AN ANALYSIS OF THE WATER POTENTIAL ISOTHERM IN PLANT TISSUE

II.* COMPARATIVE STUDIES ON LEAVES OF DIFFERENT TYPES

By I. Noy-Meir†‡ and B. Z. Ginzburg†

[*Manuscript received January* 26, 1968]

Summary

The water potential isotherms of leaves of carob (a sclerophyllic xerophyte), plane tree (a mesophyte), and saltbush (a semisucculent xero-halophyte) were measured by vapour equilibration with filter paper. The isotherm of the living tissue was partitioned into components by measuring the isotherms of killed tissue and of isolated matrix fractions. Empirical functions were fitted by regression to each of the components. The isotherm of the matrix fractions fitted best to a function of the form $\Psi = -a/w^2+b/w$ and the isotherms of killed tissue, whether before or after subtraction of the matrix, to a function of the form $\Psi = -a/w^2-b/w$. The first term indicates non-ideality of the tissue solution. The water potential difference between living and killed tissue, which is an approximation of the hydrostatic potential, was far from linear with water content; either a quadratic function or two discontinuous linear ones could be fitted to it. Negative hydrostatic potentials were measured, the highest values (20 atm) being attained in carob. A hysteretic component was measured both in the entire tissue and in the matrix fractions.

The parameters of the isotherm (the coefficients of the functions) for the leaves of the three species were compared and related to their "drought tolerance" and their ecological preferences. The leaves of the two xerophytes, carob and saltbush, can both tolerate lower water potentials than those of plane, but have very different isotherms. As a result, in saltbush most of the decrease in water potential upon drying is in the osmotic potential, while in carob a considerable part of the decrease is accounted for by the hydrostatic potential (negative turgor). This suggests that the mechanisms of adaptation to drought at the cellular level of the two species are of a different nature.

I. Introduction

The water potential isotherm relates the water potential (specific free energy of water) of a given system to its water content. It thus characterizes the water equilibrium properties of the system. The problems concerning the analysis of this isotherm in plant tissues have been introduced and its theory has been discussed in a previous paper (Noy-Meir and Ginzburg 1967).

The present paper describes the results of an experimental analysis of the isotherm for leaves of three species. The aim of the work was to assess the contribution of the various components of the isotherm in the leaves in question. Special

* Part I, *Aust. J. biol. Sci.*, 1967, **20**, 695–721.

† Department of Botany, The Hebrew University of Jerusalem, Jerusalem, Israel.

‡ Present address: Department of Geography, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600.

attention was paid to the analysis of the matrix and the problem of negative turgor (Tyurina 1957*a*, 1957*b*; Slatyer 1958, 1960, 1961; Kreeb 1961, 1963; Rehder 1961).

Ecologically diverging species were chosen for comparison, to test the suggestion that the form of the isotherm and its components are related to adaptation to drought (Tyurina 1957*b*; Weatherley and Slatyer 1957; Slatyer 1961; Jarvis and Jarvis 1963).

The theory underlining this work was considered in general terms before the experimental work was begun. But the detailed and explicit treatment of the theory reported in the first paper was undertaken only after the experiments had been concluded. This treatment revealed some additional effects which might complicate the analysis. These theoretical effects have no experimental counterpart in the work reported here, but have to be considered as possible sources of errors.

II. Methods

The determination of the water potential isotherm for living plant tissue is done by measuring water potential and water content simultaneously on a large number of samples of similar tissue throughout the desired range of water potential. Of the methods available for measuring water potential, the gravimetric (Ursprung and Blum 1916), densimetric (Shardakov 1953), and refractometric methods (Ashby and Wolf 1947) all involve direct contact of the tissue with the external measuring solution. Consequently plasmolysis occurs at low water potentials; possibly solute leakage could also occur. The various psychrometric methods (Spanner 1951; Richards and Ogata 1958; Macklon and Weatherley 1965), which are probably the most accurate, require elaborate instrumentation for each determination, which makes them impracticable and costly for experiments in which many determinations are necessary, such as the isotherm analysis. The vapour equilibration method (Slatyer 1958) seemed suitable for the conditions used in these experiments, except that a long equilibration time is necessary (24–72 hr) and changes in the tissue are possible. In principle, the equilibration time might be shortened by reducing the distance between the tissue and the solution, but in practice this reduction is limited by the risk of wetting the tissue by the solution. In contrast, it was thought that the use of a solid hydroscopic substance as the measuring system instead of a solution would allow direct contact (minimal distance) between the tissue and the measuring system. The water content of the hydroscopic solid would serve as an indicator of water potential, the water potential isotherm of the solid being used for calibration. The method was tested in a series of experiments, with filter paper as the hygroscopic solid.

(*a*) *Isotherm of Filter Paper* (*Calibration*)

The purpose of the experiment was to determine whether the water content (per dry weight) of filter paper can be a reproducible measure of water potential, and if so, to obtain a reliable curve relating the two quantities.

Each equilibration vessel consisted of the upper half of a Petri dish (5·8 cm diam.) closed by a rubber stopper, leaving an air space of 5–8 mm. In each vessel several disks of filter paper (to prevent splashing) were soaked in 5 ml of sodium chloride solution of known water potential. Above this, upon a coarse grid of PVC 2 mm thick, three disks of filter paper (Whatman No. 1, 5·5 mm diam.) were placed. The dishes were equilibrated in a thermostatic bath ($\pm 0{\cdot}05$°C; Grant Instruments) for 36–48 hr, during which the disks attained constant weight ($\pm 0{\cdot}5\%$). Each sample was then weighed within 15 sec on a Mettler H6 ($\pm 0{\cdot}1$ mg) balance (w_E), oven-dried (95°C, 12 hr), and reweighed (w_D). (Weighing at different intervals indicated that within 15 sec the moist sample lost about $0{\cdot}5\%$ of its water content, which was neglected.) The water content of the filter paper is:

$$w = (w_E - w_D)/w_D.$$

The experiment was repeated with two pretreatments of the filter paper: air dry, and wetted with water to $w_0 = 0{\cdot}50$. The results (Fig. 1) showed that the equilibrium water content of initially wet disks was 10–20% higher than that of initially dry ones which absorbed water only as vapour. The transition from the "dry" to the "wet" curve seemed to require contact with liquid water. Thus, the water content of the paper is a reproducible measure of water potential only if the initial state is constant for all samples. The air-dry state was chosen as the initial state in subsequent experiments.

