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Superimposed Amplified Exotherm Differential Thermal Analysis System[1]

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Abstract. **Superimposed Amplified Exotherm Differential Thermal Analysis System is presented as a system used for detecting low-temperature exotherms of excised dormant flower buds. This system uses thermistors that do not penetrate the tissue and a liquid freezing medium for more uniform temperatures between the sample and reference. A test chamber is presented that can be used with a nonprogrammable freezer. Tests with *Forsythia* flower buds indicate 2 exotherms, the second of which is associated with a dramatic loss of tissue viability.**

Several workers have shown that, by monitoring the temperature of plant samples during cooling, it is possible to detect freezing points of those tissues by recording sudden rises in temperature caused by the exothermic reaction of water freezing (2, 3, 4, 6, 11). The 2 simplest calorimetric techniques for the measurement of these exothermic reactions are thermal analysis and differential thermal analysis (1).

Data from thermal analysis is represented as a cooling curve of the sample temperature vs. time (1). Exotherms from thermal analysis can be easily distinguished if constant cooling rates are maintained by the use of programmable freezing units. Differential thermal analysis (DTA) utilizes two thermocouples in series, one in a reference and one in the sample (1). Data from DTA are plotted as the temperature difference between sample and reference on the ordinate versus sample temperature, reference temperature, or time on the abscissa (1). Most calorimetric techniques have utilized thermocouples, which often were inserted directly into the sample being studied (8). Quamme (7), however, has shown that ice "seeding" of the flower buds by incision and inoculation raises their killing temperatures. Proebsting and Sakai (5) have stated that, in many instances, the insertion of thermocouples into peach flower buds also resulted in raising the killing temperature during controlled cooling.

Superimposed Amplified Exotherm Differential Thermal Analysis System (SAEDTAS) is a modification of conventional DTA in that: (a) it uses thermistors instead of thermocouples, (b) it uses a liquid medium instead of air to surround the sample, (c) it does not require penetration by the temperature sensor into the sample being tested for good exotherm resolution, and (d) at the moment of ice formation, the temperature differential between the sample and liquid medium is sensed, amplified by a factor of 10, and superimposed on the medium temperature versus time curve. SAEDTAS is made up of 3 parts: differential temperature sensor circuitry, regulated power source, and the freezing units.

The differential temperature sensor is a 2-channel system (Fig. 1). Each channel consists of a bridge circuit, an input amplifier stage, and an output amplifier stage. The bridge resistors are selected so that at 0°C the output is 0.0 volts. The regulating variable resistor is adjusted for about −8 mV output when both thermistors are at −50°. As long as both thermistors are at the same temperature, the output will be a smooth curve following the change in medium temperature. When the thermistor monitoring bud temperature senses a sudden change in temperature due to exotherms, the output will reflect this change by a shift in output voltage. This shift will be followed by a return to the original curve shape. The input and output amplifiers are integrated circuits and require low power.

For the differential temperature sensor to function properly, the power source must be regulated. The regulated power supply consists of power transformer, bridge rectifier, filter, a plus 5-volt regulator, and a minus 5-volt regulator. These regulators are integrated circuits and provide good regulation for the bridge circuit and the integrated circuit amplifiers.

The lack of a programmable freezer made it necessary to develop a freezing unit that could be placed into a precooled chamber with the resulting cooling curve of the freezing unit's inner core being a reflection of how well it was insulated. This was possible because SAEDTAS was designed to detect differential temperatures in addition to recording medium temperature as a reference. This unit consisted of a large styrofoam pot filled with perlite in which a 250-ml Erlenmeyer flask filled with 95% ethanol was imbedded (Fig. 2). Positioned in the flask was a 17 mm by 120 mm test tube, which contained the liquid medium (FC-80, a nontoxic fluorocarbon having a high oxygen affinity and low freezing point, IBM, St. Paul, Minn). The liquid medium, similar to one used by Quamme et al. (8) for apple stem segments, was used to enhance thermal conductivity of the heat released by freezing of the flower bud to the live sample thermistor and to guard against temperature stratification between the live sample thermistor and the medium thermistor. The thermistor leads were expoxied to a glass stirring rod, which was placed in the test tube containing the liquid medium. The sample

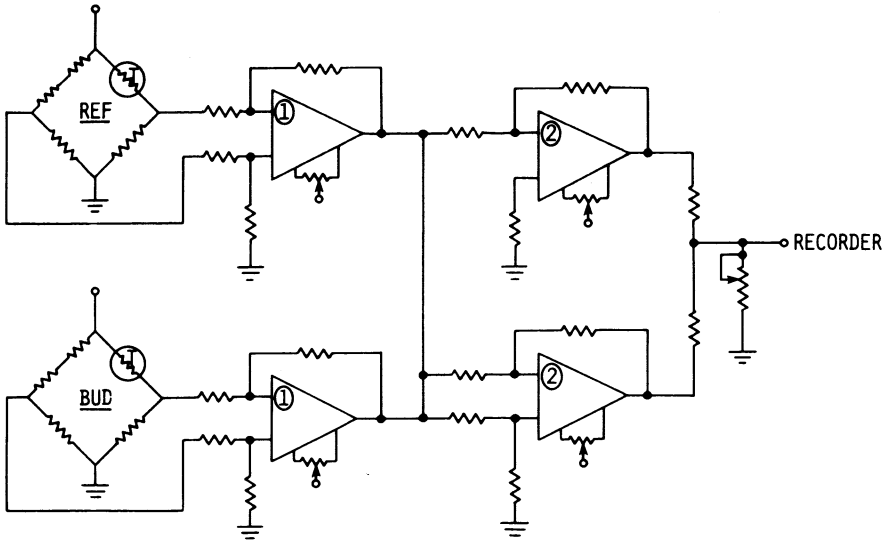

Fig. 1. Differential temperature sensor circuitry for the Superimposed Amplified-Exotherm Differential Thermal Analysis System (SAEDTAS). T, 1, and 2 represent thermistors, first and second integrated circuits, respectively.

