Vernalization Responses of *Campanula* 'Birch Hybrid'

Sonali R. Padhye[1] and Arthur C. Cameron[2]
Department of Horticulture, Michigan State University, East Lansing, MI 48824

Abstract. The objective of this study was to characterize the influence of vernalizing temperatures and durations based on different flowering responses of *Campanula* 'Birch Hybrid'. Clonally propagated plants of *Campanula* 'Birch Hybrid' were exposed to –2.5, 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, or 20 °C for 0, 3, 5, 7, 9, or 12 weeks and were subsequently grown at 20 °C in a greenhouse. *Campanula* 'Birch Hybrid' exhibited a near-obligate vernalization requirement, and all flowering responses studied were influenced by the treatment temperatures, durations, and their interactions. The minimum and maximum cardinal temperatures for vernalization were <0 °C and between 15 and 17.5 °C, respectively. The range of optimal vernalizing temperatures (T_{opt}) varied based on the flowering response assessed. For instance, T_{opt} for flowering percentage ranged between 2.5 to 7.5 °C, while T_{opt} for number of open flowers was 0 to 12.5 °C when plants were vernalized for 5 weeks. T_{opt} for flowering time also varied when analyzed as rate to flower, time to flower from the end of temperature treatments, total time to flower measured from the start of temperature treatments, and thermal time to flower. For example, after 12 weeks of treatment, T_{opt} for thermal time to flower was 0 to 2.5 °C yet shifted to 2.5 to 12.5 °C for total time to flower. Because the flowering response being assessed altered the T_{opt}, this study reiterates the significance of considering all relevant flowering responses while developing and interpreting vernalization models.

Vernalization is defined as the promotion of flowering initiated by a cold treatment (Chouard, 1960; Thomas and Vince-Prue, 1984; Vince-Prue, 1975). Vernalization is quantitative in nature, and the intensity of the response is a function of the interaction between thermoinductive temperatures and durations of exposure. Thermoinductive temperatures can vary by species (Lang, 1965) and have been described as cardinal temperatures for vernalization with minimum (T_{min}), optimum (T_{opt}), and maximum (T_{max}) values (Atherton et al., 1990; Yan and Hunt, 1999). T_{min} and T_{max} are minimum and maximum temperatures at which the rate of vernalization is zero. The range of vernalizing temperatures defined by T_{min} and T_{max} is species-dependant and varies between less than –5 °C [*Secale cereale* (Lang, 1965)] and 17 °C [*Miltoniopsis* 'Trinity' (Lopez and Runkle, 2006)], respectively. T_{opt} is a range of vernalizing temperatures at which highest vernalization response is achieved and is also species-specific. By definition, T_{opt} is relative and is based on the vernalization response being assessed. When based on flowering percentage, T_{opt} varied between –2.5 and 0 °C for *Veronica spicata* 'Red Fox' (Fausey and Cameron, 2007), 5 °C for *Dianthus gratianopolitanus* 'Bath's Pink' (Padhye and Cameron, 2008), and 11 and 14 °C for *Miltoniopsis* 'Trinity' (Lopez and Runkle, 2006).

The cardinal vernalization temperatures can also change with treatment duration, even within a species (Lang, 1965). The duration of cold treatment has a quantitative effect on vernalization response until saturation, and therefore, increasing duration of cold treatment progressively increases flower-ing until the response saturates and additional exposure to thermoinductive temperatures does not cause a further change (Lang, 1965). Optimum temperatures and durations for vernalization have been characterized for several field crops, including wheat (*Triticum aestivum*), carrot (*Daucus carota*), and onion (*Allium cepa*) (Brooking, 1996; Robertson et al., 1996; Streck, 2003; Yan and Hunt, 1999), and select potted flowering plants, including cineraria (*Pericallis ×hybrida*) and easter lily (*Lilium longiflorum*) (Lange and Heins, 1990; Streck and Schuh, 2005; Yeh et al., 1997).

