Cross-gradient Temperature Plates for Environmental Control of Growth and Differentiation

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ABSTRACT

Several simple apparatuses were constructed which permit the establishment of precise temp gradients that may be changed in a day-night sequence. Warm and cold temp-controlled water is supplied to opposite ends of a solid 0.25-inch-thick aluminum plate. Many small but significant experiments on the interactions of temp, light, and chemicals on the growth and development of microorganisms and tissue cultures can be carried out at the same time on these cross-gradient plates. Phytopathology 60:1389-1390.

Additional key words: Tilletia caries, common bunt fungus, germination, sporulation.

Temperature is a very important parameter in most pathological and physiological responses. Much recent work has shown that a change in temp of a few degrees can exert a great influence on growth, sporulation, and germination of fungi (1, 4, 5, 6, 8, 9). Most studies dealing with temp effects on microorganisms are carried out in incubators which usually have a cyclic variation of several degrees. Such studies give only limited data because, at best, they only show the effects of one alternating day-night temp regime. In attempts to improve these techniques, various devices have been used by biologists for a number of years to produce a linear gradient temp change from cool to warm conditions (2, 3, 4).

It would be a great advantage to phytopathologists if biological responses could be observed in a gradient series of day and night temp. We have recently designed and used several simple apparatuses which permit the establishment of precise temp gradients that may be changed in a day-night sequence. For example, the main plate may consist of a solid 0.25-inch-thick aluminum plate (12 $\times$ 12 inches) to which small U-channels (0.50 $\times$ 0.25 inches) are welded around the edges (Fig. 1). Warm and cold temp-controlled water is supplied to opposite ends of the plate. If a light regime is to be used in the experiment, solenoid valves wired to a time-clock control the water flow to give either the day or the night condition. The water baths can be set at any temp extremes, and a linear gradient will be established in the aluminum plate between the two extremes. The circulating water baths control the temp very precisely, and the small fluctuations that occur are dampened by the aluminum plate. The result is a very constant temp at any point on the plate.

Another example of a convenient plate that may be used for microbial experiments is illustrated in Fig. 2. Again the main plate is of 0.25-inch-thick aluminum (6 to 10 inches sq). The plate, with cover attached, is autoclaved and filled with agar medium just as petri dish cultures are prepared. After the agar has solidified and been inoculated, several plates may be placed side by side and clamped, at each end, between square aluminum tubing. The plate may be manually rotated 90° to establish cross temp gradients if desired.

When the plate temp are monitored by the thermocouples, all of the intermediate temp between the cool and warm extremes are found along the solid diagonal (Fig. 1). These remain constant irrespective of the day-night conditions. The positions located along the dotted diagonal have the greatest temp changes between the day-night condition. The corners of the cross-gradient plate represent the four extremes: cold days/cold nights, warm days/warm nights, warm days/cold nights, and cold days/warm nights. All of the intermediate conditions between these four extremes are represented on the same plate.

If long incubation periods are required with the warm end of the plate 20-30 C warmer than the cool end, water will evaporate from the warm end and condense at the cool end. To prevent or reduce this phenomenon, a thin sheet of polyester film may be placed directly over the plate. The film will permit the diffusion of O_2 and CO_2, but will inhibit the passage of water vapor.

It is easy to establish a chemical concn gradient perpendicular to a linear temp gradient, so that the entire range of concn of the chemical will be tested at each temp on the plate. Single or double chemical gradients may be established by the wedge technique of Weinberg (10) and Sacks (7). A series of holes or longitudinal grooves into the aluminum plate may also be used to separate different chemical concn on the same plate.

In studying optimum conditions required for growth, sporulation, germination, enzyme production, etc., broad limits should be used initially on the experimental plate; e.g., 5 to 40 C or 10^{-3} to 10^{-8} M concn of test chemical. If the desired response is observed on a small area of the plate, subsequent experiments can focus on those areas, expand their limits, and thus define more precisely the optimum conditions.

