COMPARISON OF WATER POTENTIALS IN LEAVES AS MEASURED BY TWO TYPES OF THERMOCOUPLE PSYCHROMETER

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[*Manuscript received April* 28, 1964]

Summary

A comparison of water potentials as measured by two types of miniature thermocouple psychrometer, when used in vapour pressure equilibration chambers containing leaf material, has been made. Significant differences were observed.

Experiments showed that these differences were caused by the liberation of heat accompanying aerobic respiration by the tissue, which raised chamber temperature above the temperature of the controlled water-bath in which the chamber was immersed.

One of the psychrometers (Spanner 1951) could be read before the non-reference junction was wetted. It was therefore possible to measure both dry- and wet-bulb temperatures of the chamber. In this way an accurate estimate of chamber wet-bulb depression could be obtained. It was also shown that chamber dry-bulb temperature was not materially different from the temperature of the surface of the leaf tissue lining the chamber.

Owing to the non-reference junction being permanently wet in the second psychrometer (Richards and Ogata 1958), the observed temperature depression was between the bath (reference junctions) and chamber wet bulb (non-reference junction). This led to spuriously low estimates of water potential.

The prevention of temperature rise within a chamber by attaching the leaf to a heat sink is demonstrated, the two psychrometers then agreeing closely. However, due to practical difficulties, the heat sink is not recommended for routine determinations.

The merits of the two psychrometers are discussed in relation to these findings, and it is concluded that the Spanner psychrometer has advantages over the Richards and Ogata instrument in the determination of leaf water potential, although it is not so simple to use.

Large changes in water potential accompanied the onset of anaerobic conditions; normal respiration seemed essential to prevent these changes, which were probably associated with impairment of semipermeability. Anaerobic conditions could arise in a few hours when the chamber was lined with more than a single layer of tissue. Smaller changes in water potential could occur in 12–24 hr, even though anaerobiosis was prevented. The desirability of reaching vapour pressure equilibrium before such changes occur is stressed. This is best achieved by completely lining the chamber with not more than a single layer of leaf tissue.

I. Introduction

The need for accurate measurements of water potential in plants has been stressed by Kramer (1963), who pointed out that plant water potential is a key property, affecting many others, such as turgor, growth, stomatal aperture, transpiration, photosynthesis, and respiration. The development of miniature thermocouple

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psychrometers by Spanner (1951) and Richards and Ogata (1958) has facilitated this measurement (Brix 1962; Ehlig 1962; Gardner and Ehlig 1963).

Principal advantages of the psychrometric technique over earlier methods are that a determination can be made with a single sample, and the tissue remains in air, whilst sensitivity is at least as good as in the earlier methods. The psychrometer is used to measure the wet bulb depression of air over a sample in a closed equilibration chamber held at constant temperature. A constant reading indicates vapour pressure equilibrium has been established between the air and the sample; the corresponding water potential is equal to that of the sample.

Spanner (1951) reported that for a chamber containing leaf material, vapour pressure equilibrium was reached in from 10 min to 1 hr and was a function of water potential. Kramer and Brix (1962), using a modified version of this couple (Monteith and Owen 1958), found equilibrium took 12–24 hr, regardless of water potential. Ehlig (1962), using the Richards couple, found 2½–6 hr necessary, depending upon the equilibrium water potential. Clearly, it would be advantageous if equilibrium could be attained in 1 hr or less.

Monteith and Owen (1958), when describing their modification of the Spanner couple for use with soils, pointed out that the presence of free water on a permanently wet junction could raise the humidity in a small space. This effect, which could only occur with the Richards couple, seemed all the more likely when working with leaf tissue. The tissue would offer greater resistance to water vapour movement than would soil, especially as the stomata would be shut in the dark equilibration chamber.

Another point of uncertainty concerned the quantity of tissue necessary. Kramer and Brix (1962) used 100 sq cm of leaf with the Spanner psychrometer, whilst Ehlig (1962), working with the Richards psychrometer, used less than half this amount in a container of similar size. A small sample would be advantageous, particularly since many leaves are not as large as 100 sq cm.

It was felt that these problems could best be resolved by a comparison of the performance of the psychrometers; accordingly this was undertaken with the object of formulating the most accurate and convenient technique. Preliminary results (Barrs 1964) showed a hitherto unconsidered and sometimes quite large error to be the liberation of heat accompanying the respiration of plant material when enclosed in the equilibration chambers. These findings are confirmed and their effects discussed.

II. Materials and Methods

Leaves of pelargonium (*Pelargonium zonale* L. cv. Paul Crampel) were the main experimental material used. However, a wider understanding of the behaviour of plant material when placed in the equilibration chambers was gained by additional experiments with leaves of American Upland cotton (*Gossypium hirsutum* L. cv. Empire), pepper (*Capsicum frutescens* L. cv. Californian Wonder), tobacco (*Nicotiana tabacum* L. cv. Hicks), pumpkin (*Cucurbita pepo* L. cv. Yates Butternut), and rock melon (*Cucurbita pepo* L. cv. Hale's Best).

The thermocouples were essentially as described by Spanner (1951) and Richards and Ogata (1958), except that chromel-P and constantan were substituted

for bismuth and bismuth–5% tin in the Spanner couple. Details of construction are shown in Figures 1*A*–1*C*. The thermocouple mounts fitted into brass caps (Fig. 1*C*) at either end of stainless steel equilibration chambers; thus two psychrometers could be compared within the same chamber containing the one leaf sample. This procedure eliminated variability due to use of paired leaf samples. However, some work was done with chambers permanently closed at one end and containing only one psychrometer.

In most experiments the walls of the chamber were lined with leaf tissue and the perforated copper tube and stainless steel wire mesh (Fig. 1*C*) were omitted, but in some work the leaf material was wrapped round the copper tube and held firmly against it by the stainless steel mesh. The tube was a tight push-fit onto the brass end caps, and formed a heat sink, minimizing temperature differences between the leaf and the chamber.

