A Computerized, Multiple-chamber Controlled Freezing System

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Additional index words. cold hardiness, freezing injury, differential thermal analysis

Summary. An inexpensive controlled freezing system that allows the temperature regime of eight modular sample chambers to be varied independently was constructed. A microcomputer-based data acquisition and control unit controls the freeze–thaw cycle in each of the chambers, as well as recording sample temperatures at desired time intervals. The computer also controls the freeze–thaw cycles in, and records data from, two differential thermal analysis units and records data from an electrical conductivity meter.

Controlled freezing chambers are used in cold hardiness and freezing injury studies because they allow investigators to maintain control over the freeze–thaw protocol of samples. Various chamber designs have been described previously (Gilreath et al., 1982; James, 1979; Schneider et al., 1958; Scott and Spangelo, 1964). In the most-basic systems, samples enclosed in insulated containers are placed into freezers to cool and are removed when the samples reach desired temperatures. With this method, accurate control of cooling and warming rates is difficult, and the range of available rates limited. Precisely maintaining samples at specific temperatures during freeze–thaw cycles is also difficult, particularly when the experimental design requires holding several samples at different temperatures simultaneously. Numerous investigations, however, have demonstrated the influence on tissue survival of cooling rates, warming rates, prefreezing temperatures, and exposure times during freezing stress studies (Finkle et al., 1974; Pogosayan, 1971; Pogosayan and Sakai, 1973; Sakai, 1965; Weiser, 1970).

Freezing studies were improved through the use of programmable freezers that provide increased control of freeze–thaw protocols. The difficulty with these units is that each freezer can be used only for a single freeze–thaw protocol at one time. When very low temperatures and slow cooling and warming rates are used, it becomes difficult to process samples from several treatments without having some samples collected later than others or stored for relatively long periods of time. Both of these practices have the potential to create artifacts in resulting data. Gilreath et al. (1982) addressed this problem by connecting eight small freezers to a computerized control system to conduct chilling requirement studies in blueberries and peaches. While the design was an improvement over previous units, the minimum temperature of the freezers used was only –18C and the maximum warming rate was limited by the rate of heat gain from the laboratory. Using individual freezers as modular sample chambers also adds bulk and expense to the system, while decreasing flexibility.

The primary objective of this project was to: a) develop an inexpensive controlled freezing system that would allow multiple samples to be exposed simultaneously to a wide range of freeze–thaw protocols; b) use a microcomputer to control and record sample temperatures; and c) incorporate the controlled freezing system into an integrated cold-hardiness laboratory system that controls and records data from differential thermal analysis (DTA) units and an electrical conductivity meter.

The controlled freezing system is divided into three components: 1) a low-temperature freezer; 2) a computer and associated data acquisition and control peripherals; and 3) modular sample chambers.

The freezer used in this system provides a temperature range of –18 to –85C. The computerized data acquisition and control system is based on the design used by Wolf and Pool (1986) to control and acquire data from differential thermal analysis (DTA) units. A low-speed, multi-function, analog / digital I / O expansion board (Dascon-1, MetraByte, Taunton, Mass.) installed in an IBM-compatible microcomputer serves as the heart of the data acquisition and control system. The board provides four analog input channels, each of which has a resolution of 12 bits (±0.5 mV / bit). There are 30 A / D conversions per second. An expansion sub-multiplexer board (EXP–16, MetraByte) was modified to fit the Dascon-1 board and provides up to 16 inputs / outputs for each of the three available Dascon-1 channels. One of the I / O channels is occupied with a thermocouple cold junction compensator located on the EXP-16. A solid-state relay board containing eight relays controlled by the I / O board regulates the sample chamber heaters. A separate relay interrupts power to all chambers if one or more overheats. A screw-terminal board, control panel with an on–off switch for each chamber, power junction box inside the freezer, thermocouple and DTA lead junction box inside the freezer, and accessory junction box on the external control panel complete the control and power portions of the system. During controlled freezing runs, a monitor shows real-time calculated and actual chamber and sample temperatures, elapsed time, and chamber status (heating, cooling, or holding). A strip-chart recorder is connected to the system to allow real-time observations of exotherms from representative samples during DTA. DTA data from other samples is simultaneously digitized and recorded by the computer. A diagram of the controlled freezing system is shown in Fig. 1.

