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A computerized, solid-state, controlled temperature gradient system for determining optimal seed germination temperatures

D.T. PRICE[1] AND C.L. LEADEM[2]

[1]*Micromet Systems Inc., 740 Millyard Street, Vancouver, B.C., Canada V5Z 4A1*
[2]*British Columbia Ministry of Forests, Research Laboratory*
1320 Glyn Road, Victoria, B.C., Canada V8W 3E7

Abstract

Temperature is a critical factor influencing conifer seed viability and germination success. The objective of this study v design and construct a controlled thermal gradient system to determine the optimal temperature conditions for seed germin and other biological processes influenced by temperature. The computer-controlled thermal gradient system consists of (eight modules each containing 16 cells arranged in a 4 x 4 matrix. Each cell measures 12.5 cm square with a 10 mm alun plate at its base, and is thermally insulated from the surrounding cells. Both heating and cooling of each cell are provided by thermoelectric heat-pump units sandwiched between the aluminum plate and a large heat-sink common to all 16 cells. The temperature of each cell is monitored with a single diode temperature sensor. The computer determines whether heating or cooling is required for each cell to achieve the user-specified target temperatures, and schedules the power needed to do so, using a proportional integral derivative (PID) control algorithm. Using this approach, temperature control consistently to within ±0.1°C has been attained. The researcher may use the computer to examine current and historical cell temperatures and to change the target temperatures as required. The software also allows optional randomized arrangements of cell temperatures, so that experimental bias can be virtually eliminated. Results of preliminary tests on white spruce (*Picea glauca* [Moench] Voss) seeds are presented.

Résumé

La température constitue un facteur critique de la viabilité et du succès de la germination des graines de conifères. Cette étude avait pour objectif la conception et la fabrication d'un système à gradient thermique régularisé servant à déterminer les conditions optimales de température pour la germination des graines et pour d'autres processus biologiques sensibles à la température.Le système à gradient thermique régularisé par ordinateur est constitué de 1 à 8 modules, eux-mêmes composés de 16 cellules qui sont disposées en un bloc de 4 cellules de côté. Chacune mesure 12,5 cm^2 et comporte une plaque d'aluminium de 10 mm à sa base; chacune est isolée thermiquement des cellules contiguës. Le chauffage et le refroidissement de chacune sont assurés par des échangeurs de chaleur thermo-électriques coincés entre la plaque d'aluminium et un gros dissipateur de chaleur commun aux 16 cellules. La température de chaque cellule est contrôlée au moyen d'une seule sonde thermique à diode. L'ordinateur détermine s'il faut chauffer ou refroidir la cellule de manière à ce qu'elle garde la température établie par l'utilisateur, et il programme la quantité d'énergie nécessaire au moyen d'un algorithme du type proportionnel, intégral, dérivé (PID). Grâce à cette méthode, il est possible de garder la température à l'intérieur d'une plage de ±0,1°C. Le chercheur peut utiliser l'ordinateur pour connaître la température des cellules ou obtenir des relevés des températures antérieures ainsi que pour ajuster la température à ses besoins. Le logiciel permet aussi d'obtenir des combinaisons aléatoires de températures, ce qui supprime pratiquement les biais expérimentaux. Les résultats d'essais préliminaires sur des graines d'épinettes blanches (*Picea glauca* [Moench] Voss) sont présentés.

Introduction

Forest geneticists and seed physiologists recognize that temperature is a critical factor influencing conifer seed viability and germination success. In B.C. alone, it has been estimated that there are almost 4000 seedlots currently in use, for which optimal germination temperatures generally are not known to better than ±5°C (Rooke 1991, personal communication). Better knowledge of temperature optima should result in higher germination success, and hence reduced seedling production costs for the nursery. By "optimum temperature" we refer to the temperature treatment at which the maximum and/or fastest seed germination occurs. The optimum temperature treatment may involve changes in temperature with time, and typically a night and day fluctuation is necessary (Heit 1958). Other treatments, such as stratification, also influence the optima for seed germination (Leadem 1986). When all possible factors are taken into account, a large number of germination tests are needed before proper understanding of the optimal germination treatment for a single seedlot can be achieved.

Standard seed germination tests typically require 21-28 days to complete (AOSA 1981, ISTA 1985), and determination of the optimum germination temperature usually requires repeating these tests at several controlled temperatures. In the past, seed researchers have had to choose between two approaches to do this. One was to use a series of controlled temperature units, such as controlled environment chambers or incubators, operating at different temperatures. The alternative was to use a thermogradient plate (e.g. Larsen 1971, Morgan 1980, McLaughlin *et al.* 1985). The former approach has the disadvantage that several units are required if acceptable results are to be obtained within a reasonable time, though samples from many seedlots can be tested at once. If budgets or work space are limited, fewer units may be used, with consecutive tests being run at different temperatures, but there is the risk that the test material will age between the beginning and end of the germination tests. Further problems include ensuring the test material is subjected to similar treatment from one controlled environment to the next, since lighting, ventilation and humidity control may differ, while human factors may also have an impact. Also, the temperature control inside a large enclosure such as a controlled environment chamber cannot be perfect. Small variations in temperature with time may be acceptable, but if consistent temperature gradients exist within the controlled environment chamber, the effect of these must be considered.

