

THE MEASUREMENT OF DIFFUSION PRESSURE DEFICIT IN PLANTS BY A METHOD OF VAPOUR EQUILIBRATION

By R. O. SLATYER*

[Manuscript received January 1, 1958]

Summary

A method for measuring diffusion pressure deficit (DPD) in plants is described and compared with the standard floating techniques. Basically the method is a modification of that described by Arceichovskij and Arceichovskaja (1931) and differs from the standard floating methods in that it involves the equilibration of tissue in vapour of known water potential rather than in solutions of known osmotic potential.

The technique involves the use of micro-desiccators in which disks of leaf tissue are arranged on small grids 5 mm above the surface of salt solutions of known water potential. Equilibration takes place in a constant-temperature water-bath, with the temperature controlled to $\pm 0.001^{\circ}\text{C}$.

In a comparison with the standard floating methods, the vapour technique is shown to have advantages in precision and reproducibility at all degrees of water stress above a few atmospheres DPD. At these low stress levels both methods appear satisfactory but, as turgor pressure falls with increasing stress, errors begin to be introduced to the floating methods by cell plasmolysis and at the stage when most of the cells in the tissue are plasmolysed the method becomes almost completely unresponsive.

I. INTRODUCTION

The measurement of diffusion pressure deficit (DPD) (Meyer 1945) in plant tissues has been the subject of a great deal of study extending from the early investigations of de Vries (1884). The principal technique which has been evolved is that which involves balancing the DPD, which is equivalent to the water potential† of the plant tissues, against the water potential (or osmotic potential) of aqueous solutions of sucrose, mannitol, or salts, using a series of solutions of differing concentration. The solution in which the tissue neither gains nor loses water from or to the solution, respectively, is considered to have an osmotic potential equivalent to the DPD of the tissue. Examples of this general technique are the cell method (Ursprung and Blum 1916) in which change in cell volume is measured, the strip or simplified method (Ursprung 1923) in which change in length or volume (Lyon 1936) is measured, and the weight method (Meyer and Wallace 1941) in which the change in weight is measured. An alternative measurement (Ashby and Wolf 1947) involves a determination of change in the concentration of the solution, using a refractometer.

Although this technique has great simplicity, the need to float tissue in the solution can introduce appreciable errors due to infiltration of the solution into the intercellular spaces, and in the case of plasmolysis, intracellular spaces, of the tissues.

*Division of Land Research and Regional Survey, C.S.I.R.O., Canberra.

†Terminology of Schofield, cited by Owen (1952).

As a result, interest has been focused on vapour pressure methods for measuring DPD. Two main techniques suggest themselves. The first is to utilize the same general procedure as has just been described, but to balance the DPD of the tissues against the water potential of vapour over a solution, instead of directly against the osmotic potential of a solution. The second is to measure a characteristic of the vapour pressure when the tissue under study is enclosed in a thermally constant chamber. An example of the first technique is the method of Arcichovskij and Arcichovskaja (1931) which measures the change in weight of samples of leaf tissue which are placed in vapour over solutions of known water potential. Examples of the latter technique are the methods of Spanner (1951) who used an application of the Peltier effect to measure wet-bulb depression and hence to compute water potential, and of Stone, Went, and Young (1950) who employed a humidity-sensing device to effectively measure water potential under conditions of extreme water stress.

The latter methods have some advantages over the former in speed and precision and it can be anticipated that, as more precise methods of hygrometry are developed, still more applications will follow. In most biological laboratories at present, however, the necessary equipment is not readily available and for this reason, and because of the simplicity of the former method, it was decided to investigate it with a view to improving its accuracy and flexibility.

II. APPARATUS AND GENERAL TECHNIQUE

The principal requirement of the apparatus was that it should provide and maintain, at the surface of the tissue under examination, a constant and known water potential in the vapour phase. This necessitated very sensitive temperature control and the use of small test chambers, so that differences in water potential at the surface of the control solution and at the tissue surface would be as small as possible.

(a) *Control of Water Potential*

(i) *Constant-humidity Chambers*.—These chambers were constructed from standard laboratory pomade jars about 6 cm high and 6 cm diameter, fitted with completely watertight rubber-lined plastic lids. The jars were filled to within 1.2 cm of the top with coarse quartz sand or glass beads to minimize splashing of solution during manipulation of the chambers. The chambers were then filled with control solution so that the solution covered the sand and reached to within 1.0 cm of the top. A small grid of nylon mesh which was to carry the plant material was then arranged 0.5 cm above the liquid surface, supported on a short (2 cm) length of 2-cm diameter glass tubing which was pushed into the quartz sand to the desired depth. The tubing also acted as a baffle to prevent splashing. A diagrammatic sketch of the completed chamber is shown in Figure 1.

(ii) *Control Solutions*.—Precise data on the aqueous vapour pressure of pure water, and of solutions of sodium chloride, are available together with data on the relative molal vapour pressure lowering $[(P_0 - P)/MP_0]$ for various concentrations of sodium chloride (Robinson and Stokes 1955). The equivalent values for the water

potential in centimetres of water were obtained from the formula provided by Owen (1952):

$$d = \frac{RT}{gM} \left(-\log_e P/P_0 \right),$$

where

d = water potential in centimetres of water,

$R = 8.314 \times 10^7$ ergs per °C per mole,

T = absolute temperature, and

$g = 980.6$ dynes per gram.

