

Comparison of the Dye Method with the Thermocouple Psychrometer for Measuring Leaf Water Potentials¹

Edward B. Knipling² and Paul J. Kramer
Department of Botany, Duke University, Durham, N. C.

Received October 27, 1966.

Summary. The dye method for measuring water potential was examined and compared with the thermocouple psychrometer method in order to evaluate its usefulness for measuring leaf water potentials of forest trees and common laboratory plants. Psychrometer measurements are assumed to represent the true leaf water potentials. Because of the contamination of test solutions by cell sap and leaf surface residues, dye method values of most species varied about 1 to 5 bars from psychrometer values over the leaf water potential range of 0 to -30 bars. The dye method is useful for measuring changes and relative values in leaf potential. Because of species differences in the relationships of dye method values to true leaf water potentials, dye method values should be interpreted with caution when comparing different species or the same species growing in widely different environments. Despite its limitations the dye method has a usefulness to many workers because it is simple, requires no elaborate equipment, and can be used in both the laboratory and field.

The thermocouple psychrometer method generally is regarded as providing the most precise measure of leaf water potential (2). However, because this method requires elaborate instrumentation and strict temperature control, it is not suitable for field work. A simpler technique for measuring water potential is the dye method in which leaves are immersed in a graded series of test solutions (7, 8). The solution whose density is not changed by osmotic water exchange with the leaf tissue is assumed to have a potential equal to that of the leaf. The density changes are detected by the rise and fall of drops of dyed test solutions placed in uncolored control solutions.

Leaf surface residues and sap from cut cells contaminate the dye method test solutions (4) and produce density changes large enough to cause some of the test solution drops to move in the wrong direction when placed in the control solutions. The following research was undertaken to assess the error caused by contamination and to compare the dye method with

the thermocouple psychrometer in order to evaluate the dye method's accuracy and usefulness.

Materials and Methods

Plant Species. Leaves of dogwood, *Cornus florida* L.; sourwood, *Oxydendrum arboreum* DC.; yellow poplar, *Liriodendron tulipifera* L.; white oak, *Quercus alba* L.; American elm, *Ulmus americana* L.; and privet, *Ligustrum japonicum* Thunb., were collected in the field. Needles of loblolly pine, *Pinus taeda* L.; white pine, *P. strobus* L.; and Engelmann spruce, *Picea engelmannii* Parry, were obtained from 3-year old potted seedlings. Leaves of tomato, *Lycopersicon esculentum* Mill.; tobacco, *Nicotiana tabacum* L.; cotton, *Gossypium hirsutum* L.; and sunflower, *Helianthus annuus* L., were collected from greenhouse-grown plants.

Dye Method. The general procedures described elsewhere (7) were followed. Each test series consisted of 6 to 10 sucrose solutions (2-5 ml each) graduated by 1 or 2 bar increments and contained in test tubes. Methylene blue dye was used to color the test solutions. Water potentials were measured both with cut pieces of leaf tissue and with whole leaves for all test species except yellow poplar, privet, cotton, sunflower, and tobacco. For determinations

¹ This work was supported in part by the Coweeta Hydrologic Laboratory, Southeastern Forest Experiment Station, United States Forest Service, and in part by National Science Foundation Grant GB-82.

² Present address: United States Army Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire 03755.

with cut leaves, interveinal tissue was cut from 6 to 10 leaves and distributed among the test solutions such that an approximately equal piece of each leaf would be represented in each solution. Pine and spruce needles were cut into 1 cm segments. For whole leaf determinations, 2 leaves were rolled together longitudinally and inserted tip first into each solution. For whole pine needles, 4 to 5 fascicles were inserted into the solutions. Because of the small size of Engelmann spruce needles, 5-cm terminal branch shoots were immersed in the test solutions. Leaf immersion times for yellow poplar, tomato, and cotton were standardized at 5 hours, and for loblolly and white pine at 24 hours. The immersion times of all other species were standardized at 8 hours (7).

Thermocouple Psychrometer. The isopiestic modification (2,3) of the Richards (11) thermocouple psychrometer was used to measure leaf water potentials free of leaf resistance error (9). To evaluate the error due to heat of respiration (1) the temperature of the psychrometer chamber was measured with a dry thermocouple inserted into the chamber after removing the isopiestic thermocouple. Alternatively, the respiration error of some samples was evaluated with a dry thermocouple junction permanently installed beside the wet junction in a Richards psychrometer. Leaf respiration errors of 0.3 to 0.9 bar and leaf resistance errors of 4 to 12% were characteristic of the species used in this study. The appropriate corrections were made to the measurements for which these errors were not evaluated directly. The corrected psychrometer measurements are assumed to represent the true leaf water potentials (2).

