

Banach spaces have been given by Edelstein but under hypotheses on the existence of strong limit points for the sequence  $\{U^n x\}$  which are difficult to verify. Cf. Edelstein, M., "On fixed and periodic points under contractive mappings," *J. London Math. Soc.*, **37**, 74-79 (1962), "On non-expansive mappings of Banach spaces," *Proc. Cambridge Philos. Soc.*, **60**, 439-447 (1964).

<sup>9</sup> *Added in proof:* After the present note was transmitted for publication, the writer received copies of the manuscripts of two unpublished papers, by W. A. Kirk, and by W. A. Kirk and R. Belluce, respectively, obtaining similar results. In Kirk's paper, it is remarked that the general line of argument is due to Brodskii and Milman [*Dokl.* (1948)], who proved Theorem 1 for isometries. It should also be remarked that in another recent note by the writer [Browder, F. E., "Mapping theorems for noncompact nonlinear operators in Banach spaces," these PROCEEDINGS, **54**, 337-342 (1965)], results are obtained which in the case of Hilbert spaces imply that the conclusion of Theorem 1 is also valid for *semicontractive* mappings, i.e., mappings  $U$  of  $C$  into  $C$  where  $Ux = S(x, x)$  and  $S$  is a mapping of  $C \times C$  into  $C$  which is nonexpansive in its first variable and completely continuous in the second variable. As we show elsewhere, this extension remains valid for Banach spaces having weakly continuous duality mappings. It is not clear at the time of writing whether this extension is also valid for the general class of uniformly convex spaces.

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### ISOPIESTIC TECHNIQUE FOR MEASURING LEAF WATER POTENTIALS WITH A THERMOCOUPLE PSYCHROMETER\*

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Measurements of the water potential of plant tissue are made with thermocouple psychrometers by enclosing the tissue and thermocouple in a small container kept at a constant temperature and determining the degree of cooling of the thermocouple as water evaporates from it and is absorbed by the tissue. It is assumed that the rate of vapor transfer is proportional to the difference in potential between the thermocouple and plant material. Rawlins<sup>1</sup> suggested that leaf water potentials obtained with Richards and Ogata psychrometers<sup>2</sup> are too high, indicating too low a water stress, because of the leaf resistance to the diffusion of water vapor. In contrast, Barrs<sup>3</sup> concluded that Richards psychrometer values, when corrected for heat of respiration,<sup>4</sup> are the same as those obtained with Spanner<sup>5</sup> psychrometers and are therefore free of leaf resistance error. The lack of agreement in these studies indicates a need for further examination of the leaf resistance error in psychrometer measurements.

The mathematics describing the diffusion process within a Richards psychrometer chamber (see below) indicates that there must be a leaf resistance error in the water potentials measured by this method. The close agreement in water potentials measured with the Richards and Spanner techniques suggests that the Spanner technique may also be in error. In this paper, we describe a new technique which is free of error attributable to leaf resistance, and this technique is used to evaluate the error in Richards and Spanner measurements.

The new technique involves the use of a modified Richards thermocouple to determine the rate of vapor movement between the thermocouple and the leaf when solutions of various potentials are present on the thermocouple. No vapor movement occurs when the solution on the thermocouple has a potential equal to that

of the tissue, and therefore leaf resistance does not affect the measurement. The theoretical basis for this conclusion can be seen from the equations that follow.

The pathway for vapor transfer between the thermocouple and the leaf can be divided into two segments. The first is the air between the thermocouple and the leaf surface. The second segment is the path between the leaf surface and the liquid phase inside the leaf. The rate of vapor transfer through the first segment may be represented by

$$\left(\frac{dm}{dt}\right)_d = \frac{-a_m}{r_a} (c_a - c_a), \quad (1)$$

where  $(dm/dt)_d$  is the rate of vapor transfer from the drop ( $\text{gm sec}^{-1}$ ),  $c_a$  is the concentration of water vapor in the air next to the drop ( $\text{gm cm}^{-3}$ ),  $c_a$  is the concentration of vapor in the air next to the tissue,  $a_m$  is the geometric mean area for flow ( $\text{cm}^2$ ), and  $r_a$  is the resistance of the air to vapor transfer ( $\text{sec cm}^{-1}$ ) at  $a_m$ .<sup>6</sup>

The calibration of Richards psychrometers shows that  $c_a - c_a$  is proportional to the potential of the calibrating solution and therefore is proportional to the concentration difference,  $c_0 - c_a$ , where  $c_0$  is the saturation vapor concentration of water at bath temperature.<sup>7</sup> Vapor transfer from the drop may then be expressed by substituting  $c_0 - c_a$  and the proportionality constant,  $L$ , in equation (1):

$$\left(\frac{dm}{dt}\right)_d = \frac{-La_m}{r_a} (c_0 - c_a), \quad (2)$$

where concentrations are expressed at bath temperature and the constant,  $L$ , may be considered to be a part of the coefficient,  $a_m/r_a$ , relating concentration differences to rate of vapor transfer.