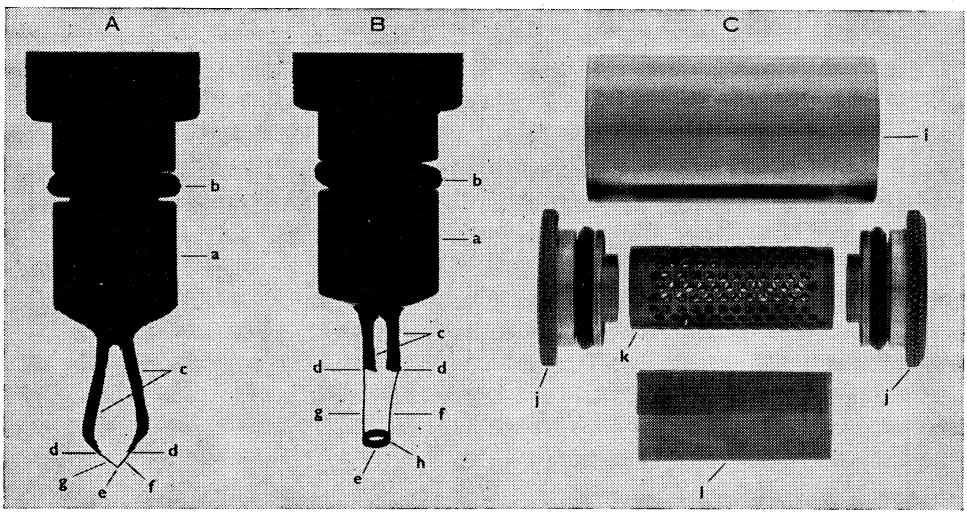

Fig. 1.—*A*, silhouette of thermocouple psychrometer, modified slightly from Spanner (1951); ×1·5. *B*, silhouette of thermocouple psychrometer, modified slightly from Richards and Ogata (1958); ×1·5. *C*, equilibration chamber and heat sink, used with one of the above psychrometers at either end; ×0·6. *a*, brass mount; *b*, O-ring seal; *c*, twin core, PVC-covered copper flex (14 by 0·0076 in.), bared in this region; *d*, reference junctions; *e*, free junction; *f*, Chromel-P 0·001 in. diameter; *g*, Constantan 0·001 in. diameter; *h*, silver cylinder; *i*, stainless steel equilibration chamber; *j*, brass end cap; *k*, drilled copper tube (heat sink); *l*, stainless steel wire mesh.

The Spanner psychrometer was read first with its free junction dry, and then with it wet. The difference between these readings was taken as the wet-bulb depression. Wetting was achieved by cooling the free junction below the dew point by passing a Peltier cooling current through the thermocouple from an external battery, causing condensation on the free junction which dried out again between readings. Readings with the Richards psychrometer were normally only made with the free junction wet as the water drop is, for practical purposes, permanent. The free junction was wetted by immersing the silver cylinder into water before inserting

the psychrometer into the chamber. On lowering the water, a drop was left behind in the cylinder. However, some results are reported using this couple with its free junction dry.

Current produced by the psychrometers was measured with a reflecting galvanometer (nominal sensitivity 315 mm/μA at 1 m) and converted to microvolts from the known total circuit resistance, which was checked frequently. The sensitivity of the galvanometer was also checked frequently, by a standard cell in series with a large resistance. High-grade, low-resistance switches were used to connect the

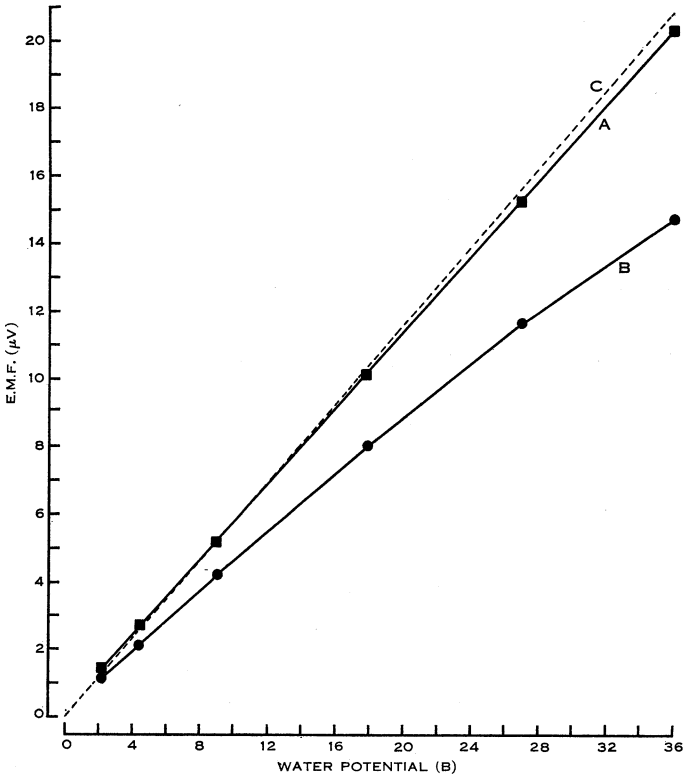

Fig. 2.—Calibration curves for psychrometers; *A*, Richards; *B*, Spanner; *C*, fully ventilated (theoretical).

thermocouples in turn to the galvanometer. The continuous output from the Richards psychrometer permitted measurement of both positive and negative deflections. Mean values were taken for greater accuracy. Owing to the ephemeral nature of its wet junction, the output from the Spanner psychrometer was measured ballistically, only maximum deflections in one direction being recorded. Supply of the cooling current to the Spanner thermocouple and reversal of the output of the Richards couple were effected by two auxiliary switches. The reference junctions of the psychrometers were held at constant temperature by immersing the equilibration chambers in a constant-temperature water-bath controlled to $\pm 0{\cdot}001$ degC, and the whole apparatus was housed in a constant-temperature room (± 1 degF).

Before use with leaf material, psychrometers were calibrated by exposure to known vapour pressures, obtained by lining the equilibration chambers with filter papers wetted with a range of sodium chloride solutions. Data from Owen (1952) were used to convert the vapour pressures of these solutions to water potentials in bars. Results for both types of psychrometer are shown in Figure 2.

III. Results

The following abbreviations are used here and in Section IV: ψ, total water potential; π, osmotic water potential; ΔT, output from psychrometer with free junction dry; $\Delta T'$, output with free junction wet. The subscripts S (Spanner), and R (Richards) indicate which instrument was used. Water potentials are expressed in bars (b).

