

A CRITICAL EVALUATION OF TISSUE-IMMERSION METHODS FOR MEASUREMENT OF PLANT WATER POTENTIAL¹

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ABSTRACT

Plant water potential was measured simultaneously by the change-in-length method and the Schardakow-dye method on segments of potato tubers. In over half of the tests, the change-in-length method underestimated the true water potential, interpreted to be the result of several sources of error inherent in the method. Therefore, the dye method is strongly recommended as the only tissue-immersion method suitable for field measurement of plant water potential.

INTRODUCTION

The level of metabolic activity in a plant tissue is affected by the energy status of water in the tissue (Vaadia *et al.*, 1961; Kozlowski, 1964; Greenway and Hiller, 1967; Hiller and Greenway, 1968). Because of this, and because water moves into and through plants along free-energy gradients (Slatyer and Gardner, 1965), it is important to be able to determine the energy status of water in various tissues and at various points in the plant. The free-energy status of water in plant cells and in tissues has been expressed in the past by several terms, most commonly by the term Diffusion Pressure Deficit, or DPD (Meyer and Anderson, 1952), but present usage favors the term Water Potential, usually designated by the symbol ψ (Kramer *et al.*, 1966). Water potential and DPD are conceptually and dimensionally the same, but opposite in sign.

Water potential can be determined most accurately by measuring the equilibrium vapor pressure (Slatyer, 1967). Several methods have been designed for estimation of plant water potential which involve measurement of equilibrium vapor pressure (Spanner, 1951; Ehlig, 1962; Lambert and Van Schilfgaarde, 1965; Lang and Barrs, 1965; Macklon and Weatherley, 1965; Boyer and Knipling, 1965; Kreeb, 1965; Manohar, 1966), but there are difficulties in using these methods (Rawlings, 1964; Barrs, 1965a, b; Rawlins, 1966), including a need for precise temperature control (Richards and Ogata, 1958; Barrs, 1965b; Lang and Barrs, 1965), difficulties which render vapor-equilibration techniques impractical for field use at present. Slatyer (1958) has developed a technique for equilibrating plant tissue in the vapor phase over solutions of known osmotic potential and then measuring the changes in weight of the tissue. While this method eliminates much of the instrumentation necessary for making accurate vapor-pressure measurements, precise temperature control still is a necessity, and it, therefore, also is a laboratory method.

Thus, from practical considerations, choice of a suitable field method for measurement of plant water potential is limited to one of the methods involving immersion of tissue segments in graded osmotic solutions (Kramer and Brix, 1965). The theory behind these methods is as follows. When strips of tissue from a leaf or other plant organ are placed in a graded series of solutions of different osmotic pressures, some strips will absorb water and some will lose water. The water potential of the tissue is assumed to be equal to the osmotic pressure of the solution (since the osmotic pressure of the solution = ψ of the solution) in which the tissue neither loses nor gains water. In practice, this situation rarely is realized, and the tissue-water potential is assumed to lie between those of the solutions in which the tissue absorbs water and those of the solutions in which the tissue loses water.

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The direction of water movement can be detected in two ways: (1) measuring change in tissue, such as length, weight, or volume, or (2) measuring change in solution concentrations. Theoretically, one should expect the same results with either type of method. The easiest way to detect changes in solution concentration is by measuring changes in specific gravity of the solution, as originally described by Schardakow (Knipling, 1967).

This paper reports the results of an experiment designed to test the hypothesis that equal values should be obtained by measurement of either tissue change or solution change.

MATERIALS AND METHODS

Solutions of known osmotic pressure were prepared with sucrose (Ursprung and Blum, 1916) and placed in test tubes. Two parallel series of tubes were prepared, covering the range of osmotic pressures from one to 12 bars, with increments of two bars between tubes. The solutions in one series of tubes were dyed with methylene blue dye, and those in the other series of tubes were used for tissue immersion. Cylinders of tissue cut from potato tubers with a No. 2 cork borer were trimmed to exactly 30 mm in length. One cylinder was placed in each tube of the non-dyed solutions.

The cylinders of tissue were removed after 2 hr, and the length of each was measured. The change in concentration of the solution in each tube was determined by placing a drop of solution from the corresponding tube in the dyed series into the test solution and observing whether the drop rose or fell in the solution. If the solution had become more concentrated, the colored drop should rise, due to its lesser density, and if the solution had become more dilute, the drop should fall, due to greater density of the drop. The water potential of the potato tuber was estimated from each of these operations, and the values obtained were designated ψ_{length} and ψ_{dye} respectively. This procedure was repeated 14 times.

RESULTS AND DISCUSSION

The results of these measurements are presented in Figure 1. All points that fell on the horizontal line through the block gave identical values with both methods. Five of the 14 measurements yielded such values. If the point was above the dissection line, the water potential, as given by the dye method, was lower than the value given by the change-in-length method. There was one such point. On the other hand, if the point fell below the dissection line, this means that the water potential given by the change-in-length method gave the lower value. There were eight such points. Thus, it appeared that, most of the time, there was a discrepancy between the two measurements when theoretically they should have been the same. This systematic error could be due to either an overestimation of the true water potential by the dye method or an underestimation by the change-in-length method. Which of these alternatives is true is important in determining which type of method, change-in-tissue or change-in-ambient-solution, is preferable for routine measurements of plant water potential.