Comparison of Methods. Measurements of water potential by the dye method and thermocouple psychrometer were made simultaneously on leaves collected as one sample. The time required to set up both methods was approximately 5 minutes. While preparing the psychrometer leaf sample, the leaves to be used in the dye method were stored in a humid chamber to minimize water loss from the leaves.

To obtain leaf tissue of different potentials, cut branches from plants growing in the field were brought to the laboratory and dried to desired levels. Greenhouse-grown plants were droughted to a given degree by withholding soil water.

Results and Discussion

Comparison of Methods. The results of the simultaneous measurements of leaf water potential by the dye method and psychrometer for some of the test species are given in figures 1 and 2. Curves of regression equations are drawn through the data. The diagonal lines represent the theoretical loci of equal measurements by the two methods. Agreement between the methods generally was within 1 to 5 bars over the water potential range of 0 to about -30

bars. Over intermediate portions of this range dye method values measured on cut leaves generally were higher (less negative) than water potentials measured with the psychrometer, but at the high and low potentials of the 30 bar range the dye method tended to indicate values too low. Whole leaf dye method values of white oak, American elm (fig 1), and tomato (fig 2) also followed this curvilinear pattern, but the relationships for whole leaves of dogwood and whole needles of loblolly and white pine (fig 1) were linear.

Not shown in this report are the water potential comparisons for privet, sourwood, and Engelmann spruce. The cut leaf and whole leaf dye method values for the latter species were similar to those for loblolly pine (fig 1) and the comparisons for privet and sourwood were similar to those of white oak (fig 1).

Even though many of the dye method—psychrometer comparisons are similar, there are important quantitative differences in the relationships. For example, at psychrometer values of -15 bars, the dye method indicated -10, -13, and -16 bars for American elm, dogwood and yellow poplar respectively (fig 1). Similar differences were found for dogwood leaves exposed to different environments. The dogwood data of figure 1 were determined with shade leaves, but comparisons also were made on sun leaves. At psychrometer values of -6 bars, the dye method indicated -7 bars for the sun leaves, but only -5 bars for the shade leaves.

Dye method values measured with whole pine needles definitely provided better estimates of the true leaf water potentials than did cut needles (fig 1), but the comparisons of cut and whole leaves of broadleaved species were variable. There is little difference in the closeness of cut and whole leaf dye method values to psychrometer values for dogwood and American elm. Whole white oak leaves (fig 1) provided better estimates than did cut leaves, but the opposite was true for tomato (fig 2) and sourwood.

Contamination of Solutions. In addition to changing the density of test solutions, leaf contaminants theoretically can change the osmotic potentials by the direct addition of cell sap and by the inversion of sucrose. Gaff and Carr (4) calculated the latter effect to be insignificant. To determine the magnitude of the former effect, the dye method was set up with sucrose solutions of -2 to -10 bars and with cut pieces of sourwood leaf tissue having a water potential of -4.5 bars and an osmotic potential of about -18 bars. After 14 hours the potentials of the test solutions were measured with the thermocouple psychrometer and found to be 0.1 to 0.3 bar lower than originally, the opposite of what would be expected for the -6 to -10 bar solutions on the basis of osmotic water loss from the tissue. However, a dye method value of about -5 bars was determined from the density changes in the solutions. Thus, it is concluded that the sourwood contaminants did not alter the potentials of the solutions enough to