(*a*) *Comparison of* ψ_S *and* ψ_R

Using the apparatus shown in Figure 1, but with the heat sink omitted, simultaneous observations were made of ψ_S, ψ_R, and ΔT_S. Results are given in Figures 3(*a*)–3(*d*) for leaves of four species.

Pelargonium was used in Figure 3(*a*) which shows that ψ_S and ψ_R became steady, after $6\frac{1}{2}$ hr, at about 4 and 2 b respectively, a discrepancy of 2 b. After 23 hr this discrepancy was eliminated and the psychrometers were in good agreement (ψ_R 5·1 b, ψ_S 5·0 b). ΔT_S, determined immediately before wetting the free junction by passing the cooling current, remained constant for $6\frac{1}{2}$ hr, finally declining to a negative value; positive values indicated that the free junction was warmer than the reference junctions, negative values that it was cooler. Figure 3(*b*) shows that rock melon leaf gave a similar result; ψ_S exceeded ψ_R by about 1 b in the initial steady state, but finally both came to approximately the same value. Again, ΔT_S indicated the free junction was initially constantly warmer than the reference junctions, and finally became slightly colder. Figure 3(*c*) shows that for tobacco leaf ψ_S was only about 0·2 b greater than ψ_R during the approximately steady state; again there was final agreement between ψ_S and ψ_R. However, ΔT_S was considerably lower than in the previous examples and did not finally reverse its sign. Figure 3(*d*) shows ψ_S and ψ_R for pepper leaf. Although the approach to equilibrium was unusually slow, ψ_S and ψ_R were at all times in close agreement. ΔT_S was so low as to be negligible.

(*b*) *Comparison of* π_S *and* π_R

Tissue used in determination of osmotic potential was first killed by freezing with solid CO_2; direct contact was avoided by wrapping the sample in aluminium foil (Ehlig 1962). Separate chambers for each psychrometer were used in these experiments.

(i) *First Experiment.*—Under these conditions no real discrepancies were observed between π_S and π_R; also ΔT_S was negligibly small. A typical result for paired samples of pelargonium leaf was: π_S 9·7 b; π_R 9·8 b; ΔT_S +0·01 μV.

(ii) *Second Experiment.*—The tissue in the equilibration chamber with the Spanner psychrometer was not frozen, but air in the chamber was replaced by nitrogen which was passed for 4 min before sealing the chamber and immersing it in the

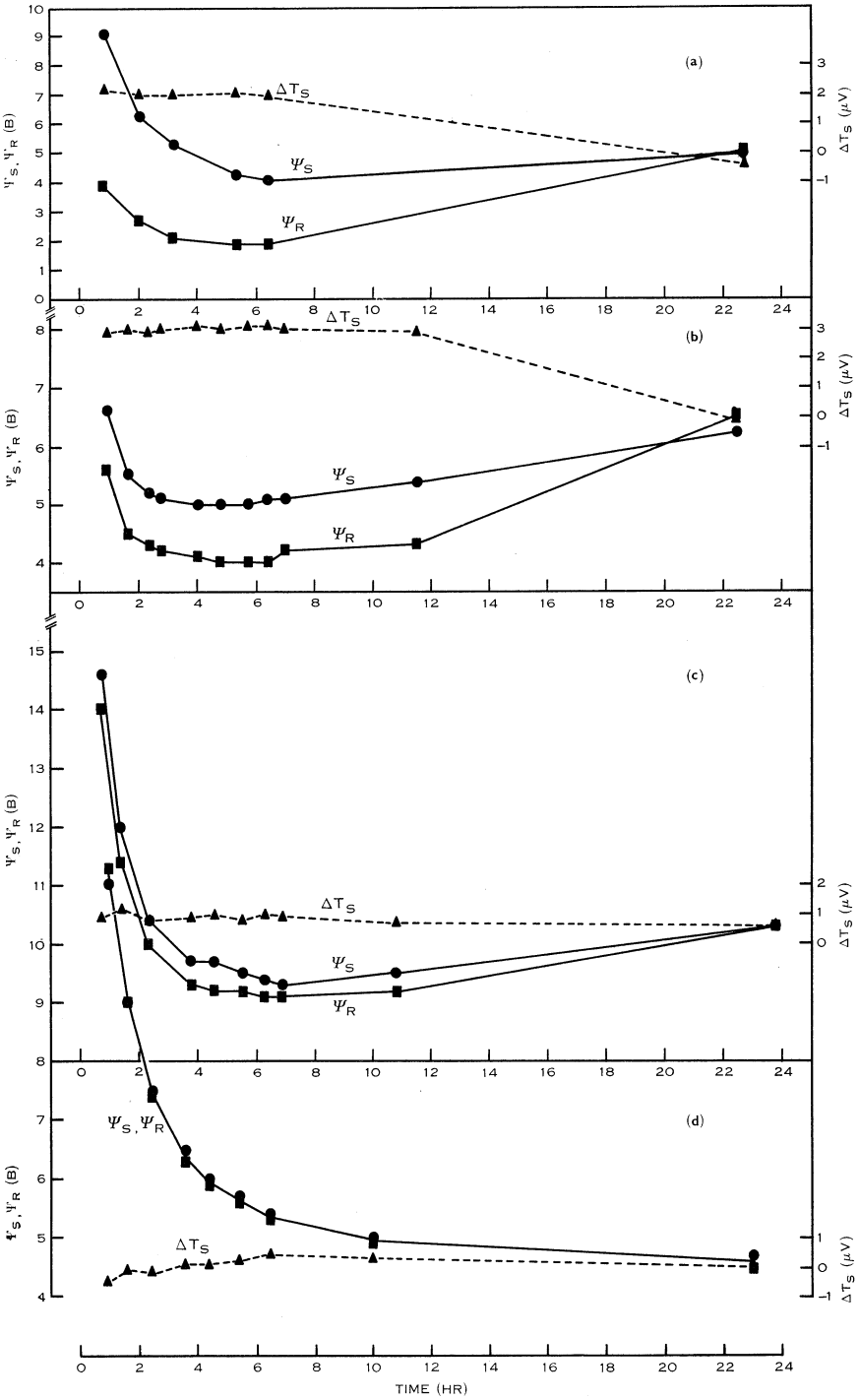

Fig. 3.—Water potentials according to Spanner and Richards psychrometers (ψ_S and ψ_R respectively), and output from dry Spanner psychrometer (ΔT_S): (*a*) pelargonium leaf; (b) rock melon leaf; (*c*) tobacco leaf; (*d*) pepper leaf.

water-bath. Tissue in the Richards chamber was frozen, as before. Again there was no discrepancy between π_S and π_R, but a small negative value of ΔT_S showed the free junction to be slightly colder than the reference. Results were: ψ_S 7·8 b; ψ_R 7·8 b; ΔT_S −0·06 μV.

