THE QUANTITY OF WATER IN THE CELL WALL AND ITS SIGNIFICANCE

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Summary

Cell wall preparations from the leaves of *Eucalyptus globulus* Labill. may absorb water equal to approximately 150% of their dry weight.

The proportion of cell wall in the leaves of this species was estimated by three different methods, and the maximum water in the cell wall was calculated to constitute approximately 40% of the water content of the leaf at full turgor.

The implications of the large fraction of water in the cell wall are discussed. It is proposed that the cell wall, not the protoplast, acts as the main pathway for extrafascicular movement of water, and that in the leaf the cell wall water operates as a buffer against loss of water from the protoplast. The buffering capacity of the water in the wall may be a factor in the "hardening off" of plants to drought.

I. INTRODUCTION

The view that the cell walls of living cells are moist is firmly established in the literature, e.g. Crafts, Currier, and Stocking (1949), Kramer (1955), Stocking (1956). The general acceptance of the osmotic theory of the water relations of plant cells, however, has focused attention on the importance of the water enclosed by cell membranes. This has served to distract attention from the water in the cell wall. In recent years, however, it has been shown that the apparent free space (A.F.S.) forms an appreciable proportion of the volume of plant tissues. The A.F.S. is thought to be largely or entirely in the cell wall (Levitt 1957; Dainty and Hope 1959*b*), and its magnitude suggests that the water content of the cell wall may be high. For example, the A.F.S. of wheat and barley roots is of the order of 25% (Butler 1953; Epstein 1955). Black *et al.* (1960) reported that the A.F.S. of bacterial spores is as high as 40% and calculated that the cell wall represented a similar proportion of the cell. Work by G. W. Scott *et al.* (1957) indicates an A.F.S. of 90% for *Ulva lactuca*, the cell walls of which are thick and gelatinous. Data are presented in this paper to show that significant quantities of water are held in the cell wall of leaves of *Eucalyptus globulus* Labill., and to support the view that such water may play an important role in the water relations of plants.

II. MATERIAL AND METHODS

Homogeneous cell wall material was prepared from the fully expanded juvenile leaves of *E. globulus*. All leaves were taken from four saplings of similar age (5 years).

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(*a*) *Preparation of Cell Wall Material*

The midribs were removed and the laminae (in which veins are not prominent) were frozen with dry ice and ground to a fine powder by hand in a mortar and pestle. The ground leaf tissue was mixed with cold water, centrifuged at 3000 r.p.m. for 20 min at 2°C, and the residue again washed with cold water. Ethanolamine was added to the residue and the mixture ground in a top-drive homogenizer and then centrifuged. This procedure removes cytoplasm from cell walls (F. M. Scott *et al.* 1956) and presumably removes any protein constituent present in the wall.

The residue was subjected to this treatment repeatedly until, on centrifuging, the supernatant ethanolamine was light in colour. The residue was then homogenized in ethanolamine with a Potter–Elvehjem pestle homogenizer. The homogenate was left at 90°C for 4–5 hr and centrifuged. The residue was washed with cold water and three times with acetone, when there was only a faint coloration of the liquid.

Finally the moist residue was poured into shallow watch-glasses and allowed to dry in air to a flesh-coloured "paper" about 1 mm in thickness.

The total nitrogen of the preparation was determined by microKjeldahl techniques and nesslerization. Distillation of ammonia was performed using the apparatus described by Archibald (1943). The protein content was estimated using a factor (wt. of protein/wt. of nitrogen) = 6 (Chibnall 1939).

(*b*) *Drying and Storage of the Cell Wall Material*

Although it was desirable to refer water contents of the preparation to the dry weight, oven drying might have modified the hygroscopic properties (Wise and Jahn 1952). Consequently drying was performed at room temperatures by means of desiccators containing a saturated solution of lithium chloride in contact with an excess of the solid salt at 21°C. The cell wall preparation was supported by wire gauze about 2 cm above the surface of the solution. The saturated solution of lithium chloride, at 20°C, maintains a relative humidity of 15% (cf. 32% for relative humidity of air over a saturated solution of calcium chloride). This is sufficiently low to avoid growth of fungi on the cell wall preparation during storage in the desiccator.

Preliminary experiments showed that water-saturated cell wall preparations reached a constant weight in these desiccators within 24 hr (2 days were usually employed), and that the ratio of the "desiccated weight" so obtained to the "oven dry weight" (90°C for 2 days) was, on the average, 1·05.