A similar equation describes the rate of water vapor movement through the second segment of the diffusion path:

$$\left(\frac{dm}{dt}\right)_a = \frac{-a_i}{r_i} (c_a - c_i), \quad (3)$$

where  $c_i$  is the concentration of water vapor inside the leaf,  $a_i$  is the area of the leaf, and  $r_i$  is the resistance of the leaf to vapor diffusion.

An equation describing steady-state vapor transfer for the entire diffusion path from the drop to the leaf can be determined by first dividing (2) and (3) by their respective coefficients relating vapor concentration differences to rate of vapor transfer. Adding the concentration differences in the two equations then gives an expression for the total concentration difference,  $c_0 - c_i$ , at bath temperature in terms of flow in the two segments of the vapor pathway. Since the rate in each segment is the same at the steady state, vapor transfer in the total pathway may be described in terms of the rate of vapor transfer from the drop. Rearranging terms:

$$\left(\frac{dm}{dt}\right)_d = \frac{-La_m a_i}{La_m r_i + a_i r_a} (c_0 - c_i). \quad (4)$$

Equation (4) may be restated in terms of water potentials<sup>8</sup>

$$\left(\frac{dm}{dt}\right)_d = \frac{-c_0 \bar{V} La_m a_i}{RT(La_m r_i + a_i r_a)} (\Psi_0 - \Psi_i), \quad (5)$$

where  $\bar{V}$  is the partial molal volume of water (liters mole<sup>-1</sup>),  $R$  is the ideal gas constant (liter bars mole<sup>-1</sup> deg<sup>-1</sup>),  $T$  is the Kelvin temperature,  $\Psi_0$  is the water potential of the thermocouple drop at bath temperature, and  $\Psi_i$  is the water potential of the leaf.

Equation (5) is the steady-state flow equation for Richards psychrometers when leaf tissue lines the walls in the chamber. It describes a straight line with a slope of  $-(c_0\bar{V}La_m a_i)/[RT(La_m r_i + a_i r_a)]$ . Since  $r_i$  is included in the slope term, it will affect the rate of vapor transfer at all points except when  $\Psi_0$  equals  $\Psi_i$  and  $(dm/dt)_a$  is zero. The point at which these two potentials are equal, the isopiestic point,

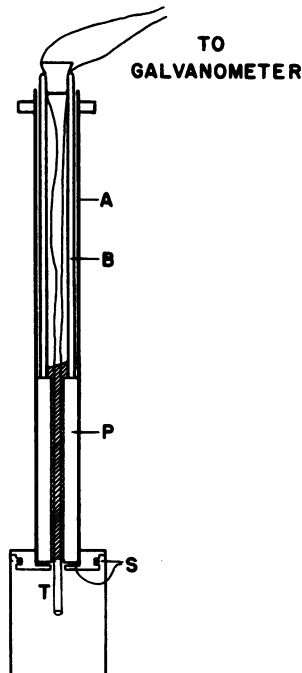


FIG. 1.—Thermocouple psychrometer for making isopiestic determinations. The psychrometer chamber and barrel are made of brass and are submerged in a constant-temperature water bath below the point A. Key to symbols: barrel, A; Plexiglas tube, B; plunger heat sink, P; diagrammatic representation of O-ring seal for chamber and soft rubber seal for plunger, S; thermocouple with ring junction, T.

may be determined by measuring thermocouple output when  $\Psi_0$  is changed. A thermocouple output of zero indicates no transfer.

An apparatus for measuring the isopiestic point is shown in Figure 1. The thermocouple is made of heavier wire (B and S gauge 38) than that of Richards and is mounted on a plunger which is sealed into the chamber by a soft rubber washer, permitting it to be removed and resealed rapidly. Leaf water potential is measured by first allowing the rate of vapor transfer between water or a solution on the thermocouple junction and the leaf tissue to reach the steady state. The plunger is then removed and rapidly replaced by a second plunger assembly with a solution of different potential on the thermocouple junction, and a second steady-state measurement is made.

