## **Relative Turgidity of Leaves:**

## **Temperature Effects in Measurement**

Abstract. The technique generally used for measuring relative turgidity may lead to large errors. This error arises when there is a difference between the in situ leaf temperature and the arbitrary constant temperature used in the determination of uptake. Similar errors may occur in methods for measuring leaf water potential.

The measurement of water status in plant leaves is of basic interest in plant environment studies. As Kramer (1) points out, the water status of leaves is a key property related to many others such as turgor, growth, stomatal aperture, transpiration, photosynthesis, and respiration. The relative turgidity technique (2, 3) has been widely used to measure leaf water status, and on occasion (4) to estimate the total water potential of leaves.

The relative turgidity (r) of leaf tissue is defined by the expression

$$r = \frac{w - w_a}{w_t - w_a} \times 100 \qquad (1)$$

where w is the initial fresh weight,  $w_d$ is the dry weight, and  $w_t$  is the experimentally determined turgid weight. In the standard technique,  $w_t$  is obtained by taking excised leaf tissue at some *in situ* temperature and total water potential and placing it in pure free water at a constant temperature (usually 20°C) until the uptake of water has satisfied the initial deficit (5). The total potential ( $\psi$ ) of water at any point in the tissue is made up of components  $\pi$ ,  $\tau$ , and P arising from osmotic, matric, and pressure effects, respectively. In the equilibrium condition,

$$\psi = \pi + \tau + P \tag{2}$$

is uniform throughout the tissue (6). An approximation of  $\pi$  may be made by using van't Hoff's law which is analogous to the gas laws; here

$$\psi = (nRT/V) + \tau + P \qquad (3)$$

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where (n/V) is the molar concentration of the solution, R is the gas constant, and T is the absolute temperature. Clearly,  $\pi$  (hence  $\psi$ ) is dependent upon temperature. Little or nothing is known about other effects of temperature, such as whether n varies with temperature-induced changes in metabolism or whether  $\tau$  or P varies significantly with T. The use of a constant temperature and the neglect of *in situ* leaf temperature in the standard  $w_t$  determination overlook the possibility of significant temperature dependence of the terms in Eq. 3.

The effect of low temperature  $(3^{\circ}C)$ on uptake has been examined (2), but only in connection with the inhibition of growth uptake (5). In some cases it was found that the initial rapid uptake (5) was also inhibited, but this was regarded as a somewhat anomalous result. Werner (7) noted an effect of temperature on uptake, but overlooked the point that the value of robtained from a  $w_t$  determination made at the arbitrary standard temperature (20°C) may be considerably different from the true value of r obtained from a  $w_t$  determination made at the in situ temperature.

Simple experiments were run to demonstrate the size of the error that may be incurred in a determination of  $w_t$  and a subsequent calculation of rwhen *in situ* temperature and temperature of leaf uptake differ. With some exceptions (5), the technique used and the preliminary tests undertaken were similar to those proposed by Barrs and Weatherley (2). These tests indicated that the effects of injection and dryweight changes were relatively unimportant and were affected little by temperature.

In order to obtain leaf material of uniform initial water status, needles of uniform size, age, vigor, and exposure were removed from a short segment of the stem of a small *Pinus radiata* tree. The tree was grown under fairly steady environmental conditions (about 20°C) for 14 hours before sampling. Usually, a different tree was used in each experiment and a sufficient number of mature needles (8 cm long)

Figs. 1-4. Effect of temperature on the uptake of water by mature needles of *Pinus radiata*, expressed as a percentage change of the initial fresh weight with time. The mean values of relative turgidity are in brackets next to each temperature. Each figure represents an experiment run on a different occasion. The LSD at the 5 percent level are indicated; (a) between temperatures within times and (b) between times within temperatures. For Figs. 1, 3, and 4 the trees were grown at 20°C, and for Fig. 2, at 35°C, for 14 hours before sampling.



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was collected to give three replicates, each of 12 needles, for each uptake temperature. Immediately following excision, the fresh weight was measured, and the cut ends of the needles were placed in sealed test tubes with 5 mm of water. These tubes were placed in water baths at selected uptake temperatures. Water uptake at the different temperatures was observed and the percentage of change of the initial fresh weight was plotted against the time during which the change occurred.

The results of the first experiment, run at 8°, 20°, and 37°C, are shown in Fig. 1; each point represents the mean value of the three replicates.

These results suggest that the neglect of a temperature change of 17°C in a standard (20°C) relative turgidity determination leads to an error of only 8 percent, a seemingly trivial error when referred to the 0 to 100 percent scale. However, it is more realistic to gauge the importance of the error by reference to the small range of values of r between full turgor and wiltingthe range over which growth and transpiration rates fall from high to very low values. Measurements by Johnston (8) indicate that pine needles wilt at a relative turgidity of about 80 percent. Thus, the 8 percent error in the full range is equivalent to a 40 percent error in the range of r that is of most interest.

The arbitrary basis for the use of an uptake temperature of 20°C was demonstrated further (Fig. 2). In this experiment a tree was held at a temperature of 35°C for 14 hours before sampling, and uptake temperatures of 20° and 35°C were used. Other work indicates that even larger errors may occur with higher temperatures and with other species (7) and conditions.