Sterile conditions can be readily maintained on the plate. The 0.25-inch-deep space on top of the plate is filled with an aqueous or semisolid medium on which or in which the biological material is placed. Media containing inorganic salts, if placed in direct contact with the aluminum, tend to etch the plate. This can be obviated by coating the plate with Teflon. Plates of

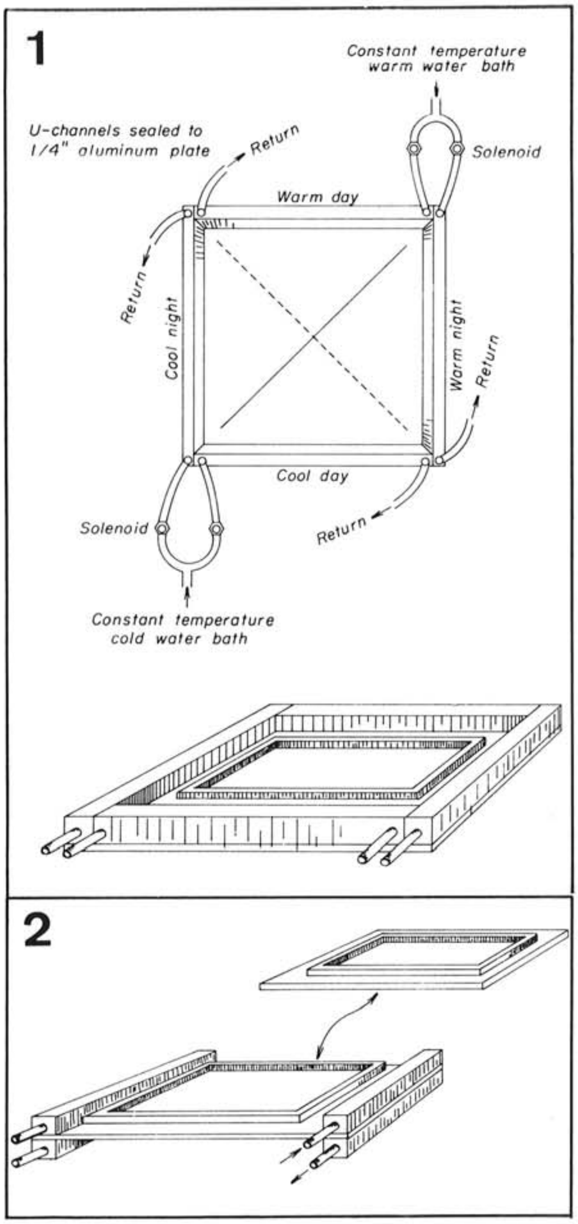

Fig. 1-2. **1)** Cross-gradient temp plate with illustrated connections. **2)** Temperature-gradient plate clamped between square aluminum tubing, through which temp-controlled water flows.

various sizes can be made to accommodate the particular biological material being investigated.

The real advantage of this cross-gradient plate is that literally hundreds of small but significant experiments on the interactions of temp, light, and chemicals on the growth and development of microorganisms and tissue cultures can be carried out on the same plate at the same time. For example: (i) The effect of light intensity (or photoperiod) can be tested over a wide range of temp; (ii) the effect of each day temp can be tested over a wide range of night temp, or the effect of heat shock (over a range of temp) followed by subsequent cooling (or heating) each locus in a second range of temp; and (iii) the simultaneous testing of a series of concn of a chemical over a wide range of temp. Biochemical manifestations of these many temp regimes may also be readily studied with these plates. For example, enzyme induction, release of extracellular enzymes, production of hormones, antibiotics, and many fermentation products all have temp optima which would be quickly found with this apparatus.

Table 1 illustrates the effect of alternating day-night temp on the germination of teliospores of *Tilletia caries* after 48 hr on water agar, on the plate illustrated in Fig. 1.

TABLE 1. The effect of day-night temp on the germination of teliospores of *Tilletia caries*. Average percentage germination of 300 spores at each locus. Twelve-hr day length under daylight fluorescent and near-ultraviolet lamps

	C								
Day temp	Night temp								
	6	9	12	15	18	21	24	27	30
6	0	0	0	4	7	3	0	0	0
9	0	5	8	15	22	18	13	4	1
12	0	7	29	39	70	60	23	6	3
15	4	16	43	53	66	53	33	14	0
18	5	31	76	73	63	45	30	9	0
21	4	19	64	53	45	36	29	13	0
24	0	11	20	32	29	18	8	0	0
27	0	5	6	11	6	7	0	0	0
30	0	1	2	0	0	0	0	0	0

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