(*c*) *Uptake of Water Vapour by the Dry Cell Wall Preparation*

Samples of the cell wall preparation were taken from storage in the desiccators, weighed, and placed in equilibration chambers containing water or sucrose solutions of various concentrations. Although the data for sucrose were obtained for other purposes, they are included here to support the results for water.

The equilibration chambers were glass phials 2 by 1 in., half-filled with filter paper saturated with solution. The cell wall preparation (approx. 1·5 by 1 cm) was supported on a tared glass plate resting on stainless steel gauze supported by the

wet filter paper (Fig. 1). The closed chambers were submerged in a stirred water-bath in which the temperature was controlled at $26 \pm 0{\cdot}02$°C.

Periodically each sample was slid off its glass plate into a tared weighing jar, weighed, and replaced in its equilibration chamber, or into a fresh chamber containing solution of the same concentration.

(*d*) *Loss of Water Vapour from the Moist Cell Wall Preparation*

Six samples of the cell wall preparation were removed from storage in the lithium chloride desiccators, and were placed in cold water in petri dishes. On contact with the water, the paper-like cell wall preparation expanded, at the same time becoming soft and difficult to handle. After 2 hr in water, the samples were manoeuvred on to small glass supports of known weight and were removed from

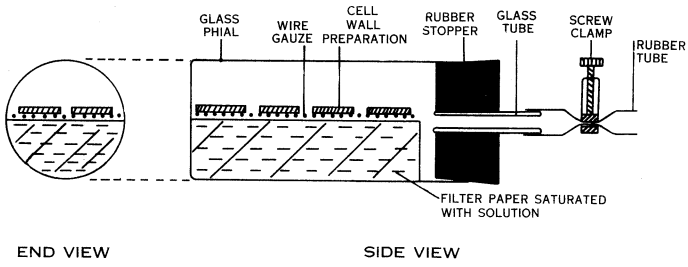

Fig. 1.—Apparatus used as equilibration chamber.

the water. The glass support and the cell wall material were carefully placed on a pad of filter paper with the support uppermost. When the circle of water on the filter paper ceased expanding, the paper was carefully peeled away from the surface of the cell wall preparation. During these manipulations loss of cell wall material was unavoidable. Nevertheless, the bulk of the sample of the cell wall preparation remained intact on the support.

Each sample, together with the glass support, was weighed in a tared weighing jar and then placed in an equilibration phial over sucrose solutions at 26°C ranging in concentration from 0 to 1·0M. The samples were reweighed at various intervals of time. Fresh equilibration phials and solutions were substituted periodically in order to avoid appreciable dilution of the solution.

The samples of cell wall material were left for 2 days in lithium chloride desiccators and then reweighed.

III. Results

Tests with iodine solution showed that the cell wall preparation contained no starch. The leaves used contained 14% protein (estimated as a percentage of the oven dry weight) but no nitrogen was detected in the cell wall preparation. The removal of all nitrogenous materials from the cell wall preparation means that any such materials which may form an integral part of the cell wall are not represented

in the preparation. Opinions differ on the degree of penetration of the cell wall by the protoplast of the plant cell and, in view of the prevailing uncertainty on this point, it is perhaps preferable to confine this study of the hygroscopicity of the cell wall to its non-nitrogenous fractions.

(*a*) *Sorption by Dry Cell Wall Material*

The results are given in Table 1, where the weights of each sample have been corrected to an initial "desiccated weight" of 100 mg. Some of these data are plotted in Figure 2.

TABLE 1

SORPTION BY CELL WALL MATERIAL

Weights of samples corrected to an initial dry weight of 100 mg. The times indicated in brackets refer to the samples over 0·2M, 0·6M, and 0·8M sucrose

Time (hr)	Concentration of Sucrose in Equilibration Chamber				
	0 (water control)	0·2M (5 atm)	0·4M (11 atm)	0·6M (18 atm)	0·8M (26 atm)
0	100·0	100·0	100·0	100·0	100·0
5 (4·5)	141·7	147·4	142·1	140·8	133·3
21·5 (21)	181·8	182·8	172·9	165·7	151·0
29·5 (28·5)	190·1	188·2	179·8	166·9	152·3
45·7	201·8	—	193·1	—	—
53·5 (52·0)	208·6	209·8	198·6	175·8	158·7
70	217·7	—	202·3	—	—
75·5	220·1	—	204·9	—	—
94	224·5	—	207·8	—	—
(118·5)	—	239·4	—	178·9	159·1
166	259·4	—	223·6	—	—
Maximum water content (% dry wt.)	159	139	124	79	59

It is clear that the cell wall possesses marked hygroscopic properties. The dry cell wall can take up more than its own weight of water vapour. As is the case in live leaf disks (Carr and Gaff 1959), the cell wall material does not attain a virtual equilibrium in a short time. There is a continuous progress towards equilibrium at a continually decreasing rate. The data show that the cell wall material may absorb water vapour equal to approximately 150% of the dry weight. This value may be lower than the water content which may obtain in the cell wall in the intact leaf, where, in addition to water held by hygroscopic forces, there would be some water held in spaces in the cell wall. Consequently, desorption from wet wall material might give a greater value for the retention of water in the wall.