As nearly as can be determined, there is only one example in the literature of the simultaneous measurement of water potential by change-in-tissue and change-in-solution. Ashby and Wolf (1947) measured suction force ($=\psi$) of several tissues by change in weight of the tissue (= change-in-length in this study) and change in refractive index of the solutions (= movement of drop in this study). The results they obtained with the change-in-weight method always gave values farther from zero than did results obtained by the refractometer method. Thus, the present results which indicate an underestimation of the true water potential by the change-in-tissue method are supported by a similar observation by Ashby and Wolf (1947). In their study with several tissues, the closest correspondence between the two values occurred in potato-tuber tissue. The difference, in that

case, was about 1.1 bar, compared with as much as 10 bars difference in *Iris* leaf tissue. They found that the cause for the differences was infiltration of intercellular spaces with solution. Thus, in tissues where there was no net gain of water, or even in those which lost small amounts of water, if there was a movement of solution into the intercellular spaces, an increase in weight of the tissue would result.

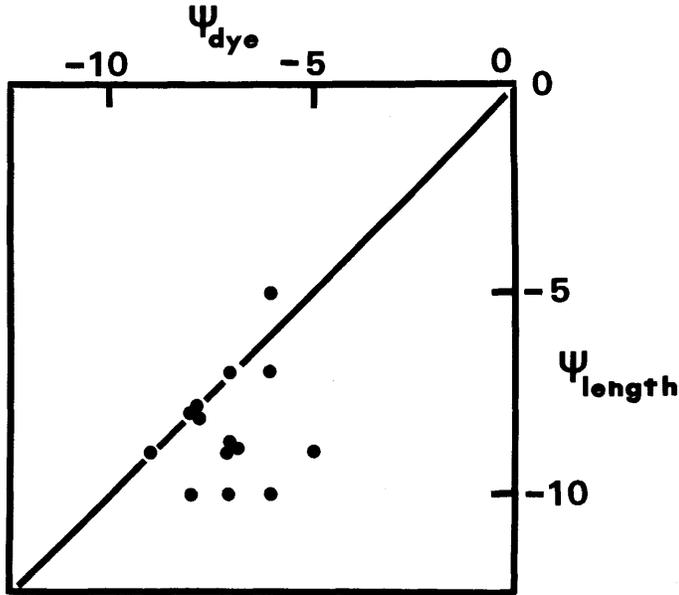


FIGURE 1. Comparison of water potential values in potato-tuber cylinders obtained with the dye method (ψ_{dye}) and the change-in-length method (ψ_{length}). All values are in bars.

Meyer and Wallace (1941), on the other hand, found that almost identical values were obtained, when changes in weight and in length of potato-tuber cylinders were determined. They did not find that movement of solutes into or out of the tissues introduced any error in the measurements. This is important because, if the sucrose is penetrating into the cells during the equilibration period, the concentration of the solution with which the tissue seems to be in equilibrium will, in fact, be higher than the true equilibrium solution, and the value of the water potential will be underestimated. That is, if the apparent equilibrium solution is one with a supposed water potential value of -10 bars, and the concentration has been decreased, due to uptake of sucrose by the tissue, so that the true water potential of the solution is -8 bars, the water potential of the tissue would be assigned a value 2 bars lower than the true water potential. Slatyer (1966) found that changes in concentration of equilibration solutions were less of a problem with potato-tuber tissue than with any other tissue tested. That is, the potato-tuber cells do, in fact, seem to be almost completely impermeable to the solute molecules in the ambient solutions, especially if mannitol was used as the osmotic agent.

There are some clear conclusions to be drawn from the evidence presented here and in other studies. When tissue strips are immersed in solutions, there will be movement of solution into the apparent free space of the tissue (Jennings, 1963), including intercellular space and cell walls. Thus, measuring changes in tissue will be subject to error introduced by this infiltration. Particularly sensitive to this will be change in weight of the tissue. Change-in-weight methods are

highly undesirable for other reasons also. For example, when tissues are removed from solution, surface moisture must be uniformly removed, and this procedure is highly variable. Unless great precautions are taken, large errors can result from unequal application of pressure when blotting tissue dry (Ashby and Wolf, 1947).

The effect of solution infiltration into tissue on changes in length will vary according to the extensibility of the tissue being examined. This should be less of a problem with storage tissue, such as potato tuber, than with strips of young leaves, for example. However, as was seen in the present study, it can be a problem even with potato tubers. Furthermore, most field measurements of plant water potential will be with leaves, rather than with storage tissues. While change in length would not be subject to the range of errors to which changes in weight are subject (including changes in weight due to respiratory loss, as well as those mentioned above), this still is an undesirable method.

Therefore, if one is forced to use a tissue-immersion method for measurement of plant water potential, and that is usually the only recourse in the field, it is recommended that the Schardakow or dye method be used. Because it is changes in solution concentration that are measured, an accurate determination of whether there has been a net transfer of pure water between the cells and the solution is possible. Furthermore, because very small differences in solution concentration can be detected by this method, it is not necessary to wait for equilibrium as in change-in-length or change-in-weight methods. All that is important in this method is to determine the direction of water movement, into or out of the tissue. As soon as enough water exchange has occurred to change the concentration of the ambient solution, the test can be made. Thus, it is a rapid method, compared to other methods involving equilibration of the tissue with the ambient solution. As a result of the short time required, the additional problem of change in test-solution concentration due to solute uptake by tissue is minimized. In addition, of all the tissue-immersion methods, it requires the least amount of material and is the easiest technique to perform in the field. Finally, when compared with the thermocouple-psychrometer measurements of water potential, the dye method has been shown to be very reliable (Kramer and Brix, 1965; Knipling and Kramer, 1967), and it has been shown to be a suitable field method (Knipling, 1967).

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