Figure 2 shows the graph obtained for a determination by the isopiestic method. To determine the line, four measurements of thermocouple output were made, one with water and three with solutions of different potential on the junction. The four values lie on a straight line, as predicted by equation (5), so that the line may be determined by a minimum of two measurements. The  $x$  intercept is represented by the determination at  $-3.9$  bars and is the isopiestic point. This value, when corrected for respiration, is  $\Psi_l$ , the true leaf water potential. The  $y$  intercept represents a typical Richards determination of  $-3.6$  bars with water on the thermocouple junction. This value is essentially  $\Psi_a$ , the water potential of the air at the leaf surface.

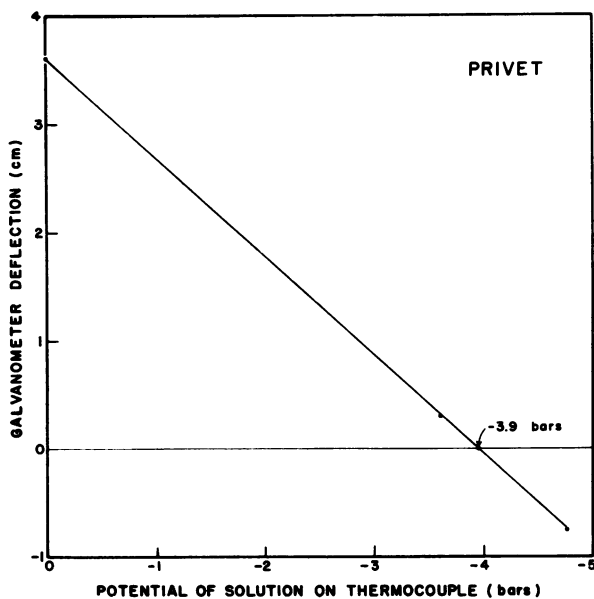


FIG. 2.—Isopiestic measurement of water potential of privet leaf tissue. The isopiestic value,  $-3.9$  bars, is shown by a determination at the  $x$  intercept. The output at the  $y$  intercept corresponds to a Richards determination of  $-3.6$  bars (galvanometer deflection =  $1 \text{ cm bar}^{-1}$ ).

The success of the isopiestic technique is strongly dependent on being able to change the solution on the ring without causing appreciable change in the water potential of the tissue or the air surrounding it. To determine whether the water potential changes when the apparatus in Figure 1 is opened, a steady-state reading was obtained with water on the thermocouple junction and privet leaf tissue in the psychrometer chamber. The plunger-thermocouple assembly was then removed for 15 sec and replaced. Figure 3 indicates that the steady-state output remained the same even though the chamber had been opened three times. Similar results have been obtained with geranium and tomato.

Since leaf resistance does not affect isopiestic values, it was possible to determine the influence of leaf resistance on Spanner and Richards measurements for leaf tissue from several plant species at various water potential levels. All three methods were used on the same sample of plant tissue by determining steady-state output with the apparatus shown in Figure 1, first with a Spanner thermocouple, then with

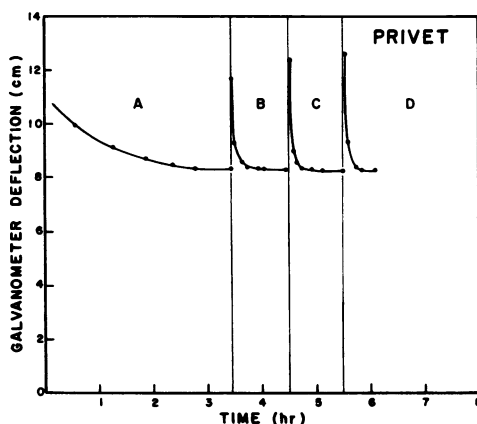


FIG. 3.—The effect of removing the plunger on the steady-state output of the thermocouple shown in Fig. 1. The initial approach to the steady state is shown at A. The plunger was then removed for 15 sec and replaced at B, C, and D.

a Richards thermocouple, and finally by the isopiestic method. The Spanner thermocouple was then placed back in the chamber to check for drying of the tissue. No drying was detected. All measurements were corrected for the heat of respiration by inserting a dry Richards or Spanner thermocouple in the psychrometer chamber.<sup>4</sup>

The results are shown in Table 1. Both the Spanner and the Richards techniques gave consistently more positive values of water potential than the isopiestic method, indicating that error due to leaf resistance is present in both techniques. Error was calculated as  $(\Psi_i - \Psi_a)/\Psi_i$  for the Richards determinations and was 4.5–12.1 per cent for all species<sup>9</sup> except geranium, which had smaller error (0–2.4%). A similar calculation was made for Spanner thermocouples, which had an error of 2.6–7.2 per cent. At potentials of about –20 bars, Richards thermocouples differed by as much as 2.6 bars from isopiestic measurements.