Different flowering responses to vernalization have been assessed and include the percentage of plants flowering in a population (Clough et al., 2001; Lange and Heins, 1990), node or leaf number at flowering (Brooking, 1996; Clough et al., 2001; Lange and Heins, 1990; Rawson et al., 1998; Robertson et al., 1996; Suzuki and Metzger, 2001), rate to flower (Streck, 2003; Yan and Hunt, 1999), time to flower (Clough et al., 2001; Rawson et al., 1998; Streck et al., 2003; Suzuki and Metzger, 2001; Yan and Hunt, 1999), thermal time to flower (Rawson et al., 1998), number of reproductive buds at flowering (Clough et al., 2001; Suzuki and Metzger, 2001), and percentage of reproductive lateral branches (Fausey and Cameron, 2007). Although many studies have determined T_{opt} based on thermoinductive temperatures, treatment durations, and one or more flowering responses listed above, to our knowledge, no study has yet characterized T_{opt} for all the listed flowering responses in one species.

Campanula 'Birch Hybrid', an interspecific cross of *Campanula portenschlagiana* and *C. poscharskyana*, is a hardy herbaceous perennial reported to exhibit a qualitative vernalization response (Enfield, 2002; Niu et al., 2004). *Campanula* 'Birch Hybrid' is a good candidate for investigating the vernalization responses because it produces large numbers of propagules from a few stockplants and can be vernalized as small clonal propagules. While stockplants are held in a controlled environment, propagation and thermoinductive treatments can be scheduled at varying times so that all treated

plants can be transferred and grown in a greenhouse simultaneously. Thus, all treated plants can be grown under the same temperature and light conditions regardless of treatment duration as demonstrated for *V. spicata* 'Red Fox' by Fausey and Cameron (2007). *Campanula* 'Birch Hybrid' has a day-neutral photoperiod response for flowering (Enfield, 2002). Niu et al. (2004) characterized the vernalization response of *Campanula* 'Birch Hybrid' to 0 to 10 °C for 0 to 8 weeks based on flowering percentage, time to visible bud and flower, number of flowering shoots, and number of flower buds per shoot. The authors concluded that temperatures of 0 to 10 °C were equally effective in promoting flowering of *Campanula* 'Birch Hybrid' after 6- or 8-week treatments. However, investigation of the responses of *Campanula* 'Birch Hybrid' to a broader range of temperatures and durations is lacking, and the cardinal temperatures for a range of vernalization responses are not established. The objective of this study was to characterize the effect of various thermoinductive temperatures and durations on different flowering responses of *Campanula* 'Birch Hybrid'. The T_{min} and T_{max} for vernalization of *Campanula* 'Birch Hybrid' were also determined, and the T_{opt} for a range of flowering responses was characterized.

Materials and Methods

STOCKPLANT MANAGEMENT AND PROPAGULE CULTURE. Clonally propagated *Campanula* 'Birch Hybrid' stockplants were grown in 13-cm square plastic containers prefilled with commercial soil-less medium (Sure-Mix; Michigan Grower Products, Galesburg, MI) and were rejuvenated by dividing and repotting as necessary during the experiment. The stockplants were grown in a controlled-environment chamber set at 22 °C under a 13-h photoperiod provided from 0600 to 1900 HR by incandescent [22% of the photosynthetic photon flux (*PPF*), 60A-130V; Philips, Somerset, NJ] and cool-white fluorescent lamps (78% of the *PPF*, VHOF96T12; Philips) ($\approx$150 μmol·m^{-2}·s^{-1} *PPF*). Stockplants were watered when necessary with acidified well water (H$_2$SO$_4$ to a titratable alkalinity of $\approx$140 mg·L^{-1} CaCO$_3$) containing nutrients (40 N, 4 P, 40 K, 5 Ca, 0.3 Fe, 0.2 Mn, 0.2 Cu, 0.03 B, 0.03 Mo, and 0.2 Zn mg·L^{-1}; MSU Special; Greencare Fertilizers, Chicago).