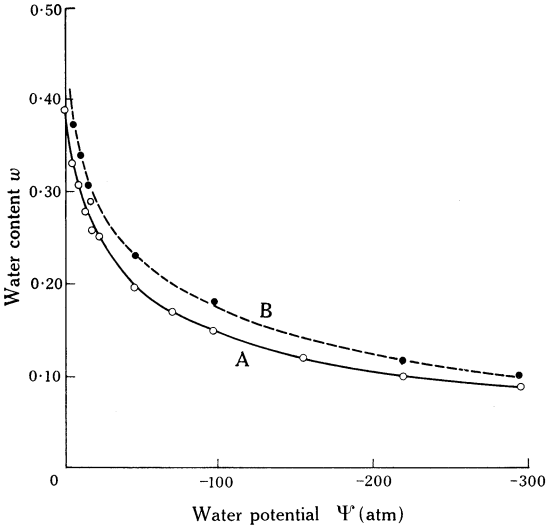

Fig. 1.—Effect of wetting on the water potential isotherm of filter paper. *A*, isotherm of air-dried paper ($w_0 = 0{\cdot}05$); *B*, isotherm of paper wetted to $w_0 = 0{\cdot}50$.

The simplest empirical equation which can be fitted to the isotherm of filter paper is of the form:

$$\Psi w^2 = -a + bw$$

or

$$\Psi = -(a/w^2) + (b/w), \tag{1}$$

where a and b are constants. A full explanation of symbols is given in the Appendix. The parameters a, b, and w_{max} ($= a/b$) resulting from a regression using this equation are given in the following tabulation (standard deviations being given in parentheses):

Temp. (°C)	No. of Points	a(atm)	b(atm)	w_{max}
28	24	3·92(±0·06)	10·67(±0·30)	0·368
25	10	4·16(±0·08)	10·71(±0·41)	0·388

The equation with the corresponding values of a and b was used for calibration in the following experiments.

(*b*) *Comparison of Tissue/Paper with Tissue/Solution Equilibration*

These experiments were designed to compare the accuracy of the tissue/solution technique with the tissue/paper technique, under similar conditions.

Leaves of carob (*Ceratonia siliqua*, a tree with rather thick and sclerophyllous leaves) were cut with a sharp scalpel into strips 3–5 mm wide and 10–20 mm long (excluding the main vein). Samples of about 0·5 g of these strips were equilibrated to different water potentials by the two methods: tissue/paper and tissue/solution.

The technical details of the equilibration were as for the calibration experiments. In the tissue/solution dishes the leaf samples were placed on a grid above the solution as before. In the

tissue/paper dishes the leaf samples were placed directly on three disks of air-dried filter paper. Different water potentials were obtained by either predrying (in air) the tissue sample or including wet filter paper (separated from both the tissue and the dry paper).

The water content of each sample was determined before equilibration (w_0) and after 24 hr (w_{24}) and again after 48 hr (w_{48}) of equilibration at 28°C. A preliminary experiment had shown that the tissue lost about 1% of its dry weight for every 24 hr of equilibration, and the values of w were corrected accordingly. The results from the two methods were compared with regard to two types of error: random error and bias due to incomplete equilibration.

(i) *Random Error.*—the standard deviation of water content was estimated from the range (Snedecor 1956) of the water content at constant values of water potential, expressed as percentages of the mean water content ("coefficient of variation"):

$$c_w = 100kr_w/\bar{w}, \tag{2}$$

where k = a constant depending on the sample size n (for $n = 2$, $k = 0{\cdot}89$; $n = 3$, $k = 0{\cdot}59$), r_w = the range of water content at constant Ψ, and $\bar{w}$ = the mean water content at the same Ψ.

(ii) *Bias Due to Incomplete Equilibration.*—The relative error (bias) in w resulting from taking w_{24} as the equilibrium value instead of w_{48} was calculated as a percentage:

$$\text{Bias} = 100(w_{48}-w_{24})/w_{48}. \tag{3}$$

With the tissue/solution method, the bias averaged 8% over the whole range, and was 10–15% at lower water potentials (−40 to −100 atm), while with the tissue/paper method, it averaged 4% and did not exceed 6%. The random error c_w, on the other hand, was about 5% for tissue/paper, and only 3% for tissue/solution. However, in contrast to the bias error, this error can be reduced by increasing the number of measurements. Since the tissue/paper method is technically simple, this is easily done.

It was therefore considered that the advantage of more rapid equilibration outbalanced the increased scatter, and it was decided to adopt the filter paper technique for measuring the water potential isotherm of leaf tissue.

(c) *Experimental Measurement of the Water Potential Isotherm of Leaf Tissue*

The experimental set-up used for the measurement of the isotherm of leaf tissue was the same as that described for the tissue/paper equilibration experiments. Each equilibration dish contained three air-dried disks of filter paper (0·5–0·6 g) and 0·3–0·6 g of leaf material. The leaves used were from three woody species with different leaf anatomies and habitat:

(1) *Ceratonia siliqua* (carob)—a sclerophyllous evergreen tree, typical of the mediterranean region.

(2) *Platanus orientalis* (plane)—a mesophyllic deciduous tree, restricted in the mediterranean region to the banks of streams.

(3) *Atriplex halimus* (saltbush)—a halophytic shrub of the semi-arid region, with semi-succulent leaves.

Two sets of measurements were performed on the two former species, and one on the lattermost. For each set of measurements, 50–200 g of adult leaves from one plant were collected, cut into strips (3–5 mm wide), and divided into the following groups:

Group 1: Living tissue (initial Ψ about −20 atm).

Group 2: Tissue killed by freezing or by ether vapour.

Group 3: Tissue dried to about $\Psi = -200$ atm.

Group 4: Isolated matrix fractions (initially wet).

Group 5: Isolated matrix fractions (initially dried to about $\Psi = -200$ atm).

The matrix fractions were prepared from killed tissue by homogenization, followed by repeated extractions of solutes by cold water and centrifugation. Separation between a cell wall fraction and a cytoplasmic fraction was attempted by repeated quick sedimentation in cold

water. It was achieved only partially, because some cells remained unbroken. An almost pure "cytoplasmic fraction" (in which starch granules and chloroplasts could be identified) was obtained from *C. siliqua and P. orientalis* leaves, but the remaining "cell wall fraction" included also unbroken cells with cytoplasmic particles (estimated between 20 and 40% of all cells). With *A. halimus* leaves such a partial separation was not obtained, and measurements were made only on the whole "matrix fraction".

Measurements were made only on the insoluble (matrix) fraction and not on the solution, since the application of the filter paper technique to the latter proved to be difficult.