Of the few designs for thermal gradient plates that have been published, most are based on a single large thermally conductive plate, heated at one edge and cooled at the other (see McLaughlin *et al.* 1985 for a review). Cooling is usually achieved by circulating a refrigerated coolant through the plate, or pipes attached to its base, while heating is provided either using heated liquid, or more commonly, by electric heating elements. Temperature sensors positioned at various points on the plate are used to provide temperature control using simple feed-back loops. The seed researcher can run germination tests on small quantities of seed at several temperatures simultaneously, thereby eliminating some of the problems of using controlled temperature units described above. However, other problems are often introduced. Firstly, since the gradient is continuous, it is inevitably true that no two points on the plate (in the gradient direction) are ever at identical temperatures. Thus the researcher must either be content with very small samples of test material, or must accept that seeds within each sample are not actually being subjected to identical temperature treatments. Secondly, because the only sources of heating and cooling are at the edges of the plate, temperature control at any point in between is necessarily imprecise.

A more recent design was significantly different. It described a seed germinator with 100 individually temperature controlled cells (McLaughlin *et al.* 1985). Arranged in a 10 x 10 matrix, each cell was surrounded by styrofoam insulation and could accommodate a 10 cm Petri dish. Instead of a continuous temperature gradient, each cell was maintained at a single uniform temperature. The germination response could then be plotted as a curve or surface over a range of clearly-defined discrete temperatures. Both heating and cooling of each cell were provided by a thermoelectric device sandwiched between the lower surface of an aluminum pedestal at the base of the cell and a larger heat-sink, common to all 100 cells. Thermoelectric heat-pumps have the advantage that reversing the current direction switches them between heating and cooling modes. However, a significant drawback is that in cooling mode, excess heat is generated, requiring external cooling of the heat-sink.

The 100-cell germinator used an electromechanical timer to control a 24-hour cycle, during which as many as six separate control temperatures could be generated. The temperature control system was inflexible because it allowed a maximum of only six predetermined temperatures per cell, and required the construction of a new circuit board if a new cell temperature regime was required. McLaughlin *et al.* (1985) suggested that the electromechanical temperature control system could be replaced by a computer, which could monitor the cell temperatures continuously, and control relays to switch the heat pumps between heating and cooling, as required. However, the authors did not consider that the computer could also be used to schedule the demand on the power supply. In their system, it was assumed that all the heat pumps would sometimes need to be powered simultaneously, so a very large power supply was needed. The complete system was therefore large and heavy, and hence very inconvenient to move.

The project reported here arose because of an urgent need to identify temperature optima for seedlots in

British Columbia. The objective was to design and construct a thermal gradient system to generate a range of discrete biologically active temperatures simultaneously. It would be similar in concept to the McLaughlin *et al.* (1985) seed germinator, but with two major improvements. Firstly, the system was to be computer-controlled to enable any cell to be maintained to within ±0.1°C for any temperature in the range 0° to 50°C. Secondly, the system would be "modularized", with each module containing only 16 cells arranged in a 4 x 4 matrix. Combined with smaller power requirements, this would make the system more flexible and portable. For studies requiring larger numbers of cells, two or more modules would be connected to the same controlling computer.

Methods

Module Construction

Each module of the thermal gradient system consists of a square enclosure (650 x 650 x 150 mm) covered by a closely fitting clear double-glazed lid, hinged at the rear. Inside are 16 square (126 x 126 mm) cells arranged in a 4 x 4 matrix. The construction of a single cell is shown in Figure 1. Within each cell, the temperature is regulated independently by sets of five thermoelectric (Peltier) heat-pump units (model CP1.0-31-05L, Materials Electronics Products Corp., Trenton, NJ), connected electrically in series and thermally in parallel (see Anon. 1985, for discussion of Peltier heat-pump principles). The specification for these units was determined from consideration of the energy exchanges expected for worst-case heating and cooling conditions, discussed in more detail below. The five heat-pump units are sandwiched between, and in intimate thermal contact with, the lower surface of a 10 mm thick anodized aluminum "test-plate" at the base of each cell, and the upper surface of a single much larger aluminum "base-plate", common to all 16 cells. The heat-pump units are arranged in an 'X' pattern for more uniform energy distribution, with plastic foam insulation filling the gaps. The thermoelectric heat-pumps are very efficient (under optimum conditions, the ratio of heat pumped to electrical energy consumed exceeds 50%), and the base-plate facilitates heat transfer from cells being cooled, to those being heated, but some excess heat is inevitably generated and must be removed. In each module, a pair of extruded aluminum cooling plates (V&V Refrigeration Ltd., Richmond, B.C.) are bolted to the lower surface of the base-plate, so that liquid coolant (normally water) may be passed through the system to maintain the temperature of the base-plate, typically at about 15°C. Aluminum pipes welded to the ends of the cooling plates protrude from the sides of the enclosure, allowing the module (or modules) to be connected to a cold water source. Normally, a flow rate of 3-4 L min^{-1} (per module) at 15°C is sufficient to dispose of all excess heat.