Sample chambers are constructed of two concentric cylinders made from aluminum irrigation pipe (7.7- and 10.2-cm outer diameters, respectively) separated and held in place by plywood bases and closed by polyurethane foam-insulated plywood lids. The aluminum walls are 1.5 mm thick and each chamber has an inner length of 26 cm. The outside of each chamber is

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Research supported by the Northwest Small Fruit Research Center and the Idaho Agricultural Experiment Station. Mention of a trade name does not constitute guarantee of a product, nor does it imply the approval of any product to the exclusion of comparable products. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement *solely to indicate this fact.*

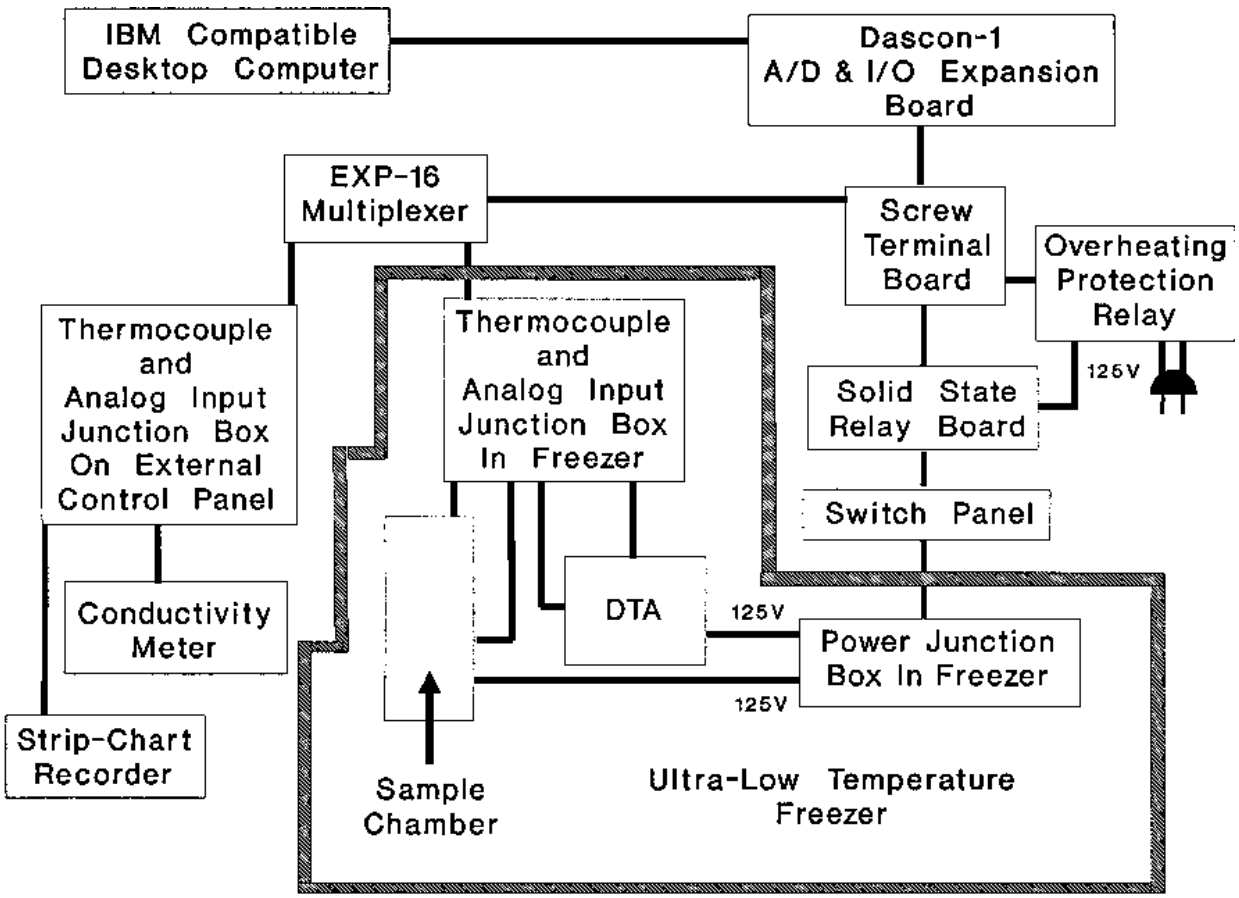

Fig. 1. *Schematic diagram of the integrated cold hardiness laboratory system.*

insulated with 2.5-cm-thick polyurethane foam held in place with fiberglass-reinforced tape. No insulation is used between the aluminum foil-wrapped samples and inner chamber wall. Two heavy wire frames attached to opposite sides of the plywood base and secured between the foam and fiberglass tape provide support for the lid hinge and stainless steel spring and hook assembly, which hold the lids securely in place. Heat to maintain desired sample temperatures is produced with high-temperature nichrome heater wire wrapped around the outside of the inner aluminum cylinder. The wire is 0.40 mm in diameter, insulated with 4.3-mm-diameter ceramic beads, and rated at 400 W. A total of 365 cm of wire is used per chamber. Two grooved, steel bars attached to the outside of the inner cylinder keep 14 wire turns spaced 15 mm apart and securely in place. The heater wire is connected to a three-wire electrical cord and plug, which connects to the power junction electrical outlet array inside the freezer. Each wire receives a line voltage of 117–125 V, 60 Hz AC power through a solid-state relay. The relay is controlled by the computer system, which regulates the amount of time power reaches the heater wire based on sample temperature.

Fiberglass insulation covers the heater wire and fills the void between the two cylinders. A 0.6-mm-diameter (24 AWG) copper–constantan thermocouple is used to monitor the chamber wall temperature, and allows for overheating protection. The soldered thermocouple tip, insulated with PVC heat-shrink tubing, is attached to the inner cylinder using a screw. If the wall temperature of any sample chamber exceeds 50C, power to all chambers is cut off by a computer-controlled relay. A similar thermocouple with an uninsulated tip is inserted through the center of the lid to monitor sample