Michoacán (*4*). His placement of the phases in time has been changed in accordance with the current dating of time markers (Mazapan figurines, Plumbate pottery, and copper artifacts) found at the site. It is also in accordance with cross-ties of a radiocarbon-dated ceramic sequence established for the geographically close site of Tizapán el Alto excavated by Meighan and Foote (*13*).

Sequences have been published for both the Autlán and the Tuxcacuesco zones. The Autlán sequence (*5*) was defined on the basis of surface surveys and cross-ties with the ceramic sequence established for the excavations at Tuxcacuesco (*14*). The Colima sequence was based on surveys, excavations of tombs (*5*), and ceramic cross-ties with the Tuxcacuesco sequence (*14*).

Kelly's sequences have been accepted without modification with the exception of the dates of a suggested correlation with the Basin of Mexico (*14*). The lower limits of her "coeval" complexes at Tuxcacuesco and Colima (Tuxcacuesco and Ortices-Chanchopa) have been pushed back in time. This has been done in order to correlate with a radiocarbon date (UCLA-1066) (*10*) from the rifled tomb at Chanchopa, near Tecomán and four radiocarbon dates from early stratigraphic levels at Morett, Colima, which have ceramic cross-ties with the Tuxcacuesco complex of the Tuxcacuesco sequence.

Both the Tuxcacuesco and Ortices complexes have been provisionally correlated with Teotihuacán III (*14*) of the Basin of Mexico, on the basis of a Thin Orange, Chanchopa tomb association. However, the Thin Orange restorable vessel came from a Chanchopa tomb with pure Chanchopa contents, while the radiocarbon-dated shell bracelet fragments came from another Chanchopa tomb with mixed Chanchopa and Ortices contents. The temporal relationship between the Chanchopa and Ortices complexes is not yet clear (*15*).

Both the middle and upper phases of the Autlán, Tuxcacuesco, and Colima sequences have been temporally modified. However, the changes are not based directly on radiocarbon dates, but on the current dating of the appearance and disappearance of such time markers as Mazapan figurines and copper artifacts.

Stratigraphic excavations were conducted in the Cihuatlán province at Barra de Navidad, Jalisco (*16*), Playa del Tesoro, Colima (*17*), and Morett, Colima (*18*) by UCLA in 1961–62. A tentative sequence has been established on the basis of preliminary ceramic analysis and 11 radiocarbon dates (*19*).

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Isopiestic Technique: Measurement of Accurate Leaf Water Potentials

Abstract. *Sunflower leaf tissue of known potential was obtained by equilibrating an interveinal leaf sample, at constant temperature in air, with a potential determined by sucrose solutions. Equilibration occurred within 17 hours. Except for one determination, all measurements of the water potential of the equilibrated samples with an isopiestic technique were within 0.1 bar of the known potential of the tissue. This finding indicates that thermocouple psychrometers can measure accurate values of water potential when an isopiestic technique is used.*

There are many methods of measuring the water potential of leaves (*1*). Often, however, different methods do not give the same answer, although water potentials may be similar (*2*). There have been studies of errors that affect measurements of water potential (*2, 3, 4*) but there are none which demonstrate that, once the errors have been corrected, the technique is an accurate or absolute measure of water potential.

When first introduced, thermocouple psychrometers were expected to provide values close to actual leaf water potentials. However, the heat produced by respiration (*5*), the adsorption of water vapor on the walls of psychrometer chambers (*6*), and the resistance of leaf tissue to vapor transfer (*3*) cause inaccuracies in determinations. Readings may be corrected for these errors, and in some instances the errors may be eliminated altogether.

A modification of psychrometer practice has been suggested which incorporates all the above corrections in a single method and is called the isopiestic technique (*4*). Basically, the method consists of finding a solution which neither loses nor gains water from the plant sample. The potential of the solution, which is known, is then equal to the potential of the

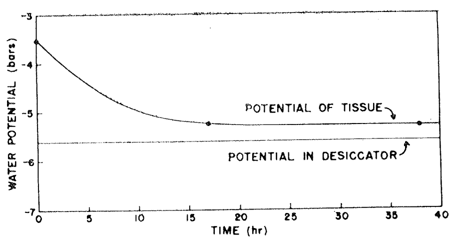

Fig. 1. Equilibration of sunflower leaf tissue in a desiccator containing water vapor with a water potential of —5.6 bars. Each point represents one determination.

sample. If the corrections applied to the technique are valid, it should be an absolute rather than relative measure of water potential; that is, the water potential of a tissue sample having a known potential should be the same as the water potential indicated by the instrument. In this report, I use the isopiestic method to show for the first time that a method of measuring leaf water potential can give absolute values.