The walls of each cell are 50 mm high, consisting of white plastic insulating foam approximately 25 mm

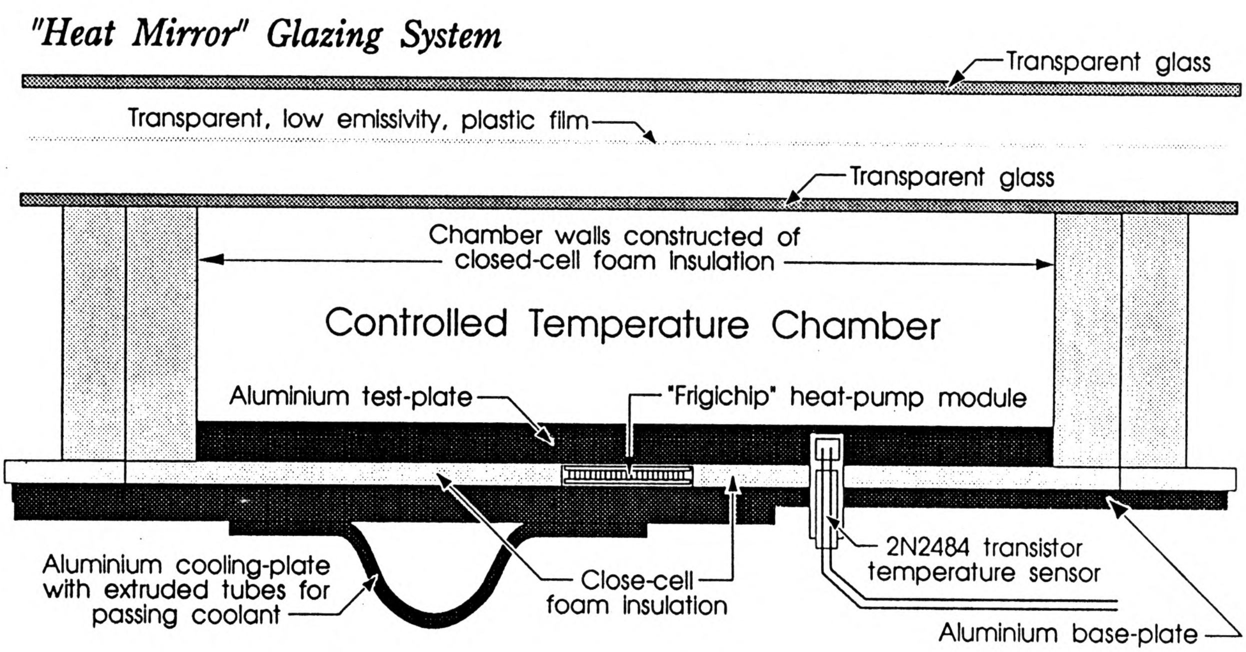

Figure 1. Cross-section through a single cell of a thermal gradient system module.

thick. When closed, the lid rests on the top edges of the cell walls, thereby insulating each cell from its neighbours, and the enclosure's surroundings. The lid features a proprietary glazing system (Heat Mirror™, Southwall Technologies Inc., Palo Alto, CA) consisting of two sheets of glass 22 mm apart, and a specially coated plastic film midway between. The plastic film filters strongly in the near infrared wave-band, thereby significantly reducing radiative heating of the cell or its contents (e.g. when used below incandescent lamps). The plastic film also reduces convective exchange between the two glass layers. The lower glass sheet is tempered for greater strength and resistance to heat stresses resulting from temperature differences between neighbouring cells.

A high current 12 VDC power supply is connected by pairs of #4 gauge welding cables to two pairs of terminals protruding from the rear of the module. Each terminal pair is mounted on a printed circuit board and allows up to 20 Ampere to be supplied to half the module (i.e. there are two such boards per module). Each circuit board carries 8 solid-state switches formed by sets of four power MOSFETs (Metal Oxide Semiconductor Field Effect Transistors), which allow power to be rapidly switched to the thermoelectric heat pumps in eight cells. Each cell typically draws about 2.2 A at 12 VDC, which the MOSFETs can dissipate without heat-sinking. The computer prevents more than eight cells from ever being powered simultaneously, so a 250 W power supply is typically needed for each module. Each of the 8 switches is controlled by two logic lines, one to determine whether the cell is powered, and the other the current direction. The 16 logic lines (two per switch) are toggled by two 8 bit ports of an 8255A programmable peripheral interface (PPI) controller, receiving its instructions and power from the computer via an "A-Bus" (Alpha Products, Darien, CT) ribbon cable connected to the board. Under computer control, each solid-state switch thus allows direct current to a single cell to be switched off or on, and in either direction. A panel of 16 bi-directional light emitting diodes (LEDs) connected across the MOSFET switches and mounted on the front of the module shows the status of the cells. When current passes in one direction, the heat-pumps cool the cell, while the corresponding LED glows green. Reversing the current causes the heat-pumps to heat the cell, and the LED glows red. When no power is supplied, the LED is off and the cell loses or gains heat as it returns toward ambient temperature (usually that of the base-plate).