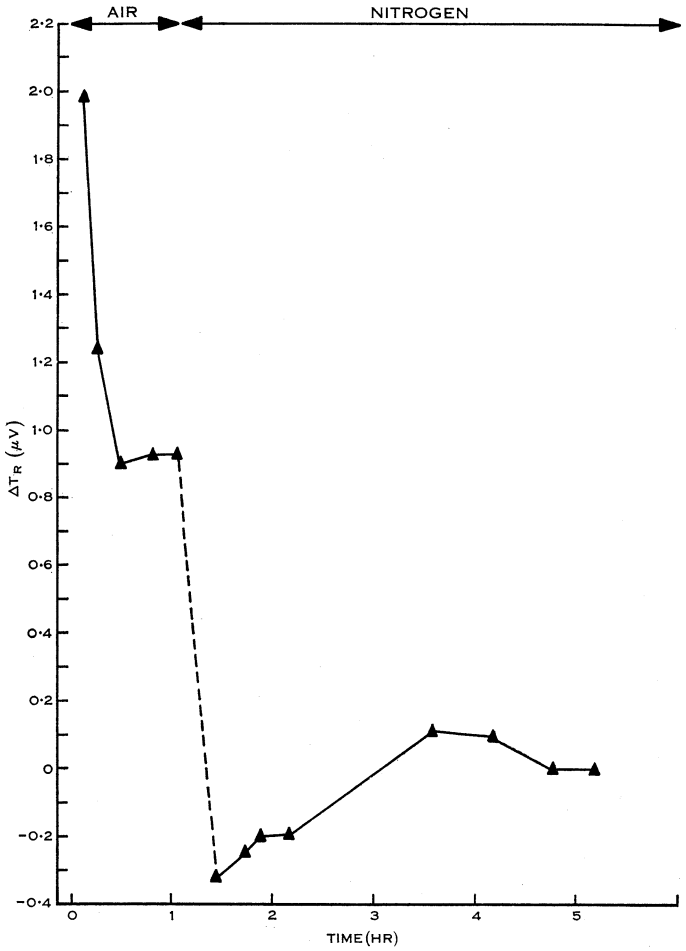

Fig. 4.—Output from a dry Richards psychrometer (ΔT_R) with a single layer of pelargonium leaf in air or nitrogen.

(*c*) *Experiments on* ΔT

The rather surprising observations of output (ΔT_S), from the Spanner couple with dry free junction [Figs. 3(*a*)–3(*c*)] were investigated further. Figure 4 shows, for a single layer of pelargonium leaf, that a similar output could be obtained from a dry Richards couple (i.e. silver ring without water drop), and also that when the air in the chamber was replaced by nitrogen, ΔT_R fell to zero after preliminary fluctuation.

Figure 5 shows that, for a double layer of pumpkin leaf in an oxygen-filled chamber, ΔT_S was maintained at an approximately constant positive value for

24 hr without sign of a decline. This contrasts with tissue in an air filled chamber, where ΔT_S declined almost continually, finally falling through zero to a slightly negative value.

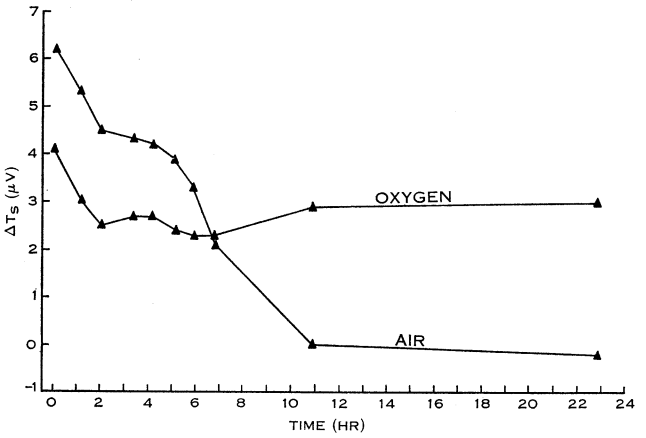

Fig. 5.—Output from a dry Spanner psychrometer (ΔT_S) with a double layer of pumpkin leaf in oxygen or air.

(d) *Effect of* ΔT_S *on* ψ_S *and* ψ_R

Figure 6 shows ψ_S and ψ_R for psychrometers in two separate chambers, each containing a double layer of pelargonium leaf; also given are the corresponding

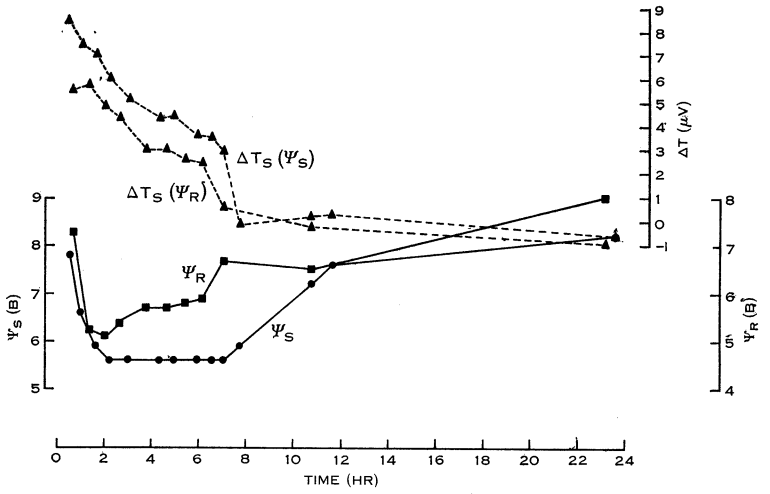

Fig. 6.—Outputs from dry Spanner psychrometers (ΔT_S) and water potentials measured either with the same instrument (ψ_S), or a Richards psychrometer (ψ_R). Two chambers, each with a double layer of pelargonium leaf.

curves for ΔT_S. The course of ΔT_S is similar in both chambers, but ψ_S and ψ_R differ in that ψ_S became constant for 5 hr whilst ψ_R showed no corresponding steady state.