(*b*) *Desorption of Wet Cell Wall Material*

The water content of the samples after imbibing water for 2 hr is quite variable but averages about eight times the "desiccated weight" of the sample (see Fig. 3). It seems probable that most of the water is present as free water occupying the spaces between the cell wall fragments. In the equilibration chambers water is lost from the samples at a rate dependent on the difference in the water activity of the sample and of the sucrose solution. The rate of water loss, therefore, remains constant until all the free water has evaporated. Further water loss is at the expense of water held by imbibitional forces. Water loss at this latter stage increases the diffusion pressure deficit of the cell wall material, and results in turn in a reduced

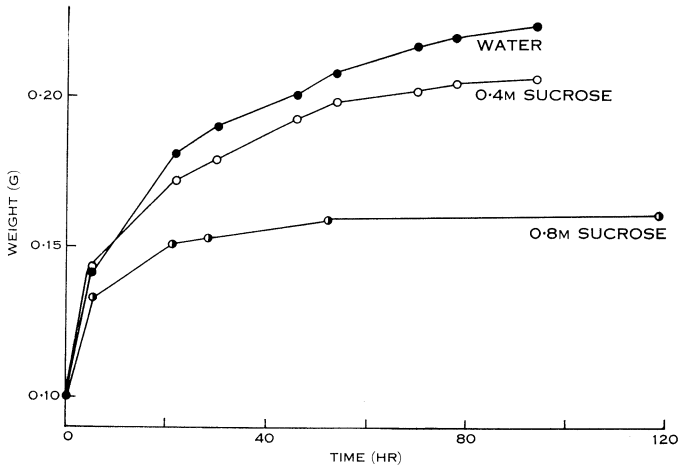

Fig. 2.—Time course of water uptake by desiccated cell wall preparations in equilibration phials containing water, 0·4M, and 0·8M sucrose. The weights of the samples have been corrected to a desiccated weight of 100 mg.

rate of water loss. The exponential loss indicative of this situation is most clearly suggested by the data for 1·0M sucrose where the loss of bound water is greatest.

The change from a constant to an exponential rate of water loss appears to occur at a water content of about 150% of the dry weight. This supports the conclusion that the maximum amount of water held in the wall is equal to approximately 150% of the dry weight of the wall material.

(*c*) *Proportion of Cell Wall Material in the Leaf of* E. globulus

Owing to the chemical complexity of the cell wall and the cell contents, it is difficult to obtain an accurate estimate of the proportion of cell wall material in a leaf even by using elaborate techniques. Three different methods were employed to obtain such an estimate.

(i) *Estimate* 1.—Freshly picked leaves, from which the midribs had been removed, were frozen with dry ice and ground to a powder. A sample of the powdered leaf was weighed in a tared, stoppered, weighing jar after the jar and its contents

had reached room temperature. The dry weight of the sample was determined after 2 days in an oven at 90°C.

The remainder of the powdered leaf was also weighed at room temperature, and transferred to a beaker containing distilled water. The mixture was boiled gently for 12 hr, then cooled, and centrifuged for 20 min at 3500 r.p.m. in tared "Nylex" tubes.

The residue was twice washed with distilled water and centrifuged. Finally, the residue was desiccated in the tubes at 90°C for 2 days and reweighed.

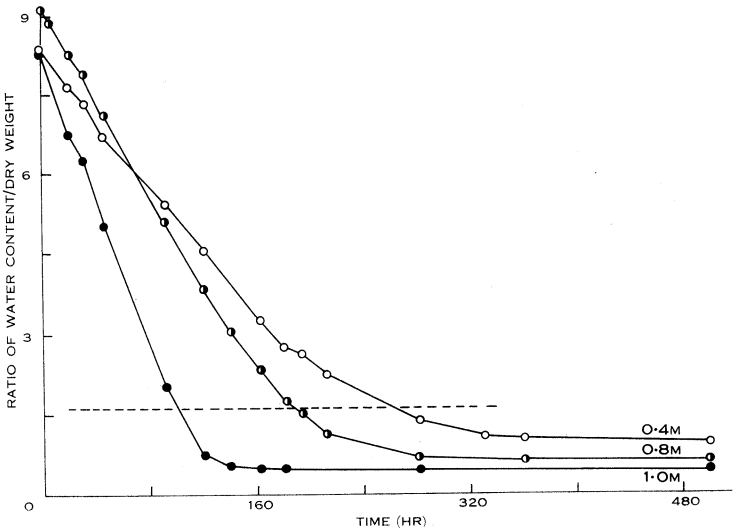

Fig. 3.—Time course of water loss from moist cell wall preparations in equilibration phials containing 0·4M, 0·8M, and 1·0M sucrose. The dotted line parallel to the abscissa represents approximately the water content at which the cell wall material is fully imbibed.