As DPD is measured by being balanced against osmotic solutions or vapour of known water potential, it should be expressed in the same terms, i.e. in centimetres or metres of water potential. However, Meyer (1945) regards DPD as a unit of pressure, conceptually and dimensionally different from a unit of potential energy, and expressible in atmospheres. Although this contention can be disputed, the use of DPD as a term in plant physiology has come into widespread use. For this reason it has been considered necessary, though undesirable, in this paper to refer to DPD

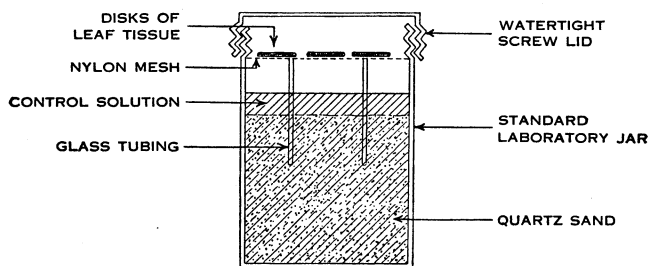


Fig. 1.—Diagrammatic sketch of constant-humidity chamber in which plant material was placed during determinations.

when discussing plant measurements, but to refer to the vapour in the controlled humidity chambers in terms of water potential. Though dimensionally incompatible, pressure and energy units can be regarded as numerically equivalent, and to this extent can be related by the expression:

$$\text{water potential (in cm)} = -1033 \text{ DPD (in atm)}.$$

The solutions were made up to the desired concentration and added to the constant-humidity jars as described above. No special arrangements were made for stirring of the solutions, but slight vibration in the water-bath assisted in the elimination of the possible concentration gradients in the solutions in each jar which could have developed through evaporation or condensation of water at the liquid surface. Best results were obtained when the solutions were replaced at the end of each determination. The temperature for all determinations was 25°C.

(b) *Control of Temperature*

For the maintenance of constant water potential in the humidity chambers, it was important that they should be placed in a temperature-controlled water-bath, where sudden changes in temperature could be eliminated and possible condensation of water at the tissue surface avoided. A water-bath arrangement as

finally utilized has enabled the control of temperature to within $\pm 0.001^{\circ}\text{C}$. It consisted of an outer tank of about 200 l. capacity in which was placed a smaller tank of about 75 l. capacity. The outer tank was controlled to better than $\pm 0.05^{\circ}\text{C}$ and this enabled the inside one to be controlled to $\pm 0.001^{\circ}\text{C}$ without undue difficulty. The whole apparatus was located in a constant-temperature room which was held at $25 \pm 0.5^{\circ}\text{C}$. Various thermoregulators were used for the inside tank, the most effective being a Fisher-Serfass "Micro-Set" differential thermoregulator operating through a Fisher-Serfass electronic relay. A "Sunvic" toluene-mercury thermoregulator operating through a proportioning head and "Sunvic" EA3T electronic relay was utilized for the outside tank. Each thermoregulator actuated a 200-W standard frosted light bulb to provide intermittent heating. Cooling, when required, was provided by a copper coil through which water flowed at about 20°C . The water pressure was controlled by a constant-head source, and rate of flow was adjusted arbitrarily by a valve on the inlet tube. Rapid circulation of water in each tank was provided by propeller or pump-type stirrers (the latter being more satisfactory) which enabled uniform temperature conditions to be maintained throughout the tank.

(c) *General Method*

The main objective of the present study was to improve the general technique of Arcichovskij and Arcichovskaja (1931) which was described above. Accordingly, a series of experiments was conducted to study the time needed for equilibration of the tissue used, and the influence of various factors which could affect the validity of the determinations. In general, several main procedures were always adhered to. Firstly, all plant material was allowed to reach temperature equilibrium in the constant-temperature laboratory before it was sampled. Secondly, the sample taken consisted of disks of tissue of varying size (see below) which were punched from the leaves using a sharp cork borer. The leaves were always selected for uniformity and representative age. After sampling, the disks were immediately transferred to tared weighing bottles and weighed on an automatic balance to 0.1 mg. They were then transferred to the constant-humidity chambers which remained in the water-bath except for the introduction or removal of samples. Each of these latter processes took about 40 sec per sample. At the end of each determination the disks were again transferred to the weighing bottles and reweighed. If any floating treatment was imposed, the disks, after removal from the liquid, were placed on filter paper to allow rapid drainage of adherent water and then dried for 30 sec between eight sheets of Whatman No. 4 filter paper under a 500-g weight. This procedure was adopted to ensure uniformity in the drying operation, a potential cause of serious weighing errors (Ashby and Wolf 1947).

The number of disks per sample varied with disk size and the degree of accuracy needed. The size of disk most frequently used was 0.7 cm diameter. Normally a sufficient number of disks was punched to bring the total weight of the sample to at least 0.10 g. Notwithstanding this provision, when larger disks were used, a minimum of six disks generally appeared necessary to obtain valid results, and a sample size of 10 or 12 disks was utilized where possible.

Several species of plants were utilized for the determinations, particularly privet (*Ligustrum japonicum* Ait. and *L. lucidum* Ait.), tomato (*Lycopersicon esculentum* Mill.), saltbush (*Atriplex nummularia* Lindl.), and sorghum (*Sorghum vulgare* Pers.). In general, the results obtained with these different tissues were very similar, and the data presented below are from privet and tomato, with which the major part of the investigations were conducted. Because the investigations took place in three laboratories over a period of more than two years, a wide variety of plants within these species was sampled. For this reason the data from different sections of the results presented are not always strictly comparable.

III. RESULTS

(a) Time Needed for Equilibration

Before directly examining the method of Arcichovskij and Arcichovskaja, it was considered important to examine aspects of water uptake and loss by leaf disks in vapour of different water potentials, so that the effect of such factors as disk size, disk placement, and water potential on the time needed for equilibration could be evaluated.