Vegetative cuttings with four to six nodes were taken starting 13 May 2003 and 17 Sept. 2003 for replications 1 and 2 of the study, respectively. Additional cuttings were taken 3, 5, 7, 9, and 12 weeks later during each replication. Cuttings were dipped in a commercial rooting hormone (Dip 'N Grow; Dip 'N Grow, Clackamas, OR) containing 1000 mg·L^{-1} indole-3-butyric acid and 500 mg·L^{-1} naphthalene acetic acid and were rooted in 72-cell trays (50-mL cell volume; Landmark Plastic, Akron, OH) containing 50% commercial peat-perlite media (Sure-Mix) and 50% coarse perlite (Therm-O-Rock; East, New Eagle, PA). Cuttings were rooted for 20 d under mist and were hand-watered for the first 4 d in a glass propagation house set at 23 °C air temperature and 26 °C media temperature, maintained by providing bottom heat, and 0.3 kPa vapor pressure deficit generated by injecting water vapor in the air. A blackout system was opened from 0800 to 1700 HR and maintained a 9-h photoperiod under natural sunlight in the propagation environment. After propagation, plants were grown for an additional 33 d in a controlled-environment chamber maintained at 20 °C under the same lighting and watering regimen as the stockplants.

TEMPERATURE TREATMENTS. For each replication, plants were transferred 57 d from the start of rooting to controlled-environment chambers set at –2.5, 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, or 20 °C for 0, 3, 5, 7, 9, or 12 weeks. Thus, each plant entered vernalization at the same stage of development. During temperature treatments, a combination of incandescent and fluorescent lamps provided 20 (–2.5 to 2.5 °C) or 100 (5–20 °C) μmol·m^{-2}·s^{-1} *PPF* for 11 h as described by Fausey and Cameron (2007). Plants were watered with acidified well water with nutrients described previously. Plants were transferred to the temperature treatments on different dates for each treatment duration such that all treatments ended on the same day (1 Oct. 2003 and 5 Feb. 2004 for replications 1 and 2, respectively).

PLANT CULTURE AND CLIMATE CONTROL. Following temperature treatments, nine plants per treatment were potted in 13-cm square plastic containers prefilled with commercial soil-less medium (Sure-Mix) and grown in a glass greenhouse set at 20 °C under a 16-h photoperiod. High-pressure sodium lamps provided supplemental lighting (150 μmol·m^{-2}·s^{-1} *PPF*) when ambient light was below 200 μmol·m^{-2}·s^{-1} and ceased after ambient light exceeded 400 μmol·m^{-2}·s^{-1}. When necessary, plants were watered with reverse osmosis water containing nutrients (125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe, 0.5 Mn, 1.0 Cu, 0.3 B, and 0.1 Mo mg·L^{-1}; MSU Special).

The greenhouse environment was regulated by a climate-control computer (model CD750; Priva, De Lier, The Netherlands). Air temperature was measured at plant height on every greenhouse bench by type E thermocouples (TT-E-40; Omega Engineering, Stamford, CT) placed in aspirated tubes, and *PPF* at plant height was measured at two locations by line quantum sensors containing 10 photodiodes (Apogee Instruments, Logan, UT). Temperature and light sensors were connected to a datalogger (CR10; Campbell Scientific, Logan, UT) and data were collected every 10 s; hourly averages were calculated and recorded in a computer. The average daily temperature (ADT) and daily light integral were calculated from the day of potting for 15 weeks and were 20.8 and 21.0 °C and 9.0 and 13.2 mol·m^{-2}·d^{-1} during replications 1 and 2, respectively.