To check these results, diffusive resistances of leaf tissue were measured and theoretical errors based on leaf resistances were calculated for Richards psychrometer measurements. The theoretical error expression was derived from equations

TABLE 1  
WATER POTENTIAL OF LEAF TISSUE MEASURED BY ISOPIESTIC, SPANNER,  
AND RICHARDS TECHNIQUES\*

| Plant        | Isopiestic potential, bars | Spanner         |          | Richards        |          |
|--------------|----------------------------|-----------------|----------|-----------------|----------|
|              |                            | Potential, bars | Error, % | Potential, bars | Error, % |
| Privet       | –21.8                      | –20.9           | 4.1      | –19.2           | 11.9     |
|              | –18.1                      | –17.3           | 4.4      | –16.0           | 11.6     |
|              | –13.7                      | –13.3           | 2.9      | –12.6           | 8.0      |
|              | –8.3                       | –7.7            | 7.2      | –7.3            | 12.1     |
|              | –4.6                       | –4.4            | 4.4      | –4.2            | 8.7      |
|              | –3.9                       | —               | —        | –3.6            | 7.7      |
| Geranium     | –8.2                       | –7.8            | 4.9      | –8.0            | 2.4      |
|              | –7.7                       | –7.5            | 2.6      | –7.7            | 0        |
| Tomato       | –8.5                       | —               | —        | –7.9            | 7.1      |
| Magnolia     | –6.5                       | —               | —        | –6.0            | 7.7      |
| Philodendron | –2.2                       | —               | —        | –2.1            | 4.5      |

\* Measurements by the three techniques were made on the same tissue sample at each water potential level. The calculation of error in Spanner and Richards techniques is described in the text.

(2) and (3), which describe the vapor transfer occurring from the drop to the air and from the air to the leaf. Since the rate of vapor transfer through the two segments of the diffusion pathway is equal at steady state,

$$\frac{La_m}{r_a} (c_0 - c_a) = \frac{a_l}{r_l} (c_a - c_l). \quad (6)$$

The concentration differences of (6) may be replaced by water potential differences.<sup>8</sup> Noting that  $\Psi_0$  is equal to zero and rearranging terms, the equation may be written

$$\beta = \frac{\Psi_l - \Psi_a}{\Psi_a} = \frac{L(a_m/r_a)}{(a_l/r_l)}. \quad (7)$$

The error expression can then be written

$$\epsilon = \frac{\beta}{\beta + 1} = \frac{\Psi_l - \Psi_a}{\Psi_l} = \frac{L(a_m/r_a)}{L(a_m/r_a) + (a_l/r_l)}. \quad (8)$$

Leaf resistances were determined under psychrometer conditions of unstirred air and of a net transfer of water vapor to the leaf. Leaf disks of 2.4 cm diameter were placed across cylindrical chambers 1 cm away from water-saturated filter paper disks of the same diameter. The chambers containing the disks were maintained at  $27.0 \pm 0.001^\circ$  for 14–15 hr prior to determining weight gains by the leaf disks. Concentrations  $c_l$  were calculated from leaf water potentials measured isopiastically on parallel samples of tissue. Using the over-all concentration difference,  $c_0 - c_l$ , and the cross-sectional area of the chamber, total diffusive resistances ( $r_a + r_l$ ) were calculated from an equation similar to (1). Diffusive resistances of the lower leaf surfaces were then calculated from the differences between the total resistances and the air resistance in the chamber. Using the diffusivity of water in air ( $0.256 \text{ cm}^2 \text{ sec}^{-1}$ ),<sup>10</sup> the resistance of the air in the chambers was calculated to be  $3.9 \text{ sec cm}^{-1}$ . This was confirmed by two determinations with NaCl solutions on filter paper disks, which indicated air resistance values of 3.9 and 4.0  $\text{sec cm}^{-1}$ .