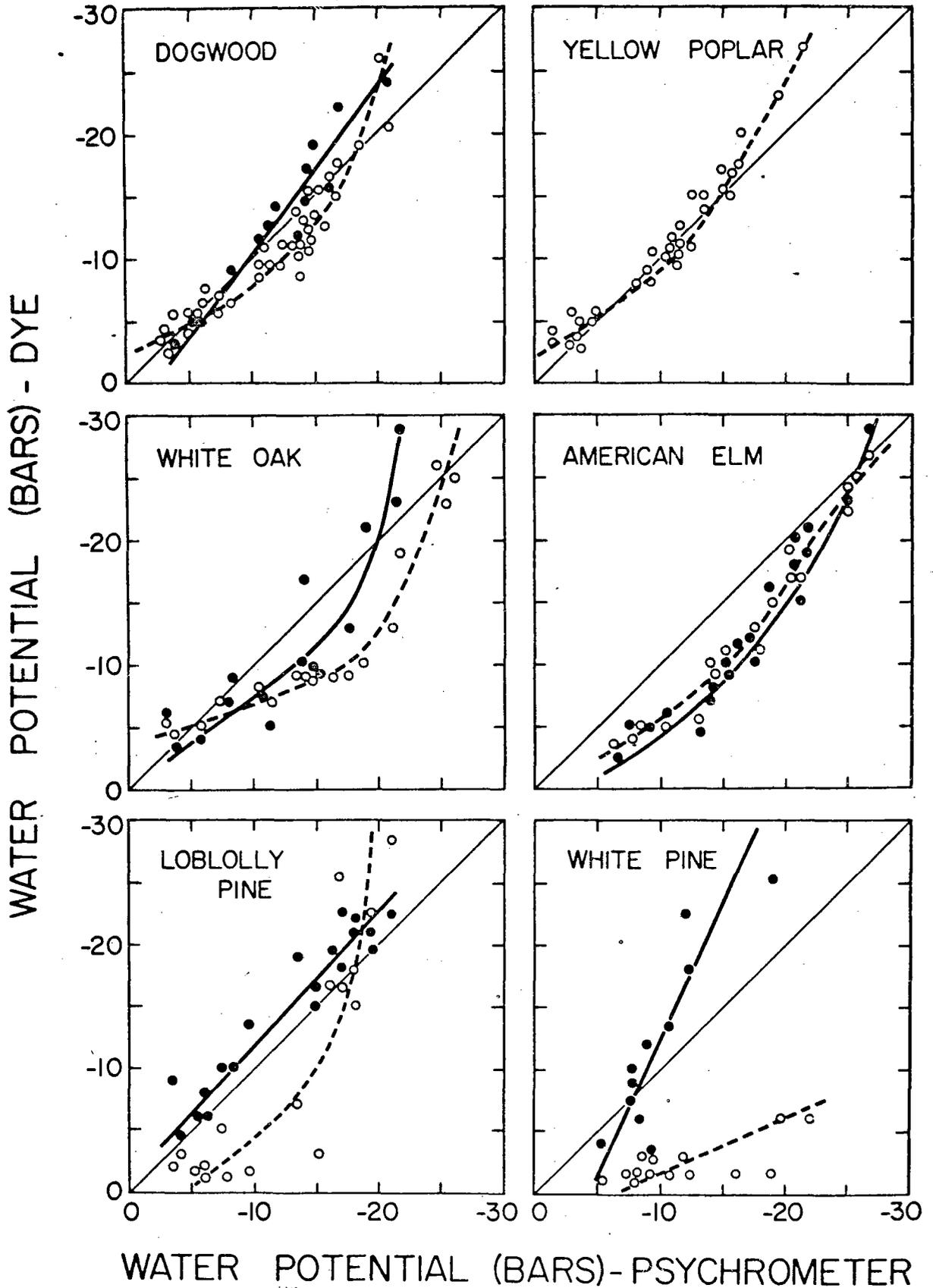


FIG. 1. Comparisons of leaf water potentials measured by the dye method and thermocouple psychrometer. Equipotential values are represented by the diagonal lines. Solid lines and circles represent dye method determinations with whole leaves; dashed lines and open circles represent determinations with cut leaves.

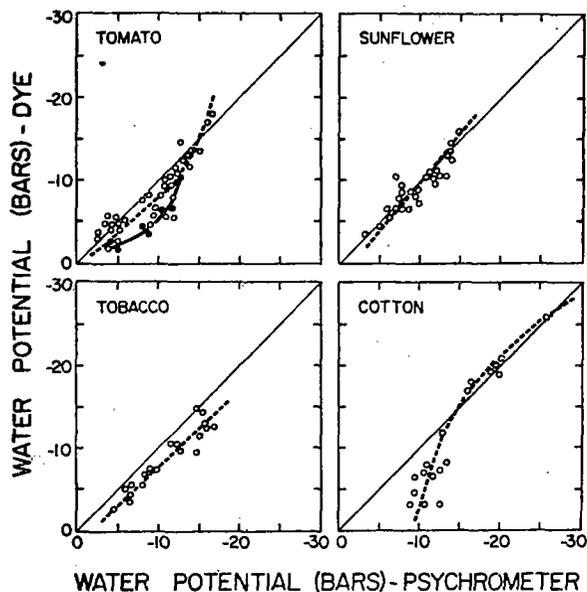


FIG. 2. Comparisons of leaf water potentials measured by the dye method and thermocouple psychrometer. Equipotential values are represented by the diagonal lines. Solid lines and circles represent dye method determinations with whole leaves; dashed lines and open circles represent determinations with cut leaves.

change the direction of the gradient between the leaf tissue and solutions.