Samples from each group were brought to final water potentials ranging from 0 to −200 or −300 atm. The period of equilibration was fixed at the time necessary to obtain about 97% equilibration. Preliminary experiments showed this to be 32 hr for carob leaves, and 24 hr for plane and saltbush leaves. Water content of the plant material and the filter paper was determined per dry weight. Subsequent floating on water to determine turgid weight and "relative water content" was not used, since this involved considerable marginal injection in carob (and washing of salts from the cell walls in saltbush?). The tissue samples in any one experiment were selected to be homogeneous, so that this further standardization probably would not have changed much. For comparison between experiments a different standardization was used (see Section IV). The water potential was computed from the water content of paper by the calibration equation (1).

The isotherm of the living tissue (group 1) was then analysed in the following way: the difference between groups 1 and 2 was taken as the hydrostatic potential; the difference between groups 2 and 4 (or 5) as the isotherm of the soluble fraction; and the difference between groups 4 and 5 as the hysteretic component of the matrix, and between groups 2 and 3 as the hysteretic component of the whole tissue. The general procedure was to fit an empirical regression equation for each curve to be subtracted and use the equation to estimate the subtracted value at each point of the curve from which subtraction was made. The computations were programmed in FORTRAN IV and performed by the IBM–3060 computer at the Hebrew University of Jerusalem.

In order to assess the effect of low water potential on the viability of the tissue, samples of living tissue which had been equilibrated to various low water potentials were equilibrated back to about −10 atm. The percentage of lethal damage was then estimated by visual inspection of the necrotic areas.

III. Results

(*a*) *Matrix Fractions*

The isotherms of all matrix fractions showed a hysteresis effect, the curve for the wet state diverging from that for the dry state [Fig. 2(*a*)]. The water content of the wet matrix was usually 20–50% above that of the dry matrix from 0 to −25 atm, the difference decreasing rapidly with decreasing water potential, becoming undetectable at about −50 atm. Once the material had been dried to −50 atm, further drying did not seem to affect the reabsorption curve. The effect is similar to the above-mentioned hysteresis in filter paper. Within the range of water potential where the two curves diverged, the results for the dry state were more reproducible than those for the wet state, and only the former are considered in the following results.

In an attempt to fit to the data a function which would express the water potential Ψ as a simple polynomial in $1/w$, Ψ was multiplied by the water content w and by its square w^2 and the values of $-\Psi w$ and $-\Psi w^2$ plotted against w. The $-\Psi w$ versus w curves [Fig. 3(*a*)] were still markedly concave; on comparing linear and quadratic regression, the "curvilinearity" or improvement by inclusion of the quadratic term (Snedecor 1956) was always highly significant ($P < 0{\cdot}01$). On the

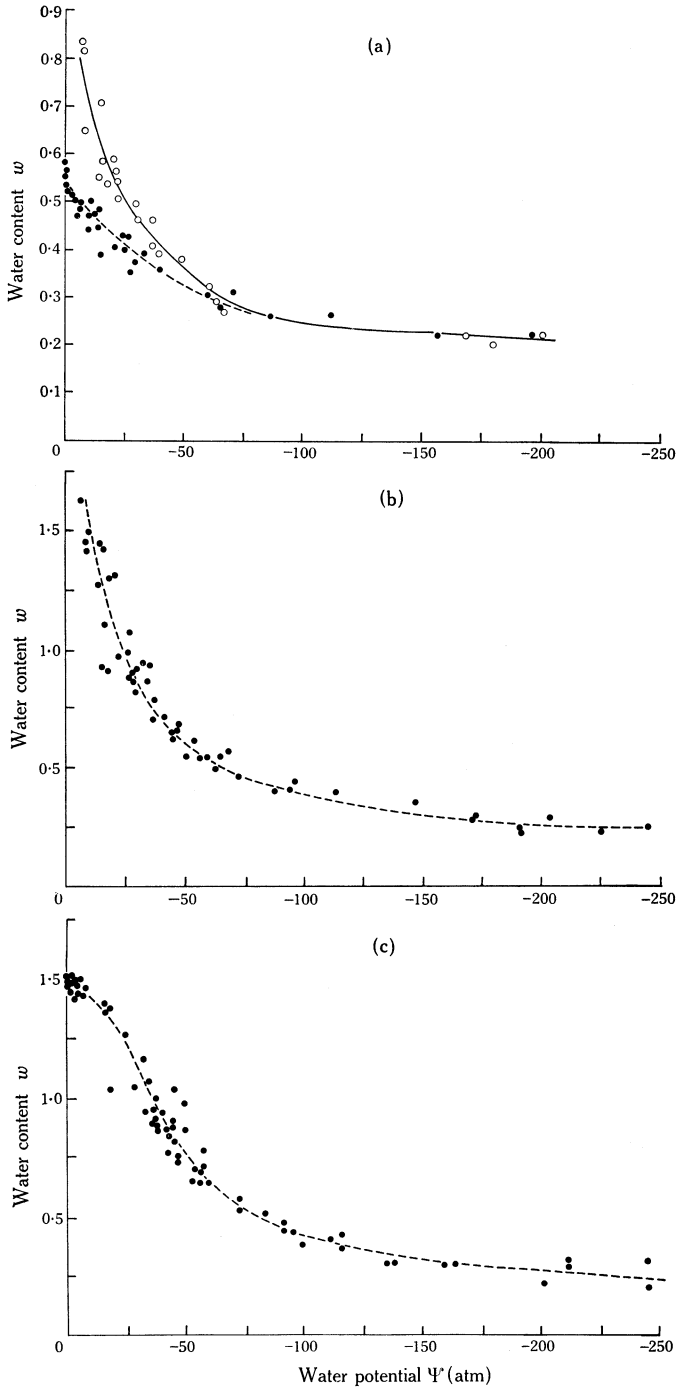

Fig. 2.—Isotherms of various tissues. (*a*) Matrix fraction from wet (○) and air-dried (●) initial states (cell wall of *C. siliqua*, experiment 1). (*b*) Killed tissue (*P. orientalis*, experiment 1). (*c*) Living tissue (*C. siliqua*, experiment 2).

other hand, the $-\Psi w^2$ versus w curves [Fig. 3(*b*)] did not show any consistent form of departure from a decreasing straight line; the linear regression was always significant ($P < 0{\cdot}01$) whilst the curvilinearity was not significant in all but two