(e) Comparison of ΔT at the Leaf Surface and at the Centre of a Chamber

For this purpose a thermocouple somewhat resembling a Richards psychrometer was specially constructed. The fine chromel-P and constantan wires (cf. Fig. 1*A*) were, however, considerably longer and simply joined to form the free junction by soldering to a small square of silver sheet. By turning the chamber from the vertical

TABLE 1

TEMPERATURES AT THE LEAF SURFACE AND IN THE AIR WITHIN AN EQUILIBRATION CHAMBER LINED WITH A SINGLE LAYER OF PELARGONIUM LEAF

Bath temperature assumed to be 25·000°C

Time	Position of Free Junction	Temp. (°C)	Temp. Difference, Leaf–Air (degC)*
1138	Leaf surface	25·022	
1140	Air	25·020	+0·002
1335	Air	25·014	
1340	Leaf surface	25·018	+0·004
1440	Air	25·017	
1445	Leaf surface	25·018	+0·001
1515	Air	25·020	
1518	Leaf surface	25·017	−0·003

* Mean temperature difference = +0·002 degC.

to the horizontal, the free junction of the thermocouple was brought from a central position in the chamber, where it was surrounded by air, into contact with the surface of the leaf lining the wall of the chamber. The output from the couple was measured in these two positions and results are summarized in Table 1. Readings

TABLE 2

OUTPUT FROM DRY PSYCHROMETERS (ΔT) EXPRESSED IN EQUIVALENT UNITS OF WATER POTENTIAL (B), IN THE PRESENCE AND ABSENCE OF A HEAT SINK

Chamber lined with a single layer of pelargonium leaf

With Heat Sink			Without Heat Sink (same leaf)		
Time	ΔT_R (b)	ΔT_S (b)	Time	ΔT_R (b)	ΔT_S (b)
1457	0·4	0·1	1615	1·6	0·3
1509	0·3	0·1	1625	1·2	0·4
1518	0·2	0	1627	1·2	—
1539	0·2	0·1	1633	1·2	0·4
1548	0·2	0			

have been converted to temperature by using a conversion factor (Korven and Taylor 1959) of 63 μV/degC and adding an arbitary 25 degC. The latter represents assumed water-bath temperature, which was held constant ($\pm$0·001 degC), at approximately that temperature. Temperature differences between leaf and air are small and on average may be considered negligible.

(*f*) ΔT, ψ_S, *and* ψ_R *as Affected by a Leaf Heat Sink*

Work in all the previous sections was carried out in the absence of the heat sink described in Section II and illustrated in Figure 1*C*. The use of the heat sink was found to be effective in reducing ΔT_S to zero, and ΔT_R to a very low figure. This is shown in Table 2 where the observed outputs have been converted to water potentials. The table also shows that without the heat sink, a considerable ΔT, larger in the case of the Richards than in that of the Spanner couple, was present. Figure 7, curve *A*, shows that when the heat sink was used, ψ_S and ψ_R were in good agreement. However, it should be noted that the experiment was set up some 12 hr before readings commenced and both ψ_S and ψ_R continued drifting slowly down

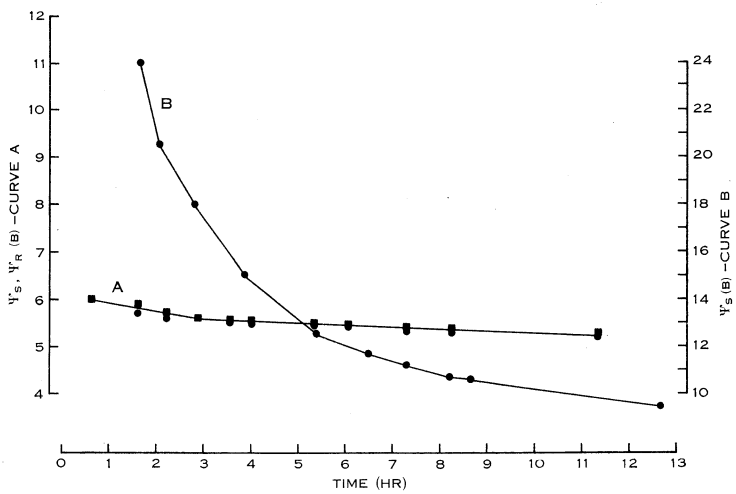

Fig. 7.—Experiments with a leaf heat sink: curve *A*, water potentials according to Spanner and Richards psychrometers (ψ_S and ψ_R), readings commenced 12 hr after setting up; curve *B*, readings, (ψ_S) only, commenced within 1 hr of setting up. Single layer of pelargonium leaf.

during the course of the day. Figure 7, curve *B*, where readings were commenced within an hour of setting up, shows how the approach to equilibrium was slowed considerably by the presence of the heat sink (cf. Fig. 6, ψ_S).

(*g*) *Effect of Oxygen upon* ψ

Two chambers, one oxygen-filled, the other air-filled, and each containing a double layer of pelargonium leaf tissue, were set up with Richards psychrometers. Figure 8 shows that ψ_R for the oxygen-filled chamber remained fairly steady, after preliminary equilibration, whereas ψ_R for the air-filled chamber followed a course very like that seen in Figure 6, with an initial decline, a slow rise, a rapid rise, and a final slow rise.

The use of oxygen was not always effective in preventing ψ from rising. Figure 9 shows this for a chamber containing a single layer of pumpkin leaf. However, the figure also shows this rise was smaller than in the case of pumpkin leaf in an air-filled chamber, and a more definite steady state was first obtained.