The data are shown in Table 2. Leaf resistances of privet and cotton<sup>9</sup> were about  $14 \text{ sec cm}^{-1}$ , which corresponded to theoretical errors in Richards determinations of about 7 per cent [calculated<sup>11</sup> from the right-hand side of equation (8)]. Tobacco, tomato, philodendron, and magnolia had leaf resistances ranging from about 19 to 23  $\text{sec cm}^{-1}$  and corresponding errors in  $\Psi_l$  of about 10–11 per cent.

TABLE 2  
DIFFUSIVE RESISTANCES OF LEAVES AND THEORETICAL ERRORS IN  
DETERMINATIONS OF LEAF WATER POTENTIAL BY THE RICHARDS METHOD\*

| Plant        | Potential, bars | Resistance, $\text{sec cm}^{-1}$ | Error, % |
|--------------|-----------------|----------------------------------|----------|
| Privet       | -18.9           | 14.1                             | 7.3      |
|              | -29.4           | 12.8                             | 6.7      |
| Cotton       | -16.1           | 13.7                             | 7.1      |
| Tobacco      | -13.2           | 19.5                             | 9.8      |
| Philodendron | -4.4            | 21.5                             | 10.7     |
| Tomato       | -10.9           | 21.8                             | 10.8     |
| Magnolia     | -21.3           | 23.1                             | 11.4     |
| Geranium     | -10.7           | 6.2                              | 3.3      |

\* Each resistance value is the mean of three observations. The errors were calculated from equation (8) (see text) for  $20 \text{ cm}^2$  of leaf.

Geranium had the lowest resistance, 6.2, which represented an error of approximately 3 per cent.

Leaf resistance may also be determined for leaf tissue in the psychrometer chamber using equation (5) for comparison with the resistances quoted in Table 2. The slope of the line defined by the equation is determined by the resistance of the leaf, since the other terms are constant for a particular psychrometer. Calculations from (5) indicate that privet leaf tissue in the psychrometer chamber has a resistance of approximately  $15 \text{ sec cm}^{-1}$ , which agrees closely with the values in Table 2.

The theoretical errors calculated from leaf resistance are approximately the same as those determined directly for the Richards technique by the isopiestic method. This is particularly apparent with geranium which had a low resistance and a correspondingly low error when measured by the isopiestic method. The theoretical errors do not agree with those of Rawlins,<sup>1</sup> who calculated errors of up to 90 per cent for philodendron on the basis of leaf permeability measurements. Our calculations indicate that the leaf permeabilities which he measured correspond to leaf resistances as high as  $1600 \text{ sec cm}^{-1}$  for philodendron. The data presented in this paper indicate a resistance for philodendron of 21.5, which is similar to that of the other species in Table 2 and also similar to that measured by other workers<sup>12</sup> for other species having closed stomata.

The error found for the Richards and Spanner methods does not agree with the conclusion of Barrs<sup>3</sup> that there is no error in these techniques. Since his comparisons were made with paired samples of tissue, variations between samples may have obscured differences between methods.

Although error is present in the Richards and Spanner techniques, it is small enough for tissues with higher potentials so that both methods remain useful for many purposes. However, it becomes quite large in the absolute sense when measurements are made on tissues with lower potentials. In these cases, isopiestic determinations should be used.

There are several advantages to the use of the isopiestic technique in addition to its lack of resistance error. For instance, calibration is unnecessary because the thermocouple is used simply as a "null point" or zero transfer detector when solutions of known potential are placed on the junction. Since the rate of vapor transfer is a straight line function of the potential difference between the thermocouple and leaf, the line may be determined by a minimum of two points and extrapolated to the isopiestic value without actually placing an isopiestic solution on the thermocouple. However, highest precision is obtained when one of the solutions has a potential which is close to that of the leaf.

The method can be used over a wide range of vapor pressures. Tissues drier than  $-30$  to  $-50$  bars have often been difficult to measure with Richards and Spanner techniques because the drop lasts only a short time and the steady state may not occur. This is not a problem with isopiestic measurements, since the potential difference between the solution and the plant tissue may be kept small.

The theory of the isopiestic technique and the error in water potential determinations with psychrometers may be summarized by the following statements:

1. The steady-state vapor transfer equation for Richards and Ogata thermocouples is the equation of a line. Leaf resistance influences thermocouple output whenever vapor transfer is not zero. The isopiestic technique, based on this equa-

tion, is free of leaf resistance error because it measures potential at zero vapor transfer.