temperature. To reduce heat loss through the thermocouple wire to the air outside of the chamber, and subsequent inaccurate sample temperature indications, the sample thermocouple wire is coiled tightly several times just above the sample. The thermocouples are calibrated between –80 and +5C using a commercial electronic thermometer. When measured at the thermocouple inputs on the multiplexer, approximately 4 mV of random, high-frequency (≈14 MHz) electronic noise is generated in the thermocouples by the computer and monitor, but virtually all of this noise is removed by differential amplifiers on the Dascon-1 board. Fluctuations in the sample temperature graphs in Fig. 3 are actual thermal fluctuations, not electronic noise. Thermistors could be used in place of the thermocouples to achieve greater sensor accuracy, but their cost is greater than thermocouples.

From one to eight chambers may be connected to the system at one time. An additional multiplexing unit could be used to add more chambers. Miniature thermocouple connectors are used in the junction boxes. Type-T connectors are used for thermocouples and uncompensated, copper–copper connectors are used for DTA and other non-thermocouple leads.

Polyvinyl chloride (PVC) electrical insulation became brittle and shattered when used at –80C, so teflon-insulated type-T thermocouple wire is now used for the thermocouples and nickel-plated copper, teflon-insulated thermocouple extension wire protected

with tinned copper overbraid is used for the DTA leads. The nichrome heater wires in the sample chambers are connected to the power junction box using heavy-duty, neoprene-insulated, three-wire electrical cords. The insulation on these wires has not shown any damage when used at –80C. A diagram of a sample chamber appears in Fig. 2.

When the controlled freezing sample chambers are not in use, the system can be used for DTA by connecting the DTA block heaters to the power junction outlets and thermal sensors to plug connectors on the junction box inside the freezer. An electrical conductivity meter used to measure tissue electrolyte leakage following freeze–thaw cycles, a pH meter, and an electronic balance can be connected to the system by means of an accessory junction box attached to the multiplexer. The desired application is accessed through computer software. No rewiring or hardware modification between applications is necessary. Two sample temperature junctions inside the freezer are connected to the external junction box, allowing sample temperature to be recorded continuously on a stripchart recorder. Likewise, the external junction box allows the output of two DTA samples to be monitored on a stripchart recorder.