Power is supplied under computer-control in pulses of approximately 0.05 s duration. The number of "on pulses" delivered in a given period therefore determines the amount of energy being supplied to the cell. For cells requiring much heating or cooling, power may be supplied more or less continuously, while those requiring only slight temperature adjustment need relatively few pulses per minute.

The temperature of each cell is measured every 10 s using a calibrated 2N2484 transistor (McNaughton 1989, personal communication) embedded near the upper surface of the test-plate. The voltage across the 2N2484 varies linearly with its temperature and, with careful calibration, it serves as a very accurate thermometer. All sensors were individually pre-calibrated in a stirred water bath over the range 0° to 60°C against a measurement-grade fine wire copper/constantan thermocouple connected to a data logger (model 21X Micrologger, Campbell Scientific Instruments, Logan, UT) operating in double-ended measurement mode. The r^2 was found to be typically better than +0.9999 with 50-100 readings over the calibration range.

The temperature sensors are connected via a 16-channel multiplexing circuit (one channel per cell) to a high quality instrumentation grade amplifier, mounted on a third circuit board located at the front of the module. A separate 500 mW power supply provides power to this analog sensing board because it is optically isolated from the computer's power supply, to eliminate digital noise that might contaminate measurements of the analog signals from the temperature sensors. For the same reason, the analog circuitry is also physically separated and electrically shielded from the digital switching circuitry at the rear of the module. The sensitivity of the sensors is about 2.2 mV °C $^{-1}$, but after amplification, the sensitivity is increased to about 200 mV °C $^{-1}$, over the range -25° to +75°C. The output voltage from the amplifier is multiplexed into an analog-to-digital (A/D) converter (ADC) circuit based on the ADC574A chip (Burr-Brown, Tucson, AZ). With the 12 bit resolution of the ADC574A, the measurement resolution is about ±0.025°C. Under computer control, the temperature of each cell is sampled, and the transistor output voltage multiplexed and amplified before being converted to a digital value by the ADC. Multiplexing and A/D conversion are controlled by the third of the 8255A interface controller's ports on one of the two digital boards at the rear of the module, while digital output from the ADC is returned to the computer via the third port of the 8255A on the other digital board. Each cell temperature measurement is completed in about 0.001 s. As many as eight modules may be connected to the computer, providing up to 128 individually controlled temperature regimes.

Energy Balance Analysis

To estimate the size of the thermoelectric heat-pump units required, a simple energy balance model was constructed. The model attempted to simulate the energy balance for a single cell in two extreme cases (Table 1). For both cases, heat exchanges between the cell and the base-plate (through the insulation separating the test-plate and the base-plate), the neighbouring cells (through the walls), and the surrounding air (through the double-glazed lid) were all estimated and summed. The net long-wave radiative exchange was also estimated from consideration of the radiative properties of the materials and suitable values for surface temperatures. In addition, for the "cooling worst-case", the short-wave radiative input from tungsten lamps was included.

Figure 2 shows that the amount of heat energy required to be pumped varies, with maximum demands under worst-case conditions of about 10 W when cooling to -5°C, and 11 W when heating to +60°C. The five thermoelectric heat-pump units used to heat and cool each cell, connected in series, are able to pump a total of about 27.5 W under optimum conditions (i.e. when the test-plate temperature is equal to the base-plate temperature). The heat-pumps decrease in efficiency as the temperature difference increases, but for the worst-case conditions identified above, the heat-pumps are specified to remove about 19 W when cooling to -5°C, and deliver 18 W when heating to +60°C, assuming the base-plate temperature can be maintained at 15°C.

Control Software

A memory-resident control program for IBM PC-type microcomputers runs continuously to maintain the temperatures of all the cells, even when the computer is being used for other purposes. It uses a closed-loop proportional integral derivative (PID) algorithm which is repeated on a 10 s cycle. For each module, the first stage in the cycle is to initialize the total energy requirement, ΣE_p, to zero. ΣE_p is used to accumulate the total energy requirement for all cells, to ensure that the total demand does not overload the capacity of the power supply.