The nitrogen content of a sample of the dried residue was determined by microKjeldahl techniques and nesslerization. Protein was estimated to compose 11·4% of the dry weight of the residue. The remainder of the residue, assumed to be mainly cell wall material, amounted to 31·7% of the dry weight of the leaf tissue, i.e. 13% of the fresh weight.

The treatment of the ground leaf tissue with boiling water, in addition to removing the non-protein materials contained in the protoplast, would remove most of the hemicelluloses and pectins from the cell wall. The estimate given here for the amount of cell wall material must, therefore, be regarded as a minimum value.

(ii) *Estimate* 2.—A freshly picked leaf was folded along its midrib and corresponding areas were cut from the two halves of the leaf. The fresh weight of each sample was recorded. One sample was crushed between two sheets of blotting-paper, under a pressure of 2500 lb/sq. in. The crushed leaf was soaked in water for a few minutes and crushed once again between fresh blotting-papers. The crushed leaf was weighed and, together with the uncrushed sample of leaf, was dried for 2 days

at 90°C. The dry weights of the samples were recorded, and the proportion of cell wall material in the leaf was calculated.

Microscopic examination of leaf material crushed in this way showed that the vacuoles of the cells had been eliminated, but that the protoplasm remained in the cell cavities. A correction was therefore applied for the weight of protein remaining. On the basis of previous experiments, the weight of protein was taken to be equal to 14% of the dry weight of the uncrushed leaf. In this way the dry weight of the cell wall material was estimated to constitute 69% of the total dry weight of the leaf, i.e. approximately 27% of the fresh weight. The water content of the crushed leaf was 52% of the initial water content.

TABLE 2

DISTRIBUTION OF TISSUES AND CELL WALL IN THE LEAF

Tissue	Cell Volume as Percentage of Total Volume of Leaf	Cell Wall Volume as Percentage of Volume of Cell	Cell Wall in Tissue as Percentage of Total Volume of Leaf
Spongy mesophyll Palisade	43	12	5·2
Xylem	3	47	1·4
Phloem	3	18	0·5
Parenchyma sheath	4	8	0·3
Sclerenchyma	1	91	0·9
Collenchyma	4	35	1·4
Epidermis	16	19	3·0
Total	74*		12·7

* Intercellular spaces = 24%; oil in glands = 2%.

(iii) *Estimate* 3.—Microtome sections of the leaves were mounted in Canada balsam and measurements were made of images of the sections projected on to a sheet of tracing paper supported on glass. The areas occupied by the various tissues of the leaf were outlined on the paper for 16 microscope fields (objective ×45, eyepiece ×12, diameter of field *c.* 0·2 mm).

The paper was cut, sorted according to the type of tissue outlined, and weighed. The weights were taken to be a measure of the relative volumes of the component tissues of the leaf.

This technique was employed to estimate the proportion of cell wall in tissues with large cells and thick walls (xylem, collenchyma, sclerenchyma). In the other tissues the proportion of cell wall was estimated from means of measurements of the dimensions of a number of cells. The standard errors of the mean length, depth, and breadth of the cells were 3% or less of the mean, those of the wall thickness 7%. Calculations were based on cylinders, spheres, or rectangular prisms according to which of these geometrical shapes the form of the average cell most resembled. The results are given in Table 2.

The volume of the dehydrated cell walls constitute [(12·7/74) × 100], i.e. 17% of the total cell volume of the leaf. In further calculations it is assumed that the dry cell wall has the same relative density as amorphous cellulose (i.e. 1·48), and that the relative density of the protoplast is approximately that of 1M sucrose (which has an osmotic pressure equal to the mean osmotic pressure of the tissue at incipient plasmolysis). Then, bearing in mind that the cell wall imbibes water equal to 150% of its dry weight, we may estimate that the dry weight of the cell wall is equivalent to approximately 16% of the fresh weight of the lamina.