To operate the controlled freezing system, the freezer is equilibrated at –80C. Samples with thermocouples inserted are wrapped in aluminum foil and placed into the chambers. The aluminum foil, which also covers the thermocouple wire coil described above, provides even heat distribution around the sample and helps hold the thermocouple in place. We found that adding insulation between the inner wall of the module and the sample greatly increased sample temperature fluctuation. The samples and chambers are equilibrated at about 4C in a refrigerator. If ice nucleation is a concern, samples are wrapped in moistened laboratory tissue, or are prepared in a cold room and inoculated with ice crystals before being equilibrated at a subfreezing temperature. When the freezer and sample chambers have equilibrated, the chambers are placed into the main freezer compartment and the thermocouples and power plugs are inserted into their junction connections.

Menu-driven software written for this application (GW-BASIC, Microsoft Corp., Redmond, Wash.) is used to set the initial holding temperature and time, cooling rate, minimum temperature, holding time at minimum temperature, warming rate, and final holding temperature for each module. Up to eight different protocols can be used simultaneously. A fill-in-the-box array is used to modify preset defaults, if desired. Once the program is activated, the computer controls the temperature of each chamber and records sample temperatures at a selected time interval. Using the cooling / warming rates and holding times selected, along with elapsed time, the software calculates the desired sample temperature for each module. If a sample temperature is below that calculated, the relay for that particular chamber is closed and electricity applied in a brief pulse to the heater wire. If the sample temperature is at or above the calculated value, the relay remains open and no heat is applied to the chamber.

