A Temperature-Gradient Plate Designed to Function Near and Below 0 C

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ABSTRACT

A modification of a temperature-gradient plate apparatus to function in the range of 10 C to -10 C is described. Growth and sporulation of fungi can be accurately evaluated at low temperatures with this system.

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Pathogenesis in fungi active during the winter is often limited to narrow temperature ranges and is thus readily influenced by minor changes in weather. Certain pathogens of winter wheat such as *Cercosporella herpotrichoides* and *Puccinia striiformis* continue to sporulate at temperatures as low as 0 C, the latter capable of sporulating under snow cover (3, 7). Bruehl & Cunfer (2) reported that virulence and production of sclerotia varied among three snow mold pathogens (*Typhula idahoensis, T. incarnata*, and *Fusarium nivale*) at temperatures of 1.5, 0, and -1.5 C.

Although critical evaluation of the effects of temperatures near and below 0 C is difficult using traditional approaches, many problems can be circumvented by utilization of temperature-gradient plates designed to provide a uniform gradient of temperatures across the surface of an aluminum plate. These plates are ideal for study of the effects of small changes in temperature (1, 4, 5, 6, 8). A major advantage of this system is that all temperatures within a particular range can be studied simultaneously, thus eliminating much of the variability and time involved in separate evaluation of each temperature. Adaptation and slight modification of the temperature-gradient plate apparatus designed by Trione & Leach (8) provides a versatile instrument for low temperature studies (10 C to -10 C).

The aluminum plate used in our system is ca. 30 cm square and 1 cm thick, and is provided with a 20-cm square working surface enclosed by a 1 cm high retaining wall to hold the incubation medium. The plate is tightly clamped at opposite ends to two 2- to 3-cm square, 40- to 50-cm long tubular aluminum bars with inlet and outlet fittings of copper tubing. Circulating thermostatically controlled fluids through the bars creates a linear temperature gradient across the plate. Incorporation of a 2- to 5-gal insulated reservoir into each circulating system greatly reduces temperature fluctuations. Inexpensive commercially available thermos jugs are easily adapted for this purpose. The gradient plate and attached bars are set into a styrofoam block and operated in a cold room at an ambient temperature of 3-5 C.

To create a gradient through 0 C, one bar is connected to a thermostatically controlled bath circulating water above ambient temperature at the upper end of the desired range. The other bar is connected to a refrigeration unit circulating 95% ethanol cooled to below the desired range. A sealless circulating pump should be used in the ethanol system to minimize the fire hazard. A 50% solution of ethylene glycol antifreeze proved unsatisfactory as a coolant because increased viscosity at low temperatures blocked circulation. Placement of a thermostat (± 0.5 C accuracy) in the circulating system, external to the refrigeration unit, allows critical adjustment of the temperature at the "cold" end of the plate. This thermostat should be wired to the refrigeration unit through a solenoid to avoid burning out its contacts.

The working surface of the plate within the retaining walls can be filled with agar or wet sand as an incubation surface. Plate temperatures are recorded by embedding thermocouples in the medium (Fig. 1). A hood of mylar film with 3-cm high metal sides designed to cover the working surface is sealed to the plate with Vaseline to eliminate water loss while allowing some air exchange. A styrofoam hood covering the entire plate

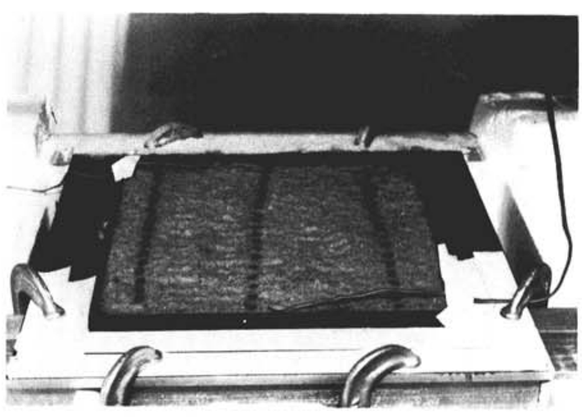

Fig. 1. Temperature-gradient plate showing wet-sand working surface with rows of mycelial-agar plugs and attached thermocouples.

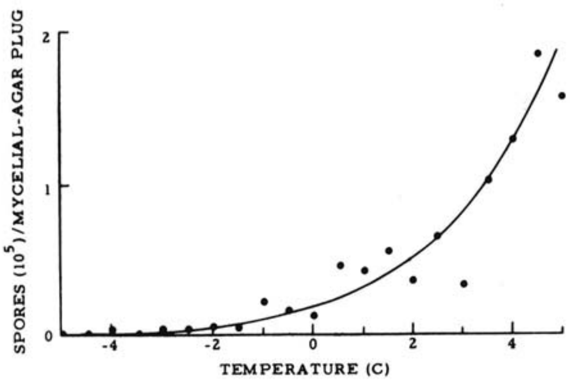

Fig. 2. Sporulation by *Cercosporella herpotrichoides* Fron above and below 0 C.

further minimizes the influence of ambient temperature.

Once set up, the plate is adjusted to the desired range by manipulation of the thermostat controlling temperatures of the circulating fluids. There is usually a 4- to 6-C difference between the temperatures of the circulating fluids recorded in the reservoirs and temperatures measured at the extremes of the plate's working surface. Since temperature gradients attained with this apparatus remain nearly linear both above and below 0 C, plate temperatures can be accurately calculated when only the extremes are measured. Figure 2 illustrates the precise evaluation of fungal activity near the freezing point obtained with this apparatus.

A slight modification of this apparatus allows evaluation of the effects of the duration of fluctuating night and day temperatures. This is accomplished by adding, immediately adjacent to the warm bar, a third aluminum bar connected to the sub-0 C ethanol system through a solenoid valve. This solenoid and the circulating pump of the warm bar are connected to alternate poles of a time clock. In this manner, the two adjacent bars function alternately at intervals set on the time clock. The cold bar at the opposite end is allowed to function continuously; thus, during a "night" period a constant low temperature is created across the plate by having both ends cooled to the same temperature. During the "day", switching on the warm bar creates a gradient. Under these conditions, it is necessary to use a 20-30% concentration of ethylene glycol antifreeze in the warm system to prevent the solution from freezing while setting in the warm bar during the night cycle. The range of the day gradient can be adjusted by changing the temperature of the water bath or by using only certain portions of the plate. A wet sand substrate is most satisfactory when working at fluctuating temperatures below 0 C, since agar disintegrates under freezing and thawing conditions.

Further modifications of this apparatus would allow users to obtain almost any combination of constant and gradient temperatures near 0 C.

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