IV. Discussion

(a) *Water Content of the Cell Wall*

The three separate estimates of the dry cell wall content of the lamina are 13, 27, and 16% of the fresh weight of the lamina. The difference between the first two estimates is probably due mainly to loss of pectins and hemicelluloses from the cell wall during boiling. Bishop, Bayley, and Setterfield (1958) report that, on a dry weight basis, at least 51% of the cell walls of the parenchyma of *Avena* coleoptiles is a mixture of hemicelluloses. It is not unreasonable, therefore, to expect leaf parenchyma cells to contain some hemicelluloses.

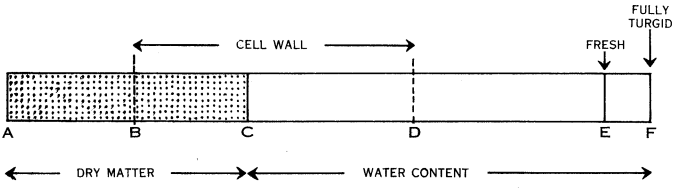

Fig. 4.—Relative quantities of dry matter and water in the leaf of *E. globulus*. *AC*, dry weight of leaf; *BC*, dry weight of cell wall material; *CE*, water content of a freshly picked leaf; *CF*, water content of a fully turgid leaf; *CD*, water content of the fully imbibed cell walls.

In addition, the presence of appreciable amounts of starch in the plastids would inflate the first and second estimates, despite the fact that the leaves were picked during the morning, to reduce the importance of this source of error.

For the purposes of estimating the quantity of water in the cell walls we take the mean of these three estimates, i.e. the dry weight of the wall (*BC* in Fig. 4) is 0·19 times the fresh weight of the lamina (*AE*). Since the water content of the fully imbibed wall (*CD*) is 1·5 times the dry weight of the wall (*BC*), the water content of the wall (*CD*) is 0·28 times the fresh weight of the leaf (*AE*). This is equivalent to approximately 0·4 times the water content at full turgor (*CF*).

A number of papers may be cited in support of the estimate of the hygroscopicity of the cell wall. Data demonstrating sorption and desorption of water vapour by various types of wood have been summarized by Stamm (1952). Jute absorbs water up to 34% (Pfuhl 1888), and manilla hemp and piassaba fibres take up water amounting to nearly 50% of the dry weight (Wiesner 1921). Cohn (1892) recalculated data

of other authors and obtained values for the water content of lignified tissues ranging from 31% of the tissue dry weight in *Pinus sylvestris* wood to 59% in oak sapwood, and reaching 120% in *Juncus* phloem fibres.

Christensen and Kelsey (1958) have studied the sorption of water vapour by the major components of the wood of *Eucalyptus regnans*. Various methods of extraction were used, and the hygroscopicity of the components was found to vary according to the extraction technique employed. Lignin can take up water equivalent to approximately 25% of its dry weight; hemicellulose takes up 75–100%, and holocellulose 33–52%. As might be expected then, lignified cell walls are less hygroscopic than other walls. For example, drying in air reduces the volume of phloem walls in potato stolons by 50% (Crafts 1931) and shrinks cambium cell walls to one-third their original thickness (Preston and Wardrop 1949). Hansteen-Cranner (1914) reported that preparations of the cell wall material of the pith from turnip petioles take up water equal to 170% of the dry weight of the wall material.

Cohn (1892) found that in four different species the water content of collenchyma cell walls was as high as 165–245% of the wall dry weight.

Hansteen-Cranner's data for pith and Cohn's data for collenchyma are comparable with the value obtained for the cell wall preparation from leaves of *E. globulus*.

Scarcely any published data are available on the hygroscopic properties of the cell walls of leaf tissue. One would expect that the hygroscopicity of this poorly lignified material would be considerable. Hartel (1951) investigated the swelling capacity of oven-dried leaves and concluded that it was due mostly to the cell wall material.

The high proportion of cell wall water in leaves of *E. globulus* is associated with a relatively high percentage dry weight of leaf (41% of the fresh weight), but this value is not exceptionally high for eucalypts (Blagoveshchensky and Bogracheva 1955). It is probable that similar amounts of cell wall water occur in the leaves of many plants.

(*b*) *Water Movement in the Mesophyll*

It has been pointed out by van den Honert (1948) that, in comparison with other resistances to water movement in the plant, the resistance offered by the protoplast is very large. Consideration of this, and of the large proportion of water in the cell wall, has led us to the view that extrafascicular water movement occurs mainly through the cell wall, and involves movement of capillary-condensed water. That is, the protoplast may not lie on the direct path of the transpiration stream.