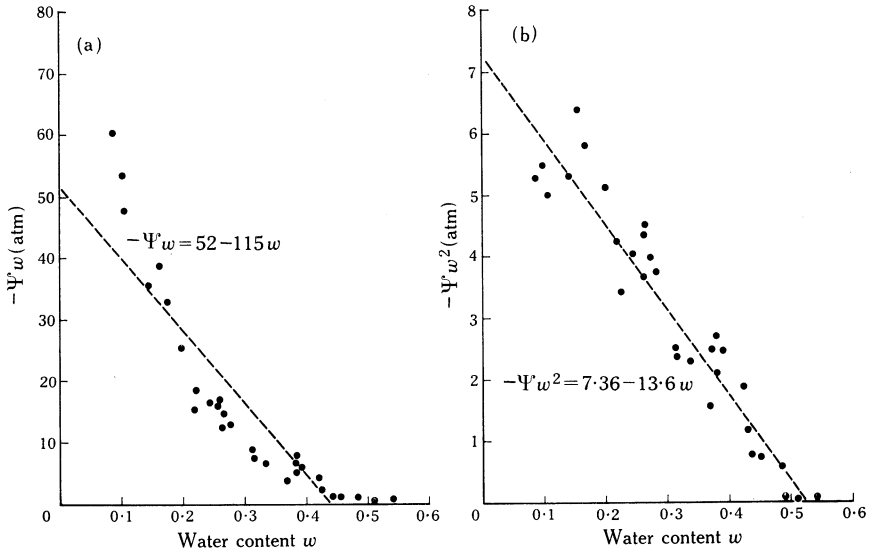

Fig. 3.—Isotherm of a matrix fraction (cell wall of *C. siliqua*, experiment 2) in two graphical representations, and the corresponding regression lines. (*a*) Shows concavity; (*b*) no curvilinearity.

cases, where it was marginally significant ($P < 0{\cdot}01$). The data could therefore be represented by either of the equations:

$$-\Psi_m w_m = a_m + b_{m_1} w_m + b_{m_2} w_m^2, \tag{4}$$

or

$$-\Psi_m w_m^2 = a_m + b_m w_m, \tag{5}$$

where Ψ_m = water potential of matrix fraction and w_m = water content of matrix fraction per dry weight of fraction. The latter, simpler equation was chosen. It is equivalent to:

$$\Psi_m = -(a_m/w_m^2) - (b_m/w_m). \tag{6}$$

The coefficient a_m was always positive, and can tentatively be interpreted as a measure of the "specific hydrophily" of the matrix. The coefficient b_m was always negative; $-b$ can likewise be interpreted as a measure of the rigidity of the matrix, which limits its swelling. The maximal water content at $\Psi = 0$ is:

$$w_m^{\max} = -a_m/b_m. \tag{7}$$

Comparison of the parameters of the isotherm of the different matrix fractions fitted by equation (6) are given in the following tabulation. Results from two

experiments are given for *C. siliqua* and *P. orientalis*. Standard deviations are given in parenthesis:

Species	Fraction	a_m (atm)	b_m (atm)	w_m^{max}
C. siliqua	Cell wall	7·8–12·3	14·5–21·1(0·9–1·4)	0·53–0·58
	Cytoplasmic	7·9–10·8	15·6–23·6(2·5–1·9)	0·50–0·46
P. orientalis	Cell wall	8·4–9·5	18·9–13·4(1·9–2·4)	0·45–0·71
	Cytoplasmic	8·3	16·3(1·3)	0·51–0·65
A. halimus	Matrix (unseparated)	19·7	12·3(1·0)	1·60

These results show that there is no clear-cut difference between the "cell-wall" and "cytoplasmic" fractions taken from the same leaves. This result, which is somewhat surprising in view of the presumably different composition and structure of the two fractions, raises the question as to what extent the fractions have been modified by the method of preparation. They also show that the matrix fractions of *C. siliqua* and *P. orientalis* have similar isotherms, while *A. halimus* matrix has a higher a_m and lower b_m coefficient than the two others, and hence a much higher maximum water content.

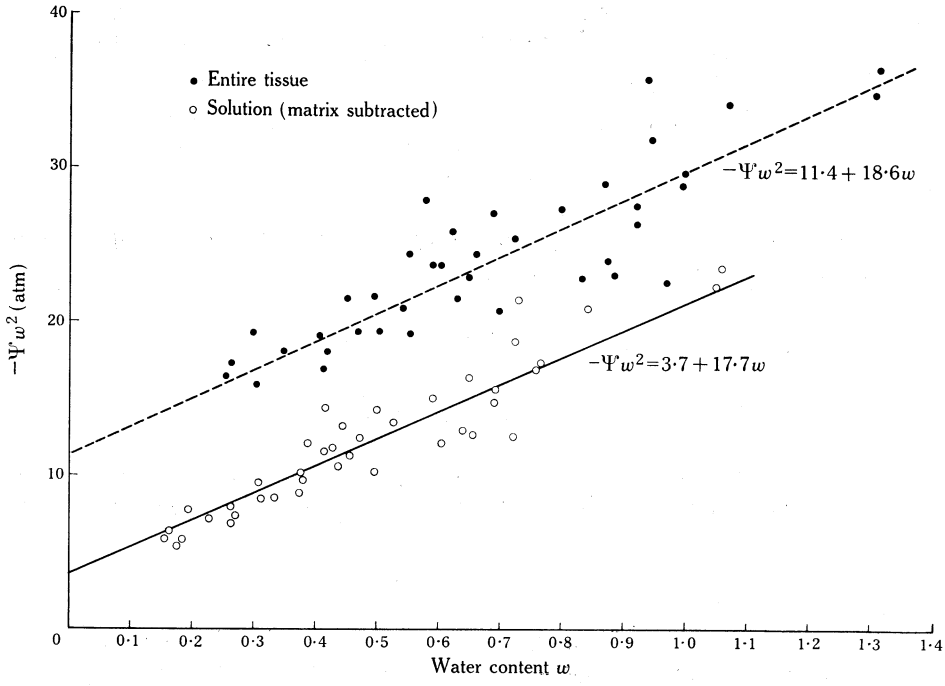

Fig. 4.—Isotherm of killed tissue (*P. orientalis*, experiment 2) in the $-\Psi w^2$ versus w plot before and after subtraction of matrix water. Lines fitted by regression.

(*b*) *Killed Tissue*

The isotherm of the killed tissue in the $-\Psi$ versus w_m plot was a typically concave one [Fig. 2(*b*)]. As with the matrix, the Ψw versus w plot still gave a concave curve with a significant quadratic term. The $-\Psi w^2$ versus w plot again gave a good

fit to a straight line (Fig. 4) (this time an increasing one) without significant curvilinearity ($P \geqslant 0{\cdot}01$). The results were therefore fitted by regression to an equation similar to (5) or (6), this time both a and b being positive coefficients (Table 1).

