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Plant Moisture Stress: A Portable Freezing-Point Meter Compared with the Psychrometer[1]

J. W. Cary and H. D. Fisher[2]

ABSTRACT

A small portable instrument for measuring the freezing-point depression of plant tissue has been developed for field use. The instrument is easy to operate and can be constructed from materials costing less than $100.

Moisture stress measurements made with the freezing-point meter on a variety of plants were compared with vapor pressure psychrometer measurments. Variation between duplicates in the freezing point averaged 1.2 bars, but differences between stress measurements made with the psychrometer and freezing-point instrument averaged 2.6 bars.

Additional key words: **Freezing-point depression, Diffusion pressure deficit, Plant water relations.**

THE moisture stress in living plant material may be measured with a vapor pressure psychrometer (Rawlins, 1966), a pressure chamber (Boyer, 1967), moisture exchange reactions (Knipling and Kramer, 1967; Slatyer, 1967), electrical conductivity (Kreeb and Bogner, 1967), and by relative water content (Slatyer, 1967).

Of these methods, the vapor pressure psychrometer is considered to be the most accurate, but it is time-consuming, requires painstaking attention, and uses expensive electronic equipment. The pressure chamber technique can be adapted for field use, but is limited to specific types of plant material. Of the water exchange-type measurements, the vapor transfer methods are slow and must be carried out in the laboratory, and the liquid exchange methods are subject to errors arising from solute exchange across the plant cell membranes.

Relative water content methods are subject to the same errors as the liquid exchange tests. Electrical conductivity measurements have received only limited attention and appear to present a number of problems which must be overcome before receiving general acceptance.

An alternative way to measure energy status of water is by freezing-point depression. This technique is well known and has been widely applied (Abele, 1963), though not to measure water stress in plant material. Being a laboratory procedure, it has had no advantage over the vapor pressure psychrometer; however, the recent development of solid state cooling devices suggested to us that a portable freezing-point depression meter might be constructed. Because ice first forms in the intracelluar spaces, the initial freezing-point depression should depend upon the activity of the external cell water rather than the osmotic solution in the cells. After the ice crystals have grown for a few minutes or after thawing occurs, some cell membranes rupture and the turgor pressure component of total stress begins to disappear. Consequently, freezing-point depression measured immediately after ice crystals begin to form in plant tissue in the field could give on-the-spot estimates of plant water stress.

There is a real need for this type of information. The past several years have seen rapid progress in characterizing microclimate, yet we have very little knowledge of how the physical environment affects plant-water relations. Moisture stress has important consequences on the biochemistry of plants (Slatyer, 1967), but we have almost no information on hourly, or even daily, moisture stress levels in field plants under natural field conditions.

PROCEDURES AND EQUIPMENT

The portable freezing-point meter is shown in Fig. 1. Its basic component is a small freezing chamber mounted on the cold side of a Peltier battery. A cross-sectional schematic diagram of this chamber is shown in Fig. 2. A leaf sample with 4 or 5 cm^2 of surface is folded several times and wedged firmly between thermistors T_1 and T_2. These thermistors are encased in slender glass rods. After inserting the sample, the stopper is placed in the top of the freezing chamber and the sample allowed to cool below the freezing point. The toothpick tipped with ice crystals is then moved so that the crystals briefly come into contact with the leaf tissue. This induces rapid freezing in the sample. Its temperature rises to a steady value determined by the freezing point of the plant fluid and the heat transfer properties of the chamber.

