A Versatile Temperature Control System for Cooling and Freezing Biological Materials[1]

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Abstract. **A precise temp control apparatus is described which is useful for studying plant responses between $+50^{\circ}$ and -100°C.**

Several cooling systems have been devised which utilize the vaporization of liquid nitrogen for freezing biological materials (1, 3, 4). In this report, we describe how automatic proportional control devices developed for physical engineering applications can be used to provide a wide range of cooling and thawing rates for biological studies. We have found the apparatus to be especially useful for freezing hardy plant tissues where precise control of temp between $+50^{\circ}$C and -100° is desired.

The control system consists of a programmer, recorder, time proportioning controller and solenoid valves. In the configuration shown in Fig. 1 the sample temp is detected by a thermocouple. This signal is amplified by the recorder and relayed to the controller. The controller also receives a signal from the programmer as it reads the temp program you have provided. You can make programs for any arbitrary temp vs. time profile by simply drawing them with a stylus on plastic cards which are then mounted in the programmer. The controller compares the sample and programmer signals and generates a power signal. This signal is used to rapidly open or close a solenoid valve which regulates the flow of cold nitrogen vapor or cooled air to the sample or to turn an electric heater on or off. The controller power signal can be used to control sample temp in a variety of ways since it performs a time proportion function which simply regulates the on and off duration of cooling or warming cycles.

Fig. 2 illustrates the components of the control system and several of the configurations that we have used for controlled freezing of plant samples. Panel A of Fig. 2 shows the 2 alternative recorder controller configurations which were tested. One system was a combination of an Electronic 19 recorder and an Electropulse controller (Honeywell Control Systems, Minneapolis, Minn.) (See Fig. 1). In the other system these 2 components were replaced with a Twintrol Model 630 controller (Research Incorporated, Minneapolis, Minn.). A Datatrak programmer (Research Incorporated, Minneapolis, Minn.) was used with both of the recorder controller systems. Numbers (1, 2, 3, 4, and 5) in Fig. 2 designate the input and output signals. The advantage of the Twintrol controller lies in its ability to accept a signal directly from the sample thermocouple and to produce independent cooling and heating signals. The unit is also available with rate or time proportioning on either or both of the heating and cooling channels.

Fig. 2B, C, D illustrate 3 of the ways in which the control systems were used to cool plant material. In Fig. 2B the controller signal was used either to control the on-off cycle of an electric heater or the opening and closing of a solenoid valve which regulated the discharge of liquid nitrogen vapor under low pressure into a test chamber. The test chamber was a styrofoam ice chest and samples were enclosed in plastic bags to avoid direct contact with the nitrogen atm. A heater was used to accelerate warming.

Fig. 2C illustrates another configuration where the control system was used to control the flow of pressurized liquid nitrogen vapor through a heat exchanger placed between the nitrogen tank and the solenoid valve. This system was used to cool plant material in contact with a metal block heat sink or on a microscope stage.

In Fig. 2D two solenoid valves in parallel, one opening when the power was on (A), and the other (B) opening when the power was off, were used to control the proportion of air passing through either a cold loop of copper tubing immersed in a dewar flask of unpressurized liquid nitrogen or a hot loop which was warmed with an electric heater. In this case the hot and cold streams of air were mixed and discharged through a manifold into a styrofoam test chamber in which intact plants and plant parts were subjected to controlled freezing. A drying material (Linde Molecular Sieve, Union Carbide Co.) was used to dry the air and prevent icing of the cooling coil. Mechanical dryers are also available for providing dry air (2).

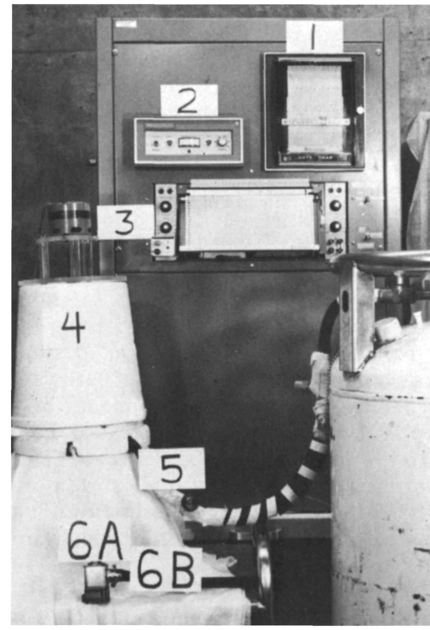

Fig. 1. The components of the control system: 1) The programmer, 2) electropulse controller, 3) recording potentiometer, 4) test chamber with mixing fan on top, 5) low pressure liquid nitrogen supply line from tank, 6A) solenoid valve, and 6B) signal power leads to the solenoid from the controller.