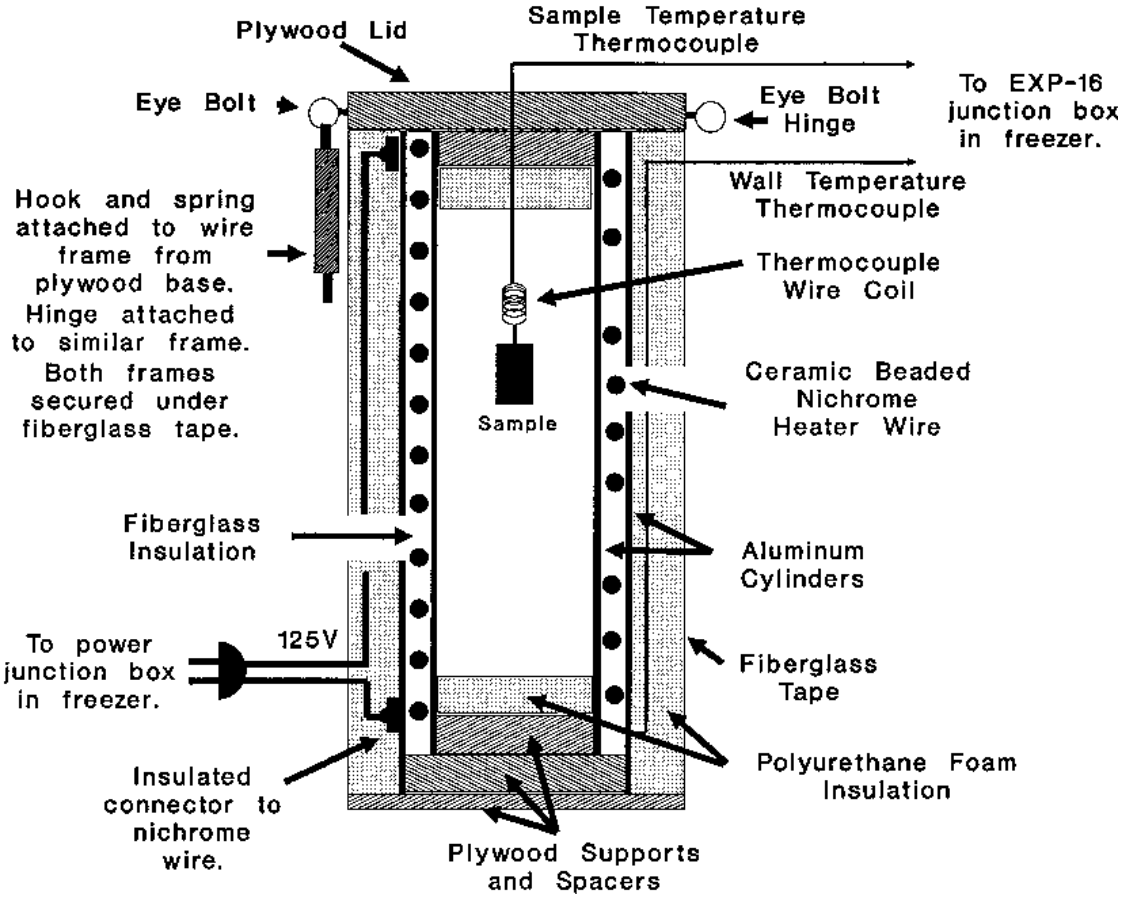

Fig. 2. *Schematic diagram of a modular sample chamber.*

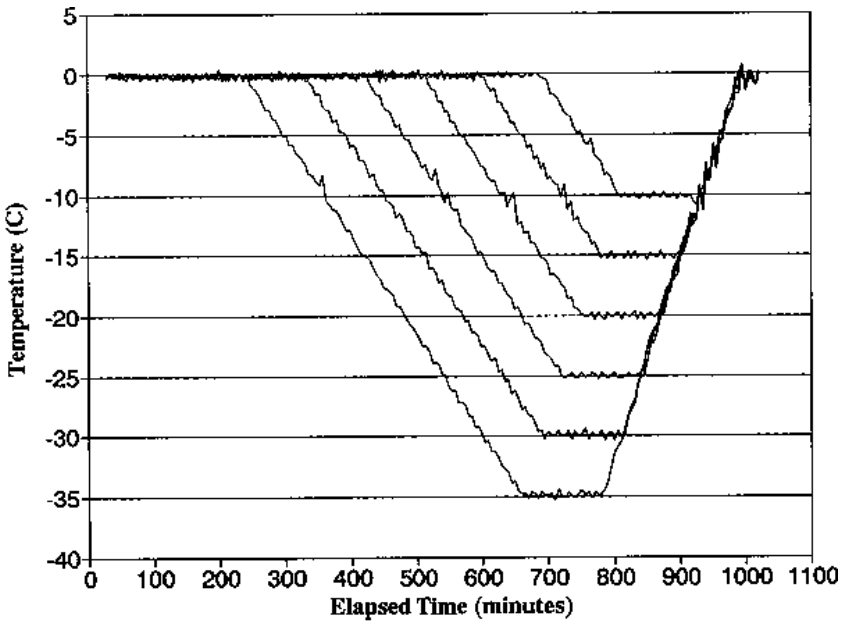

Fig. 3. *Sample temperatures of dormant raspberry canes cooled at 5C/h and warmed at 10C/h. Variations in sample temperatures in the –5 to –15C range are exotherms caused by ice formation in the samples.*

Sample temperatures from a controlled freezing run are shown in Fig. 3. Six raspberry floricane samples were cooled at 5C / h to minimum temperatures of from –10 to –35C. Each sample consisted of a bundle of five 2-cm-long internode sections banded together and wrapped in aluminum foil. Samples were held at their minimum temperatures for 2 h and then warmed to 0C at 10C / h. Initial holding times at 0C were staggered so that all samples reached their final holding temperature of 0C simultaneously. This protocol is used to eliminate artifacts caused by some samples remaining thawed longer than others, and makes it possible to load all samples into the freezer in the late afternoon, and have them reach their final holding temperatures by the beginning of the next work day.

The system described above could be improved by using proportional voltage control on heater inputs to reduce heating overshoot and the minor sample temperature fluctuations shown in Fig. 3. Thermistors would give greater temperature sensor accuracy, but are significantly more expensive than the thermocouples described here. An uninterruptible power supply (UPS) backup to the computer would help avoid data and sample loss in the event of brief power outages.

Samples of the software used for the controlled freezing chambers and electrical conductivity meter may be obtained by writing to D.L. Barney.

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Comparison of Packing Systems for Injury and Bacterial Soft Rot on Bell Pepper Fruit

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Additional index words. *Erwinia carotovora, Capsicum annuum,* chlorination, storage, postharvest handling, pathology

Summary. Commercial packing lines in Sampson County, N.C., were surveyed during two growing seasons to study handling methods on susceptibility of bell pepper fruits (*Capsicum annuum* L.) to bacterial soft rot (*Erwinia carotovora* subsp. *carotovora*). Samples were taken from two field packers and one packing house in 1991 and from two field packers and four packing houses in 1992. One field packer and one packing house were common to both years. Fruits were either inoculated with bacteria or untreated and stored at 10 or 21C. Damaged fruits were counted and classified as crushed, cut, bruised, abraded, and other injuries. Fruit

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