was not the case with pepper.

To measure ψ_s , the plug (K) of the sample cell was fitted around the petiole, and a seal made between petiole and plug by lightly tamping Q-compound through the outer ends of the milled slot. The O-ring (J) was fitted using a jig, and the rest of the apparatus assembled as already described. After obtaining a practically

constant output, water was added to the soil. In all, six experiments were done with cotton and two with pepper plants.

In three of the experiments, the approximate superficial density of a leaf lamina of the plant was determined by β -ray gauging by the method of Mederski (1961). The gauge was calibrated with aluminium foil of known superficial density. Also, in five of the experiments, a leaf adjacent to that in the water-potential apparatus

TABLE 4
WATER POTENTIALS IN PLANTS

Expt. No.	Plant	Ambient Temperature (°C)	Apparatus Temperature (°C)	Water Potential (J.kg ⁻¹)			
				Wilted Plant (ψ_g)	Detached Leaf of Wilted Plant	Rewatered Plant (ψ_g)	Detached Leaf of Rewatered Plant
13	Cotton	32	26	-3600			
14	Cotton	32	29	-2500			
15	Cotton	37	26	-2740	-2660		
16	Cotton	36	24	-4470	-4450		
17	Cotton	34	26	-2450	-2180	-1110	-1240
18	Cotton	31	26	-3840	-3650	-1250	-1140
19	Pepper	29	26	-1550	-1360	-790	-460
20	Pepper	28	25	-1680			

was detached from the plant just before rewatering, and in three experiments, 2½–7½ hr after rewatering. A water-bath at 25°C and psychrometers of either the Spanner or the Richards and Ogata type (Barrs 1965) were used to determine the water potentials of these leaves.

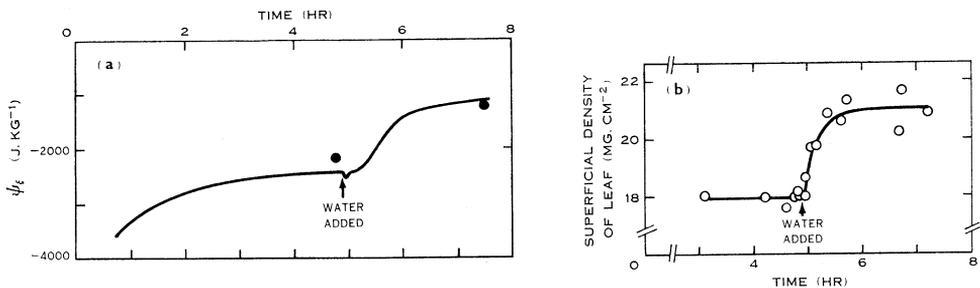


Fig. 4.—Effect of watering a wilted cotton plant upon (a) the water potential in the xylem (ψ_g) and (b) the superficial density of a leaf. ● Water potentials determined in a water-bath of leaves detached from the plant at the times indicated.

(b) Results

Values of ψ_g obtained just prior to and some time after watering are given in Table 4, together with water potentials determined in detached leaves of the same plant.

Figure 4 shows how ψ_{ξ} (taken from a recorder trace) and the superficial density of a leaf of the same cotton plant varied with time (expt. 17). Water potentials determined on two leaves detached from the plant are shown at the times of their excision. This experiment was chosen for illustration because it showed simultaneously the three parameters which were measured. Experiments 13–18 all showed the sharp “knee” 20–30 min after adding water, while all experiments in which superficial densities were measured showed the almost immediate increase in superficial density on adding water.

Pepper plants behaved differently, as is shown in Figure 5. First the approach to equilibrium was slower and secondly the change in slope on adding water was smaller.

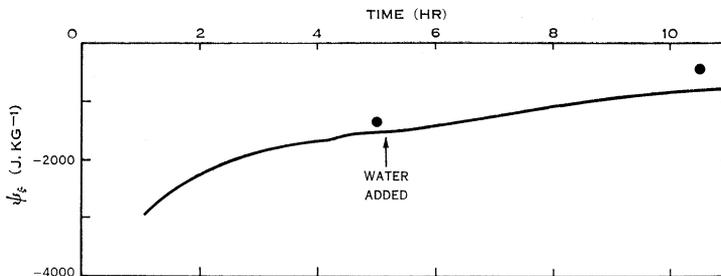


Fig. 5.—Effect of watering a wilted pepper plant upon the water potential in the xylem (ψ_{ξ}). ● Water potentials of leaves detached from the plant.

(c) Discussion

Since no other reliable method is available for measuring the water potential in the xylem of an intact plant, there are no values with which the present results may be compared. However, the ψ_{ξ} values for cotton changed in the expected direction upon watering a droughted plant, and followed the change in superficial density of the leaves. We believe that ψ_{ξ} is a good approximation to the water potential in the xylem of cotton plants.

The close agreement between the ψ_{ξ} values and water potentials of detached leaves may mean that a satisfactory estimate of the water potential in the xylem can be obtained by measuring detached leaves; however, the close agreement may not be general, for the cotton plants in the experiments described were probably transpiring at a relatively low rate. Furthermore, recent work with tobacco plants by Rawlins (1963) has indicated a large difference in water potential between stem and leaf.

The method can, in principle, be applied to all plants having a suitably long petiole and broad leaf. However, leaves of the second plant tested, namely pepper, were very slow to equilibrate with an enclosed space, either when detached or attached to the plant, and the ψ_{ξ} values for the plant did not increase rapidly after rewatering. Thus for pepper relatively quick changes of water potential in the xylem can probably not be followed.

Differences between the ambient temperature and that within the apparatus (Table 4) did not cause great differences between the ψ_{ξ} values and the water potentials of detached leaves. Consequently, it is believed that varying the ambient temperature does not introduce major errors in ψ_{ξ} .

The errors arising from the respiration of cotton and pepper leaves were relatively low, and have been neglected in the present experiments. However, it should be noted that the apparatus is equally compatible with Spanner-type psychrometers, which are recommended where large errors caused by respiration are present.

V. ACKNOWLEDGMENTS

We acknowledge with thanks construction of the apparatus by Messrs. J. J. Bleijie and R. Trewin, the growing of the plants by Mr. T. C. Mitchell, assistance with a major part of the experimental work by Mr. W. van Aken, and Mr. E. S. Trickett's advice on the measurement of microvoltages. We are grateful for comments by Mr. E. T. Linacre and Dr. J. H. Palmer.

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