The thermistors operating in the circuit (Fig. 3) cause the microammeter to register the sample's temperature. This circuit consists of two wheatstone resistance bridges which use the 50 μA meter as a galvanometer. The meter may be connected into either bridge through switch S_1. The switch S_2 adjusts the temperature span of the thermistors in the left bridge. When opened, the μA meter will be on scale at all temperatures. When

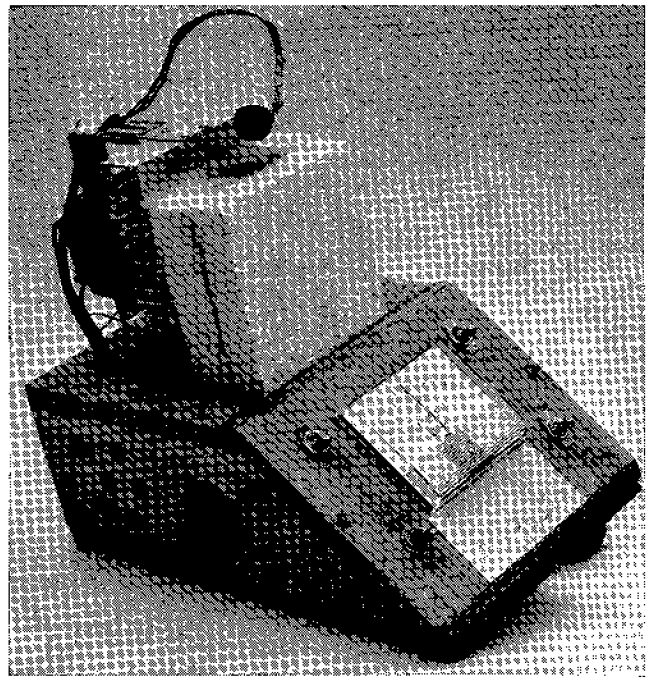

Fig. 1. Photograph of the portable freezing-point unit.

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[2] Research Soil Scientist and Mathematician, respectively, Snake River Conservation Research Center, Kimberly, Idaho 83341.

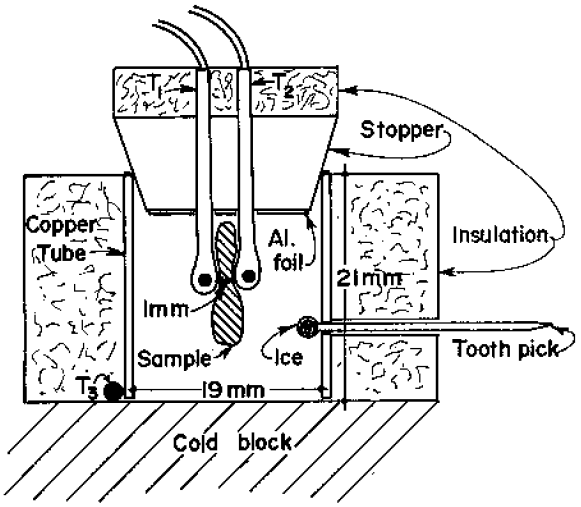

Fig. 2. A cross-sectional diagram of the freezing chamber of the freezing-point apparatus. T_1, T_2, and T_3 are thermistors shown in the circuit diagrammed in Fig. 3.

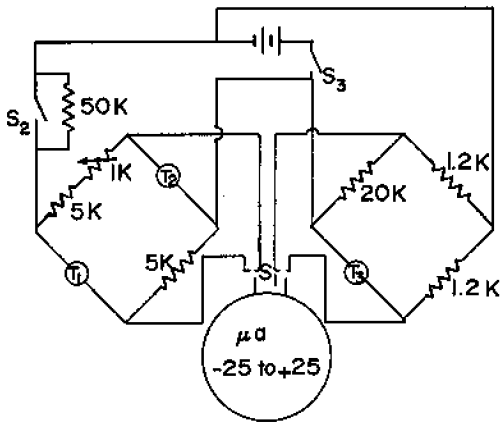

Fig. 3. A circuit schematic for the freezing-point depression instrument.

