MEASUREMENT OF LEAF WATER POTENTIAL
BY THE DYE METHOD

By

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Abstract. The dye method for measuring leaf water potential is simple, inexpensive, and suitable for both laboratory and field work. Leaves are immersed in a graded series of solutions, and the solution which neither gains nor loses water is assumed to have a water potential equal to that of the leaf. Although limited by certain inherent errors, the dye method can be used to measure relative values and changes in potential. Specific procedures are described for the successful use of the method.

There are few techniques for measuring leaf water potential (Kramer, Knipling, and Miller 1966) that are truly suitable for field work. The recently described pressure chamber method appears promising for some species (Scholander et al. 1965, Boyer 1967, Waring and Cleary 1967). The dye method, also known as the Shardakov (1948) or density method, is another useful technique because it is simple and requires no elaborate or expensive equipment. The dye method has been described elsewhere, but no comprehensive discussion is readily accessible to many workers. This report examines the method and describes specific procedures and precautions that need to be taken for its successful use.

In the dye method (Fig. 1) samples of leaf tissue are immersed in a graded series of solutions of known water potential contained in test tubes. The leaf water potential is assumed to lie between the solutions in which the leaf samples absorb water and those in which the samples lose water. The directions of this water exchange are determined from the resulting density changes in the test solutions. Each test solution is colored lightly with a small amount of a powdered dye such as methylene blue or methyl orange. Drops of the colored solutions are then introduced with medicine droppers into the centers of the corresponding members of a parallel series of uncolored control solutions. The drops fall if the test solutions have been concentrated and rise if the solutions have been diluted. In case the leaf sample has a water potential equal to that of one of the solutions used, there theoretically is no water exchange and no

density change, and hence the colored drop diffuses outward in all directions.

The purpose of the dye is to make the test solution drops visible when they are placed in the control solutions. The small amount of dye used to color the solutions lightly does not significantly alter their densities.

The precision of measurement by the dye method depends on the size of the water potential increment between test solutions. It is possible to use increments as small as 0.5 bar, but in order to have a workable number of solutions (generally 6 to 10) bracketing an unknown leaf water potential, 1- to 5-bar increments often are the only reasonable choices.

Test solutions generally are made with sucrose or mannitol. When sucrose is used, it is suitable to use the commercial (trade) type. The solutions of known
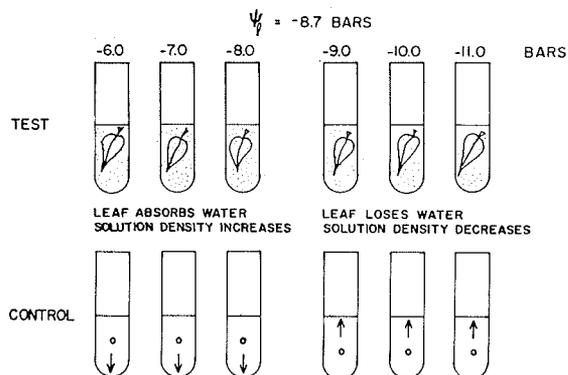

Fig. 1. Diagram representing dye method for measuring leaf water potential. The measured water potential is taken to be between the solutions in which the colored test solution drops rise and fall, i.e., —8.5 ± 0.5 bars.

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water potential can be prepared according to tables of Walter (1931) or Ursprung and Blum (1916). The amount of solution used in each test tube should be enough to cover the leaf sample. Test solution volumes of about 2 ml in 13- by 100-mm test tubes are suitable for most water potential determinations with small pieces of leaf tissue, but larger volumes generally are necessary when whole leaves are used. The volume of the control solutions should be about 5 ml in order to have a sufficient depth in which to observe the direction of drop movement.

The amount of leaf tissue used per unit volume of solution should be as large as is feasible without restricting access of solution to the tissue and without damaging the tissue when immersing it into the solution. An exactly equal amount of leaf tissue is not needed in each solution because the method detects the direction of water exchange, not the amount of exchange. However, it is advisable to use approximately the same amount of tissue in each solution and from one water potential determination to the next.

The use of whole leaves in the dye method is convenient because of the ease with which they can be sampled and inserted into the solutions. In addition, the water potentials measured with whole leaves are in some cases more accurate than those measured with cut leaves. This appears to be true for conifer needles, but the comparisons for broadleaved species are variable (Knipling and Kramer 1967). In some cases the cutting of leaf tissue is desirable because it facilitates water exchange and permits the variability in water potential among a sample of leaves to be represented in each solution. Often the use of cut leaves is necessary when the leaves are large or the supply limited.