DATA COLLECTION AND ANALYSIS. The number of nodes on each plant was counted at the start and end of temperature treatments. The time to develop one node during temperature treatment was computed and its reciprocal was used as the node development rate for regression analysis using PROC REG in SAS (version 9.1; SAS Institute, Cary, NC). Node development data from the two replications were not significantly different and therefore were pooled in the analysis. The base temperature for node development and the thermal time (°C d) required for developing one node were estimated from the linear regression as described by Roberts and Summerfield (1987).

The date of first open flower was recorded and the number of days from the end of temperature treatments to first open flower was reported as time to flower. Plants not having an open flower after 105 d in the greenhouse were considered vegetative. Rate to flower was computed by taking the reciprocal of time to flower and the rate to flower of vegetative plant was reported as zero. Because vegetative plants were eliminated from flowering time analysis, even though the same dataset was used to calculate rate to flower and time to flower, the analyses of these two responses differed for the treatments that did not achieve 100% flowering. Total time to flower was computed as days from the start of temperature treatments to first open flower. The thermal time (°C d) to flower of individual

flowering plants was calculated as accumulation of ADT above the estimated base temperature for node development from the beginning of temperature treatments to the day of first open flower.

The number of vegetative and reproductive lateral branches was counted 1 week after first open flower and percentage of reproductive lateral branches was computed for each plant. For each plant, the number of open flowers was counted 1 week after the first open flower was recorded. Additionally, during replication 1, for every 4th, 5th, and 6th plant to flower in each treatment group, the number of reproductive buds was recorded 1 week after the first open flower.

Days to flower, rate to flower, thermal time to flower, percentage of reproductive lateral branches, number of open flowers, and number of reproductive buds were analyzed using PROC MIXED of SAS, and the least significant difference procedure was used for paired comparisons with 0.05 as the value for significance. The experiment was completely randomized with temperature and duration as treatment factors in a factorial arrangement. Pooled data from both replications were analyzed for all response variables except number of open flowers. Number of open flowers differed significantly between the two replications and hence was analyzed separately.

Results

SURVIVAL AND LEAF DEVELOPMENT DURING TEMPERATURE TREATMENT. All plants treated at ≥ 0 °C survived and showed no visual symptoms of injury during their subsequent growth. After exposure to –2.5 °C, 36% and 27% of *Campanula* 'Birch Hybrid' plants died during replications 1 and 2, respectively, and several surviving plants showed varying degrees of visual symptoms of freezing injury such as water-soaked areas and necrosis. Therefore, all data from the –2.5 °C treatments were eliminated from the analyses.

During temperature treatments, time to develop one node decreased in a nonlinear fashion with increasing temperature (Fig. 1A) and averaged 50.8 and 7.5 d at 0 and 20 °C, respectively. The node development rate fit a linear regression model [$r^2 = 0.98$, significant at $P = 0.001$ (Fig. 1B)] and, by solving this regression model, the base temperature and the thermal time (°C d) for node development were estimated to be –2.9 °C and 151.5 °C d, respectively.

FLOWERING RESPONSE. No plants flowered without temperature treatment (Fig. 2) and only 1 of 90 plants flowered after treatment at 17.5 or 20 °C (Fig. 3A). Based on flowering percentage (the presence of at least one open flower per plant), *Campanula* 'Birch Hybrid' vernalized between 0 and 15 °C and the response was quantitative. For example, after treatment at 15 °C for 5, 7, and 12 weeks, flowering percentage was 0%, 39%, and 94%, respectively. Only 28% and 11% of plants flowered after 3 weeks at 0 and 5 °C, respectively. Complete (100%) flowering of *Campanula* 'Birch Hybrid' was achieved after 5 weeks at 2.5 to 7.5 °C, 7 weeks at 0 to 7.5 and 12.5 °C, and 9 and 12 weeks at 0 to 12.5 °C, while $\geq 85\%$ of plants flowered after 5 weeks at 0 and 10 °C, 7 weeks at 12.5 °C, and 12 weeks at 15 °C.