The following meter component descriptions are listed for the convenience of the reader and in no way imply preferential endorsement of the manufacturer by the United States Department of Agriculture: T_1, T_2 — VECO 31A13 thermistor 1 KΩ @ 25 C; T_3 — VECO 35A8 thermistor 5KΩ @ 25 C; 1 K — Bourns 1 KΩ 10-turn potentiometer; S_1 — DPDT toggle switch; S_2, S_3 — SPST toggle switches; Battery — 2 Mallory RM-4R 1.35-V mercury cells; Meter — Knight 25-0-25 μA 3½" panel meter; Resistances are ¼-watt, 10% tolerance.

Peltier cells may be obtained from such companies as: Materials Electronic Products Corp., 990 Spruce Street, Trenton, N. J.; General Instrument Corp., P. O. Box 544, Hicksville, N. Y.; Cambridge Thermionic Corp., 445 Concord Avenue, Cambridge, Mass.; and others.

closed, the μA meter has a full scale expanded range of approximately −4 to +1 C. A DPDT switch may be wired into this bridge to replace the thermistors T_1 and T_2 with a pair of 2.5 K resistors for quick circuit tests.

The bridge on the right (Fig. 3), containing thermistor T_3, is used to monitor the temperature of the cold block. The cold block is operated such that the sample temperature will fall at a rate of about 1 μA per second when S_2 is in the expanded scale position. The block temperature is held constant to within ± 2 μ amps by adjusting the DC voltage across the Peltier cooling cell. This may be accomplished by manually switching series power resistors in approximately 0.1 Ω steps. These resistors can be purchased commercially or made from heavy nichrome wire.

The output of the bridge is set with the 1 KΩ variable resistor. To make this setting, a piece of paper towel saturated

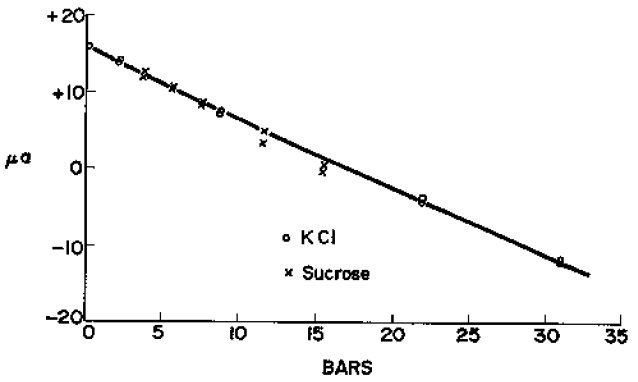

Fig. 4. Calibration curve for the freezing-point moisture stress meter.

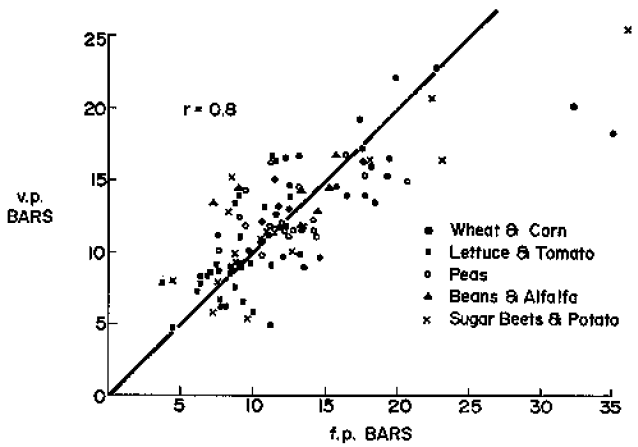

Fig. 5. A comparison of plant moisture stress measurements made with the vapor pressure psychrometer and the freezing-point apparatus. The solid line was drawn with a 1:1 slope.