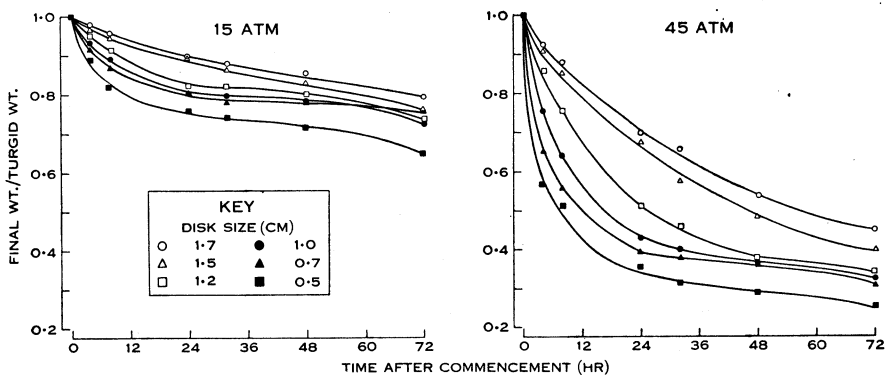


Fig. 2.—Effect of size of disk on rate of loss of water from tomato leaf tissue, in vapour of water potential equivalent to 15 atm and 45 atm DPD.

(i) *Effect of Disk Size.*—To investigate aspects of this effect, disks of various sizes, and hence of varying cut surface/total surface ratios, were used to determine the rate of loss of water in vapour of different water potential. Only the rate of loss was examined because of the difficulty in establishing uniform stress for the commencement of uptake. The disks were punched from unwilted plants, floated in water for 8 hr, dried, weighed, and placed in the appropriate constant-humidity chambers. In Figure 2, data for tomato tissue in water potentials equivalent to DPD's of 15 and 45 atm are presented which illustrate the pattern of response observed in all species at different water potentials.

The data show a rapid initial decline in weight followed by a transition to a final stage characterized by a slight but continuous weight loss. This final stage persisted to the end of the experimental period and probably reflected losses due to

respiration. The data also show that as disk size increased the rate of change in ratio of the final weight/original turgid weight increased, so that the final stage was reached fastest in the smallest, and slowest in the largest disks. This suggests that most of the water exchange takes place through the cut surfaces of the disks, the greater the cut surface/total surface ratio, the faster the rate of exchange.

Since the speed with which equilibration occurs is an important factor to be considered in assessing the effectiveness of vapour methods for measuring DPD, one of the objectives of this test was to indicate a disk size which would enable equilibration, or the attainment of the final stage, in the shortest period of time, but would not result in a degree of tissue damage severe enough to affect the final value. The 0.5-cm disks did not appear to satisfy the latter provision as they reached final values significantly lower than those of the other groups. Disks of greater than 1.2 cm diameter clearly failed to satisfy the former provision. Disks of the intermediate group were therefore indicated and after extensive preliminary tests with the species listed above, disks of 0.7 cm diameter were adopted, except where otherwise stated, for the remainder of the determinations reported in this paper.

(ii) *Effect of Water Potential*.—Two series of experiments were conducted in order to determine the rate of water uptake by, and loss from, leaf disks in vapour of different water potential.

In order to secure data on rates of both uptake and loss, branches were removed from the plants to be sampled and allowed to wilt very severely in the greenhouse. They were then brought into the constant-temperature room, allowed to equilibrate with room temperature, and two series of disks were punched from representative leaves. After weighing, one series of disks was immediately placed in the constant-humidity chambers; the other series was floated on water for 8 hr, dried, weighed, and then placed in the chambers. This enabled sorption* curves to be obtained from the former series and desorption curves to be obtained from the latter. The results for tomato and privet, for a range of water potentials, are shown in Figure 3. The sorption curves are not strictly comparable because it was not possible to create the same degree of stress in each species before sampling. The maximum DPD which could be developed in tomato without damage was about 40–50 atm; in privet, on the other hand, it was possible to develop well over 90 atm. As a result no data are given for tomato above 45 atm and in each species the rate of uptake is partly a reflection of the different initial degree of stress. The desorption curves are comparable because of the preliminary period of floating which enabled the disks to reach full turgor before being placed in the chambers.

From Figure 3 it is evident that the general rates of uptake were similar in both species, although slightly slower in privet. This also applied to rates of water loss, but this process was much more rapid. In general, with all the species tested, there was a tendency for water exchange to be slower in species with well-developed cuticles. As in the previous series of tests, final steady equilibrium values were not obtained, all the curves showing a slow decline after the final stage had been reached.

*Because both adsorption and absorption processes operate in the uptake of water by plant material, the term sorption is used in this paper to indicate uptake of water and desorption to indicate loss of water.

The rate at which the final stage was reached was markedly affected by water potential, particularly at values equivalent to DPD's of less than 15 atm. With the uptake curves, in the 0 atm treatment, the final stage did not appear to be reached in either species even after 72 hr. As the water potential increased negatively the time required decreased, but even in the most extreme cases more than 48 hr elapsed. In the loss process, which was much more rapid, the final stage was reached after about 24–32 hr at water potentials corresponding to DPD's in excess of 15 atm and after a little over 32 hr at lower values. Apart from differences in rate of equilibration, it is interesting to note that the final value obtained on the sorption curves always appeared to be less than the value on the desorption curve. This was possibly due to hysteresis and is discussed in more detail below.

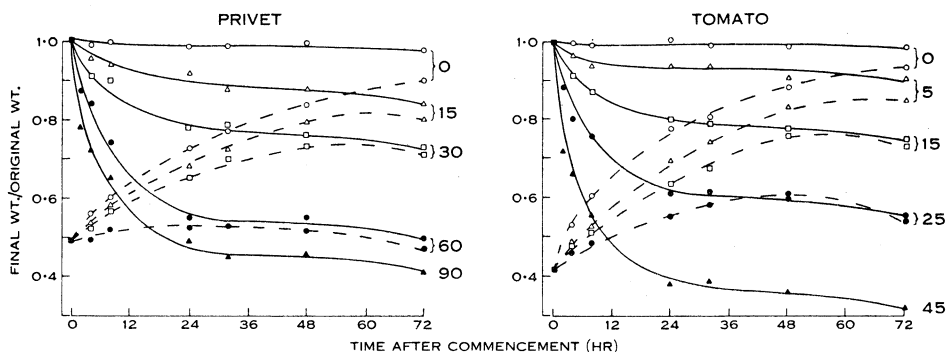


Fig. 3.—Change of weight of disks of privet and tomato leaf tissue in vapour of different water potential, in relation to time allowed for exchange of water by the disks; continuous lines represent loss, broken lines represent uptake. Water potential (in atm equivalent DPD) is indicated at end of each set of curves.