TABLE 1

PARAMETERS OF THE EQUATION $-\Psi w^2 = a+bw$ FOR THE ISOTHERMS OF WHOLE KILLED TISSUE, SOLUTION (= BULK OSMOTIC), AND BULK MATRIC POTENTIAL

Standard deviation of b given in parenthesis

Species	Killed Tissue		Solution		Bulk Matric	
	a(atm)	b(atm)	a(atm)	b(atm)	a(atm)	b(atm)
C. siliqua	8·5–15·4	7·2–13·8(13·0–5·5)	1·6—4·2	10·2–17·1(0·8–4·0)	6·9–11·2	−3·0– −3·3
P. orientalis	11·4–10·6	18·6–26·1(1·4–1·5)	3·7–4·4	17·7–22·8(1·0–1·1)	7·7–6·2	0·9–3·3
A. halimus	115·0	138·4(13·8)	63·0	135·0(12·3)	5·2	3·4

The isotherm of the killed tissue, $\Psi_d(w_d)$, was then partitioned on the water content axis by reference to the isotherm of the isolated matrix fractions. At each point (Ψ_d, w_d) the water content of the matrix fractions (w_m) at the same Ψ was calculated by using the equations for the corresponding fractions. w_m was multiplied by the ratio of the dry weight in the fraction to the total dry weight. The water potential Ψ was then plotted against the difference $w_s = w_d - w_m$, to give the isotherm of the "soluble fractions" of the tissue. (Actually, w_s thus calculated might differ from the water content of the isolated soluble fraction measured at the same water potential, if there is any interaction between the two fractions.) This new "solution" isotherm, $\Psi(w_s)$, again became nearly linear on the $-\Psi w^2$ versus w plot (Fig. 4) and was fitted to the equation:

$$\Psi_s = -(a_s/w_s^2) - (b_s/w_s). \tag{8}$$

Comparison of the resulting coefficients (Table 1) to the one corresponding for the whole tissue shows that subtraction of the matrix water resulted in a considerable decrease in a, and either no change or a slight rise in b. In all cases a is still above zero, though not always significantly. For an ideal solution, $a = 0$; therefore $-b_s/w_s$ and $-a_s/w_s^2$ can be interpreted as the ideal and non-ideal components of the osmotic potential of the soluble fraction of the tissue respectively (Noy-Meir and Ginzburg 1967).

The isotherm of the killed tissue usually showed a hysteresis after drying to about −200 atm. The effect was larger than could be accounted for by the hysteresis of the isolated matrix. This suggests that drying to such low potential had an irreversible effect on the properties of the soluble fraction, either directly or through interaction with the matrix.

It was attempted to partition the isotherm of the killed tissue also on the water potential axis, i.e. into the "bulk" osmotic and matric potentials of the whole tissue (Noy-Meir and Ginzburg 1967). The "direct" approach—reference to the isotherm measured on the isolated matrix—seemed to be meaningless in this case, since hydration was unlimited for the whole killed tissue but limited for the matrix;

the resulting "matric potential" would have been zero except at very low w. Instead, the isotherm of the soluble fraction, $\Psi(w_s)$, which had been derived by subtraction of the matrix on the w-axis, was treated as the bulk osmotic component of the isotherm of the whole tissue $\Psi_\pi(w)$. The bulk matric component was then calculated by subtraction of $\Psi_\pi(w)$ from the total isotherm $\Psi_d(w)$ on the Ψ-axis (see Fig. 4):

$$\Psi_\tau(w) = \Psi_d(w) - \Psi_\pi(w). \tag{9}$$

The osmotic and matric potentials thus calculated are not necessarily equal to those which would have resulted from measuring the isotherm of the isolated soluble fraction and subtracting it on the Ψ-axis. They are probably approximately equal, but will differ according to the extent of "overlapping" between matrix water and solution. Since the equations fitted to $\Psi_d(w)$ and $\Psi_\pi(w) = \Psi(w - w_m)$ were of the same general form, the coefficients of a similar equation for the matric potential:

$$\Psi_\tau = -(a_\tau/w^2) - (b_\tau/w) \tag{10}$$

could be calculated simply by subtraction of the coefficients:

$$a_\tau = a - a_s, \tag{11}$$

and

$$b_\tau = b - b_s. \tag{12}$$

The results (Table 1) show that the bulk matric potential is dominated by the $-(a/w^2)$ term, b_τ being usually very small.

(c) *Living Tissue*

The isotherm of the living tissue had the typical S-shape [Fig. 2(c)]. At each point (Ψ, w), the value of Ψ_d (the water potential of the killed tissue) was calculated from w and the corresponding equation $\Psi_d = \Psi_d(w)$, and subtracted from Ψ. The difference, assumed to be the hydrostatic potential $\Psi_p = \Psi - \Psi_d$, was plotted against w.

In all of the resulting curves (e.g. Fig. 5) Ψ_p passed from positive to negative values with decreasing w, usually reached a minimum, and increased again to near zero with further decrease in w. This last part of the curve, beyond the minimum, corresponded to the increasing lethal damage observed in the tissue. The gradual reversal of the hydrostatic potential from negative to zero in this range is most readily interpreted as being associated with gradually increasing mortality of cells. This range was not included in the following analysis of Ψ_p. Even in the range where no damage to the tissue could be detected, Ψ_p was not linear with water content. The results could be fitted either by a quadratic function:

$$\begin{aligned} \Psi &= [\epsilon^0 + k(w - w^0)/w^0][(w - w^0)/w^0] \\ &= kw^2/(w^0)^2 + [w(\epsilon^0 - 2k)/w^0] - (\epsilon^0 - k), \end{aligned} \tag{13}$$

where ϵ^0 = elastic modulus at $\Psi_p = 0$, and

$$k = \mathrm{d}\epsilon/\mathrm{d}[(w - w^0)/w^0],$$

or by two discontinuous linear functions:

$$\Psi_p = \epsilon_1(w-w^0)/w^0 \text{ for } \Psi_p > \Psi_p^c,$$
$$\Psi_p = \epsilon_2(w-w^0)/w^0 \text{ for } \Psi_p < \Psi_p^c, \tag{14}$$

where Ψ_p^c = transition value of Ψ_p. The points were too scattered for a distinction between the two models to be made. The parameters resulting from the two