In the second stage, the computer instructs the A/D circuit to use the 16 channel multiplexer to step through the cells of each module to read the sensor voltages, one by one. After amplification, each voltage is digitized by the ADC574A, and the digital output

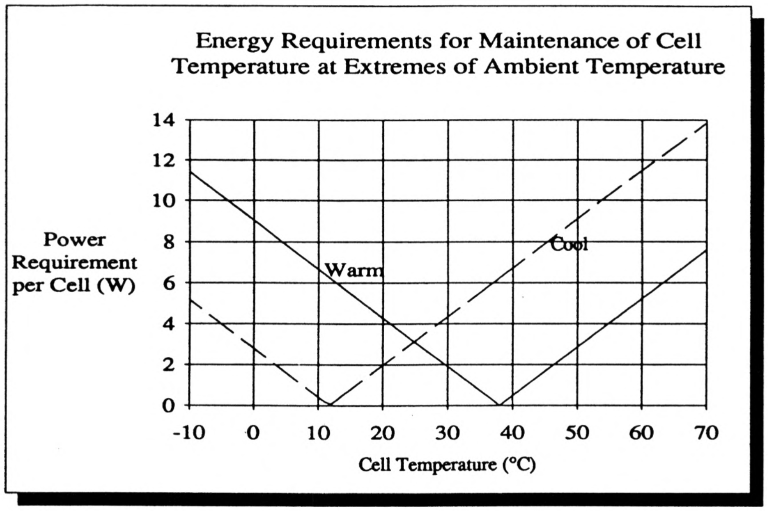

Figure 2. Summary of results of energy balance analysis for module. The two lines represent the range of energy input required to maintain the cell at the indicated temperature. "Warm" and "Cool" refer to the ambient conditions, as given by Table 1a.

Table 1a. Summary of values of variables used in energy balance analysis. This table gives the worst-case control plate temperatures. The results for these and other temperatures at 5°C intervals are shown in Figure 2.

Variable	Cooling Worst-case	Heating Worst-case
Incident short-wave radiation	500 W m^{-2}	0 W m^{-2}
Temperature of surroundings	50°C	20°C
Ambient air temperature	35°C	15°C
Temperature of lid	40°C	10°C
Temperature of base-plate	15°C	15°C
Temperature of neighbouring cells	50°C	0°C
Plate control temperature	-5°C	60°C

returned to the computer via the A-Bus, where it is converted to a temperature reading using the calibration coefficients determined for the temperature sensors. The sensor calibration coefficients are read in from an external data file when the program is loaded, so these may be changed easily. The target temperature, T_t, set by the researcher, is compared with the actual temperature just measured, T_p, and the error, e_0, is calculated as $(T_t - T_p)$. The priority for heating or cooling each cell is assessed on the basis of the magnitude of e_0. In the third stage, on completion of the measurements, the computer sorts the cells in descending order of priority (i.e. their absolute temperature errors). The error is also used to calculate the energy required (positive or negative) over the next 10 s period to bring the actual test-plate temperature to the target temperature and maintain it there, using a PID equation of the form:

$$E_p = K_p [e_0 + K_i e_i + K_d(e_0 - e_1)] \qquad (1)$$

where Ep is the energy required for the next 10 second control period in Joules; K_p, K_i and K_d are the proportional, integral and differential coefficients (derived empirically) and e_0, e_1 are the temperature error terms for the current and previous 10 s control periods, respectively. The term e_i is the integrator error, which varies depending on how close the actual temperature is to the target temperature. When the error is greater than 5.0°C, e_i is set to zero, so that the energy requirement is calculated only as a function of the error (proportional term) and of the rate of change in the temperature error (the derivative term), determined as (e_0-e_1). When the magnitude of e_0 falls below 1.0°C, the integral term is activated, and e_i is calculated as the sum of the temperature errors $(e_i = e_i + e_0)$. As the control algorithm "seeks" the required control temperature, e_0 varies between positive and negative, and the sum e_i will tend to a small value fluctuating about zero. For temperature errors in the range 1.0 to 5°C, e_i is left unchanged. This means that when the actual temperature is approaching the target, e_i is set to 0, until the magnitude of the error falls below 1.0°C. Conversely, if the temperature starts to drift away from the target, there is a broader band over which the integral term actively influences the temperature control.

Once E_p has been calculated, the energy requirement is doubled if cooling is required, (i.e. if $E_p < 0$) in recognition of the fact that the thermoelectric heat-pumps are approximately half as efficient in cooling mode. Finally, ΣE_p is accumulated by summing the absolute value of E_p for all cells in the system. When totalled, ΣE_p is compared to that available from the power supply, and if the latter is exceeded, the energy requirements for all cells are scaled down proportionately. Note that the PID control algorithm allows cooling to continue even when the temperature has fallen below the target (or heating when the temperature is above the target). The ideal situation occurs when the heat supplied or removed by the heat-pumps exactly matches the heat being lost or gained over the same period. In reality, external perturbations, the pulsed power input, and limits on temperature measurement precision, prevent a perfect balance from being achieved continuously, but the PID algorithm adjusts frequently enough to obtain satisfactory results.