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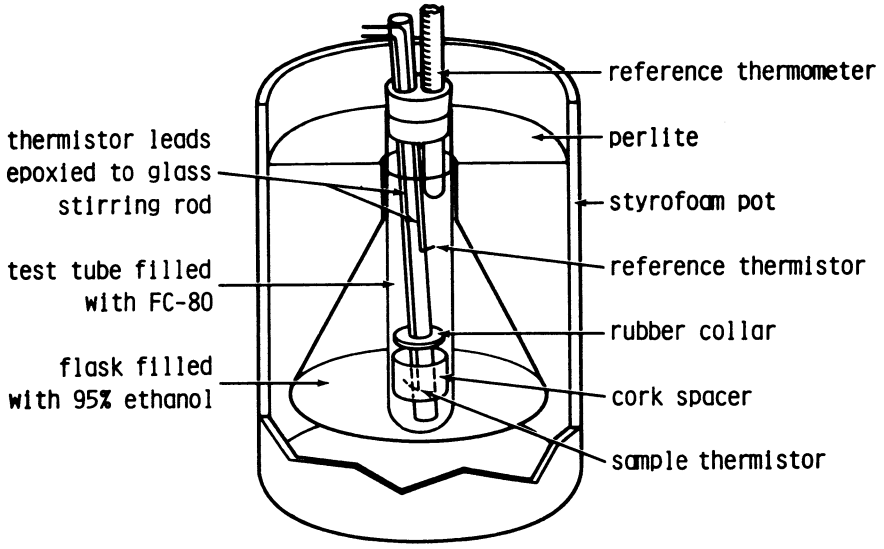

Fig. 2. Diagram showing the construction of a freezing unit of SAEDTAS.

thermistor was surrounded by a cork spacer which was covered by a rubber collar. A small amount of the cork was removed to make space for the thermistor and the bud to be tested. Five buds have been accommodated at one time by removing more of the cork. It should be possible to test larger buds as well, as long as they will fit into the cork near the thermistor.

Operation of SAEDTAS was test by using *Forsythia* 'Sunrise' flower buds that were taken from hardwood cuttings obtained from container-grown shrubs being overwintered in storage at 4–6°C. After removal from the shrubs, hardwood cuttings were placed immediately in polyethylene bags and stored in darkness at 4° until buds could be excised just before testing. SAEDTAS freezing units also were stored at 4°. When a freezing test was to be made, flower buds were excised at random from hardwood cuttings with at least 2 mm of flower bud pedicel attached (7) and positioned individually in contact with the sample thermistor. Freezing units then were placed in a cryostat, which had been cooled to −30°C. Subsequent cooling curves were recorded with Microsen 1300S-XMK recorders.

SAEDTAS freezing curves of single 'Sunrise' *Forsythia* flower buds exhibited a broad exotherm from −8 to −10°C and a second sharp exotherm near −23 (Fig. 3). This second sharp exotherm is associated with a dramatic loss of tissue viability as verified by a refined triphenyl tetrazolium chloride reduction assay (9) and direct measurements of flower bud respiration after cooling using a differential respirometer (unreported data). This pattern of freezing curve kinetics is similar to that of acclimated peach flower buds (1) and is thought to represent the following general sequence: supercooling, intercellular freezing (first exotherm) deep supercooling, intracellular freezing (second exotherm), death (10).

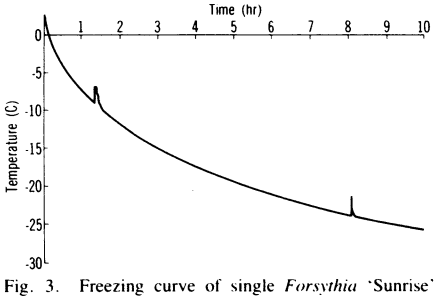

Fig. 3. Freezing curve of single *Forsythia* 'Sunrise' flower bud as recorded by SAEDTAS showing the first broad exotherm from −8 to −10°C and a second sharp exotherm near −23°.

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Evaluation of Apricot Flower Bud Hardiness Using a Computer-assisted Method of Thermal Analysis[1]

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Abstract. **A method for large scale evaluation of flower bud hardiness in apricots using a thermal analysis system interfaced to a computer is described. The technique measures the heat released during the lethal freezing of supercooled water within the bud primordia. Nine thermoelectric junctions wired in series were used to monitor the temperature of 10 individual buds. Bud temperature was scanned every 30 seconds and the data recorded on magnetic tape. The data were subsequently transferred to a minicomputer which analyzed and stored data and produced graphics. Computer assisted thermal analysis can accommodate a large number of samples and simplifies handling and storage of data. This technique has applications as a research tool, for determining critical bud temperatures and in screening selections from a breeding program.**

The dormant flower buds of several important horticultural crops supercool and thereby avoid freezing injury (1, 2, 4). Thermal analysis studies have demonstrated the occurrence of a low temperature exotherm resulting from the freezing of supercooled water. Quamme (4) demonstrated that the temperature at which this exotherm was initiated was closely correlated with the temperature of freezing injury in several species of *Prunus*. Thermal analysis has been employed primarily in research on the nature of freezing in-

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