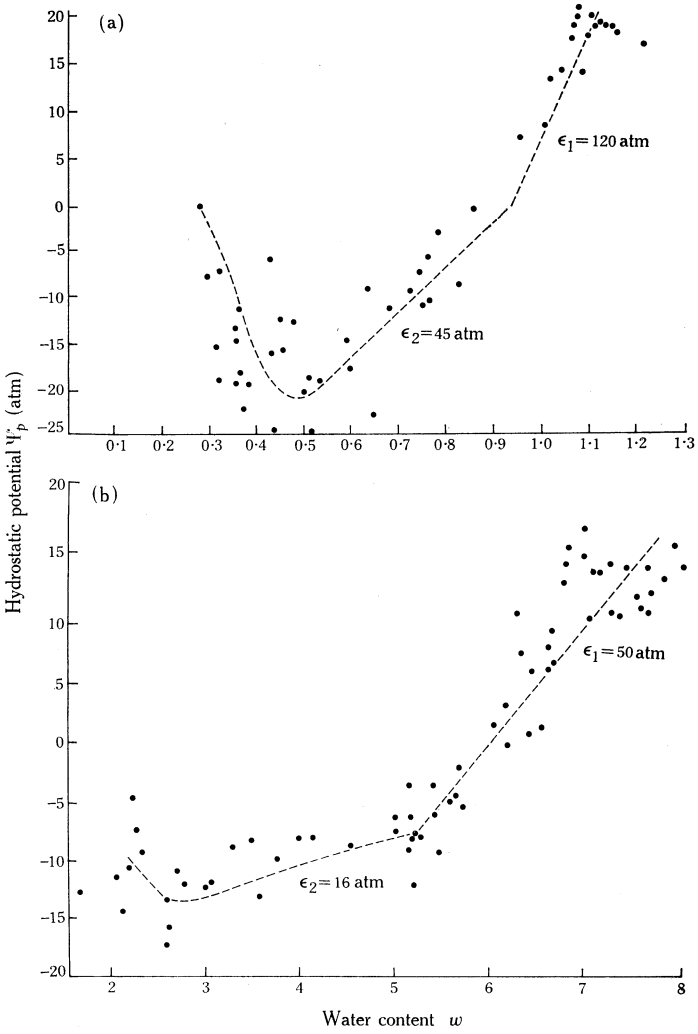

Fig. 5.—Hydrostatic potential versus water content. (*a*) *C. siliqua* (experiment 1); (*b*) *A. halimus*.

alternative types of equation are given in the following tabulation:

Species	w^0	Quadratic Parameters		Linear Parameters		
		ϵ^0(atm)	k(atm)	ϵ_1(atm)	ϵ_2(atm)	Ψ_p^c(atm)
C. siliqua	0·95–1·30	70–75	40–75	120–130	45–35	0–0
P. orientalis	1·32–1·37	55–50	60–95	105–100	30–22	0–2
A. halimus	6·0	45	50	50	16	−8

Since Ψ_p was related to the total w, these results apply to the average hydrostatic potential of the whole tissue, including the matrix. When Ψ_p was related to the "solution water" w_s only, assuming that for the matrix $\Psi_p = 0$, the slope of the $\Psi_p(w)$ curve and hence the value of ϵ increased markedly, tending to infinity near "full turgor". This result suggests that the latter assumption is wrong, and at least part of the matrix water is affected by the hydrostatic potential.

(*d*) *Lethal Damage*

From curves relating visible lethal damage to the water potential to which the tissue had been equilibrated three points were determined: initial damage (Ψ^i), 50% damage (Ψ^{50}), and 100% damage (Ψ^{100}). Assuming that the decline of negative Ψ_p is associated with lethal damage, the values of the total Ψ were also determined at the point where Ψ_p reached its minimum (Ψ^n), and the point where it reached zero again (Ψ^r). Both values, especially the latter one, are very crude estimates due to the large scatter. These values of Ψ are given in the following tabulation:

Species	$-\Psi^i$ (atm)	$-\Psi^{50}$ (atm)	$-\Psi^{100}$ (atm)	$-\Psi^n$ (atm)	$-\Psi^r$ (atm)
C. siliqua	60–50	90–80	150–130	77–67	140–120
P. orientalis	45–50	60–70	80–100	50–53	80–80
A. halimus	60	120	$\geqslant 150$	114	160

The results suggest great variability in the ability of cells within each tissue to stand low water potentials, especially in saltbush, where there was a difference of about 100 atm between initial damage and 100% damage. The point where Ψ_p reaches zero again is usually close to the point of 100% visible damage. The point of minimal Ψ_p is always between initial and 50% damage.

IV. Discussion

(*a*) *General Features of the Results: the Components of the Isotherm*

There was a noticeable effect of the matrix on the water potential isotherm in all three species examined; at −20 atm matrix water was between 7 and 26% of the total water content and matric potential was about the same fraction of total water potential. The water potential of the matrix was found to be dominated by a term inversely proportional to the square of water content, while that of the solution

was inversely proportional to water content proper. Hence the relative effect of the matrix increased with decreasing Ψ, and at -100 atm the fraction of matrix water was 12–33%. The cell wall contributed approximately 60% of this fraction. In the most sclerophyllous leaf examined, carob, the cell water fraction was only 15–20% as compared to 30–40% measured for *Eucalyptus* by Gaff and Carr (1961). This would still be enough to produce the "buffer effect" of the cell wall suggested by Gaff and Carr (1961). However, the conditions necessary for this effect are not only a high water capacity of the cell wall, but also its ability to maintain steep gradients of Ψ by increasing resistance when dry (Shimshi 1963).

A combination of both properties might be responsible for the "time drift" in water potential measurements (Rehder 1961; Kramer and Brix 1965) and for very low values of Ψ obtained by short-time measurements (e.g. Tyurina 1957*a*, 1957*b*).

Since isotherm measurements were made on the isolated matrix, but not on the isolated solution, it was not possible to evaluate the effects of solute–matrix heterogeneity and interaction (Noy-Meir and Ginzburg 1967). For this particular set of measurements at least, partition of the total water content into matrix water and solution water was more satisfactory and straightforward than partition of total water potential into matric and osmotic components.

It is not clear to what extent the hysteresis cycle found in isolated matrix affects the water relations of the whole tissue. It could, however, explain discrepancies between water content of vapour-equilibrated and liquid-equilibrated tissues at equal water potential (Macklon, cited by Weatherley 1965), and the fact that 100% "relative water content" (or "relative turgidity") has rarely, if ever, been measured (Slatyer and Barrs 1965).

Turning to the isotherm of the killed tissue, the most striking result is the consistent deviation from ideal solution behaviour, even after subtraction of the matrix water. The deviation term (proportional to the square of concentration), accounts for 7–16% of the total osmotic potential at -20 atm. This term is probably mainly associated with the presence of proteins and other large "hydrophilic" molecules in the cell solution, since pure solutions of these substances are known to show large effects of this form. However, the measurements and calculations by which the isotherm of the solution has been found in this experiment cannot exclude the possibility that matrix–solution interaction is responsible for the effect.