(h) Comparison of Two Richards Psychrometers with Different-sized Wet Junctions

The psychrometers were used in separate chambers but with material from the same cotton leaf. One psychrometer had a large silver cylinder, approximately 1 mm

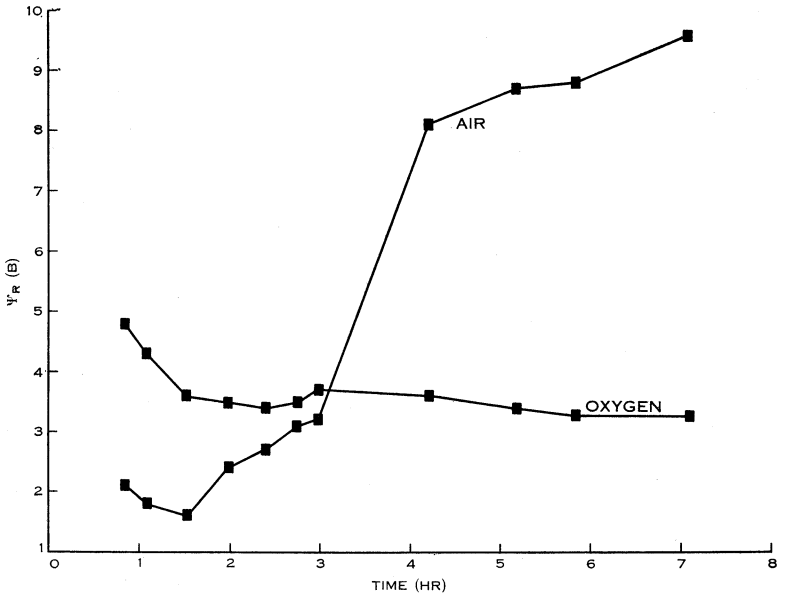

Fig. 8.—Effect of oxygen or air on water potential (ψ_R) in chambers containing double layers of pelargonium leaf.

high by 3 mm in diameter, holding a water drop weighing 0·0024 g; the other had a normal silver cylinder 2 by 0·5 mm, holding a water drop weighing 0·0008 g. The water potentials in two experiments with cotton leaves were: 16·3 b (large drop), 16·2 b (small drop); 15·4 b (large drop), 15·3 b (small drop). These results show

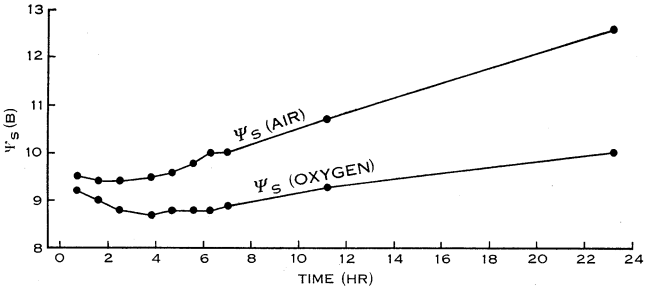

Fig. 9.—Effect of oxygen or air upon water potentials (ψ_S) in chambers containing a single layer of pumpkin leaf.

that there was no tendency for the larger drop to give a lower water potential, and hence no measurable tendency for humidity to be raised by the larger drop, although this possibility had been raised in general terms by Monteith and Owen (1958).

IV. Discussion

The results in Section III(a) show that ψ_S may exceed ψ_R for some hours whilst the output from the thermocouples remains relatively constant [Figs. 3(a)–3(c)] but that finally this discrepancy disappears. However, Figure 3(d) shows the complete absence of the discrepancy during the whole course of the experiment. Inspection of these results will suggest a relation between ΔT_S and the discrepancy between ψ_S and ψ_R. For the period when output is steady, high ΔT_S is associated with a large discrepancy [Figs. 3(a) and 3(b)], but when ΔT_S is small, the discrepancy is small [Fig. 3(c)]; finally when ΔT_S is effectively zero, the discrepancy is no longer apparent [Fig. 3(d)]. Also, in any one experiment, the final disappearance of the discrepancy occurs when ΔT_S declines to a very low value [Figs. 3(a), 3(b)]. Other points of note are that the Richards psychrometer seems to be more affected by these changes in ΔT_S than the Spanner instrument and that ΔT_S often finally changes sign. Knowing the direction of deflection of the galvanometer during calibration of the psychrometers, it was possible to deduce that in all cases where intact leaf tissue was placed in the chambers and ΔT_S was measurable, the free junctions were initially warmer than the reference junctions, though often finally becoming slightly colder.

The discrepancies noted above between ψ_S and ψ_R were not found in comparisons of π_S and π_R [ψ Section III(b)]. When the tissue was killed by freezing with dry ice (experiment 1), ΔT_S became negligibly small and ψ_S and ψ_R were in very good agreement. Experiment 2 shows that this agreement was maintained when the tissue in one chamber was exposed to anaerobic conditions. In this case a small ΔT_S was recorded, such that the free junction was colder than the reference junctions. However, ΔT_S has presumably not affected ψ_S, since ψ_S and ψ_R agree.

Experiments with nitrogen (Richards couple) and oxygen (Spanner couple) show respectively that ΔT was reduced to zero (Fig. 4) or maintained at a constant level for at least 24 hr (Fig. 5). The experiment with nitrogen also shows, in the initial portion of the curve where the chamber was air-filled, that a large ΔT_R could be observed with the Richards psychrometer when its free junction was dry. This demonstrated that the earlier observations of ΔT_S with the Spanner couple were not artefacts associated with the use of this type of psychrometer. The experiment also showed that nitrogen caused the free junction to become cooler than the reference junctions for a time.

It has already been noted that there is no ΔT in the presence of tissue killed by freezing. To this may be added the fact that no ΔT was found during calibration of the psychrometers with salt solutions. Together with the dependence of a demonstrable ΔT on the presence of living tissue, its inhibition by nitrogen, and its prolongation by oxygen, these observations lead to the conclusion that ΔT is caused by the liberation of heat accompanying aerobic respiration. The decline of ΔT after tissue has been in the chambers some time suggests the onset of anaerobiosis. This is supported by the observation that ΔT frequently becomes slightly negative after leaf material has been in the chambers for 24 hr, since this was also found to occur when air was replaced by nitrogen [Section III(b)(ii) and Fig. 4]. It may be that under these conditions processes requiring inflow of energy to the leaf, predominate.

