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https://hdl.handle.net/2324/8227

出版情報：BIOTRONICS. 26, pp.31-37, 1997-12. Biotron Institute, Kyushu University
バージョン：

BIOTRONICS **26**, 31–37, 1997

A COMPUTER-CONTROLLED SEED GERMINATOR FOR REALISTIC TEMPERATURE RESPONSE STUDIES*

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(Received August 26 1995; accepted March 24, 1997)

ALM D. M., GARVES, R., STOLLER E. W. and WAX L. M. *A computer-controlled seed germinator for realistic temperature response studies.* BIOTRONICS **26**, 31–37. The construction and operation of a computer-controlled seed germinator that implements realistic diurnal temperature regimens is described. A key feature of the apparatus is the ability to easily program sinusoidal fluctuations that seeds experience in the soil rather than the square waves typical of many germination cabinets. The apparatus consists of 16 independently-controlled, 25 cm-tall insulated aluminum germination chambers that each hold up to 15 plastic Petri dishes. When programmed for a gradual diurnal fluctuation in temperature, the chamber has little variation in temperature regimen from the center to the top or bottom of the chamber, with a small phase shift in the time when the minimal and maximal daily temperatures occur. Thus, five treatments with three replicates can be placed in each chamber, providing the ability to conduct a five species × 16 temperature regimen experiment.

Key words: thermocouple; thermoelectric apparatus; temperature fluctuation; jimsonweed; *Datura stramonium* L., cocklebur; *Xanthium strumarium* L.

INTRODUCTION

The apparatus described herein was designed to provide realistic diurnal temperature regimens and to reduce the time required to measure response of seed germination to temperature as it interacts with temperature fluctuation. Most germination studies define temperature fluctuation as the difference between a daytime and nighttime temperature, with a step-change between the two. Seeds in natural seed banks, however, experience a more slowly varying diurnal temperature regimen, typically a somewhat sinusoidal curve (*8*). Therefore, a germinator that closely mimics these fluctuations would likely elicit germination responses experienced by seeds in their natural habitat.

McLaughlin *et al.* (*6*) constructed a seed germinator that allows 100 separate sinusoidal temperature regimens. Operation and construction of this apparatus,

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however, was complicated and expensive. Their basic strategy was to use one thermoelectric cooling couple for each petri dish. The design that we describe uses two thermoelectric cooling couples, one below and one above a stack of 15 petri dishes, thereby reducing the cost per petri dish. In addition, our apparatus is more flexible and easier to control, as it is regulated by a short computer program. We first describe constuction and operation of the germinator and then present a comparison of germination at constant temperature compared to that with the temperature fluctuating sinusoidally on a 12 h period with an amplitude equal to half the mean.

MATERIALS AND METHODS

Germinator design and construction

The 16 individual germination chambers were constructed from 25 cm long sections of aluminum irrigation pipe that were 10 cm in diameter. The top third of the pipe was cut and an additional section of pipe was used to fashion an internal sleeve to provide support and thermal conduction between the top and bottom portions of the chamber; This resulted in an internal diameter of about 9.6 cm, which was sufficient to accommodate 9.3 cm diameter petri dishes. Petri dishes were stacked in the chamber; the stack was wrapped with a single piece of thin metal stapping, which was used to easily insert or remove the entire stack for examination. The ends of the chamber were enclosed by a 2 mm-thick aluminum plate machined to fit into the pipe openings and secured with small screws. These top and bottom plates served as the conductive surface on which thermoelectric cooling couples were mounted; and a larger (1.5×6×6 cm) rectangular aluminum heat-sink block was fastened to the other side of each cooling couple. Cooling fluid was circulated in parallel through the heat sink blocks of all the chambers and into a water bath maintained at 10℃ (Figure 1).

With thermoelectric cooling couples, reversing the direction of the current causes a reversal in the direction of heat movement. Current flowed through the thermoelectric couples whenever the chambers were operating and the temperature was controlled by toggling the direction of current with a double-pole, double-throw mechanical relay (Table 1) that was driven by a smaller, digitally-controlled relay (Figure 2). The digital relays comprised a relay board (MEM-32; Table 1), which was connected to a bus expansion board (Metrabus Driver; Table 1). The bus expansion board was installed in an IBM PC computer with an 8088 microprocessor (Figure 1). Setpoint temperatures were monitored at the inner surface of the chamber end plates with a copper-constantan thermocouples connected to one of the 40 channels of two 20-channel data acquisition boards (MTherm-20; Table 1), which were also connected to the Metrabus Driver board. A minor calibration of the desired vs. the achieved setpoint temperature was necessary, so petri dish temperatures were measured independently of the control apparatus with two 12-channel data loggers (CR-21, Campbell Scientific). Turbo Pascal (Borland International, Scotts Valley, California) source code for the control program is available upon request from the