(iii) *Effect of Disk Placement.*—Because differences have been observed, in floating experiments, between disks placed with their adaxial surface uppermost, as distinct from those with abaxial surface uppermost, the possible influence of disk placement on rate of change of tissue weight, and on the final value obtained, was investigated. As before, only the loss curve was studied because of difficulties in standardizing the degree of stress before uptake. Disks were punched from unwilted plants and after floating and reweighing were arranged in the controlled humidity chambers so that one series had the adaxial sides of the disks uppermost and the other the abaxial sides uppermost. No significant differences were observed (at $P = 0.10$) regardless of the water potential involved or species used.

(b) Utilization of Method for DPD Measurements

(i) *Time Needed for Determinations.*—Because final equilibrium values are extremely difficult to obtain, some arbitrary time limit must be set for the duration of DPD determinations. Such a period should be as short as possible, so as to minimize effects of respiration and because of the convenience, experimentally, of a rapid determination, but should be of adequate duration to minimize the effects of

differential rates of sorption and desorption, so as to provide a sensitive estimate of DPD.

In order to determine an appropriate period for the determination, samples were taken from plants which had previously been subjected to moderate-severe wilt. This procedure ensured that an appreciable water deficit developed in the tissue so that a significant amount of both uptake and loss could be expected during the determinations. After introduction to the constant-humidity chambers, different sets of samples were removed after 4, 8, 24, and 48 hr. The results obtained with privet and tomato leaf tissue, which were similar to those from the other species, are given in Figure 4.

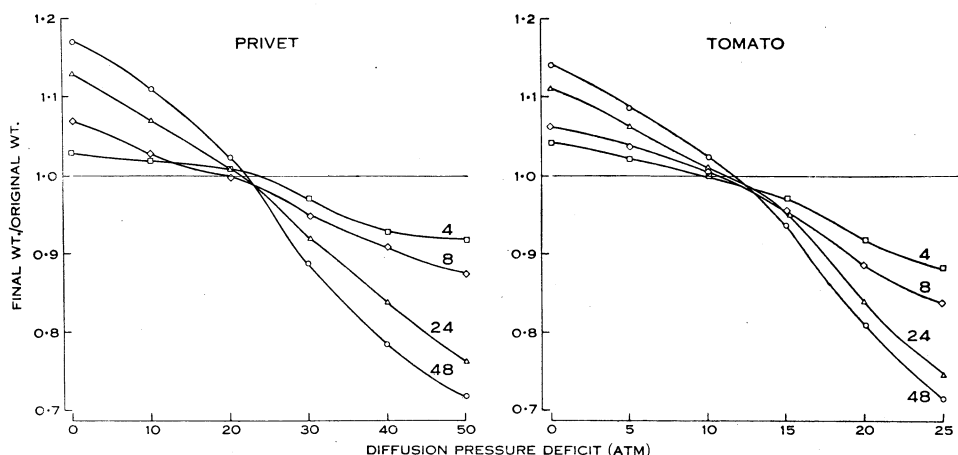


Fig. 4.—Influence of time allowed for determination of DPD of privet and tomato leaf tissue on the final value obtained. Time allowed (hr) shown on curves for each determination.

The data indicate that although a significant estimate of DPD could be made after 4 hr, the value obtained tended to drift slightly with time. This appeared to be due primarily to slower initial rates of sorption, since with increasing time proportionally more uptake occurred and the point of intersection shifted along the line of final weight/original weight = 1.0. The difference between the various estimates can be seen to be of the order of 2–3 atm.

Although this suggests that as long a period as possible should be allowed for the determination, the data show that the uptake in the 24–48-hr period represents only a minor proportion of the total uptake, so that the advantages of extending the determinations beyond 24 hr are limited. Also, any undesirable effects due to respiration can be expected to become more pronounced with increasing time. For these reasons a 24-hr period was adopted as a standard time for the determination and has been found satisfactory in extensive laboratory use of the method.

(ii) *Factors Influencing DPD Value Obtained.*—From the data just presented it appears that the main factors which affected the value obtained for DPD in any one determination were those affecting the shape of the uptake and loss curves and so influencing the point of intersection with the line of final weight/original

weight = 1.0. As suggested above, the relatively slow rate of uptake was probably the primary factor in this regard.

In order to secure data on the extent to which these factors operated, it was decided to compare the results obtained with the standard method with those obtained when the tissue was first floated to turgor and then dehydrated to various degrees. This latter procedure should, in effect, examine only the desorption curve and so the amount by which the standard gain and loss curve diverged from it should be indicated.

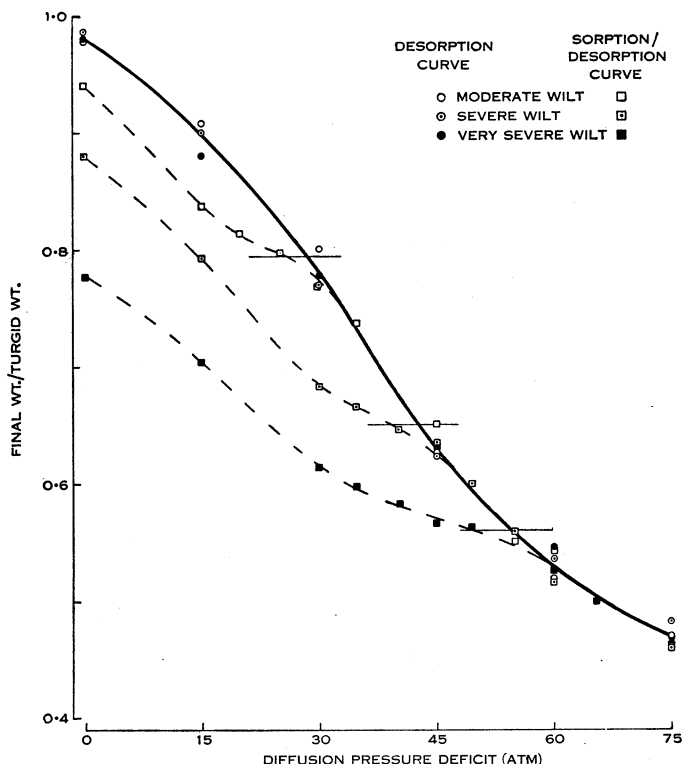


Fig. 5.—Comparison of value obtained for DPD of privet leaf tissue when determination is conducted by standard sorption/desorption technique, with value obtained from desorption curve after tissue has first been floated on water and then dehydrated to known degrees. Tissue first subjected to moderate, severe, and very severe wilting to develop a wide range of DPD's.