Theoretically, only a short leaf immersion time should be necessary for a water potential measurement by the dye method. This is because the drop movement is sensitive to extremely small concentration differences (0.0005M sucrose), and therefore, detectable density changes should be established in the solutions long before complete equilibration. The only effect of longer immersion times should be to change the distinctiveness of drop movement. However, there also is a change
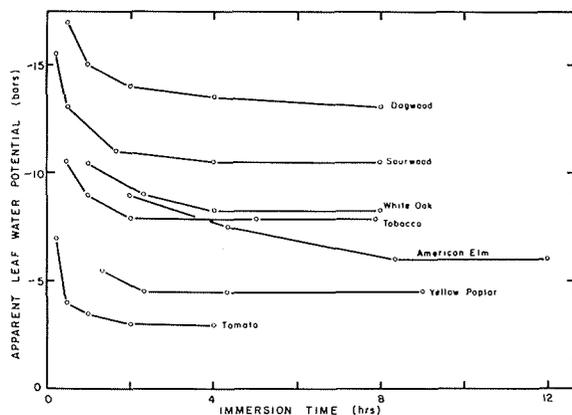

Fig. 2. The effect of progressively longer immersion times on the leaf water potentials of cut leaf tissue measured by the dye method. Species: dogwood, *Cornus florida* L.; sourwood, *Oxydendrum arboreum* DC.; white oak, *Quercus alba* L.; American elm, *Ulmus americana* L.; yellow poplar, *Liriodendron tulipifera* L.; tomato, *Lycopersicum esculentum* Mill.; tobacco, *Nicotiana tabacum* L.

in the measured water potentials with time (Fig. 2). Characteristically, the values measured after about 30 min are 2–5 bars lower (more negative) than the final, stable values reached after 2–8 hr. However, water potential measurements of conifer needles continue to change for about 24 hr. Since the final values provide the best estimates of water potential (Rehder and Kreeb 1961, Lemee and Gonzalez 1965), the proper length of immersion for a particular species should be determined prior to using the dye method for routine measurements.

Numerous explanations have been offered for the changing values with increasing immersion time. Walter and Ellenberg (1957) suggest that the initial values are those of epidermal cells, whereas later readings represent an approach toward the higher water potentials of interior cells. However, it is questionable if such a large water potential gradient exists within leaves. Furthermore, with cut leaf tissue, one would expect interior cells to equilibrate as rapidly as surface cells.

Other workers (Lemee and Gonzalez 1965, Brix 1966) suggest that the changing values reflect a gradient of water potential created by cutting the leaf tissue, the initial values representing the osmotic potential of cells along the cut edges where turgor pressure has been eliminated. Although this induced gradient may contribute to the changing values, it does not fully account for the initial values because they generally are lower (more negative) than the leaf osmotic potential. For example, the osmotic potential of dogwood leaves was accurately measured with a thermocouple psychrometer (Ehlig 1962) to be —15.3 bars. The water potential measured on a parallel sample of leaves by the dye method was —24 bars after 10 min immersion and —17 bars after 30 min immersion (Fig. 2). Further evidence that the initial dye method values are not determined by the osmotic potential of cut cells is indicated by the fact that the changing values also are observed with uncut leaves.

The major cause of the erroneous dye-method values measured after short immersion times appears to be the adsorption of water onto the leaf surfaces (Rehder 1961, Schlafli 1964). This extraction of water from the solutions occurs within the first few minutes of immersion and increases the density of all test solutions before osmotic water exchange takes place. Thus, drops from the test solutions having potentials lower than that of the leaf move in the wrong direction when placed in the control solutions. After progressively longer immersion times, the values indicated by the dye method approach the true leaf water potential as osmotic water loss from the tissue compensates for the initial density changes. The effect of adsorbent leaf surfaces on solution densities can be simulated by placing filter paper in sucrose solutions.

In most cases leaf surface residues and sap from cut and damaged tissues, which contaminate the test solutions (Gaff and Carr 1964, Brix 1966), probably contribute to the abnormal density increases of the solutions during the early period of immersion. Also, the subsequent leakage of low density contaminants from the leaf tissue probably helps compensate for the initial density changes.

Once dye-method values reach a stable level after an extended immersion time there still is a residual error in the measurements. The contamination of the solutions appears to be the major source of error (Brix 1966, Knipling and Kramer 1967), although the uptake of solutes by the leaf tissue, which decreases the densities

of the test solutions, contributes to the total error (Goode and Hegarty 1965, Brix 1966). The direction of the contamination error depends on the density of the contaminants relative to the density of the solution having a potential equal to that of the leaf. When the contaminants are more dense than the equipotential solution, the dye method indicates a value lower (more negative) than the true leaf water potential; when the contaminants are less dense, the method indicates a value higher than the true potential.

The final size of the contamination error depends on the extent which osmotic water gain by or loss from the leaf tissue compensates for the contamination density changes during complete equilibration. In this regard, succulent-leaved species such as tomato, tobacco, and yellow poplar, which undergo relatively large changes in water content per unit change in water potential, generally have smaller contamination errors than leaves of woody species such as white oak and American elm.

To assess the total error in dye-method values, the method has been compared with the thermocouple psychrometer method, which is considered to measure leaf water potentials accurately (Boyer 1966). Values measured by the two methods on the same leaf material generally have agreed within 3 bars, although the values have differed by as much as 5–8 bars for some species (Kramer and Brix 1965, Knipling and Kramer 1967).

