Controlled-temperature Treatments with Low-cost, Off-the-shelf Equipment for Bud or Seed Forcing Experiments

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Abstract. Inexpensive plug-and-play temperature controllers have recently become available. These allow a chest freezer to be programmed easily to hold a desired set point across a range of biologically relevant temperatures. Installation can be completed in a few minutes using consumer-grade chest freezers. We used these temperature controllers to create five temperature-controlled chambers at 12, 14, 16, 18, and 20 °C. We demonstrated the use of these temperature controllers with two biologic assays: floral budbreak of peach [Prunus persica (L.) Batsch] stem cuttings and germination of sunflower (Helianthus annuus L.) seeds. We used the budbreak and germination rates at multiple temperatures to estimate base temperatures and thermal time requirements for development.

Characterizing the regulation of development by temperature requires controlled exposure of replicate plants (whole or in part) to multiple temperature environments simultaneously. Experiments with seeds or other small plant parts can be performed on a thermal gradient table, which can generate many temperatures simultaneously (Welbaum et al., 2016). However, experiments involving larger plant parts, such as cut stems used in forcing experiments of woody perennials, require temperature control of a larger three-dimensional volume, such as an environmental chamber (Anzanello and Biasi, 2016; Primack et al., 2015). Inexpensive access to the number of environmental chambers needed for the parameterizing temperature response curves for development is not common and can limit the scope of experiments. Modifications of consumer-grade freezers or refrigerators to bypass built-in temperature controllers with programmable controllers have been used for a variety of applications, but require a minimum level of technical ability to wire and install safely (Hutten-Czapski, 2017). Newly available plug-and-play temperature controllers allow conversion of a standard chest freezer into a controlled-environment chamber in minutes, with no custom modification.

Our objective in this study was to demonstrate that inexpensive, easy-to-use temperature controllers are able to provide reliable set temperatures for the detailed observation of developmental rates in response to different temperature treatments. We used the observed developmental rates at 12, 14, 16, 18, and 20 °C to estimate a thermal time parameter (base temperature) for the development of two different developmental events: floral budbreak in peach [Prunus persica (L.) Batsch] and seed germination in sunflower (Helianthus annuus L.). Developmental rates were evaluated at the temperatures mentioned to avoid the confounding influence of chilling temperatures on peach floral bud development, which are most effective between 4 and 8 °C (Anderson et al., 1986). Floral budbreak in Prunus sp. is commonly assumed to have a base temperature for development of 4 °C, but this assumption has not been widely tested across diverse species or varieties (Anderson et al., 1986). Recent work demonstrated genotypic variation in the base temperature for vegetative budbreak within fruit tree species, demonstrating the need to develop methods to test our assumptions with widespread screening of germplasm (Anzanello and Biasi, 2016). A base temperature of 6.7 °C is often used in germination modeling in sunflower, but varieties have been shown to have lower base temperatures (Khalifa et al., 2000).

Results and Discussion

All chambers showed a consistent oscillation representing the on/off cycles determined by the temperature controllers (Fig. 1). With the cooling differential set at 0.5 °C, freezers were only supplied power when the temperature reached the set point + 0.5 °C; the power was turned off when the temperature fell below the set point. Average chamber temperatures (11.8, 13.6, 15.9, 17.8, and 20.1 °C), monitored by data loggers, were slightly different from the temperature controller set points (12, 14, 16, 18, and 20 °C) despite placement of the data loggers near the

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controller temperature probe (Fig. 1). This was a result of a slight overshooting of the temperature past the set point on the cooling cycle. Observation of actual average temperature in each chamber could be used to adjust the temperature set point to achieve the desired environment.

The effectiveness of the chambers to investigate the effect of temperature on developmental rates was assessed with two biological assays: budbreak progress of peach floral buds warm-forced at different temperatures and sunflower seed germination (hypocotyl emergence from media). As expected, development at each temperature followed a sigmoid-shaped curve (Fig. 2). Both budbreak and germination showed a clear temperature-dependent effect on development, with each decrease in temperature slowing development from the previous temperatures (Fig. 2).

The minimum (or base) temperature for development is an important tool for modeling plant phenology. The linear relationship between development rate and temperature at suboptimal temperatures can be used easily to calculate an estimated base temperature for that developmental response (Anzanello and Biasi, 2016; Trudgill et al., 2005). We calculated the hours to reach median (50%) budbreak/germination at each temperature treatment by linear interpolation between the observations bracketing the median value. Expressing hours to median budbreak/germination at each temperature as a rate (per hour) produced a linear relationship with temperature (Fig. 3). Extrapolating this linear relationship to the point where development rate is expected to be zero (i.e., solving for \( x \) where \( y = 0 \)) provided an estimate of the minimum or base temperature required for development (Fig. 3). We used our data to calculate a base temperature of 4.75 °C for median floral budbreak in peach variety 'A209' (Fig. 3). This is close to the commonly used base temperature for warm-forcing of blooms in Prunus sp. of 4 °C (Anderson et al., 1986). The linear relationship up to 20 °C also indicates that we had not yet reached the optimum temperature for floral development in peach, which has been reported to be 25 °C (Anderson et al., 1986).

We calculated a base temperature for the sunflower seeds used in this experiment to be 6.3 °C, which falls within the range of reported base temperatures (3.3 to 6.7 °C) for sunflower varieties (Khalifa et al., 2000).

The ITC-308 is available through online retailers for about US$40 as of 2018. In the configuration we used in our study, with 17.5-ft³ freezers, the total cost of each chamber with all components mentioned in Materials and Methods was about US$718 as of 2018, of which the predominant expense was the chest freezer at US$500. Costs per chamber will be variable because consumer-grade chest freezers come in a wide variety of sizes. The desired chamber volume is dictated by the number and physical size of the samples to be tested. If either of these is small, costs per chamber can be reduced significantly by decreasing the size of the chest freezer used. Likewise, the electrical costs and electrical circuit demands of these chambers are dependent on the ambient temperature, internal temperature setting, and/or size of the chest freezer used. As with any electrical appliances, care should be taken to avoid exceeding the circuit capacity if all appliances draw current simultaneously.

These controllers will allow horticulturists, agronomists, foresters, and educators to design and perform experiments when multiple controlled-temperature environments are required without access to specialized facilities or skills. Off-the-shelf, easy-to-use components offer the potential to expand greatly the community of researchers who are able to incorporate temperature physiology into their investigations of plant development and phenology—particularly workers outside of traditional research institutions. Without humidity control or high-intensity lighting, these controllers will be of the most use in warm-forcing experiments such as budbreak or germination, when photosynthetic growth is not
required. Lighting with light-emitting diodes for photoperiod signaling can be added easily if desired. Finally, chamber temperatures should be validated independently of the controllers to allow adjustment of set points for accuracy.

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