For the remainder of the current cycle, and for the short period required to measure the temperatures and allocate energy for the next cycle, power is supplied to the heat-pumps in 0.05 s pulses approximately 18.2 times per second, using the IBM PC's timer interrupt. A pulse is the shortest period for which the cells may be switched on or off, with each 10 s cycle divided into 182 pulses. Each time the PC's timer issues an interrupt, the appropriate 8255A logic lines are set for the duration of the next pulse for all the connected

Table 1b. Summary of constants used in energy balance analysis. The results of the analysis are shown in Figure 2.

Variable	Value	Units	Source
Short-wave transmissivity of lid	0.48		Southwall
Albedo of plate surface	0.6		Estimate
Long-wave emissivity of plate surface	0.1		Estimate
Long-wave emissivity of walls	1		Estimate
Thermal conductivity of foam walls	0.0433	$W\ m^{-1}\ °C^{-1}$	BXL Plastics
Thermal conductivity of lid (cooling)	2.1	$W\ m^{-1}\ °C^{-1}$	Southwall
Thermal conductivity of lid (heating)	1.82	$W\ m^{-1}\ °C^{-1}$	Southwall

modules, before control is returned to the foreground task. Energy pulses from the power supply are allocated to the cells in decreasing priority order, with the highest priority cells receiving the most pulses. The computer schedules the available pulses to ensure that of the 16 cells within each thermal gradient module, no more than eight are powered simultaneously (so the total current drawn from the power supply never exceeds 20 A per module). Once the cells have reached their target temperatures, this is not normally a significant restriction, but under certain conditions (e.g. when all the cells are undergoing a step-change in temperature) more power may be required than the power supply can provide. In this event, the energy requirements for all cells are scaled down by the ratio $E_{max}/\Sigma E_p$, where E_{max} is the maximum energy that the power supply can provide. After a few 10 s cycles have elapsed, the cells with larger temperature errors will have been warmed or cooled closer to their target temperatures, so the priority order will change and other cells will then receive their power allocations.

The remaining function of the resident control program is to monitor the system clock and record 30 minute temperature averages, minima and maxima on disk, at half-hourly intervals. The 10-second temperature readings are stored for one minute, and then averaged. These one-minute averages are stored for 30 minutes, with the 30-minute averages being updated every minute. On the half-hour, the current 30-minute averages are written to the disk, together with the 30-minute maxima and minima. Data are stored sequentially in a binary file until all available records are occupied. At this point, the oldest data are overwritten, and the file becomes a ring buffer operating on a "first-in, first-out" principle. The maximum size of the data file may be easily adjusted, so that the user may decide for how long data will be retained.

A transient program provides access to the control program when the system is operating. When the user runs the transient program, it searches memory for the presence of the resident control program, and assuming the latter is located, it then "eavesdrops" on the data being collected and stored. In this way the transient program allows the user to view current temperatures, modify control parameters, and access temperature data previously stored on disk, without affecting execution of the control program. All data recorded in the binary data files can be recalled and displayed as a 24-hour or 7-day graph on the monitor screen. By default, the most recent data for a selected module are displayed, but the user may examine older data by moving backwards through the file.

The transient program also allows the researcher to set the temperature of the cells individually, or as a complete experiment involving all 16 cells within each module. For the individual cells, in addition to constant, the temperature can be made to vary as one of three simple functions of time (step, ramp or sinusoid). In the latter cases, the mean temperature, the wave amplitude and the durations of the day and night periods can all be controlled. When setting up an experiment involving all 16 cells, the researcher can set the range of temperatures, and select from one of the four time functions to be used by all the cells. One may also select an experimental design, for which four choices exist: the one-way gradient replicates four temperatures in four cells with the coldest at one side of the module and warmest at the other; the two-way gradient generates 16 temperatures with the coldest cell at one corner and the warmest at the opposite corner; the Latin square replicates four temperatures in four cells using a randomized Latin square design; the random square generates 16 randomly arranged temperatures. The randomized designs would normally be used since these help to eliminate external bias in the germination tests. (It is even possible for the technician doing the germination assessments to be kept ignorant of the temperature treatments being applied!) Systematic designs may be useful for demonstration purposes, or for routine tests when experimental rigour is not essential.

Virtually any combination of experimental design and individual cell temperature functions can be handled because the control parameters for each cell can be customized after an experimental design has been selected. In addition, further wave functions and experimental designs could be added to the control software quite easily, if the need arose. The only limitations imposed are by the rating of the power supply and the amount of cooling that can be provided. For example, it is not possible to maintain all 16 cells at 0°C simultaneously if the coolant temperature is 15°C. This should not be a problem for most purposes, since the objective of the thermal gradient system is to provide a range of biologically significant temperatures. Even this limitation could be overcome by heating or cooling the coolant and/or increasing the size of the power supply.