Both Spanner (1951) and Richards and Ogata (1958) stress the need for very accurate temperature control when using their psychrometers, and they have implicitly assumed that this is adequately provided for by immersing the equilibration chambers in a water-bath controlled to $\pm 0{\cdot}001$ degC. This ensures the absence of any temperature gradient between the reference and free junctions apart from that due to wet-bulb depression, during calibration, or when the psychrometers are used with soil. However, the present results show that introduction of living leaf material, by liberating heat as it respires, may warm the air in the chamber and also the free junction. The massive copper leads prevent the reference junctions from being heated, and they remain at bath temperature. Hence the temperature difference measured when the free junction is wet is in fact bath temperature minus wet-bulb temperature; the required difference being, of course, chamber dry-bulb minus chamber wet-bulb temperature. Since chamber temperature is usually above bath temperature (by ΔT), the measured wet-bulb depression is an underestimate of the true wet-bulb depression, and water potentials derived from these depressions will be correspondingly low.

This analysis applies particularly to the Richards psychrometer where the free junction is permanently wet, masking any ΔT that may be present. In the case of the Spanner psychrometer, ΔT may be measured and allowed for by making two readings, first with the free junction dry and then with it wet, giving chamber temperature (ΔT) and apparent chamber wet-bulb depression ($\Delta T'$), respectively. By adding ΔT to $\Delta T'$ (or subtracting if the chamber is cooler than the bath), the true wet-bulb depression may be obtained. This depression should strictly only be referred to bath temperature $\pm\Delta T$. However, provided ΔT is small, there is no appreciable error in referring it directly to bath temperature. In this analysis it has been assumed that chamber air temperature, as measured with the free junction of the psychrometer dry, is equal to the temperature of the leaf surface lining the chamber. Evidence supporting this assumption has been given in Table 1.

Provided ΔT is not unduly large, readings of the Spanner psychrometer used in this way should give correct wet-bulb depressions and hence correct water potentials. If vapour pressure equilibrium has been attained, changes in ΔT should not affect ψ_S. The Richards psychrometer, on the other hand, would not be expected to give a steady wet-bulb depression in the presence of changing ΔT, even though vapour pressure equilibrium had been attained. Every change in ΔT would be matched by a corresponding change in $\Delta T'$ and hence in ψ_R; the net result would be that ψ_R would follow as a mirror image of ΔT, increasing as ΔT decreased, and vice versa.

The experimental results shown in Figure 6 confirm that the two psychrometers do behave differently in the presence of changing ΔT, and in fact, behave in the way suggested above. ΔT_S follows a similar path in both chambers; an initial curvilinear decline, followed by a sharp drop to approximately zero, and a final low negative reading. ψ_S and ψ_R both exhibit the typical initial decline as vapour pressure equilibrium is approached. ψ_S then remains constant for the next seven readings spread over 5 hr, indicating that vapour pressure equilibrium has been reached and is maintained, although ΔT_S continues to fall. ψ_R shows no corresponding steady state; instead it rises from a minimum value of $5{\cdot}1$ b as ΔT_S continues to decline, and

inspection of the two curves will show ψ_R following as a mirror image of ΔT_S even to the extent that it remains constant for the brief period ($3\frac{3}{4}$–$4\frac{1}{2}$ hr) when ΔT does so.

The final marked rises in water potential shown in Figure 6 occur when ΔT_S declines to zero or becomes slightly negative. An earlier result (Fig. 4) has suggested that this decline occurs when aerobic respiration ceases, and Section III(*b*)(ii) shows that under completely anaerobic conditions, the total water potential, ψ, becomes equal to the osmotic potential, π. It seems that aerobic respiration is necessary to maintain cellular organization, and that when it stops or falls too low, structural breakdown commences. Presumably semipermeability of cellular membranes is lost, leading to direct exposure of cell contents to the air in the chamber.

It might be argued that errors arising from the use of the Richards psychrometer could be avoided by taking readings with the free junction alternately dry and wet, in the same way as has been suggested for the Spanner psychrometer. However, it would be difficult to accomplish this without withdrawing the psychrometer from the chamber to wet or dry the free junction. Unless ΔT remained constant during the time that the psychrometer took to re-establish equilibrium with the air in the chamber, the sum of ΔT and $\Delta T'$ might not in fact equal the true wet-bulb depression. An alternative approach would be to use two Richard psychrometers, one dry, the other wet, to determine ΔT and $\Delta T'$ respectively. If their calibrations were in good agreement they could be combined electrically, thereby automatically giving the correct wet-bulb depression, even though ΔT might change sign.

The feasibility of this approach was illustrated by an experiment in which material from the same pelargonium leaf was placed in two separate chambers, one containing a single Spanner psychrometer, the other containing two Richards psychrometers, the first with free junction wet, the second with free junction dry. The Spanner psychrometer gave ψ_S 6·6 b, the Richards psychrometers gave ψ_R (wet) 5·2 b; ψ_R (dry) 1·3 b; or a corrected ψ_R (wet+dry) of 6·5 b, in good agreement with ψ_S.

Care would need to be taken that the wet and dry free junctions were reasonably far apart, since observations suggest the wet junction of the Richards psychrometer has an appreciable sphere of influence. This is illustrated in Figure 10, obtained when calibrating a Richards and a Spanner psychrometer in the same chamber, with the free junctions 3 mm apart. A definite negative ΔT_S was obtained, and this was proportional to the concentration of the calibrating solution.

This technique would call for the construction of twice the number of psychrometers otherwise necessary, and a consequent double number of calibrations. Since frequent calibration checks are also necessary (Ehlig 1962), the total extra labour would be considerable. The use of the Spanner psychrometer seems preferable, not only because fewer psychrometers are necessary, but also because the same thermocouple is used to measure both ΔT and $\Delta T'$.

The analysis given above would account for the discrepancies between ψ_S and ψ_R noted in Figures 3(*a*)–3(*c*), and also for their ultimate disappearance, but not for the final rise in ψ_S. This final rise is less marked than in Figures 6 and 8. It probably represents a response to partially anaerobic conditions, since in these

experiments the chambers contained only a single leaf ayer compared with the double layer of Figures 6 and 8. However, Figure 9 shows that a similar final rise in ψ_S occurred even in an oxygen-enriched atmosphere. Hence a small final rise may be the result of causes other than the onset of anaerobic conditions; possibly the isolation of the leaf material from the plant may lead to inevitable changes in water potential after 12–24 hr. If this is so then some care may need to be exercised in the use of vapour-exchange techniques to ensure equilibrium is reached and measured before such effects become apparent. The effect may well be species dependent, since for pepper leaf ψ_S showed no sign of rising after 24 hr [Fig. 3(*d*)].