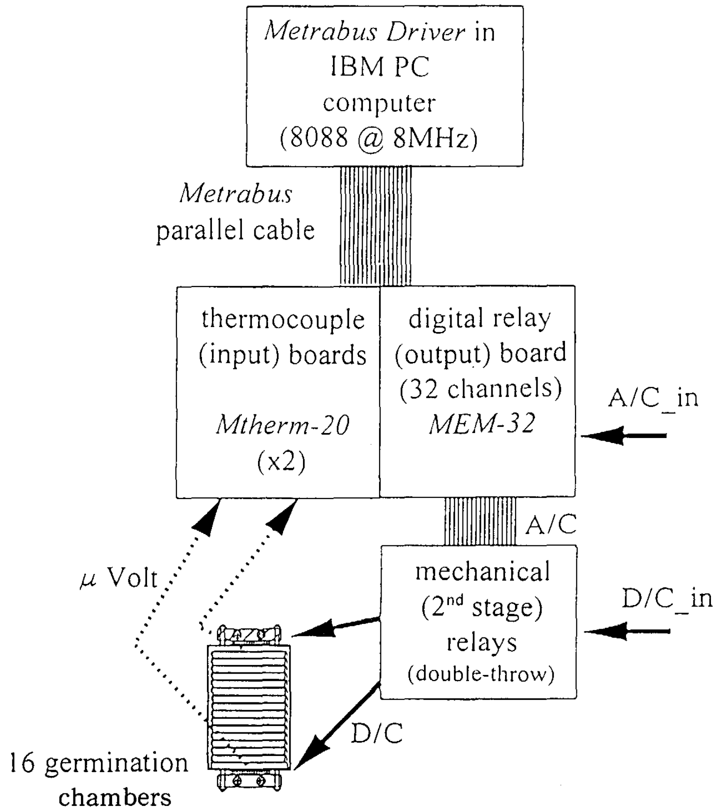

Fig. 1. Control schematic for the temperature-controlled germination apparatus. See Table 1 for parts and costs list. The battery chargers used as the D/C power source supplied a total of approximately 70 DC Amps at 6.5 volts; or 2.2 Amps per thermoelectric chip ("Frigichip", see Table 1).

first author.

Gemination experiment

Seeds were collected on the University of Illinois Agronomy South Farm, Urbana, Illinois. Seeds were harvested in September, 1992 and stored on the laboratory shelf until the experiment began in December, 1992. Twenty seeds of jimsonweed *Datura stramonium* L. in one petri dish per replicate and 10 seeds of cocklebur *Xanthium strumarium* L. in two petri dishes per replicate (*1*) with three replicates resulted in nine petri dishes per chamber. (There were actually 15 petri dishes in each chamber, but two species were completely dormant and are not reported.) All petri dishes were removed from a chamber during counting, which took about three minutes per chamber per day. Data are presented as % germination and coefficient of velocity (CV), where $CV=100$ $[\Sigma N_i/\Sigma N_i T_i]$ and N is the number of seeds germinated on day i and T is the number days from sowing. This value reflects the number and speed of germination events (*7*).

Table 1. Major Parts and Costs List for the Computer-controlled Seed Germinator.*

Description	Cost**	Vendor***
MDB-64 Metrabus (ISA slot) Driver Card [1]	$ 199	MB
MTherm-20 Temperature Boards [2]	1,550	MB
MEM-32/w Relay Board [1]	799	MB
M-10-4-7 cable [1]	80	MB
Frigichip 44910 Thermoelectric Modules [32]	730	Me
Battery Chargers [5]	250	SC
KHP17AI1Electrical Relays [32]	320	PB
	Total* 3,978	

*Other parts were classified as minor, because either we had them on hand as surplus, they are typically available in most laboratories, or the costs were comparatively minor. Minor parts included: an IBM PC w/8088 at 8 MHz; a water bath with pump; tygon tubing to distribute coolant; aluminum irrigation pipe; aluminum plating, various electrical wires and copper/constantan thermocouple wire. **Cost is for price per item times the number of items indicated inside the brackets beside the description. ***Vendor codes are: MB=Metrabyte Corporation, 440 Myles Standish Blvd., Taunton, MA 02780; Me=Melcor Thermoelectrics, 990 Spruce Street, Trenton, New Jersey 08648; SC=Schumacher Electric Corporation, Chicago, IL 60126; PB Potter and Brumfield.