Performance Tests

As an initial test of the system, a simple seed germination experiment was conducted on white spruce (*Picea glauca*) (Fig. 3). Two 800-seed samples were drawn from a single seedlot, of which one sample was stratified by soaking in water for 24 h, then chilled for 3 weeks at 2°C before being placed in the thermal gradient module. A single thermal gradient module was programmed to generate eight constant temperatures from 10° to 38°C in intervals of 4°C, each replicated twice and randomly distributed within the module. Each sample of 800 seeds was separated at random into 16 subsamples of 50 seeds. Each subsample was then placed in a transparent plastic 12 cm square seed germination tray, scattered evenly on a substrate of one layer of "Kimpak" absorbent paper covered with 2 layers of Whatman No. 1 filter paper, then moistened with 50 ml deionized water. Each tray was covered with a close-fitting lid, randomly allocated a number between 1 and 16 for easy identification and placed in the corresponding cell of the thermal gradient module. Germination assessments were made three times per week (Monday, Wednesday and Friday) for a period of two weeks. At each assessment, the number of germinated seeds was recorded, after which they were removed. Any mouldy seeds were also noted and removed. A seed was considered to be completely germinated when the radicle had extended to four seed-coat lengths. After testing the unstratified seeds, the experiment was repeated with the stratified sample.

Results and discussion

Germination Experiment

Figure 3 shows the distribution of germination percentage (during the two weeks of each test) as a function of temperature. Unfortunately, a problem in the control software caused the 30°C treatment to fail 7 days into the second test, so that the two cells operated at full power for several hours, resulting in all the chitted and germinating seeds being killed. This explains the apparently lower germination for stratified seeds at this temperature. Otherwise, the results clearly

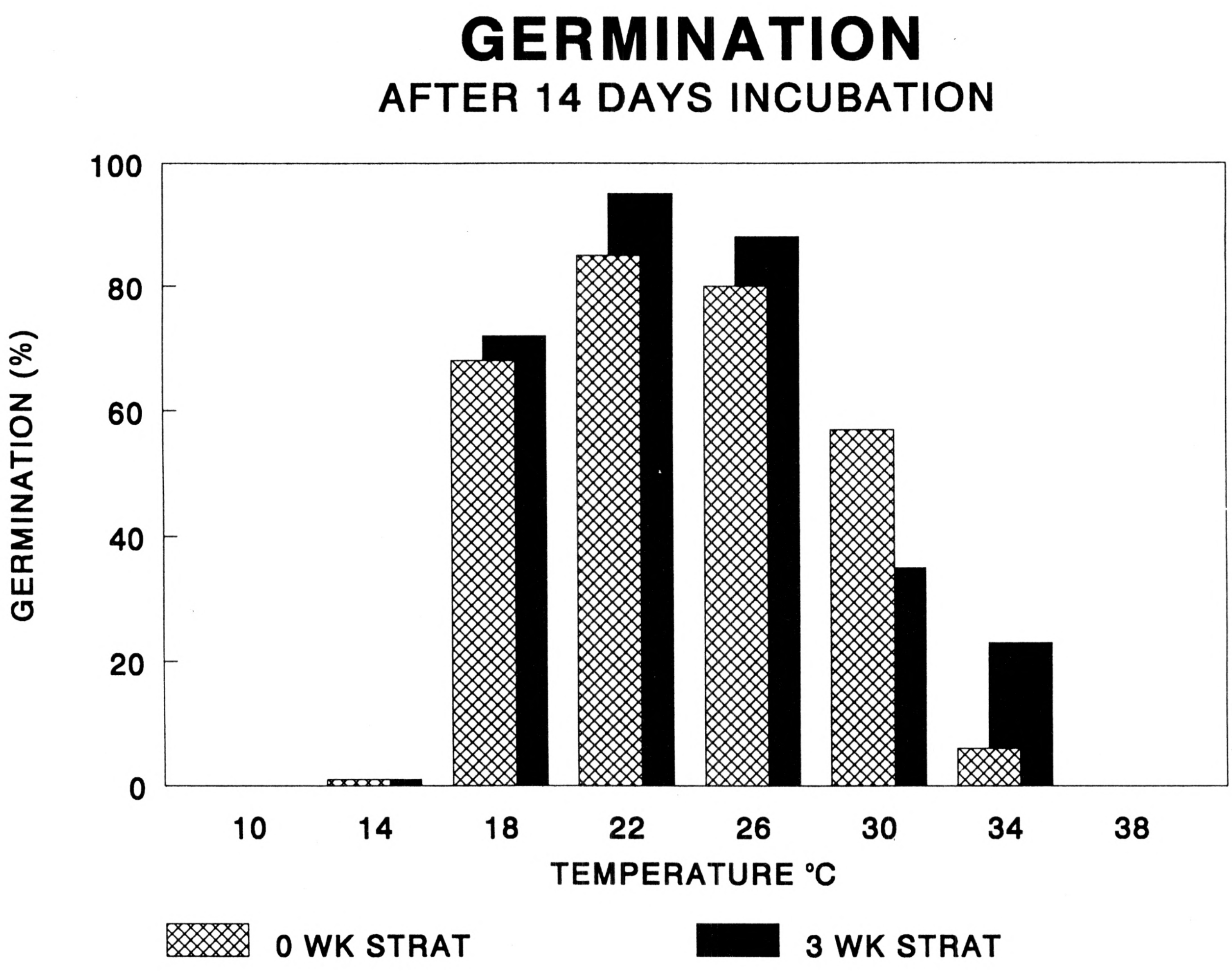

Figure 3. Germination of white spruce (*Picea glauca*) seeds after a 14-day incubation period, as a function of temperature determined using the computer-controlled solid-state thermal gradient system.