The correctness of this view depends on whether or not the resistance to water movement of the path via the protoplasts in the leaf is greater than that via cell walls. It is quite clear from permeability studies that the protoplast presents a considerable resistance to the movement of water. Values for the water permeability of cells have been assembled by Bennet-Clark (1959) and range from 0·02 to 1 μ min^{-1} atm^{-1}, although values as high as 18 μ min^{-1} atm^{-1} have been reported for *Nitella*. Mercer and Clark* found that the permeability of isolated tonoplasts plus vacuoles was up to 10 times the permeability of isolated protoplasts.

* Reported in Dainty and Hope (1959*a*).

Information on the relative resistances of the protoplast and the cell wall is meagre. The clearest demonstration of the lower resistance of the cell wall is presented by Levitt, Scarth, and Gibbs (1936), who found that the water permeability of free protoplasts of onion epidermis was the same as that of protoplasts enclosed in their cell walls. Wartiovaara (1944) reported that the permeability of isolated protoplasts of *Tolypellopsis* to deuterium hydroxide was 1·6 cm/hr compared with 1·04 cm/hr for intact cells. In considering this and other experiments in which isotopically labelled water was employed, the recent discovery by Hübner (1960) that extremely rapid loss of labelled water to the atmosphere occurs during manipulation of tissue segments, must be borne in mind. This would invalidate absolute values such as those of Wartiovaara. Nevertheless, comparative studies would still be valid where standardized techniques were employed.

Kramer (1932) found that the movement of water into hollow pawpaw petioles is considerably greater along a gradient of hydrostatic pressure than along one of osmotic pressure. This result indicates that mass flow of water (presumably through the cell walls) took place far more readily than osmosis and diffusion through the protoplasts. Similar results were obtained by Mees and Weatherley (1957*a*, 1957*b*), who concluded that appreciable mass flow of water across the cortex of tomato roots can be induced by hydrostatic pressure. Following an investigation of the effects of an increased suction tension on the passage of water through roots of *Vicia faba*, Hylmö (1958) concluded that the movement of water obeyed the Hagen–Poiseuille law for mass flow. Hylmö further deduced from deviations from this law (due to the occurrence of the Erbe phenomenon) that the width of the pores involved in the mass flow at tensions greater than 1·8 atm was of the same order as the width of the interfibrillar spaces in the cell wall as determined by X-ray, gas flow, and electrical conductance techniques, as well as by measurements made using the electron-microscope.

In regard to water movement in the intact plant, Strugger (1949) demonstrated that certain dyes which are not accumulated by the cells are swiftly transmitted along the walls of the cells of rapidly transpiring plants, and claimed this as evidence of an extrafascicular pathway of water movement.

The quantity of water which may pass through transpiring leaves may be so large that the transmission of most of the water through the protoplasts appears improbable. In species of *Eucalyptus*, for example, average rates of 50–120% of the fresh weight of the leaf per hour have been observed by Blagoveshchensky and Bogracheva (1955) in Russia at midday during summer.

(*c*) *Buffering Capacity of the Cell Wall*

The presence of considerable quantities of water in the cell walls of the intact leaf and the relatively high resistance of the protoplast to water movement raise the possibility that the hydrated cell wall serves as a buffer against loss of water from the protoplast during temporary adverse conditions. The drying wall would absorb water from wherever water was most readily available, i.e. from the xylem, via the walls of the intervening cells, rather than from the protoplast. During short

periods of dryness the water content of the cell wall may decrease, while the water status of the protoplast remains unchanged. In addition, the thicker the cell wall, the greater would be the buffering effect of the wall against transient drying forces.

Under steady-state conditions equilibrium would be reached between the water potentials of the cell wall, cytoplasm, and vacuole. However, methods developed recently for rapidly recording climatic factors have shown that, in nature, these factors are constantly changing, i.e. periods of steady state are rare (e.g. Swinbank 1958).

The relative contribution of the various tissues in *E. globulus* leaves to the total cell wall volume are given in Table 2. Together the epidermis and chlorenchyma, i.e. the surface from which evaporation of water occurs, compose two-thirds of the total cell wall. An increase in the thickness (and buffering capacity) of these walls would be reflected in an increase in the dry weight of the tissue. The greater dry weight/fresh weight ratios which are characteristic of plants of drier habitats, according to Pettersson and Gray (1958), may reflect only a greater amount of cell wall material in the leaves of species in drier habitats.

The results of several workers suggest that the buffering capacity of the wall water may be a factor in "hardening off" of plants when exposed to dry conditions. This may occur through an increased production of hemicellulose and pectic substances, coupled with a decrease in protein synthesis (see Clements 1937; Nezgovorova 1957; Prusakova 1960).