Even though the dye method appears not to measure true leaf water potentials, it can be used to measure relative values and changes in potential. However, values determined on different species should be compared with caution because of differences in the relationships of dye-method values to the true leaf water potentials.

In addition to the procedures already discussed, there are certain practices that are helpful or necessary in conducting the dye method. It is essential that all glassware be clean and dry to prevent any alteration of the solutions. The solutions should be relatively fresh so as not to be contaminated with fungi. When the stock solutions are contained in plastic wash bottles, they can be rapidly and accurately dispensed to a given level in the test tubes. The test and control solutions always should be dispensed at the same time and from the same stocks to insure equal densities at the start of a determination.

To reduce the adverse effects of contamination, Brix (1966) modified the procedure for obtaining the control solutions. Since most contamination takes place in the first 30 min of immersion, Brix excluded its effect by measuring the density changes occurring thereafter. In practice, he decanted off portions of the contaminated test solutions to use as controls. Similarly, Gaff and Carr (1964) suggested that the effects of contamination could be reduced by renewing the test solutions with fresh, uncontaminated solutions after an initial short period of immersion.

In some cases contamination can be reduced by washing the experimental leaf material several or more hours prior to sampling. When cut tissue is used in the dye method, the cutting should be done with a sharp razor rather than with a cork borer or knife to minimize damage to the leaves. When practical, interveinal leaf tissue should be used to minimize leakage of free water into the solutions from the cut vascular elements. Glass rods aid in pushing the leaf samples into the test solutions without damaging the tissue.

During the leaf-immersion period the test tubes should be stoppered to prevent density changes in the solutions caused by evaporation. Density changes also arise from temperature differences, and the test and control solutions should be kept together during the immersion period. Extra-long medicine droppers (about 5 inches) aid the insertion of the colored test solution drops into the control solutions. A separate dropper should be used for each pair of solutions. The visibility of drop movement can be enhanced by placing a white background on the test tube rack.

LITERATURE CITED

Boyer, J. S. 1966. Isopiestic technique: measurement of accurate leaf water potentials. Science 154: 1459–1460.

——. 1967. Leaf water potentials measured with a pressure chamber. Plant Physiol. 42: 133–137.

Brix, H. 1966. Errors in measurement of leaf water potential of some woody plants with the Schardakow dye method. Canada Dept. Forestry Publ. 1164. 11 p.

Ehlig, C. F. 1962. Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiol. 37: 288–290.

Gaff, D. F., and D. J. Carr. 1964. An examination of the refractometric method for determining the water potential of plant tissue. Ann. Bot. N. S. 28: 351–368.

Goode, J. E., and T. W. Hegarty. 1965. Measurement of water potential of leaves by methods involving immersion in sucrose solutions. Nature 206: 109–110.

Knipling, E. B., and P. J. Kramer. 1967. Comparison of the dye method with the thermocouple psychrometer for measuring leaf water potentials. Plant Physiol. 42: 1315–1320.

Kramer, P. J., and H. Brix. 1965. Measurement of water stress in plants. In Methodology of plant ecophysiology. 1962 Proc. Montpellier Symp., UNESCO, Arid Zone Res. XXV: 343–351.

Kramer, P. J., E. B. Knipling, and L. N. Miller. 1966. Terminology of cell water relations. Science 153: 889–890.

Lemee, G., and G. Gonzalez. 1965. Comparison de methodes de measure du potentiel hydrique (tension de succion, DPD) dans les feuilles par equilibre osmotique et par equilibre de pression de vapeur. In Methodology of plant eco-physiology. 1962. Proc. Montpellier Symp., UNESCO, Arid Zone Res. XXV: 361–368.

Rehder, H. 1961. Saugkraftmessungen on: Stachys silvatica im frischen und welden Zustand. Ber. Deut. Bot. Ges. 74: 84–92.

Rehder, H., and K. Kreeb. 1961. Vergleichende Untersuchungen zur Bestimmung der Blattsaugspannung mit der gravimetrischen Methode und der Schardakow-Methode. Ber. Deut. Bot. Ges. 74: 95–98.

Schlafli, A. 1964. Über die Eignung der Refraktometer- und der Schardakowmethode zur Messung osmotischer Zustandgrossen. Protoplasma 58: 75–95.

Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148: 339–346.

Shardakov, V. S. 1948. New field method for the determination of the suction pressure of plants. Dokl. Akad. Nauk SSSR 60: 169–172. (In Russian).

Ursprung, A., and G. Blum. 1916. Zur Kenntnis der Saugkraft. Ber. Deut. Bot. Ges. 34: 525–539.

Walter, H. 1931. Die Hydratur der Pflanze. Gustav Fisher, Jena. 161 p.

Walter, H., and H. Ellenberg. 1957. Ökologische Pflanzengeographie. Fortschr. Bot. 20: 100–117.

Waring, R. H., and B. D. Cleary. 1967. Plant mois-ture stress: evaluation by pressure bomb. Science 155: 1248-1254.