For these experiments, small branchlets of similar size and age were detached from sample plants and allowed to wilt to different stages so that different initial DPD's were developed. Two sets of disks were punched from the leaves and weighed in tared weighing bottles; one set was then placed immediately in containers comprising a range of known water potentials, the other was first floated in water for 8 hr and then dried, reweighed, and placed into a similar range of containers. The samples remained in the containers for 32 hr and were then removed and reweighed.

The results were plotted as final weight/turgid weight and are given for privet in Figure 5. Turgid weights for the samples not pre-floated were estimated from the original fresh weights, using the fresh weight/turgid weight ratios of the pre-floated samples. Short horizontal lines have been drawn across each curve at the level of the original fresh weight, so that the DPD can be read off at the point of inflexion.

The data indicate quite marked differences in the value obtained for the DPD, depending on the method used; the values obtained by using the desorption curve alone being up to 5 atm greater than those from the standard curve.

Although part of this difference might be attributed to incomplete desorption, it is apparent from the marked divergence of the two curves, which took place as soon as the sorption phase was introduced, that most of the difference seems to be caused by slow rates of sorption.

It is also possible that hysteresis effects could have contributed to the result. Although no direct evidence is available for such an explanation, the data of Figure 5 support such a contention. For instance, in the humidity chambers representing DPD's close to those existing in the tissue when sampled, the slower rates of uptake should not have been of significance in determining the final values obtained, since equilibrium should have been reached within the period allowed. Yet in such instances the final weight/turgid weight values on the sorption curves were significantly below those on the desorption curve. Such a phenomenon could be due to hysteresis, and on the basis of other studies with plant material (Rao, Rao, and Rao 1949) and with wood (Kelsey 1957) the effects of hysteresis could be expected to be of similar magnitude to the differences observed here. These points will be considered in the subsequent discussion.

From Figure 5 it is also interesting to observe the uniformity in all curves in the desorption phase. This suggests that there was little permanent effect on osmotic pressure of the tissue sap (and hence on the DPD/water content relationship) caused by the initial wilting or the pre-floating, and would tend to rule out the effects of such changes in the interpretation of the data in the diagram.

(c) Comparison of Vapour Equilibration Method with Floating Methods

Crafts, Currier, and Stocking (1949) comment that the "simplified" or "strip" and "weighing" methods of measuring DPD (Ursprung and Blum 1930) are those most widely used at the present time. Yet it is undeniable that they are most unsatisfactory in all tissues when water stress becomes severe and even at quite low DPD's with fairly rigid tissue. In order to compare these established methods with the vapour method, comparative experiments were conducted using the normal techniques for the strip and weighing methods, and the standard technique as described in this paper for the vapour method (i.e. using 0.7-cm disks, with 24 hr equilibration, and assessing DPD as the estimated point at which there was no gain or loss of weight by the tissue). For the weighing method, 0.7-cm disks were used and change in weight was assessed after 2 hr floating in graded solutions of NaCl. For the strip method, different techniques had to be employed for the tomato and privet tissue. For tomato, 0.5 by 3.0 cm strips were used, change of length being measured after 2 hr floating, with a microscope and stage micrometer. For privet, change in

length was negligible and change in thickness was measured instead (Ursprung and Blum 1927) using a paper thickness measuring device, reading to 0.01 mm. The results are shown in Figure 6 for determinations made on tissue previously wilted to a slight, moderate, and severe extent.