In the last decade, the ratio relative turgidity, i.e. the water content of a tissue/the water content at full turgor (Weatherley 1950), has found increasing use as an index of the balance between gain and loss of water by leaves. Both the name and the definition imply that only the water content of the protoplast is involved. This implication is unfortunate, since the water content of the wall must represent a significant proportion of the water content at full turgor. Indeed it seems probable that the recorded fluctuations in relative turgidity (82–92% in *Coffea arabica* (Dias and Contreiras 1958); 80–95% in potato plants (Werner 1954)) represent mainly variations in the water content of the cell walls. Slatyer (1955) records similar fluctuations in relative turgidity in cotton, peanuts, and grain soybean while the soil was moist. However, as the soil dried values of relative turgidity as low as 60% were obtained. In the latter case, the water content of the protoplast would probably be affected.

V. Acknowledgments

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VI. References

Archibald, R. M. (1943).—Quantitative microdetermination of ammonia in the presence of glutamine and other labile substances. *J. Biol. Chem.* **151**: 141–8.

Bennet-Clark, T. A. (1959).—Water relations in cells. In "Plant Physiology." (Ed. F. C. Steward.) (Academic Press, Inc.: New York.)

BISHOP, C. T., BAYLEY, S. T., and SETTERFIELD, G. (1958).—Chemical constitution of the primary cell walls of *Avena* coleoptiles. *Plant Physiol.* **33**: 283–9.

BLACK, S. H., MACDONALD, R. E., HASHIMOTO, T., and GERHARDT, P. (1960).—Permeability of dormant bacterial spores. *Nature* **185**: 782–3.

BLAGOVESHCHENSKY, A. V., and BOGRACHEVA, T. N. (1955).—Contribution to the study of the water relations of *Eucalyptus*. (In Russian.) *Fiziol. Rast.* **2**: 221–7.

BUTLER, G. W. (1953).—Ion uptake by young wheat plants. II. The "apparent free space" of wheat roots. *Physiol. Plant.* **6**: 617–35.

CARR, D. J., and GAFF, D. F. (1959).—The role of the cell wall water in the water relations of leaves. "Proceedings of the Madrid Symposium on Water Relationships in Arid and Semi-arid Conditions." (U.N.E.S.C.O.)

CHIBNALL, A. C. (1939).—"Protein Metabolism in the Plant." (Yale Univ. Press: New Haven, Conn.)

CHRISTENSEN, G. N., and KELSEY, K. E. (1958).—Sorption of water vapour by the constituents of wood. *Aust. J. Appl. Sci.* **9**: 265–82.

CLEMENTS, H. F. (1937).—Studies in drought resistance of the soybean. Res. Stud. St. Coll. Wash. No. 5. pp. 1–16. and 81–98.

COHN, J. (1892).—Beiträge zur Physiologie des Collenchyms. *Jb. wiss. Bot.* **24**: 145–72.

CRAFTS, A. S. (1931).—Movement of organic materials in plants. *Plant Physiol.* **6**: 1–41.

CRAFTS, A. S., CURRIER, H. B., and STOCKING, C. R. (1949).—"Water in the Physiology of Plants." (Chronica Botanica Co.: Waltham, Mass.)

DAINTY, J., and HOPE, A. B. (1959*a*).—The water permeability of cells of *Chara australis* R. Br. *Aust. J. Biol. Sci.* **12**: 136–45.

DAINTY, J., and HOPE, A. B. (1959*b*).—Ionic relations of cells of *Chara australis*. I. Ionic exchange in the cell wall. *Aust. J. Biol. Sci.* **12**: 395–411.

DIAS, M. A. F. R., and CONTREIRAS, J. (1958).—Aspectos fisiólogicos da imunidade-susceptibilidade do cafeeiro à *Hemileia vastatrix*. Determinação da turgidez relativa em cafeeiros imunes e susceptìveis. Rev. do café Português. No. 11. (Junta de exportação do café.)

EPSTEIN, E. (1955).—Passive permeation and active transport of ions in plant roots. *Plant Physiol.* **30**: 529–35.

HANSTEEN-CRANNER, B. (1914).—Über das Verhalten der Kulturpflanzen zu den Bodensalzen. III. Beiträge zur Biochemie und Physiologie der Zellwand lebender Zellen. *Jb. wiss. Bot.* **53**: 536–98.

HARTEL, O. (1951).—Über die Quellbarkeit pflanzlicher Membranen. *Phyton* **3**: 69–83.