In any case, the relatively large effects of matrix and "non-ideality" which have been measured indicate that it is unwarranted to assume ideal osmotic behaviour for tissue solutions, and certainly for killed tissue (without correction for matrix) of leaves, without an experimental check with the specific material used. For instance, Weatherley's (1965) finding (for *Ricinus*) that the water potential of leaves killed after desiccation increased with decreasing water content by more than expected from an ideal solution, might have other explanations than "mobilization of vacuole solutes" during desiccation. The latter process certainly cannot explain the departure from ideality in the present experiments, since the tissue was killed before it was desiccated. However, if such a process had occurred, it might have affected the comparison between living and killed tissues at lower Ψ.

Some cryoscopic measurements which were made to check this point showed no consistent difference in osmotic potential between extracts of turgid tissue and

desiccated tissue, after dilution to equal water content per dry weight of tissue. It was thus assumed that the total amount of solutes per dry weight of tissue was constant and independent of water content throughout the experiment.

The difference between the isotherms of living and killed tissues was therefore primarily interpreted as the hydrostatic potential. The observed changes of this component with water content are consistent with the following model: as the water content of the leaf falls below the level w^0, the cell walls pass from being stretched outward to being pulled inward. Their elastic resistance to this inward pull generates a negative turgor within the cell, which increases as the cell shrinks (though the elasticity modulus is lower than in the range of positive turgor), and reaches −12 atm in plane and −21 atm in carob.

At some point the negative turgor "collapses" to zero in some of the cells, probably by penetration of air between the cell wall and the protoplast, while in other cells it continues to increase. As the water content decreases, the number of cells in which the negative turgor collapses increases; the average negative turgor passes through a maximum and then decreases, until it reaches zero and stays zero, when all cells have collapsed. The collapse of negative turgor is correlated with the death of cells, but it could be either the cause or the effect of death.

It is necessary, however, to be cautious in equating the difference in Ψ between living and killed tissue with turgor, since, in theory, other effects may contribute to this difference. A probable effect is the change in the average osmotic potential due to mixing of the solutions from the various phases, varying in composition and concentration, when the tissue is killed (Noy-Meir and Ginzburg 1967).

In particular, mixing of cytoplasmic proteins with vacuole solutes might be expected to cause interactions which could affect the osmotic and the matric potential. Also, the existence of an active process which maintains a gradient of water potential between the cells and the exterior, and which is abolished by killing the tissue, cannot be certainly excluded.

In these experiments, the effects mentioned are indistinguishable from turgor. Thus, the observed values of negative turgor should be considered as including a possible uncertainty, the direction and magnitude of which can only be guessed. In carob, at least, the maximal values of measured negative turgor are so high (−21 atm) that it seems unlikely that they have arisen entirely by other effects.

In the following section effects of possible uncertainties on the hydrostatic potential will be neglected.

(*b*) *Comparison of Isotherms of the Different Species and Correlation with their Drought Adaptation*

Once the isotherm and its components have been fitted by empirical equations, in which the meaning of the various terms and coefficients is generally understood, the isotherms of the different species can be compared by comparing the coefficients. However, since the water-content parameter used (w) was calculated per dry weight, the coefficients for the osmotic and the matric (a_s, b_s, a_τ, b_τ) components were relative to dry weight as well. It seemed that the comparison would be more meaningful if the coefficients were related to some standard state defined by the isotherm itself.

Therefore w was substituted by $z = w/w^0$, where w^0 is the water content at the point of "zero turgor", i.e. where $\Psi_p = \Psi - \Psi_d = 0$ (this normalization was preferred to the more usual practice of relating to the water content at "full turgor", since the measurements at the latter point were less accurate). Similarly, w_s was replaced by $z_s = w_s/w_s^0$. By this substitution, the coefficients for the ideal osmotic, non-ideal osmotic, and average matric components become simply the values of these components at $\Psi_p = 0$; the coefficients for the hydrostatic component remain unchanged.

The values of some of the coefficients of the isotherm and some other parameters are compared in Table 2 for the leaves of the three species. The two values for *C. siliqua* and *P. orientalis* were obtained in different sets of measurements.

TABLE 2

COMPARISON BETWEEN SOME OF THE PARAMETERS OF THE ISOTHERM FOR THE THREE SPECIES

Water potentials and elasticity moduli are expressed in atmospheres

Parameter	*C. siliqua*	*P. orientalis*	*A. halimus*
Water/dry matter			
w^0	0·95–1·30	1·32–1·37	6·0
w_s^0	0·70–1·05	1·08–1·12	5·5
Matrix			
w_m^{max}	0·50–0·54	0·48–0·67	1·60
Matrix/solution			
$(w_m/w)^0$	0·19–0·26	0·18–0·18	0·08
Ψ_τ^{*0}	5·3–5·5	4·1–4·7	3·0
Ψ_π^{*0}	12·6–15·6	15·5–19·0	24·5
Solution			
Ψ_π^0	17·9–21·1	19·6–23·7	27·5
$\Psi_{\pi I}^0$	14·6–17·3	16·4–20·2	25·4
$\Psi_\pi^0 - \Psi_{\pi I}^0$	3·3–3·8	3·2–3·5	2·1
Elasticity			
ϵ_1	120–130	100–105	50
ϵ_2	35–45	22–30	16
ϵ_1/Ψ_π^0	6·2–6·7	4·2–5·9	1·8
ϵ_2/Ψ_π^0	2·0–2·1	0·9–1·2	0·6
Critical values			
$-\Psi_p^n$	19–21	7–12	14
$-\Psi_\pi^n$	48–56	38–46	100
$-\Psi^n$	67–77	50–53	114
$d^n[= (w^0 - w^n)/w^0]$†	0·50–0·54	0·39–0·42	0·68

† Relative water deficit at $\Psi_p = \Psi_p^n$.

The rank of "drought tolerance" (as measured by the water potential at the "critical point" of maximal negative turgor or about 30% visible damage) was the same as the rank of aridity in the natural habitat of the species even though the leaves were taken from plants growing in similar conditions on the university campus in Jerusalem: *A. halimus–C. siliqua–P. orientalis*. This suggests that drought tolerance may be of adaptive value, and that its phenotypic expression is at least partially independent of the environment.

However, for all the parameters derived from the analysis of the isotherm itself the rank is different: *C. siliqua–P. orientalis–A. halimus*. Thus there is no simple correlation between any parameter of the isotherm, and the ability of the tissue to survive low water potentials.

The relative effect of the matrix on the isotherm, whether measured as matrix water or matric potential, decreases slightly from *C. siliqua* to *P. orientalis*, and considerably from *P. orientalis* to *A. halimus*. Certainly, this is not due to the matrix of the first two being more "hydrophilic", since the specific water-retaining capacity of the matrix (as measured by $w_m^{\max}$) is in fact highest for *A. halimus*. The differences in the relative effect of the matrix seem rather to be dominated by differences in the relative amounts of solutes and matrix, which are reflected also in the ratio of water to dry weight (w^0) or the "succulency" of the tissue. The differences in the effect of the non-ideal term on the osmotic potential, too, might be associated with the differences in the matrix:solutes ratio.