2. The Richards and Ogata technique contains a resistance error of approximately 4.5–12 per cent. The Spanner technique contains a resistance error of 2.5–7.5 per cent. For many studies, this amount of error may be tolerated.

3. Error measured directly by the isopiestic technique is the same as the theoretical error calculated from leaf resistance measurements for Richards and Ogata thermocouples.

4. In addition to resistance error, the isopiestic, Richards, and Spanner techniques are affected by respiration error, but each type of measurement may be corrected by measuring chamber temperature with a dry junction, as Barrs<sup>4</sup> has suggested.

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<sup>1</sup> Rawlins, S. L., *Science*, **146**, 644 (1964).

<sup>2</sup> Richards, L. A., and G. Ogata, *Science*, **128**, 1089 (1958).

<sup>3</sup> Barrs, H. D., *Science*, **149**, 63 (1965).

<sup>4</sup> Barrs, H. D., *Australian J. Biol. Sci.*, **18**, 36 (1965).

<sup>5</sup> Spanner, D. C., *J. Exptl. Bot.*, **2**, 145 (1951); Monteith, J. L., and P. C. Owen, *J. Sci. Inst.*, **35**, 443 (1958).

<sup>6</sup> The geometric mean area,  $a_m$ , is  $4\pi r_d r_c$ , where  $r_d$  and  $r_c$  are the radii of the drop and chamber, which are considered to approximate spheres. The resistance of the air (diffusion path length divided by the diffusivity of water vapor in air) applies only at  $a_m$ . See Crank, J., *The Mathematics of Diffusion* (Oxford: Clarendon Press, 1956), p. 84; McAdams, W. H., *Heat Transmission* (New York: McGraw-Hill, 1954), p. 15.

<sup>7</sup> The proportionality constant,  $L$ , is evaluated by determining  $c_0$ ,  $c_d$ , and  $c_a$  in the psychrometer chamber for solutions of known potential. The concentration  $c_0$  may be obtained from tables giving saturation vapor concentrations of water at various temperatures. The concentration  $c_d$  is determined similarly from saturation values at drop temperature (see ref. 1). The concentration  $c_a$  is calculated from the potential of calibrating solutions (see ref. 8).

<sup>8</sup> The exponential relationship between water vapor concentration and water potentials higher than  $-200$  bars may be estimated by the first two terms of the exponential series,  $\exp x = 1 + x + \dots$ , so that

$$c = c_0 \exp \frac{\bar{V}\Psi}{RT} = c_0 \left( 1 + \frac{\bar{V}\Psi}{RT} \right), \quad (9)$$

where  $c_0$  is the saturation vapor concentration at the temperature  $T$ . The concentration difference  $c_0 - c_1$  of equation (4) therefore may be expressed as:

$$c_0 - c_1 = \frac{c_0 \bar{V}}{RT} (\Psi_0 - \Psi_1). \quad (10)$$

<sup>9</sup> Species used: privet, *Ligustrum japonicum*, Thunb.; cotton, *Gossypium hirsutum*, L.; tobacco, *Nicotiana tabacum*, L.; tomato, *Lycopersicon esculentum*, Mill.; philodendron, *Philodendron hastatum*, C. Koch & Sello; magnolia, *Magnolia grandiflora*, L.; geranium, *Pelargonium zonale*, L.

<sup>10</sup> Perry, J. H., *Chemical Engineers Handbook* (New York: McGraw-Hill, 1950), p. 539.

<sup>11</sup> Characteristics of our psychrometers: chamber approximates a sphere with 1.2-cm radius; spherical water drop with 0.1-cm radius; 1.1-cm diffusion path;  $r_a = 4.3 \text{ sec cm}^{-1}$ ;  $a_m = 1.5 \text{ cm}^2$ ;  $L = 0.32$ .

<sup>12</sup> Kuiper, P. J. C., *Meded. Landbouwhogeschool Wageningen*, **61**, 1 (1961); Slatyer, R. O., and J. F. Bierhuizen, *Australian J. Biol. Sci.*, **17**, 115 (1964); Slatyer, R. O., and O. T. Denmead, *National Symposium on Water Resources, Uses and Management*, Aust. Acad. Sci., C2 (1963); Van Bavel, C. H. M., F. S. Nakayama, and W. L. Ehrlter, *Plant Physiol.*, **40**, 535 (1965).