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AN INEXPENSIVE METHOD OF DETERMINING PLANT MOISTURE STRESS USING FREEZING-POINT DEPRESSION[1]

H. D. FISHER[2]

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The measurement of freezing point depression as a method of determining osmotic potentials in solutions has been used for some time. Recently, this same principle has been applied to the determination of moisture stress in plant leaves. The advent of efficient solid state Peltier cooling units has made possible the construction of portable field freezing-point meters of relatively low cost and acceptable accuracy (1).

Some problems still exist with these units. The first is that the temperature control circuit for the Peltier battery is relatively complex, requiring some skill on the part of the person constructing its. Next is the fact that the units are not, strictly speaking, portable since they require a large 12-volt DC current and so are restricted to use in a relatively small area around a vehicle whose battery can supply this current. Lastly, the price of parts for these units varies from $200 to $250, which becomes prohibitive if a number of units is desired.

The unit described here eliminates these problems by use of a different cooling method. The Peltier cooling battery is replaced by a can of pressurized Freon 33[3] refrigerant. The freezing chamber is a piece of ¾-inch O.D. copper tubing inserted through and soldered onto the side of a large metal snap-on cap for standard-size aerosol cans. This type of cap is used by several companies packaging aerosol products and is approximately the same diameter as the can. A baffle is installed inside the tubing to prevent direct contact between the refrigerant and the plant sample being frozen. A lever and plunger arrangement (Fig. 1) allows precise introduction of the refrigerant into the freezing chamber. The temperature-sensing circuit is exactly the same as that used in previous models.

The pressurized refrigerant used in this instrument is readily available from electronics supply houses or local electronics stores where it is referred to as "circuit cooler" and is used to locate defective electronic components. Between 20 and 50 samples can be run with one can of coolant, depending upon the temperature of the samples and the ambient temperature. The cost per can is less than $2.00. Obviously, more samples can be run in a laboratory where the ambient heat load is low, or if the samples are run in rapid succession so that the chamber does not have time to warm between samples.

Operation of this unit is essentially the same as for previous models of the freezing-point meter (3). The sample is cut to size and wedged tightly between the two thermistors mounted through a #3 rubber stopper. The stopper is inserted into the end of the sample chamber and refrigerant introduced into the chamber to cool it. With some experience, the operator can cool the sample to near freezing point in a minimum time without overshoot. Since the refrigerant boils at approximately −36°C, it is easy to cool the sample far below its freezing point, completely sinking the heat released when the sample freezes. If the sample is not overcooled during the initial cooling stage, it is simple to maintain an even rate of cooling down to the freezing point. It has been the author's experience that this system does not produce erroneous data; it either gives reproducible results or no indication of the freezing point at all due to overcooling of the sample.

Unlike previous freezing-point meters, this unit requires no initiation of freezing since there is almost invariably a formation of ice crystals on the inside of the sample chamber due to local-

[1] Contribution from the Northwest Branch, Soil and Water Conservation Research Division, Agricultural Research Service, USDA; Idaho Agricultural Experiment Station cooperating.

[2] Electronics Engineer, Snake River Conservation Research Center, Kimberly, Idaho 83341.

[3] Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the listed products by the U. S. Department of Agriculture.

