Abstract. An inexpensive chamber for controlled freezing of large container-grown plants up to 2 m in height was constructed using liquid nitrogen as a refrigerant. A microcomputer-based system was developed to control the cooling sequence and to collect data on tissue temperature, air temperature, and exotherms. Versatile software was written that allowed the programmed rate of temperature drop to be based on either tissue temperature or air temperature.

Freezing chambers are useful for studying plant cold hardiness because they can provide controlled stress temperatures without having to rely on nature. They also can be used to control the rate of temperature decline, to establish different temperature regimes, and to subject plants to desired temperatures during different stages of plant growth. Many chambers have been portable and designed for freezing whole trees in the field without damage to root systems. Most chambers have relied on mechanical refrigeration and were expensive. The vaporization of liquid nitrogen as a method of cooling has been used (6, 8). Recently, computers and electronic hardware have been used to record and control the rate of cooling (4).

The objective of this investigation was to design and construct a versatile controlled-freezing system for studying cold injury of intact container-grown peach trees. The project involved the integration of a cooling system, using liquid nitrogen, and a microcomputer control system. The chamber was constructed of wood and insulated with Styrofoam. It consisted of four identical wall sections (≈120 × 240 cm) and a separate top (Fig. 1). The wall sections had an inner and outer wall of 0.64-cm-thick plywood attached to a wood frame. Glued to the outer wall of the sections and inside their frames were three sheets of 5-cm-thick Styrofoam, resulting in a total insulating value 4.5 °C·m·2-W¹1 (R = 25.5 °F·ft·2-Btu¹1). The construction provided space between the Styrofoam and the inner wall for circulation of air when the chamber was in use. Styrofoam (three sheets) was used to protect root systems from cold temperatures. All wood to wood and wood to Styrofoam joints were sealed with caulking. Wall sections were attached to one another with 10-cm brass door hinges. The top was similarly constructed. The door had four casters underneath to aid in opening and closing. Closed cell foam rubber strips were placed around the inside of the door to assure tight sealing. For closure purposes, threaded rods at four positions were placed through holes of steel plates attached to one wall section then passed through other steel plates attached to the door. The door could then be closed tightly by threading a nut on one end of the rod, which already had a nut welded on the other end.

For air circulation within the chamber, vents were cut in the inner walls. To provide air circulation, a hole was cut in the center of the inner plywood sheet of the top and an exhaust fan rated to move 6.8 m³ of air per min (Pamotor model 7606) was attached. The air was pulled from the inner chamber into the space in the top.

Cooling was provided by the vaporization of liquid nitrogen in the chamber. The liquid nitrogen was supplied by a 160-liter tank (Union Carbide POL model LS-160S) equipped with a low-pressure liquid outlet and pressure relief valve. From the tank, the liquid nitrogen moved through 1.6 cm (o.d.) copper tubing, which was reduced to 1.0 cm (o.d.) before being connected to a solenoid valve (Skinner model V52DB2100). The copper tubing connected to the outlet side of the solenoid valve, then passed through the back wall section of the chamber and extended into the inner chamber space. The copper tubing in the inner chamber was crimped at the end, and had 10 equally spaced holes on the upper side 4.5 cm apart and 0.5 cm in diameter. Any liquid nitrogen that did not vaporize immediately was caught in an aluminum pan suspended beneath the copper tubing. All copper tubing outside of the chamber was insulated with 2.6 cm of foam rubber.
Fig. 1. Diagram of freezing chamber. Arrows indicate air circulation pattern. (A) fan, (B) liquid nitrogen outlet and catch-tray, (C) Styrofoam, (D) inner walls, (E) holes in inner wall for air circulation, and (F) door.

Fig. 2. Diagram of electronic control system for freezing chamber.