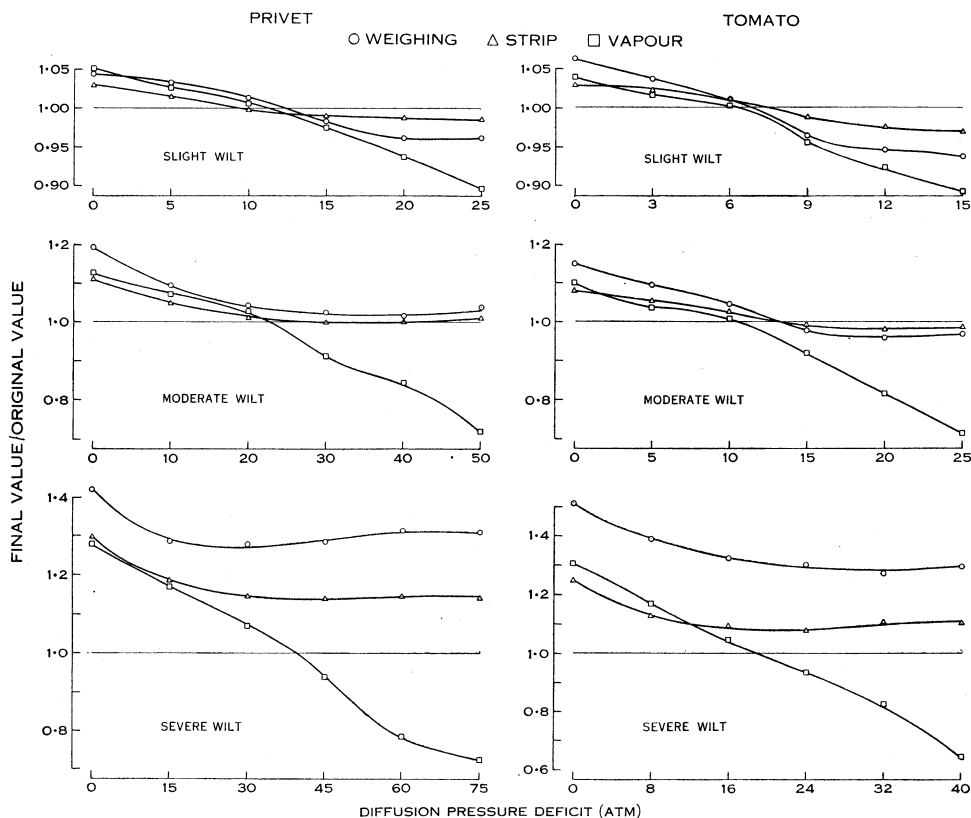


Fig. 6.—Comparison of standard vapour equilibration technique for determination of DPD with strip and weighing methods. The privet and tomato leaf tissue used was first subjected to slight, moderate, and severe wilting to develop a range of DPD's. The data are expressed as relative change in weight, length, or volume.

From the diagram it can be seen that in the slight wilt treatments, all methods gave estimates agreeing to within 2 atm, for both species. As the severity of wilting increased, however, the efficacy of the floating methods decreased, so that at moderate wilt no estimate was obtained by these methods for privet, and the estimate for tomato was much higher than that obtained by the vapour method. With the severe-wilt treatment no estimate was obtained, for either species, with the floating methods.

A probable explanation for this lack of effectiveness of the floating methods as stress increases is that infiltration of the external solution into the cells of the tissue takes place whenever the original DPD of the tissue is higher than that which

would exist at a state of incipient plasmolysis. Under these conditions it could be expected that, if the concentration of the external solution were greater than that of the plasmolytic value of the cell sap, infiltration by the solution would take place through the cell walls and each cell would swell to its shape at incipient plasmolysis. Some penetration could also possibly occur into the vacuole, and the final equilibrium situation would probably be one in which the protoplast became dehydrated to an extent such that its osmotic pressure was similar to that of the external solution and the remainder of the cell was filled with the external solution. If, at the beginning of the determinations, the surrounding solution were of lower concentration than the osmotic pressure of the cell sap, continued swelling would be expected until the DPD of the cells balanced that of the external solution.

These phenomena could be expected to result in the situation as portrayed in the diagram, where it can be seen that at DPD values in excess of the plasmolytic value of the cell sap (represented by moderate and severe wilt), tissue weight and volume increased in each of the floating treatments. The fact that an estimate was obtained for tomato at moderate wilt was possibly due either to the fact that the DPD was not quite at the plasmolytic value, or that the cell walls in tomato were less rigid than those of privet and tended to collapse to some extent in plasmolysing solutions, resulting in some loss of weight and volume.

In the determinations in which estimates of DPD were obtained by the floating methods, the values were always higher than those obtained by the vapour method. This could be attributed to the fact that, in any one tissue, cells with a range of osmotic characteristics exist and those with sap of low concentration would be plasmolysed by solutions in which most of the other cells would still retain some turgor. As further change in the dimensions of a cell virtually ceases with onset of plasmolysis, this would result in the final dimensions or weight of the tissue being greater in the floating treatments than in vapour and would, as a result, tend to progressively overestimate DPD as the number of plasmolysed cells increased.

These various explanations were supported by a demonstration in which turgid disks of tomato and privet leaf tissues were dehydrated, some by being floated in solutions of known osmotic potential and others by being placed in vapour of known water potential. The results are given in Figure 7. The dehydration periods were 2 hr for the floating treatments and 32 hr for the vapour treatments. Because the use of NaCl as a plasmolysing agent might be questioned due to its tendency to infiltrate into the vacuole in the floating treatments, data are presented for both NaCl and mannitol, the latter being widely accepted for this purpose. The highest concentrations of mannitol it was possible to maintain were of 25 atm osmotic potential.

From the diagram it can be seen that as the DPD approached the value at incipient plasmolysis (estimated from cryoscopic determinations as 11.5 atm for tomato and 21.5 atm for privet) the two curves diverged, and that in the case of the floating disks no further decrease, in fact a slight increase, was evident after this point was reached. These features support the explanation, provided above, of progressive plasmolysis of the cells of the tissue with increasing concentration of the surrounding solution, and confirm that change in cell size is slight after plasmolysis. The slight increase in weight in the solutions of highest concentration, if significant,

could be explained on the basis of infiltration of the external solution into the vacuole, which could cause an increase in the osmotic pressure of the cell sap and an associated slight increase in cell volume at incipient plasmolysis. These results would appear to confirm that floating methods can only be expected to operate at DPD's up to those at incipient plasmolysis, and that errors will begin to be introduced as soon as some cells of the tissue reach this value.

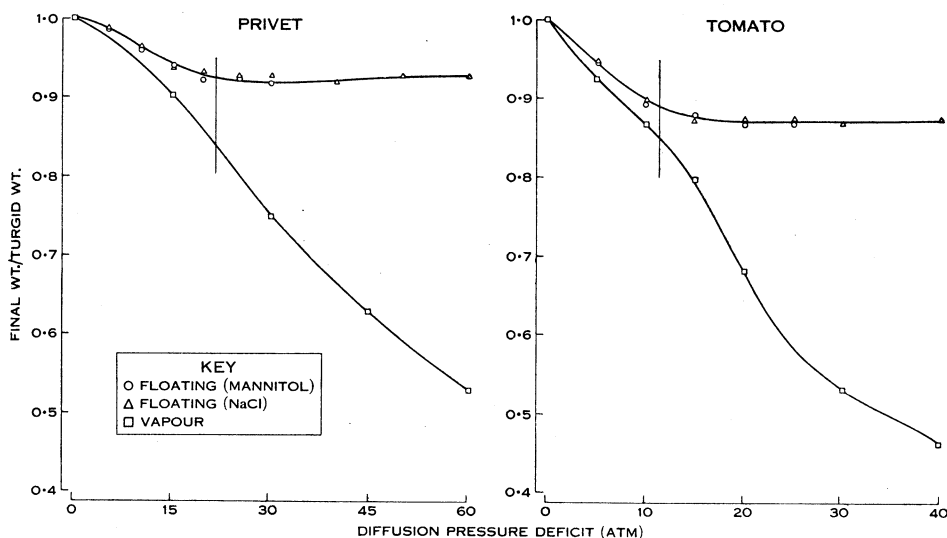


Fig. 7.—Dehydration of turgid disks of privet and tomato leaf tissue in water vapour of different water potential and in graded solutions of mannitol and NaCl.