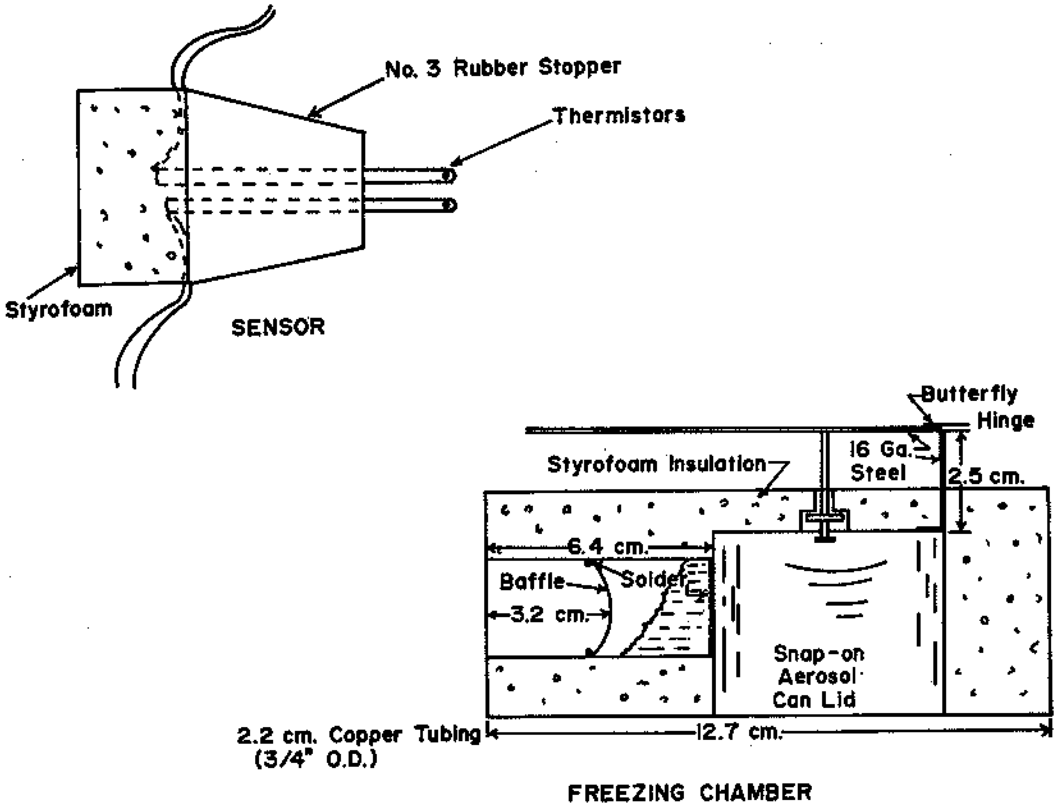

FIG. 1. Cross-sectional view of sample-freezing chamber and sample-holder.

ized cold spots where liquid refrigerant has been blown around the sides of the baffle. If, with extremely dry samples, it is necessary to initiate freezing, the sample need only be touched to the sides of the chamber. Care should be taken not to touch the sample to the bottom of the chamber because there may be liquid refrigerant there, resulting in instantaneous freezing and obscuring of the freezing point.

The meter and switches are mounted on the metal box while the other circuit components are placed inside the box, preferably inside a Styrofoam block for better thermal isolation. The can of refrigerant is inserted through a hole in the top of the box, so that it rests on the bottom, and clamped into place. When the sample chamber assembly is snapped onto the top of the can, the unit is ready for operation. If the can nozzle does not align correctly with the end of the sample chamber, it may be necessary to bend a short piece of the plastic tubing supplied with the coolant to direct the spray more directly down the tube.

When compared with a freezing-point meter using a Peltier cooling battery and sink temperature control, the freon-cooled freezing-point meter results compared within ±1.5 bars on identical samples from the same leaf taken at the same time. Previous results indicate that the variation between identical leaf samples may be ±1 bar, even with a vapor pressure psychrometer (3). The freon-cooled unit exhibited a greater temperature dependance of the calibration curve relating microamperes and bars tension.

One major difference between the unit described here and the Peltier-cooled freezing-point meter is the relationship between the temperature of the sample and that of the sample-chamber walls. In the Peltier-cooled units, the walls of the chamber are at a lower, and more constant, temperature than the plant sample being tested and act as the heat sink. In the unit described here, both the sample and the chamber walls are cooled by the refrigerant, but the chamber walls are also being constantly warmed by conduction through the insulation from the ambient. Because of the difference in masses between the two, the chamber walls are always warmer than the sample. When cooling is stopped, as when the samples begin to freeze, there could be some heat radiation to the sample from the walls, which would be detected in addition to the heat of fusion. This results in a higher freezing point for the sample than indicated by the Peltier-cooled units, the difference being dependent upon the temperature of the chamber walls and hence upon the ambient temperature. When measured, the temperature of the chamber walls remained relatively constant for a given ambient temperature, regardless of the rate of cooling of the sample, which would explain why readings are reproducible at a given temperature while not agreeing between two different ambient temperatures.

As an example of this, 0.2 N KCl gave an indication of $+3.5$ μa at an ambient temperature of 17°C and the chamber temperature was -7.6°C, while the same solution at 30°C gave $+10.0$ μa and the chamber temperature was 0°C. The difference between standard solutions run at different ambient temperatures and a standard calibration developed in the laboratory at 23°C was quite constant over the stress range 0 to -40 bars, simplifying interpretation of the data.

Two methods of interpreting the data are available. In the first, a calibration curve is run at a known constant temperature. Two standards are run at the ambient temperature in which samples are being taken and the difference between these standards and those used to determine the calibration curve is subtracted (or added) to the readings from the samples. The stress of the samples is then read directly from the calibration curve. The second method is to generate a family of calibration curves for different chamber temperatures (as determined by a thermistor bridge). The bridge reading is then recorded with the sample reading and the correct curve used to reduce the data.

In view of the temperature dependence of the readings, the temperature of the freezing chamber should be monitored by using a calibrated thermistor attached to the outside of it. This thermistor is incorporated into another wheatstone bridge in the instrument, in addition to the sample temperature sensing bridge, as shown in Reference 3. The dependence of the readings upon ambient temperature was found to be approximately 0.4 bar/°C change in ambient temperature.

The inherent advantages of this instrument far outweigh the disadvantage of greater temperature dependence. For approximately $70.00, a simple, easy-to-operate system can be constructed which is inherently portable, weighing 2 pounds, and completely self-contained.

SUMMARY

Utilization of the principles of freezing-point depression of plant materials and modern solid state cooling devices has led to the development of portable freezing-point meters for field use. The unit described in this paper eliminates the construction difficulties encountered with earlier units, particularly in the electronic control circuitry, while reducing the cost per unit to approximately $70.00, or one-fourth of the cost of previous models. Certain problems, such as temperature dependence of the readings, become more pronounced with this unit, but are easily overcome. If care is taken in gathering the data, the results compare favorably with other freezing-point meters.

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