The working ranges of the control systems are from $+50^{\circ}$C to below -100°. The accuracy of temp control is difficult to express since temporal and spatial factors vary with the desired application. In our studies air temp were controlled in small chambers with an accuracy of $\pm 0.2^{\circ}$ at a single point and between different points. The proportional control systems also afford rapid response capabilities. This combination of accurate temperature control and rapid response as well as the wide range of sample configurations and operating temp possible, make this type of system very useful for many biological applications.

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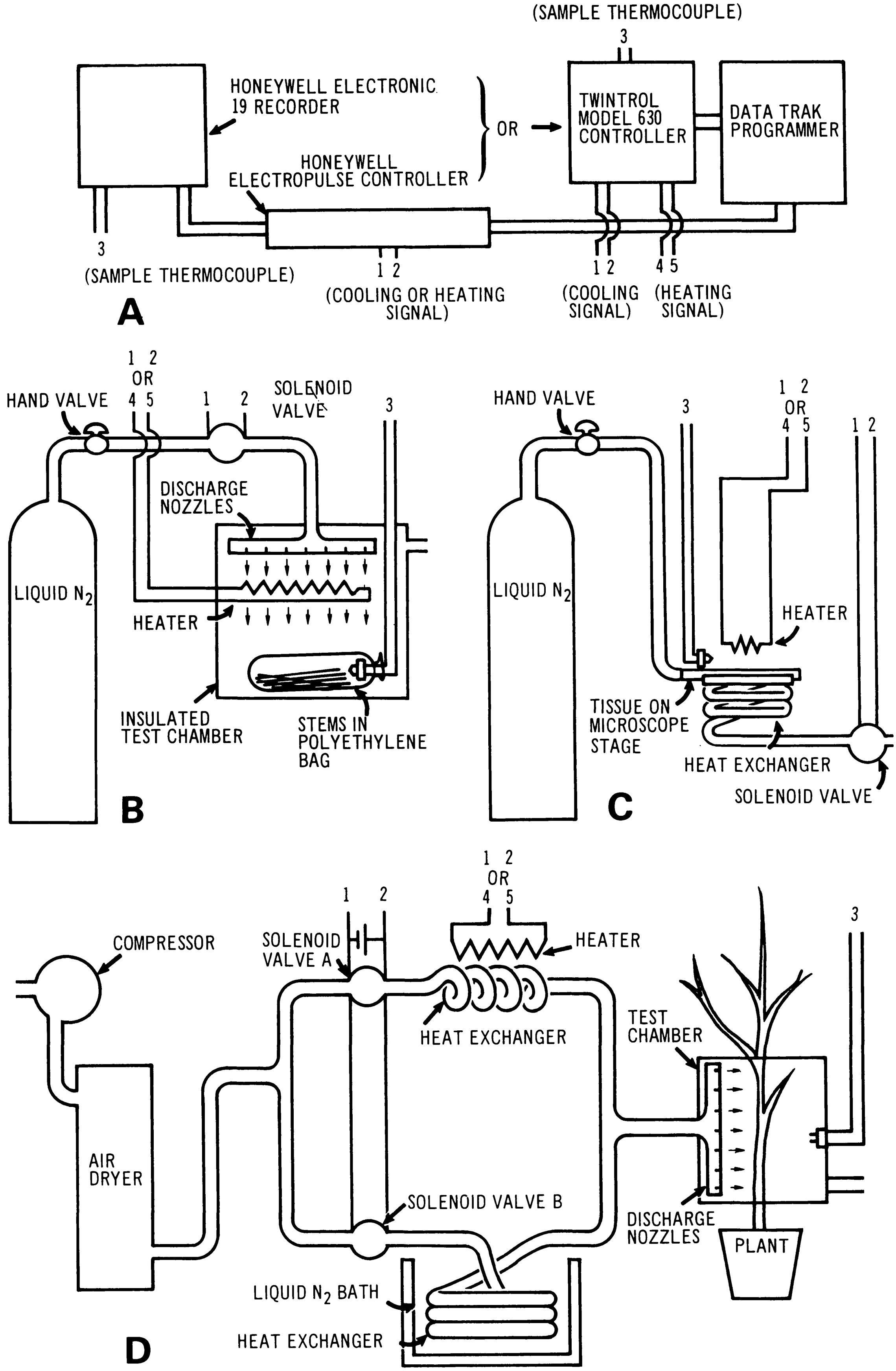

Fig. 2. Several control configurations used to freeze plant materials: A) The control components (numbers 1, 2, 3, 4 and 5 designate input and output signals); B) A system for direct vaporization of pressurized liquid nitrogen in a test chamber; C) The use of pressurized nitrogen to cool plant material in contact with heat exchanger; D) A system for cooling or warming a stream of air using unpressurized liquid nitrogen as a coolant. Air is valved either through the hot side, valve A, or the cool side, valve B, as the controller calls for heating or cooling.