The size of the temperature effect varies (Figs. 3 and 4, droughted trees). Whereas the first phase of uptake (5)at 20°C is about the same in each experiment, the uptake at 35°C differs considerably. The reason for such marked variability has not been investigated. It is clear that a difference between uptake and in situ temperatures often has a large effect on the uptake of water and consequently also on the relative turgidity. This problem is probably of most concern in studies in which the diurnal course of plant water status is to be followed and when there is a large change in leaf temperature during the day.

In practice, the error could be minimized by use of several water baths or

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incubators held at different temperatures so as to cover the range of *in situ* leaf temperatures encountered. The  $w_t$ determinations could then be made in the bath or incubator at a temperature nearest that of the measured in situ leaf temperature.

Measurements of  $\psi$  and other water stress indices are also customarily run at a standard temperature. Thus, these measurements are probably subject to error due to the neglect of the temperature effect. Direct proof of this and an elucidation of the mode of action of the temperature effect have been delayed by difficulties encountered here and by others (9) in the development of adequate methods for measuring  $\psi$ .

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## **References and Notes**

 P. J. Kramer, Agron. J. 55, 31 (1963).
H. D. Barrs and P. E. Weatherley, Australian J. Biol. Sci. 15, 413 (1962).
B. D. Millar, Australian J. Agr. Res. 15, 95 (1964). 85 (1964).

4. P. E. Weatherley and R. O. Slatyer, Nature 179, 1085 (1957).

- 5. The uptake of water by excised leaf tissue consists of an initial rapid phase, followed by a slower steady uptake lasting as long as the tissue remains healthy. It is genthe usual relation is the analysis of the second se a result of the difference in water potential between the tissue and pure free water), and that uptake due to growth commences only after the conclusion of the initial phase. This implies that growth, with its associated uptake of water, cannot commence until the tissue is fully turgid. However, as leaf tissue generally grows at somewhat less than full turgor, this supposition appears to be un-realistic. Yemm and Willis (10) assume that growth uptake is steady throughout. By extrapolating the linear portion (second phase) of the curve back to zero time, the intercept obtained leads to the value of  $w_t$ . This is the procedure adopted here.
- 6. W. R. Gardner and C. F. Ehlig, *Plant Physiol.* 40, 705 (1965).
- 7. H. O. Werner, Res. Bull. Nebr. Agr. Exp. Sta. 176 (1954).
- 8. R. D. Johnston, Australian J. Bot. 12, 111 (1964).
- 9. S. L. Rawlins, Science 146, 644 (1964); J. E. Box, Jr., Agron. J. 57, 367 (1965); J. R. Lambert and J. van Schilfgaarde, Soil Sci. 100, 1 (1965).
- 10. E. W. Yemm and A. J. Willis, New Phytol. 53, 373 (1954).
- 11. I thank Mr. G. A. McIntyre of the Division of Mathematical Statistics, CSIRO, for statistical advice.

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## Ferrosilite III: A Triclinic Pyroxenoid-Type Polymorph of Ferrous Metasilicate

Abstract. The relationships between the triclinic unit cell of ferrosilite III and those of pyroxmangite, rhodonite, and wollastonite lead to the hypothesis that this polymorph of ferrous metasilicate has a pyroxenoid-type crystal structure with single silicate chains that repeat after every nine silicon tetrahedra. Vector relations between the triclinic cell and an apparent pseudomonoclinic cell support this hypothesis. Although the proposed silicate chain has a longer repeat length than any now known, it represents a logical extension of those found in other pyroxenoids and suggests that even longer repeat lengths may yet be found among phases with pyroxene compositions.

Ferrosilite, the iron analog of the common pyroxene enstatite (MgSiO<sub>3</sub>), does not exist in nature; but two polymorphs having the ferrous metasilicate (FeSiO<sub>3</sub>) composition have been synthesized at high temperatures and pressures by Lindsley et al. (1) and Akimoto et al. (2). These workers have investigated the stability relations of the monoclinic form and the higher temperature orthorhombic form (3, 4), and x-ray data suggest that these polymorphs are isostructural with clinoenstatite and orthoenstatite, respectively (5).

In several experiments made in the low-pressure region of what is thought to be the protoferrosilite stability field (3), the phase present after quenching could not be identified by its powder diffraction pattern. Precession photo-

graphs of single crystals of this material, termed ferrosilite III, showed that it possesses triclinic symmetry but that it exhibits a pseudomonoclinic unit cell similar in size to that of diopside (CaMgSi<sub>2</sub>O<sub>6</sub>). According to Lindsley, the stability field of ferrosilite III has not been determined; although the synthesis is reproducible in his apparatus, he believes that this phase forms during the quench. The single-crystal x-ray photographs described in this report are also reproducible; five crystals from different experiments have been examined, all of which yield the same diffraction patterns.

The crudely prismatic shape of several single crystals made it relatively easy to orient them on the precession camera, but in almost all cases the first