The osmotic potential itself (measured at $\Psi_p = 0$) increases gradually from *C. siliqua* through *P. orientalis* to *A. halimus*, whilst the elasticity modulus (for both positive and negative turgor) changes in the opposite direction. The combined effect is demonstrated by the marked differences in the ratio ϵ/Ψ_π^0. Since ϵ and Ψ_π^0 are the parameters which determine the partition of the isotherm between the hydrostatic and osmotic components (excluding the matrix), the proportion of a given change in water potential accounted for by a change in the hydrostatic component depends on this ratio. This is reflected by the partition of the water potential at the "critical point", where the ratio of hydrostatic to osmotic potential is highest for *C. siliqua*, and lowest for *A. halimus*.

Assuming that the parameters of the isotherm have adaptive significance these results lead to the following hypothesis.

Low water potential can cause damage and death to plant cells by increasing either negative turgor or solute concentration (or both) which damage the cell by different mechanisms; some tissues are more susceptible to one kind of damage, some to the other. The elasticity of the cell wall (or rather its value relative to the amount of solutes) has a regulatory effect, dividing the total external "load" of water potential between the osmotic and hydrostatic components; it has adaptive significance if it diverts most of the rise in the cell to that component which damages this cell least.

If *P. orientalis* is taken as the mesophytic reference species, then *A. halimus* and *C. siliqua* represent two different, contrasting mechanisms of adaptation to xeric conditions at the cellular level. In *A. halimus* the normal metabolism and structure of the protoplast are probably sensitive to negative turgor (large cells) but less sensitive to high solute concentrations (stable proteins?). The cell walls are not rigid (low elasticity modulus) and cause the osmotic potential to decrease steeply, and the hydrostatic potential to decrease slowly, with decreasing water potential. In *C. siliqua*, on the other hand, protoplasts can probably stand high "tensions" (small cells; strong adhesion to wall?) but are more sensitive to solute concentration; the rigid walls cause concentration to rise slowly and the negative turgor steeply. The quantity and hydrophily of protoplasmic matrix may be another regulatory device of this kind, although less important in the species examined. The analysis

of the water potential isotherm can indicate the probable nature of the drought-adaptive mechanisms of a given tissue, but to prove the mechanism more direct experiments have to be made.

V. References

Ashby, E., and Wolf, R. (1947).—*Ann. Bot.* (N.S). **11**, 261.
Gaff, D. F., and Carr, D. J. (1961).—*Aust. J. biol. Sci.* **14**, 299.
Jarvis, P. G., and Jarvis, M. S. (1963).—*Physiologia Pl.* **16**, 501.
Kramer, P. J., and Brix, H. (1965).—*Arid Zone Res.* **25**, 343.
Kreeb, K. (1961).—*Planta* **56**, 479.
Kreeb, K. (1963).—*Planta* **59**, 442.
Macklon, A. E. S., and Weatherley, P. E. (1965).—*J. exp. Bot.* **16**, 261.
Noy-Meir, I., and Ginzburg, B. Z. (1967).—*Aust. J. biol. Sci.* **20**, 695.
Rehder, H. (1961).—*Ber. dt. bot. Ges.* **74**, 84.
Richards, L. A., and Ogata, G. (1958).—*Science, N.Y.* **128**, 1089.
Shardakov, V. S. (1953).—"The Water Regime of Cotton." (Mem. Acad. Sci. Rep. Uzbek Tashkent.)
Shimshi, D. (1963).—*Pl. Physiol., Lancaster* **38**, 713.
Slatyer, R. O. (1958).—*Aust. J. biol. Sci.* **11**, 349.
Slatyer, R. O. (1960).—*Bull. Res. Coun. Israel* **80**, 159.
Slatyer, R. O. (1961).—*Arid Zone Res.* **16**, 137.
Slatyer, R. O., and Barrs, H. D. (1965).—*Arid Zone Res.* **25**, 331.
Snedecor, G. W. (1956).—"Statistical Methods." pp. 37 and 454. (Iowa State College Press)
Spanner, D. C. (1951).—*J. exp. Bot.* **2**, 145.
Tyurina, M. M. (1957*a*).—*Fiziologiya Rast.* **4**, 361.
Tyurina, M. M. (1957*b*).—*Bot. Zh.* **42**, 1035.
Ursprung, A., and Blum, G. (1916).—*Ber. dt. bot. Ges.* **34**, 525.
Weatherley, P. E. (1965).—*Symp. Soc. exp. Biol.* **19**, 157.
Weatherley, P. E., and Slatyer, R. O. (1957).—*Nature, Lond.* **179**, 1085.

Appendix

List of symbols

a coefficient of water potential component dependent on w^{-2} (atm) (a_m for matrix, a_s for solution, a_τ for bulk matric potential);

b coefficient of water potential component dependent on w^{-1} (atm) (b_m for matrix, b_s for solution, b_τ for bulk matric potential);

k coefficient expressing the change of the elasticity modulus with relative water content (atm);

w water content (grams of water/grams dry weight of tissue, matrix fraction, or filter paper);

w_m water content of matrix fraction;

w_s water content of solution fraction;

w^0 water content of living tissue (measured as total water/total dry weight) at $\Psi_p = 0$ ("incipient plasmolysis");

w^n water content at minimum $\Psi_p (\Psi_p = \Psi_p^n)$;

$w_m^{\max}$ maximal water content of matrix fraction (at $\Psi = 0$; per dry weight of fraction);

ϵ elasticity modulus (atm) (ϵ^0 at $\Psi_p = 0$, ϵ_1 for positive turgor, ϵ_2 for negative turgor);

Ψ water potential (atm) (when without subscript, usually total water potential);

Ψ_m water potential of matrix;

Ψ_d water potential of killed tissue;

Ψ_s water potential of solution;

Ψ_p hydrostatic potential (Ψ_p^n, minimum Ψ_p);

Ψ^n water potential of tissue at the point where the hydrostatic potential is at its minimum (total water potential at $\Psi_p = \Psi_p^n$);

Ψ^r water potential of tissue at the point where the hydrostatic potential returns to zero;

Ψ_π osmotic potential (Ψ_π^0 at $\Psi_p = 0$, $\Psi_{\pi I}^0$ "ideal" osmotic term, Ψ_π^{*0} bulk osmotic potential at $\Psi_p = 0$, Ψ_π^n osmotic potential at $\Psi_p = \Psi_p^n$);

Ψ_τ matric potential (Ψ_τ^{*0} bulk matric potential at $\Psi_p = 0$).