HONERT, T. H. VAN DEN (1948).—Water transport in plants as a catenary process. *Disc. Faraday Soc.* **3**: 146–53.

HÜBNER, G. (1960).—Zum Wassertransport in *Vicia faba*. *Flora* **148**: 549–94.

HYLMÖ, B. (1958).—Passive components in the ion absorption of the plant. II. The zonal water flow, ion passage, and pore size in roots of *Vica faba*. *Physiol. Plant.* **11**: 382–400.

KRAMER, P. J. (1932).—The absorption of water by the root systems of plants. *Amer. J. Bot.* **19**: 148–64.

KRAMER, P. J. (1955).—Water content and water turnover in plant cells. In "Encyclopedia of Plant Physiology." Vol. I. (Springer-Verlag: Berlin.)

LEVITT, J. (1957).—The significance of "apparent free space" (A.F.S.) in ion absorption. *Physiol. Plant.* **10**: 882–8.

LEVITT, J., SCARTH, G. W., and GIBBS, R. D. (1936).—Water permeability of isolated protoplasts in relation to volume change. *Protoplasma* **26**: 237–48.

MEES, G. C., and WEATHERLEY, P. E. (1957*a*).—The mechanism of water absorption by roots. I. Preliminary studies on the effects of hydrostatic pressure gradients. *Proc. Roy. Soc.* B **147**: 367–80.

MEES, G. C., and WEATHERLEY, P. E. (1957*b*).—The mechanism of water absorption by roots. II. The role of hydrostatic pressure gradients across the cortex. *Proc. Roy. Soc.* B **147**: 381–91.

NEZGOVOROVA, L. A. (1957).—The effects of the water relations of plants on the introduction and distribution of carbon in the process of photosynthesis. (In Russian.) *Fiziol. Rast.* **4**: 440–9.

PETTERSSON, M. L. R., and GRAY, G. E. J. (1958).—Further inherent characters of plant species of wetter and drier habitats. *Nature* **182**: 450–1.

PFUHL, T. (1888).—"Die Jute und ihre Verarbeitung." (Berlin.)

PRESTON, R. D., and WARDROP, A. B. (1949).—The submicroscopic organisation of the walls of conifer cambium. *Biochim. Biophys. Acta* **3**: 549–59.

PRUSAKOVA, L. D. (1960).—Influence of water relations on tryptophane synthesis and leaf growth of wheat. (In Russian.) *Fiziol. Rast.* **7**: 170–80.

SCOTT, F. M., HAMNER, K. C., BAKER, E., and BOWLER, E. (1956).—Electron microscope studies of cell wall growth in the onion root. *Amer. J. Bot.* **43**: 313–24.

SCOTT, G. W., VOE, R. DE, HAYWOOD, H., and CRAVEN, G. (1957).—Exchange of sodium ion in *Ulva lactuca*. *Science* **125**: 160.

SLATYER, R. O. (1955).—Studies of the water relations of crop plants grown under natural rainfall in Northern Australia. *Aust. J. Agric. Res.* **6**: 365–77.

STAMM, A. J. (1952).—Surface properties of cellulosic materials; In "Wood Chemistry." Vol. 2. (Eds. L. E. Wise and E. Jahn.) (Reinhold Publ. Co.: New York.)

STOCKING, C. R. (1956).—Hydration and cell physiology. In "Encyclopedia of Plant Physiology". Vol. 2. (Springer-Verlag: Berlin.)

STRUGGER, S. (1949).—"Prakticum der Zell-und-Gewebephysiologie der Pflanzen." (Springer-Verlag: Berlin.)

SWINBANK, W. C. (1958).—Turbulent transfer in the lower atmosphere. "Proceedings of the Canberra Symposium on Climatology and Microclimatology." (U.N.E.S.C.O.)

WARTIOVAARA, V. (1944).—The permeability of *Tolypellopsis* cells for heavy water and methyl alcohol. *Acta Bot. Fenn.* **34**: 1–22.

WEATHERLEY, P. E. (1950).—Studies in the water relations of cotton. I. Field measurements of water deficits in leaves. *New Phytol.* **49**: 81–97.

WERNER, H. O. (1954).—Influence of atmospheric and soil moisture conditions on diurnal variations in relative turgidity of potato leaves. Res. Bull. Univ. Nebraska Coll. Agric. Exp. Sta. No. 176.

WIESNER, J. (1921).—"Rohstuffe des Pflanzenreichs." (Engelmann: Leipzig.)

WISE, L. E., and JAHN, E. EDS. (1952).—"Wood Chemistry." Vol. 2. (Reinhold Publ. Co.: New York.)