with 0.2 N KCl is positioned between thermistors T_1 and T_2. It is allowed to cool to approximately −3 C, which gives a meter reading of −10 to −15 μA, depending again upon the particular characteristics of the thermistors. At this point the ice crystals on the toothpick are tapped against the sample, crystalization occurs, the sample and the thermistors warm, and the μA meter reading rises to a stable peak. The 1 KΩ resistor is used to set this peak at any convenient point on the scale. We chose 7.5 μamps, which gives a pure water freezing point at 16 μamps and the calibration curve shown in Fig. 4. The instrument is then ready for use for plant samples. Each sample is folded so that it fits firmly between the thermistors. The plant material must not touch any part of the chamber except the thermistors. It will ordinarily take 2 or 3 minutes to cool the sample to −3 C and about a minute more to set the ice crystals and record the maximum μA meter reading. The system is somewhat dependent on ambient temperature and so should be checked against the standard 0.2 *N* KCl solution whenever fluctuations approach 10 C. When operating at temperatures in excess of 35 C, evaporative cooling of the heat sink fins may be needed to maintain the required sample cooling rate.

In order to test the operation of the instrument, stress measurements were checked against those given by the vapor pressure psychrometer using different species of plants grown in pots in the greenhouse. Four samples were taken from each pot, two for the psychrometer and two for the freezing-point apparatus. The psychrometer was of the Peltier-type described by Zollinger et al. (1966) using six chambers per thermocouple. Two chambers contained calibrating solutions and four chambers contained plant samples, so there were two calibration points for each thermocouple in every run.

RESULTS

The results are summarized in Fig. 5. Each of these 105 points is the average of duplicates measured with the vapor pressure psychrometer plotted against the

Table 1. Variation in plant water stress measurements within and between the freezing point and vapor pressure methods.

Plant	Average difference in bars		
	Between methods	In v. p. duplicates	In f. p. duplicates
Peas	2.1	1.1	1.2
Wheat	4.0	0.6	1.5
Lettuce	2.2	1.4	1.2
Corn	2.3	1.7	1.2
Potato	2.9	0.9	1.1
Beans	2.3	0.2	0.8
Alfalfa	2.2	---	1.4
Beets	3.2	0.6	1.8
Tomato	2.3	1.4	0.6
Mean	2.6	1.0	1.2

Table 2. Moisture stress measurements made with the psychrometer on sugar beet leaf samples from field plots in August 1967. The data pairs are from duplicate samples collected adjacent to one another. The three plants were sampled on different days.

Sample	Moisture stress, bars		
	Plant 1	Plant 2	Plant 3
Old leaf tip	12.8	14.4	13.9
	12.6	14.6	14.4
Mature leaf tip	19.0	19.6	18.8
	18.0	21.2	17.2
Young leaf tip	17.0	18.0	20.9
	18.0	19.2	16.8
Crown leaf tip	12.8	13.6	18.6
	12.8	14.2	15.4

average of duplicates measured with the freezing-point meter. All observations are reported; no data were omitted unless there was an obvious malfunction such as a psychrometer chamber leaking and filling with bath water. The correlation coefficient for all these data is 0.8. The only apparent trend in systematic error was that the freezing-point meter tended to predict higher stresses than the psychrometer above 23 bars. While most of the data below this range lies within ± 2 bars of the 1:1 slope line, there was still a significant number of observations outside this margin.

Table 1 summarizes the variation both within the two methods of measurement and between the two methods for a particular plant species tested. Duplication between supposedly identical plant samples was best with the vapor pressure psychrometer. However, freezing-point measurements seldom showed variation between duplicate samples greater than 2 bars. Exceptions to this were noted when using thick outer leaves from cabbage plants and leaves from rose bushes. These two types of plant material did not work well in the freezing-point apparatus. While the average variation between duplicates was only about 1 bar, the average difference between stress measurements made by the two different instruments was 2.6 bars. This indicates that measurements made with the vapor pressure psychrometer and freezing-point depression apparatus are not influenced by water stress in plant material in exactly the same way. Additional variables must be involved.