RESULTS AND DISCUSSION

If a daily extremum (maximum or minimum) setpoint was cooler than room temperature due to the thermal resistivity of the stack of petri dishes inside each chamber, then the extreme temperature achieved at the center of the stack was slightly warmer than that at the top or bottom where the setpoint sensors were located. Likewise, if the setpoint was warmer than room temperature then the extreme achieved at the center of the stack was slightly cooler than that at the top or bottom. To compensate for this tendency, the chambers were calibrated by determining the relationship between the programmed extreme temperature and the actual extreme reached on average among the petri dishes. The correction was obtained by applying a linear equation to the desired setpoint to calculate the setpoint to be programmed:

$$SP_{\mathrm{PROG}}=TR..+1.114\ (SP_{\mathrm{ACHV}}-T_{\mathrm{room}}) \qquad [1]$$

where SP_{PROG} is the setpoint programmed into the computer, SP_{ACHV} is the actual setpoint achieved and T_{Room} is the room temperature (25℃). This resulted in a precision among petri dishes of ±1.7℃ at a 5℃ setpoint and ±0.8℃ at a 45℃ setpoint. The precision at 25℃ was about 0.5℃. The accuracy of the thermocouple data acquisition board is also about 0.5℃ (MTherm-20 user manual, Metrabyte Corporation, Table 1). In light of the need to remove the petri dishes from the chamber for counting and expose them to room temperatures on a daily basis, a more detailed study of precision was not warranted. Indeed, the chambers are not designed for state-of-the-art precision but for rapid analysis of field samples with realistic (except for the brief counting period) temperature

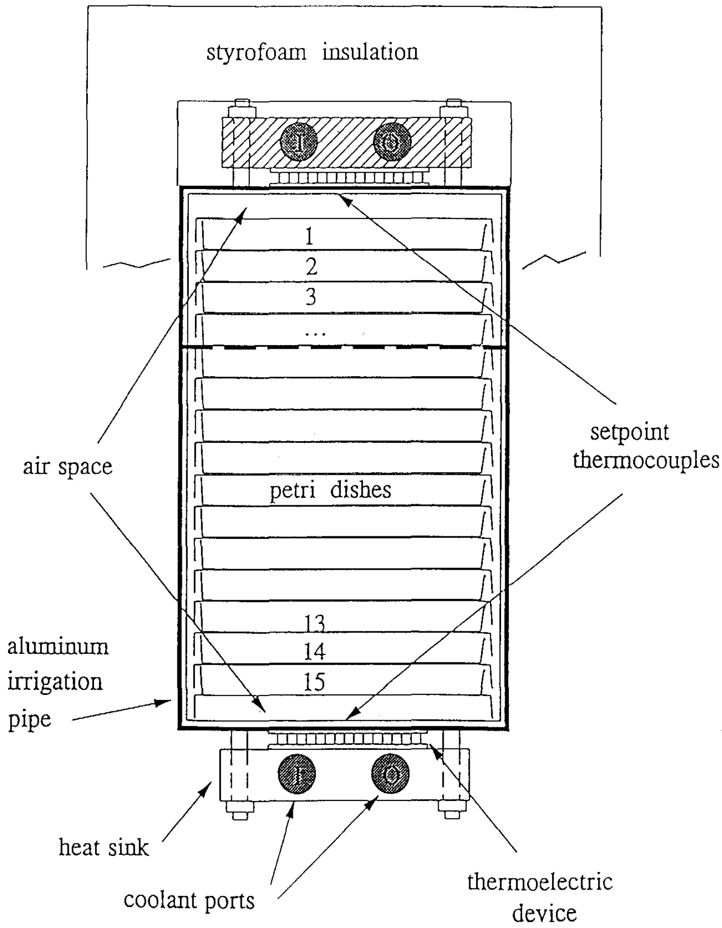

Fig. 2. Illustration of one of the 16 germination chambers. The thick, dashed horizontal line represents where the chamber is opened for installing/removing petri dishes. The heat sinks are aluminum blocks that were drilled and tapped to connect to an antifreeze solution maintained at 10℃ and circulated in parallel through all heat sinks. A petri dish lid was used to form the bottom air space, which was necessary to increase temperature precision among petri dishes within a single chamber.

regimens.