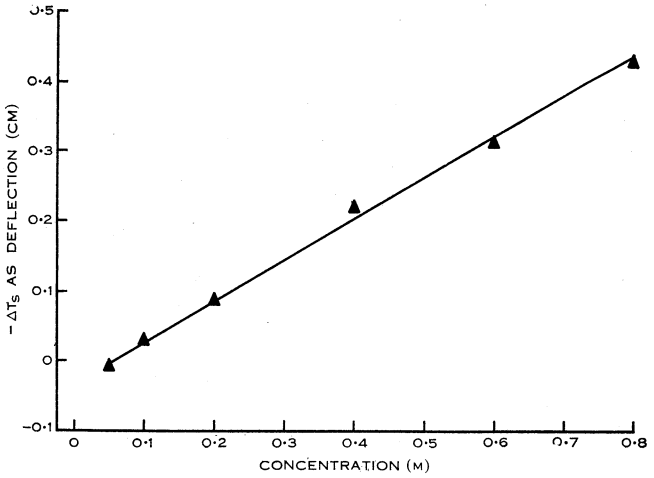

Fig. 10.—Effect of concentration of calibrating solution on output (ΔT_S) from a dry Spanner thermocouple.

Further support for the view that discrepancies between ψ_S and ψ_R are caused by ΔT arises from the use of the heat sink which effectively eliminated ΔT (Table 2), and also the difference between ψ_S and ψ_R (Figure 7, curve A). However, Figure 7, curve *B*, shows that the time required to reach equilibrium is very considerably increased, partly because a smaller quantity of leaf tissue is used (the leaf being wrapped round the $\frac{3}{4}$-in. copper sink instead of lining the 1-in. chamber) and also, presumably, because the heat sink and stainless steel gauze offer additional resistances to vapour diffusion. The work involved in drilling the heat sink is considerable, also the need to ensure good thermal contact between the leaf and the sink would probably result in damage to wilted tissue. For these reasons, it is felt that the heat sink is not a practical solution to the problems raised by the effects of ΔT.

Although the Spanner psychrometer seems preferable to the Richards instrument when working with biological material which can cause significant ΔT effects, it does, however, suffer from a number of disadvantages. Thus, the output is lower (roughly 0·3 μV/bar against 0·5 μV/bar for the Richards couple), and not linear.

These points are illustrated in Figure 2 which shows also that the Richards instrument approximates quite closely to a fully ventilated psychrometer. Nevertheless, it has been found possible to read outputs to an accuracy of 0·1 b with either

couple, see for example, Figure 7, curve *A*. The need to arrange for the passage of the cooling current through the Spanner couple increases the difficulty of setting up for automatic reading and recording. A more serious disadvantage is that it is difficult to make frequent readings at low water potentials. This is because the free junction must be completely dry in order to determine the psychrometer zero, before wetting it to determine the wet-bulb depression: the time taken for the junction to dry again after the previous reading may be long—with water potentials of about 0·5 b up to an hour may be needed. In practice such low water potentials do not normally arise in plant leaves and this difficulty is not important. Monteith and Owen (1958) have shown that there is also an upper limit to the usefulness of a psychrometer which employs the Peltier cooling effect. At high water potentials, about 100 b, the relation between wet-junction depression and water potential cannot be followed because the free junction does not remain wet long enough to permit a reading to be taken. Again this difficulty lies outside the range required when working with all except the most xerophytic plants.

In conclusion, the Spanner psychrometer has been found preferable to that of Richards when working with plant material, since it can be used to take into account the departure of chamber temperature from bath temperature caused by metabolic activity, whereas a single Richards psychrometer cannot. As a general rule, all exposed surfaces of the chamber should be lined with a single layer of leaf tissue to ensure rapid equilibration. Use of more tissue is not recommended, since heating of the chamber is thereby increased, and secondary effects from the quicker onset of anaerobic conditions may lead to the tissue becoming moribund with associated changes in water potential.

V. Acknowledgments

Thanks are due to Dr. R. O. Slatyer, Division of Land Research and Regional Survey, CSIRO, for suggesting the problem, and to Mr. E. R. Hoare, Officer-in-Charge, Irrigation Research Laboratory, CSIRO, for encouragement and provision of facilities. Mr. G. E. Plant gave invaluable technical assistance.

VI. References

Barrs, H. D. (1964).—Heat of respiration as a possible source of error in the estimation by psychrometric methods of water potential in plant tissue. *Nature*: **203** (4950): 1136–7.

Brix, H. (1962).—The effect of water stress on the rates of photosynthesis and respiration in tomato plants and loblolly pine seedlings. *Physiol. Plant.* **15**: 10–20.

Ehlig, C. F. (1962).—Measurement of energy status of water in plants with a thermocouple psychrometer. *Plant Physiol.* **37**: 288–90.

Gardner, W. R., and Ehlig, C. F. (1963).—The influence of soil water on transpiration by plants. *J. Geophys. Res.* **68**: 5719–24.

Korven, H. C., and Taylor, S. A. (1959).—The Peltier effect and its use for determining relative activity of soil water. *Canad. J. Soil Sci.* **39**: 76–85.

Kramer, P. J. (1963).—Water stress and plant growth. *Agron. J.* **55**: 31–5.

Kramer, P. J., and Brix, H. (1964).—Measurement of water deficit in plants. In "Proceedings of the UNESCO Conference of Methods in Plant Ecophysiology". Montpellier, 1962. (In press.)

MONTEITH, J. L., and OWEN, P. C. (1958).—A thermocouple method for measuring relative humidity in the range 95–100%. *J. Sci. Instrum.* **35**: 443–6.

OWEN, P. C. (1952).—The relation of water absorption by wheat seeds to water potential. *J. Exp. Bot.* **3**: 276–90.

RICHARDS, L. A., and OGATA, G. (1958).—Thermocouple for vapor pressure measurement in biological and soil systems at high humidity. *Science* **128**: 1089–90.

SPANNER, D. C. (1951).—The Peltier effect and its use in the measurement of suction pressure. *J. Exp. Bot.* **2**: 145–68.