DISCUSSION

When compared to the vapor pressure psychrometer, the freezing-point apparatus has several distinct advantages. It is inexpensive, requiring less than $100 for all of its components. It is simple to operate and relatively foolproof. A technician can be trained to operate it in a few minutes, and no special techniques for sample preparation or handling are needed. In addition to being portable, it is much faster than the psychrometer. A measurement may be completed in 5 minutes or less, while vapor pressure techniques require at least a 3-hour period for temperature equilibrium and voltage measurements. On a per-day basis, a technician can produce ten times as many stress measurements with the freezing-point apparatus as with the psychrometer. In addition to being expensive, the psychrometer requires much more training time for the technician. The psychrometer is not portable and samples must be transported from the field into the laboratory. Because of temperature changes in transit, it is sometimes difficult to prevent water loss from the sample through condensation on the sides of the chamber used for transport. This can create an error, as can any vapor sinks resulting from surface contamination on the plant samples, such as dust or salt deposits (Klepper and Barrs, 1968).

While the freezing-point apparatus is not subject to these particular errors, it does have some serious shortcomings. One is the temperature dependence of water stress in the sample, since temperature-induced changes of water energy are not the same for all solutes. Consequently, if a calibration curve for potassium chloride at 25 C is used, errors may result from extrapolating back 25 C from the freezing point, particularly when organic solutes with a different temperature dependence than KCl are involved.

Another problem may arise from the concentration of solutes around the ice crystals as they grow in plant material. There is some evidence that ice crystals first appear in intercellular spaces, though in succulent plants the site of this crystal initiation appears to be somewhat random (Idle, 1966, and Meryman, 1956). As the plant solution freezes, the solutes are excluded from the ice phase and concentrate around the periphery of the growing crystals. The concentration of this solution depends upon the freezing rate, the diffusion coefficient of the solutes, and the physical characteristics of the plant tissue such as membrane permeabilities. It is possible that these effects combine to cause a significantly different freezing-point temperature than that of the same osmotic pressure KCl solution suspended in the absorbent paper used to calibrate the instrument.

With the exception of one recent paper (Klepper and Barrs, 1968) it has been generally supposed that the vapor pressure psychrometer, properly and painstakingly operated, will produce the most accurate measurements of plant-water stress. The question is: Will the freezing-point method give sufficiently accurate results so that its advantages of simplicity, portability and speed may be utilized in the field? To answer this, one must look at the natural variation that may occur between duplicate psychrometer measurements made on plant samples collected from the field. Some limited data are summarized in Table 2. These data suggest that one must expect at least a 1-bar variation between duplicates and that a variation of several bars from leaf to leaf will occur. Variations of several bars from plant to plant may be common. Thus the lower accuracy of the freezing-point method may be compensated for to some extent by its ability to provide a greater number of observations per unit time.

Two other methods of measuring plant moisture stress — the pressure apparatus and liquid exchange

reaction — have been compared to the psychrometer. Boyer (1966) found that the pressure chamber yielded values within ± 2 bars of psychrometer measurements for sunflower and yew. In rhododendron, water potential measurements varied by as much as 6.5 bars. These variations were noted even after correcting for the osmotic pressure of the xylem fluid which required the use of the psychrometer. Klepper and Barrs (1968), using the psychrometer and pressure chamber to measure stress in cotton, found differences exceeding 6 bars. Consequently, the pressure chamber alone might at best be expected to produce water potential measurements within ± 3 bars of psychrometer observations. This method is also limited by the kind of plant material that can be sealed into the pressure chamber and does not have the potential for measuring the osmotic pressure component. Knipling and Kramer (1967) published a comparison between liquid exchange and thermocouple psychrometer methods of measuring leaf water stress. They found in several plant species a variation of from 1 to 5 bars between psychrometer values and their water exchange method. The bulk of their data showed scatter somewhat similar to that in Fig. 5. The exchange method and related techniques, such as correlating relative water content to stress, are subject to serious problems caused by imperfect semipermeable membranes in the leaves (Slatyer 1966). In conclusion, it appears that the freezing-point method for making quick measurements of moisture stress in the field offers as much possibility for development as any other technique presently available.

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