To determine the densities of contaminants from leaves having a wide range of water potential, cut leaves of dogwood were immersed in 2 ml of distilled water in a manner as if the dye method were being conducted. After 8 hours the densities of the contaminated water were determined by placing lightly colored drops into a series of dilute sucrose solutions of known density. The densities increased as leaf water potentials decreased (fig 3) because the cell sap was concentrated by leaf dehydration. The abrupt increase in density at a water potential of about -16 bars is attributed to the nonlinear relationship between leaf water content and water potential (6).

Because the contaminants were diluted considerably by the water, the measured densities only indicate relative density changes caused by dogwood leaves of varied water potentials. However, these relative densities can be used to estimate the actual densities of the leaf contaminants. Contaminants have no effect on dye method values when their density is equal to the density of the solution having a potential equal to that of the leaf. The close agreement of dye method and psychrometer measurements for dogwood leaves at about -4 bars (fig 1) suggests that contaminants from leaves of this potential had approximately the same density as the equipotential sucrose solution. At this potential in figure 3 a solid line has been drawn from the origin and

extrapolated through the contamination density curve. Based on the density of $1.00025 \text{ gm cm}^{-3}$ representing a -4 bar sucrose solution, the ordinate can be considered to represent the relative densities of sucrose solutions having potentials equal to those of the leaves which produced the contaminants. The portions of the contamination density curve above the solid line indicate leaf water potential ranges in which the dye method will measure values which are too low (too negative) because the contaminants will increase the density of the solution in which there is no osmotic water exchange with the leaf tissue. The densities of some of the solutions having potentials lower than the equipotential solution also will be increased by the contaminants, but these changes are partially compensated for by osmotic water loss from the leaf tissue. In the water potential range where the density curve is below the solid line (fig 3), the dye method will indicate values which are too high.

The solid line drawn from the origin of figure 3 also can be considered as the theoretical line of equal water potential measurements by the dye method and psychrometer. This gives meaning to the apparent similarity of the contamination density curve (fig 3)

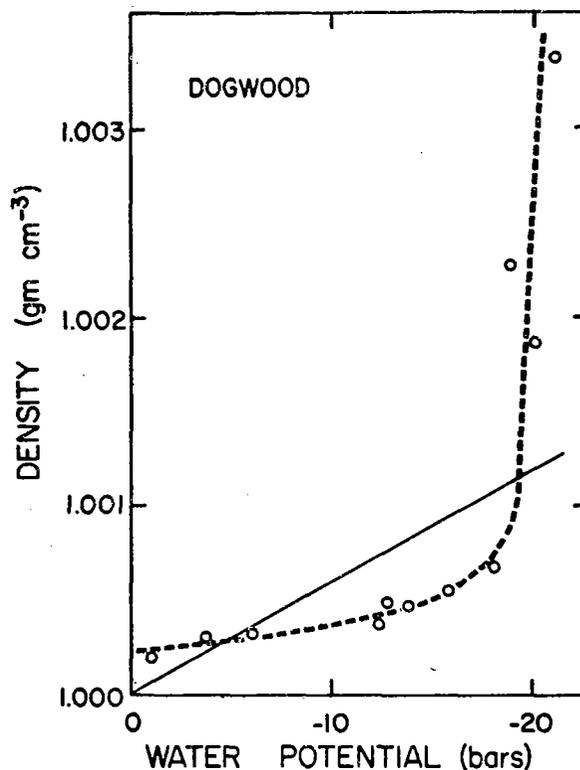


FIG. 3. Contamination of distilled water by cut leaves of dogwood of varied water potentials. Each point is the mean of 3 determinations of contamination density. The dashed curve represents the relative densities of contaminants released into dye method solutions. The diagonal line represents the relative densities of sucrose solutions which have potentials equal to those of the leaves.

and the dye method, psychrometer comparison for cut leaves of dogwood (fig 1). Note that the equipotential lines intersect both curves at about -19 as well as at -4 bars. This agreement suggests that contamination is the factor responsible for the pattern of dye method values of cut leaves of dogwood.