Monitoring of the temperature within the freezing chamber, the control of the solenoid valves, and storage of input and output data were accomplished by a computer and interfacing hardware system (Fig. 2). Temperatures inside the chamber were measured using eight cooper-constantan (Type T) thermocouples wrapped in a grounded aluminum foil shield. They were connected to a remote analog to digital I/O module (ADC-1, Remote Measurement Systems, Seattle, Wash.), which performed the necessary input signal amplification and A/D conversions. The ADC-1 was powered by a +5-V (direct current) power supply (SOLA type 130C), which also powered the General Electric 4N33 optoisolator interface for the solenoid valve.

Inputs to the ADC-1 were used to sense temperatures within the chamber while an output from the ADC-1 was used to regulated the flow of liquid nitrogen to the chamber. Actual A/D conversions were performed by a 13-bit converter over an input voltage ranged of ±0.4 V. With this input voltage range and 13-bit conversion resolution, the system could detect 0.1-mV changes in inputs. All thermocouple signals were amplified ×10 before A/D conversions were performed. Amplification of thermocouple signal allowed the system to detect temperature changes as small as 0.26°C. A temperature sensor in the ADC-1 was used to determine the temperature of the reference junction in the thermocouple.

The ADC-1 was connected to a Columbia MPC-VP microcomputer through an RS-232C interface. The Columbia microcomputer contained 256 KRAM and two disk drives. A program to enable the computer to control the entire system was written in Microsoft Interpretive BASICA (Microsoft, Bellevue, Wash.). This program consisted of six sections: the precooling segment, the temperature at which the controlled rate of temperature decline began, the rate of temperature decline, the desired minimum test temperature, the length of time this temperature was to be maintained, and the data retrieval segment. Temperatures 55°C below ambient temperatures were attainable. Temperature at any given time throughout the chamber varied <1°C.

Trees used in all studies were ‘Loring’/‘Haliford’ grown outdoors for 1 year in 12-liter containers. Following leaf drop they were held in a cooler at 4°C until used in testing. In each test, 10 trees were cooled to each temperature. Thermocouples were inserted into the cambium of tree trunks.

The computer program was designed to be flexible and easily adapted to the user’s established parameters. Tree tissue temperature closely followed the programmed rate of temperature fall when mean tree tissue temperature was the controlling parameter in the computer program (Fig. 3). Once the desired temperature was reached, tree temperatures were maintained for the desired amount of time. The air temperature in these tests was always below the tree tissue temperature during the period of temperature fall; that is, a lag in tissue temperature. During the final 60 min that the temperature remained constant, air and tissue temperatures were similar. Larger fluctuations occurred in the mean air temperature than in the mean tree tissue
Fig. 3. Example freezing curve using tissue temperature for controlling system.

Fig. 4. Example freezing curve using air temperature for controlling system.

temperatures. The air temperature dropped sharply at −2.5°C and did not return to the slope of the programmed temperature fall rate until −4.5°C. An exotherm occurred as bulk water in the tissue froze, releasing latent heat and causing the system to require colder air to keep the tissue temperature drop consistent.

Additional tests were conducted in which the mean air temperature controlled the computer program (Fig. 4). Mean air temperature did not follow the programmed rate of temperature fall as closely as mean tree tissue temperature did when it controlled the computer program. Mean air temperature never deviated more than 0.5°C from the programmed temperature. Mean tree tissue temperature drop again lagged air temperature during the entire test. The mean tree temperature moved away from the slope of the temperature fall line at −2.5°C, then resumed a cooling rate similar to the programmed cooling rate until the minimum programmed temperature was reached. This deviation resulted from release of latent heat during the bulk water exotherm.

The freezing chamber materials cost about $350, and the electronic hardware (excluding the computer) cost about $400, making the entire system much less expensive than mechanical freezing chambers available on the market today. The amount of liquid nitrogen necessary for each of the tests conducted in this investigation cost about $15. The only electrical requirements were a 120-V alternating current outlet and a 5-V direct current power supply.

The system was efficient and inexpensive to build and operate. The electronic hardware made temperature monitoring accurate and precise. The computer program was flexible and easily modified to any test parameters desired. The rate of cooling of tree tissue closely followed the programmed rate of cooling, yet air temperature and other media temperatures could be monitored. This system could be adapted for studying cold injury of relatively large specimens of many species.

Literature Cited