The results of an experiment comparing constant and fluctuating temperature regimens are shown in Figure 3. There was little difference in the rate of germination, as measured by the coefficient of germination velocity (*7*), between *Xanthium* and *Datura*, or between fluctuating and constant diurnal temperatures (Figure 3a). The final percent germination, however, increased substantially under fluctuating temperatures (Figure 3b). In addition, fluctuating temperatures tended to reduce the breadth of the response curve by eliminating germination at the highest and lowest mean diurnal temperatures used in this study (Figure 3). These results are consistent with those of others (*1, 2, 3, 4, 9*).

The germination apparatus described here is very flexible and comparatively

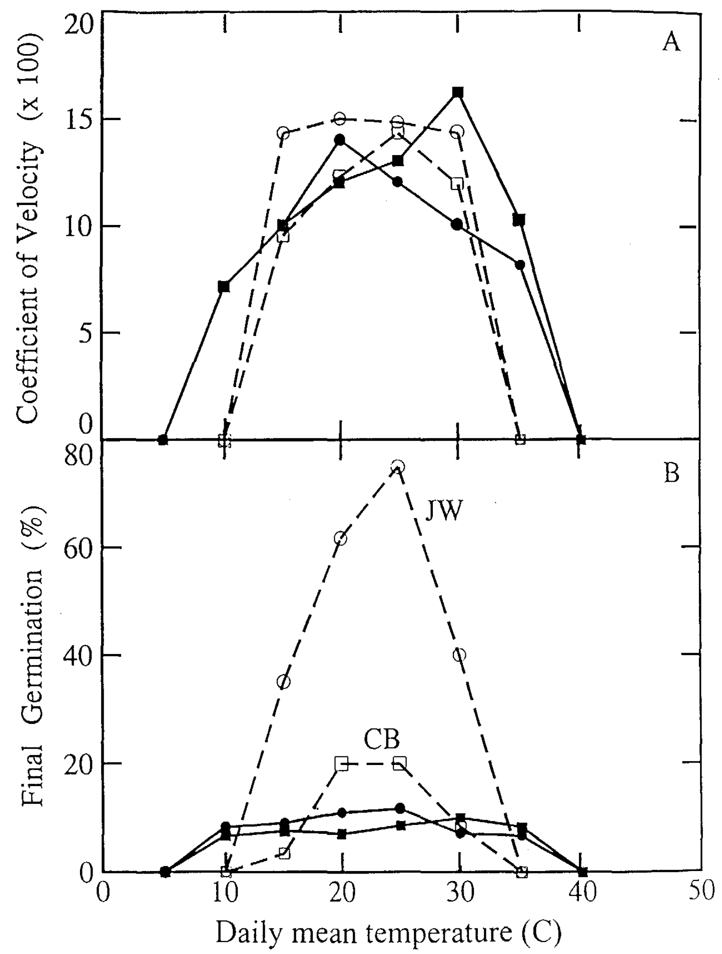

Fig. 3. Coefficient of velocity (A) and percentage germination (B) for *Datura stramonium* (circles) and *Xanthium strumarium* (squares) under constant (closed symbols, solid lines) or fluctuating (open symbols, dashed lines) diurnal temperature setpoints. Temperature fluctuation was 50% around the mean (shown on the abscissa) with minima occurring at 0600 and maxima at 1800.

(*6*) inexpensive to build; materials costs are given in Table 1. One drawback is the need to expose the seeds to room temperature during counting. A possible solution to this is to add another chamber and a thermo-controlled surface on which to place each petri dish during counting; these could be programmed to match the temperature in the chamber being counted and would minimize temperature disturbance to the point of triviality. Another drawback is the absence of any control of gaseous or light environments, both of which can affect seed germination (*2, 9*); slow venting, and fiber optic modifications could remedy this. However, opening the chambers and Petri dishes daily to obtain germination counts helps maintain the gaseous environment.

An interesting experiment would be to mimic the soil-depth effect by programming the top of the chamber to fluctuate substantially —e.g. an amplitude of 50% of the mean— while holding the bottom of the chamber constant at the diurnal mean temperature. This would create a damping of temperature fluctuation with depth, as seen in the field (*1, 5, 8*). Alternatively, the top and bottom of the chamber could be separated to create 32 separate chambers with high precision. In this case, a single petri dish could be placed in contact with the setpoint thermocouple, similar to McLaughlin et al. (*6*), and essentially any temperature function could be programmed with little effort. This apparatus should prove useful in conceiving and implementing future germination studies.

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