Further evidence that contaminants account for the differences between dye method and psychrometer measurements of dogwood leaf water potential is given by dye method measurements with solutions of mannitol, sucrose, and raffinose (fig 4). Because these solutions differ considerably in density, contaminants from leaf tissue of a given water potential affect them quite differently. For example, contaminants having a density equal to that of a sucrose solution are about 1.7 times denser than a mannitol solution of the same potential, but only about 0.6 times as dense as a raffinose solution of the same potential. These differences are reflected by the dye method data in figure 4. The leaf contaminants appear to have densities between those of mannitol and sucrose solutions except at a water potential of about -3 bars where the contaminants are more dense than all 3 equipotential solutions.

The water potential comparisons for cut needles of white pine and cut leaves of cotton and tobacco deviate from the basic curvilinear trend, but the differences appear to be caused by species-specific anomalies. The insensitivity of the dye method for cut white pine needles (fig 1) may have been caused by free water leaking directly from cut vascular elements. This also may account for the overestimation by the dye method of the high leaf water

potentials for cut needles of loblolly pine (fig 1) and for cut leaves of tomato (fig 2).

The poor agreement of cotton values (fig 2) at potentials higher than -12 bars is attributed to a factor influencing both methods. When cotton leaf tissue of high potential is enclosed in a psychrometer chamber, the leaf surface becomes wetted with excretions of low potential from salt glands. This surface wetting also was observed when the bases of detached cotton leaves stood in sucrose solutions having potentials as low as -8 bars. Such excretions from cotton leaves immersed in dye method solutions decrease the densities of the solutions and cause the potentials measured to be too high.

Kramer and Brix (8) found dye method values of tobacco to vary about 0.5 bar from leaf water potentials measured with Spanner psychrometers (13). In the present study the dye method values for tobacco (fig 2) are about 2 bars higher than the psychrometer potentials which probably are too low. Prior to reaching steady state thermocouple output, the tobacco leaf tissue began to deteriorate, and the psychrometer readings abruptly increased and approached values corresponding to the osmotic potentials of the tissue. The psychrometer values recorded were those measured just before the abrupt change.

On the basis of high density leaf surface contaminants, dye method values of uncut tissue would be expected to underestimate psychrometer values over the entire range of water potentials. Loblolly pine exhibits this trend, and white pine and dogwood (fig 1) deviate from this expected pattern only slightly. However, the whole leaf curves of American elm, white oak (fig 1), tomato (fig 2), and sourwood deviate considerably, indicating potentials that generally are higher than those measured by the psychrometer. The high dye method potentials of the latter 2 species may have been caused by low density contaminants secreted into the solutions from glandular hairs on the leaf surfaces and midveins. The subsequent slow rate of water uptake by the whole leaves may have prevented complete compensation of the contamination density changes.

The higher than expected dye method potentials of whole leaves may indicate that the psychrometer measurements of some species are incorrect. The leaf surfaces may provide sinks for water that cause the rate of water vapor transfer from the thermocouple to remain too high even at the apparent steady state, thus causing the potentials measured to be too low (10). Although this effect appears negligible for sunflower (2), it might be important with woody species which have rough, dry leaf surfaces. If surface vapor sinks are a source of error in psychrometer measurements, they probably have a progressively smaller effect as the internal water potentials of the drying leaves approach the low potentials of the dry surfaces. This may explain why the dye method—psychrometer curves of whole leaves of oak and elm (fig 1) approach and cross the equipotential lines at the lower leaf water potentials.

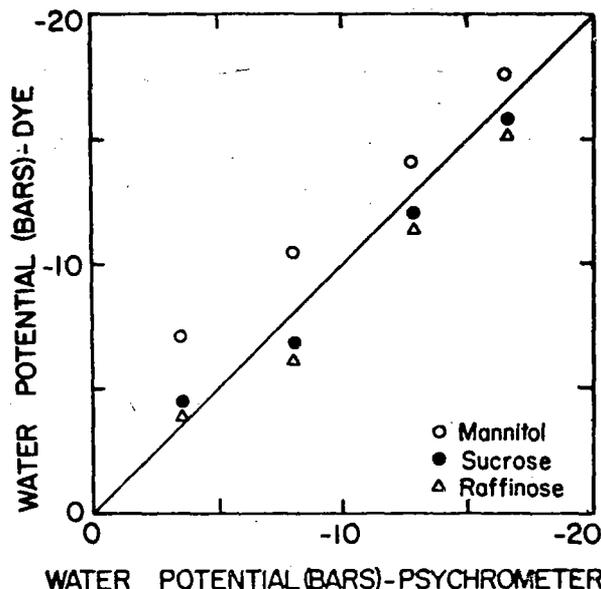


FIG. 4. Comparison of leaf water potentials of dogwood measured by the dye method and thermocouple psychrometer. Equipotential values are represented by the diagonal line. The dye method was conducted with solutions of mannitol, sucrose, and raffinose.