IV. DISCUSSION

The primary factors influencing the accuracy of the vapour method for measuring DPD appear to be the difficulty of obtaining steady equilibrium values and the marked differences in rates of sorption and desorption.

The data of Figures 2 and 3 demonstrate that the rate of water exchange between the disks and the surrounding vapour shows a characteristic approach to an equilibrium condition, rate of water exchange decreasing as the DPD of the tissue approaches that of the vapour. Instead of reaching a steady value, however, continued loss of weight occurs at a slow, but fairly steady rate. This is particularly noticeable in the sorption curves of Figure 3, and is probably attributable to the effects of respiration. If so, it may continue at a changing rate throughout the determination, depending on the water content of the tissue at any one instant (Iljin 1957), and could introduce a potential source of error by causing changes in dry weight and hence in the osmotic characteristics of the tissue. However, experimental data shows that tissue refloats on water after dehydration regains almost exactly the same weight as the original turgid weight before dehydration. Since any significant change in the osmotic characteristics of the tissue would alter the volume of water in the tissue at full turgor, this suggests that any carbohydrate transformations which take place during dehydration are of small extent, or are

reversible, and that the dry weight losses caused by respiration do not unduly influence the osmotic characteristics.

The lack of attainment of stable equilibrium values means that an arbitrary time must be set for the determination. As DPD is estimated as being equivalent to the water potential of the vapour in which the tissue would neither gain nor lose weight, such a procedure in itself does not introduce errors, but the relatively slow rates of sorption compared to desorption tend to affect the estimate obtained.

The data of Figure 5 provide evidence as to the divergence of the sorption parts of three curves from an overall desorption curve and indicate that at the DPD's concerned, differences of up to 5 atm were obtained between estimates made from the desorption curve and those from the standard curve. Although this difference appeared to be primarily due to slow rates of sorption, it is possible that incomplete desorption may also have been of influence and both these factors could have been complicated by hysteresis effects.

Although it has not been possible to obtain direct evidence of hysteresis in these experiments, hysteresis is a property of porous and colloidal materials and has been identified in living and dead plant material (Rao, Rao, and Rao 1949; Kelsey 1957). In both instances cited, the divergence of the sorption and desorption parts of the hysteresis loop amounted to a difference of up to 2-3 per cent. water content, at relative vapour pressures equivalent to the DPD's used in this paper. In the experiments presented in Figure 5 a difference in water content of this magnitude would represent a DPD difference of up to 5 atm, a value sufficient to explain the discrepancy observed. While this deduction is speculative, it must be recognized that hysteresis is almost certainly of influence in causing differences between sorption and desorption curves.

Whether or not hysteresis is of influence, it is apparent from Figure 5 that the standard method for measuring DPD will always tend to provide an underestimate due to the relatively slow rates of sorption. This feature appears to be extremely difficult to eliminate. A possible alternative is to use the desorption curve alone, as in Figure 5. Such a procedure has the advantage of considering only the sorption part of the curve, and it also enables the simultaneous measurement of relative turgidity* and DPD and the development, if desired, of relative turgidity/DPD relationships for the tissue under study (Weatherley and Slatyer 1957). However, this method also has several disadvantages. These are primarily associated with the need to obtain final equilibrium values in order to obtain an accurate estimate of DPD. As has been mentioned above, such values were not obtained in this series of experiments, although the final stage was reached in most of the desorption curves in about 32 hr. If the final stage does not represent an equilibrium condition an overestimate of DPD would be obtained. Furthermore, if hysteresis is of significance, as seems probable, an estimation of DPD made on the desorption curve would only apply if the plant as a whole were in a desorption phase when sampled. Although this no doubt applies during the period of increasing transpiration each morning,

*"Relative turgidity" has been defined by Weatherley (1950) as the ratio of the amount of water present in a quantity of tissue when sampled compared with the amount present in the same quantity of tissue when fully turgid.

it is probable that for most of the day the plant, under normal conditions, is in a sorption phase.

For these reasons, it seems preferable to use the standard method for DPD measurement, except where it is especially desired to establish relative turgidity/DPD relationships for different plant tissues. The extent of the error involved in determinations with the standard method will vary with the relative rates of sorption and desorption and with the possible influence of hysteresis. If hysteresis occurs to the extent observed by Rao, Rao, and Rao (1949), the divergence between the sorption and desorption portions of the hysteresis loop could vary from zero at zero DPD to as much as ± 3 atm at the highest DPD's employed. Although this order of variation is high, it would not be confined to vapour determinations, but would apply to any determinations of DPD in which sorption and desorption processes are involved.

The standard method therefore tends to provide an estimate of DPD midway between the sorption and desorption curves, although as mentioned above the relatively slow rates of sorption will normally cause an underestimate. In practice, the values obtained, by comparison with estimates from floating methods, have been found to lie within a range not greater than -3 to $+1$ atm about the true value. This error can be reduced further by using a series of constant-humidity chambers, each of which differ by only 2–3 atm water potential from the next, so that the sorption and desorption parts of the curve can be well defined and any misleading value identified.