demonstrate that stratification broadens the range of temperatures optimal for germination, as well as generally increasing the germination percentage. Both germination tests indicate that the optimum temperature for germination of the white spruce seedlot we investigated lies somewhere between 18° and 26°C. A further experiment could now be carried out to investigate the range between, say, 19° and 26°C, in increments of 1°C. When properly calibrated, the thermal gradient system is certainly capable of resolving differences in germination temperature as small as 1°C.

System Performance

After fixing the obvious problems (such as the software bug mentioned above), the module electronics and system control software have been found generally to work very well. As a safety precaution, a routine has since been added to the software to prevent cells from being powered when the measured temperature falls beyond the range -20° to +70°C. Examination of large quantities of constant temperature data collected by the system show that over a 30 minute interval, the actual cell temperature recorded by the computer rarely differs from the target temperature by more than ±0.1°C. When the target temperature is itself changing with time, with the exception of an imposed step change, control is invariably kept within ±0.3°C over the specified operating range (0° to 50°C). Since it is impossible for the temperature to change immediately in response to an imposed step change, the performance specification for a step change is of interest. Complete adjustment to a large change can take more than an hour, but for changes of the order of 10°C, 90% of the adjustment usually takes place within 5 minutes when cooling, and 2.5 minutes when heating. The PID control algorithm necessarily involves some compromise, because more rapid change could be brought about at the cost of increased overshoot and slower settling time at the new target temperature. We are currently pursuing the use of an adaptive control algorithm to increase the speed of temperature adjustment, without impairing the precision of control.

Because the computer schedules the distribution of power to the cells, it allows a smaller power supply to be used more efficiently than the one used in the McLaughlin *et al.* (1985) seed germinator. In their system, a 2.1 kW (-7 to +7 VDC) unregulated power supply was constructed to allow each of 100 cells to draw about 15 W. In our system, each cell draws about 27.5 W (producing faster temperature adjustment), but since all cells do not need to be powered continuously, the computer enables 128 cells to be powered by a commercially available 2 kW (+12 VDC) regulated supply.

Calibration

After the first module was constructed, it was found that the temperature calibration coefficients determined for the individual sensors did not produce satisfactory agreement with independent measurements of the surface temperatures of the test-plate. The reason for this probably lay in the accuracy with which the transistor offset voltage and amplifier gain could be set on the analog circuit board. A technique for *in situ* calibration was required in any case, since the sensor calibrations should be checked at intervals. Of three techniques tried, the most successful used the control software to maintain constant temperatures for several hours so that the mean control temperature could be adequately compared with that recorded by an independent sensor. This approach took advantage of the extremely good temperature control capability of the module electronics and the PID algorithm. To obtain the calibration data, a calibration mode was added to the control program to generate temperatures of -5°, +15°, +35° and +55°C in a Latin square design, in four six-hour phases, on a repeating 24-hour cycle.

To measure the temperature of the cells, fine wire thermocouples were attached to the surfaces of the test-plates using adhesive tape, and connected to a datalogger, certified accurate to within ±0.5°C (Campbell Scientific model 21X). Using this technique, approximately 40 paired temperature comparisons could be obtained within 24 hours, for which a regression equation could be derived. The regression equation typically produced a standard error for the y-estimate of less than 0.3°C. Given the accuracy of the datalogger, this means that the absolute accuracy of temperature measurement is at worst, ±0.8°C, but we feel it will normally be within ±0.5°C. Further planned refinements to the temperature-sensing circuitry and better calibration techniques should improve this specification.

Other Applications

The system could potentially be used in several disciplines outside seed physiology, wherever researchers wish to determine the temperature optima for small-scale biological phenomena. These include: egg-hatching (e.g. for invertebrates and fish), embryology, genetics, tissue culture, aquatic and soil invertebrate biology, mycology, bacteriology and virology. For these purposes, various design options have been considered. For example, it would be an easy matter to replace the flat plate at the base of each cell with a machined or cast aluminum block, designed to accommodate several small test-tubes or scintillation vials, instead of a single flat-bottomed tray or dish. This would allow temperature control to be provided all around the container and its contents, rather than just at the base.

Summary

The computer-controlled thermal gradient system allows researchers to generate virtually any conceivable combination of temperature regimes, constant or fluctuating, within the range -10° to +60°C. It should therefore enable seed researchers, producers and nursery growers to determine optimal conifer seed germination temperatures quickly and cheaply. Its modular construction should allow limited space and funding to be used more effectively, since not all applications will require large numbers of temperatures to be generated simultaneously. Other applications for the system are possible.

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