Solute Uptake. In addition to the error caused by contamination, the uptake of solutes by leaf tissue has been suggested as a source of error in water potentials measured by solution immersion methods. Goode and Hegarty (5) imply that the actual removal of solutes from test solutions decreases their densities and causes the dye method to indicate values that are too high. If this occurs, it should be more pronounced with cut leaves than with whole leaves. However, the dye method potentials measured on whole leaves of some test species were about the same as or higher than those measured on cut leaves.

According to Slatyer (12), the solute entry error is not the direct result of the accumulation of solutes in the tissue or of their removal from the solutions, but is due to membrane leakiness and an increase in the effective osmotic potential of the solutions. The solution which appears to be in equilibrium with the tissue actually is more concentrated than the true equilibrium solution. Thus, solution immersion methods are expected to measure potentials which are lower than those measured by vapor equilibration techniques. However, most of the dye method data in this study indicate the opposite, that is, potentials which are higher than those measured by the psychrometer.

Because plant cell membranes are more permeable to sucrose than to mannitol, Slatyer (12) also suggests that leaf water potentials measured in sucrose solutions would be lower than those measured in mannitol. However, the comparison of these 2 solutions with dogwood leaves (fig 4) indicates the opposite. Furthermore, the relative position of these data as well as the basic pattern of the dye method and psychrometer comparisons seems to be adequately explained by the effects of contamination. In this regard it is concluded that the uptake of solutes by leaf tissue does not cause a significant error in dye method values. If the error is present in the data, the differences between the dye method and psychrometer values may be attributed to the net effects of contamination and solute uptake.

Conclusions

Even though the dye method appears not to indicate true leaf water potentials, there is a characteristic pattern of changing values with progressive leaf dehydration. Hence, the method can be used to measure relative values and to indicate differences in leaf potential. However, because of species differences in the relationships of dye method values to true leaf water potentials, dye method values should

be interpreted with caution when comparing different species or the same species growing in widely different environments. Despite its limitations the dye method is useful to many workers because it is simple and easily learned, requires no elaborate or expensive equipment, and can be used in both the laboratory and the field.

Literature Cited

1. BARRS, H. D. 1965. Comparison of water potentials in leaves as measured by two types of thermocouple psychrometers. *Australian J. Biol. Sci.* 18: 36-52.
2. BOYER, J. S. 1966. Isopiestic technique: measurement of accurate leaf water potentials. *Science* 154: 1459-60.
3. BOYER, J. S. AND E. B. KNIPLING. 1965. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. *Proc. Natl. Acad. Sci.* 54: 1044-51.
4. GAFF, D. F. AND D. J. CARR. 1964. An examination of the refractometric method for determining the water potential of plant tissue. *Ann. Botany N. S.* 28: 351-68.
5. GOODE, J. E. AND T. W. HEGARTY. 1965. Measurement of water potential of leaves by methods involving immersion in sucrose solutions. *Nature* 206: 109-10.
6. KNIPLING, E. B. 1967. Effect of leaf aging on water deficit - water potential relationships of dogwood leaves growing in two environments. *Physiol. Plantarum* 20: 65-72.
7. KNIPLING, E. B. 1967. Measurement of leaf water potential by the dye method. *Ecology* (in press).
8. KRAMER, P. J. AND H. BRIX. 1965. Measurement of water stress in plants. In: *Methodology of Plant Eco-physiology*. 1962. *Proc. Montpellier Symp., UNESCO, Arid Zone Res.* XXV: 343-51.
9. RAWLINS, S. L. 1964. Systematic error in leaf water potential measurements with a thermocouple psychrometer. *Science* 146: 644-46.
10. RAWLINS, S. L. 1966. Theory for thermocouple psychrometers used to measure water potential in soil and plant samples. *Agr. Met.* 3: 293-310.
11. RICHARDS, L. A. AND G. OGATA. 1958. Thermocouple for vapor pressure measurement in biological and soil systems at high humidity. *Science* 128: 1089-90.
12. SLATYER, R. O. 1966. An underlying cause of measurement discrepancies in determinations of osmotic characteristics in plant cells and tissues. *Protoplasma* 62: 34-43.
13. SPANNER, D. C. 1951. The Peltier effect and its use in measurements of suction pressure. *J. Exptl. Botany* 2: 145-68.