Sunflower leaf tissue of known potential was obtained by equilibrating an interveinal leaf sample at constant temperature in an atmosphere that had a water potential which was determined by the vapor pressure of sucrose solutions (*7*). The equilibration chamber was an aluminum desiccator submerged in a constant-temperature bath (28° ± 0.0005°C). The air inside the chamber was stirred with a Teflon-coated magnet, rotated by a magnetic stirrer mounted upside down above the bath. The sucrose solutions covered the bottom and were absorbed by filter paper which extended 2 cm upward along the sides of the desiccator. The rest of the desiccator wall and top was coated with vaseline to reduce water adsorption.

The equilibration procedure consisted of placing a sunflower leaf in a pressure chamber (*8*) and bringing its water potential within 2.5 to 3 bars of the desired potential by forcing sap out of the petiole with pressure applied to the leaf blade. Interveinal samples from the leaf were then placed on the wall of the desiccator and held there by the vaseline which coated the walls. The contact between wall and tissue kept the leaf near bath temperature. A thermocouple was mounted in the side of the desiccator to measure small deviations between bath and leaf temperature, and the junction was held against the leaf surface by a small piece of tape. A second thermocouple was mounted in the top to measure air temperature inside the desiccator. After the leaf sample was in place, the desiccator was closed, submerged in a water bath, and the air inside was stirred. Equilibrium was reached in 17 hours (Fig. 1), but tissue usually remained in the chamber for 36 to 40 hours.

The water potential of the tissue at equilibrium in the desiccator was calculated from a slightly modified form of the familiar equation relating vapor pressure and potential:

$$\Psi_w = \frac{R\ T}{\overline{V}} \ln p/p_0$$

where Ψ_w is the water potential (bars), R is the gas constant (liter bars mole^{-1} deg^{-1}), $\overline{V}$ is the partial molal volume of water (liters mole^{-1}), T is the Kelvin temperature, and p is the vapor pressure of the sucrose solution at bath temperature. Since leaf temperature was warmer than bath temperature by as much as 0.007°C, p_0 was taken as the vapor pressure of pure water at the temperature of the plant tissue.

After equilibrium was reached, the tissue was transferred to a psychrometer chamber, and an isopiestic measurement was made. The measurement consisted of two consecutive determinations, first with water on a thermocouple that was inserted into the psychrometer chamber and then with a sucrose solution on a second thermocouple. The potential of the sucrose solution was close to that of the leaf tissue. Thermocouple output was plotted as a function of the potential of the water or solution on the thermocouple, and the line was extrapolated to zero output. The potential at zero was taken as the potential of the leaf tissue.

The tissue covered the wall and bottom of the psychrometer chamber (the top was coated with vaseline) so that adsorption of water on the walls did not affect the measurement. All determinations were corrected for heat of respiration. Sampling and transferring of the tissue between desiccator and psychrometer were carried out in a humid chamber.

Every isopiestic determination except one was within 0.1 bar of the water potential of the tissue (Fig. 2). There was no systematic error in the measurements (*5*). The one instance in which the potential of the tissue

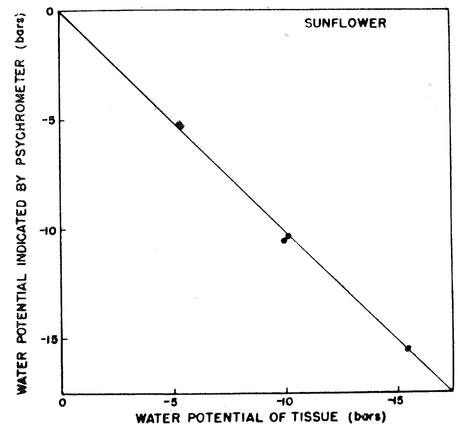

Fig. 2. Comparison of water potential values obtained by isopiestic technique with the water potential of sunflower leaf tissue that had been equilibrated in an atmosphere of known potential. The equipotential line is represented by the diagonal in the figure. Each point represents a single determination with the psychrometer.

differed from that indicated by the instrument (0.3 bar difference) was attributable to slight water losses during transfer of the tissue from the desiccator to the psychrometer chamber. Thus, the psychrometer indicates absolute water potentials and should measure absolute potentials in the intact plant as long as water loss or gain by the sample is negligible during transfer from the plant to the psychrometer. Since psychrometer values were accurate whether leaf water potentials were −6 or −16 bars, accuracy is evidently not affected by the water status of the tissue and the straight line formed by the data probably may be extrapolated to zero potential.

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