The comparative study between the standard floating methods for measuring DPD and the vapour method was of considerable value in determining the reasons for the decreasing effectiveness of the former methods as the DPD approached the value at incipient plasmolysis. The explanation provided, that when plasmolysis occurs in any one cell change in weight or volume of the cell virtually ceases, appears to be logical and satisfactory. In any one piece of tissue, cells with a range of osmotic characteristics exist, and as these progressively become plasmolysed the sensitivity of the floating methods can be expected to decrease until the whole tissue becomes plasmolysed, when the methods are virtually ineffective. It could be expected that in cells which did not readily plasmolyse and in which the walls collapsed readily with contraction of the cytoplasm, some continued loss of weight and volume might occur. No evidence of this was observed in the present experiments. On the other hand, the slight increase in weight and volume observed in some instances, where tissue was in hypertonic solutions, suggested that the external solution was to some extent entering the vacuole, raising the osmotic potential of the vacuolar sap, and hence raising the volume and weight of the tissue at incipient plasmolysis.

Despite the fact that these methods were insensitive at DPD's greater than at the plasmolytic value, at lower DPD's they gave good agreement with the vapour method, and at DPD's of only a few atmospheres, where the floating methods are most sensitive, it is probable that they are of greater accuracy.

A final point in connection with the data of Figure 7 is that the gradual divergence of the dehydration curves obtained in vapour and in solution demonstrates that the "minimum cell volume" method of measuring osmotic pressure (Ursprung

1923) could be subject to important errors. This method contends that osmotic pressure of the cell sap is isotonic with the most dilute solution in which a segment of tissue can be dehydrated to its minimum volume (or some other dimension). It depends on the curve of decreasing volume against increasing concentration having a sharp flex point from which the critical concentration can be estimated. The data in Figure 7 indicate that not only would the gradual shrinkage of the tissue prevent a sharp flex point being obtained, but also that the progressive plasmolysis and infiltration of the cells would tend to shift the point at which the minimum value was reached, increasing the value obtained.

V. ACKNOWLEDGMENTS

The bulk of the investigations reported in this paper were conducted in the C.S.I.R.O. laboratories at Canberra. However, the studies were initiated at the University of Nottingham and much of the work needed to develop the technique was conducted at Duke University, North Carolina. Grateful acknowledgement is made to Dr. P. E. Weatherley and to Dr. Paul J. Kramer, of the respective institutions, for their helpful suggestions and discussion, and additionally to Dr. Kramer for financial assistance during the period which the author spent at Duke University.

VI. REFERENCES

- ARCICHOVSKIJ, V., and ARCICHOVSKAJA, N. (1931).—Untersuchungen über die Saugkraft der Pflanzen. II. Die gravimetrische Methode der Saugkraftmessungen an den Blättern. *Planta* **14**: 528–32.
- ASHBY, E., and WOLF, R. (1947).—A critical examination of the gravimetric method of determining suction force. *Ann. Bot., Lond. (N.S.)* **11**: 261–8.
- CRAFTS, A. S., CURRIER, H. B., and STOCKING, C. R. (1949).—“Water in the Physiology of Plants.” (Chronica Botanica Co.: Waltham, Mass.)
- ILJIN, W. S. (1957).—Drought resistance in plants and physiological processes. *Annu. Rev. Pl. Physiol.* **8**: 257–74.
- KELSEY, K. E. (1957).—The sorption of water vapour by wood. *Aust. J. Appl. Sci.* **8**: 42–54.
- LYON, C. J. (1936).—Analysis of osmotic relations by extending the simplified method. *Plant Physiol.* **11**: 167–72.
- MEYER, B. S. (1945).—A critical evaluation of the terminology of diffusion phenomena. *Plant Physiol.* **20**: 142–64.
- MEYER, B. S., and WALLACE, A. M. (1941).—A comparison of two methods of determining the diffusion pressure deficit of potato tuber tissue. *Amer. J. Bot.* **28**: 838–43.
- OWEN, P. C. (1952).—The relation of germination of wheat to water potential. *J. Exp. Bot.* **3**: 188–203.
- RAO, K. S., RAO, M. B., and RAO, B. S. (1949).—Drought resistance in plants in relation to hysteresis in sorption. I. Hydration and dehydration of the leaves of certain drought resistant and drought sensitive plants. *Proc. Nat. Inst. Sci. India* **15**: 41–9.
- ROBINSON, R. A., and STOKES, R. H. (1955).—“Electrolyte Solutions”. (Butterworths Scientific Publications: London.)
- SPANNER, D. C. (1951).—The Peltier effect and its use in the measurement of suction pressure. *J. Exp. Bot.* **2**: 145–68.
- STONE, E. C., WENT, F. W., and YOUNG, C. L. (1950).—Water absorption from the atmosphere by plants growing in dry soil. *Science* **111**: 546–8.
- URSPRUNG, A. (1923).—Zur Kenntnis der Saugkraft. VII. Eine neue vereinfachte Methode zur Messung der Saugkraft. *Ber. deutsch. bot. Ges.* **41**: 338–43.
- URSPRUNG, A., and BLUM, G. (1916).—Zur Methode der Saugkraftmessung. *Ber. deutsch. bot. Ges.* **34**: 525–39.

- URSPRUNG, A., and BLUM, G. (1927).—Eine Methode zur Messung der Saugkraft von Hartlaub. *Jb. wiss. Bot.* **67**: 334–46.
- URSPRUNG, A., and BLUM, G. (1930).—Zwei neue Saugkraft-Messmethoden. *Jb. wiss. Bot.* **72**: 254–334.
- VRIES, H. DE (1884).—Eine Methode zur Analyse der Turgorkraft. *Jb. wiss. Bot.* **14**: 427–601.
- WEATHERLEY, P. E. (1950).—Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytol.* **49**: 81–97.
- WEATHERLEY, P. E., and SLATYER, R. O. (1957).—Relationship between relative turgidity and diffusion pressure deficit in leaves. *Nature* **179**: 1085–6.