The percentage of reproductive lateral branches was also quantitatively influenced by temperature treatment and increased with temperature treatment duration for 7 to 9 weeks at 0 to 15 °C (Fig. 3B). Although several individual plants produced 100% reproductive lateral branches, no treatment
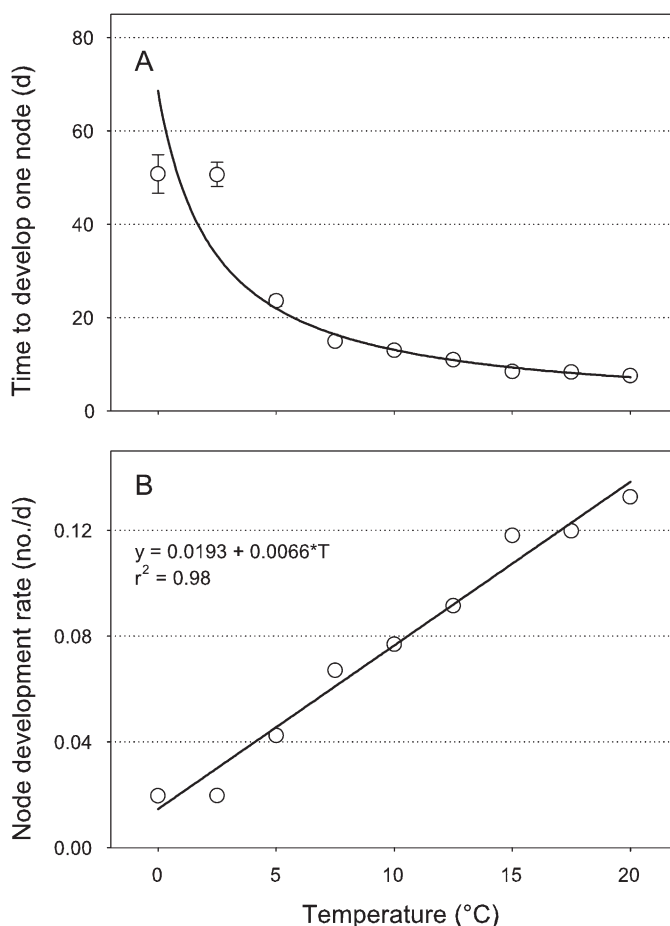

Fig. 1. Days per node (**A**) and node development rate (**B**) of *Campanula* 'Birch Hybrid' propagules as influenced by temperature treatment. Node development rate was calculated as the inverse of days per node. Open circles represent mean ± SE and solid lines represent the regression equations generated using 722 observations from two replications.

combination resulted in 100% reproductive lateral branches on all plants. Treatments that resulted in at least 75% reproductive lateral branches were 7 weeks at 5 and 7.5 °C and 9 weeks at 0, 2.5, and 10 °C. Percentage of reproductive lateral branches of the two plants that flowered after 17.5 and 20 °C treatments were 0.13 and 0.31, respectively, and were statistically similar to that of vegetative plants (0%). After treatments at 0 to 12.5 °C for ≥ 7 weeks, all plants had >50% reproductive lateral branches.

NUMBER OF OPEN FLOWERS AND REPRODUCTIVE BUDS. The number of open flowers counted 1 week after first open flower significantly differed between the two replications, but similar trends were noted between the datasets (Fig. 4, A–D). The number of reproductive buds was counted only in replication 1 and in some treatments was over 1000 per plant (Fig. 4, E and H). Treatment temperature, duration, and their interaction significantly influenced the number of reproductive buds and open flowers. The number of reproductive buds and open flowers after the 3-week treatment was statistically similar to the 0-week treatment. Overall, in both replications, the number of open flowers after the 5-week treatment at 0 to 12.5 °C was similar (Fig. 4A), while the number of reproductive buds dropped sharply at 12.5 °C and above. After the 7-week
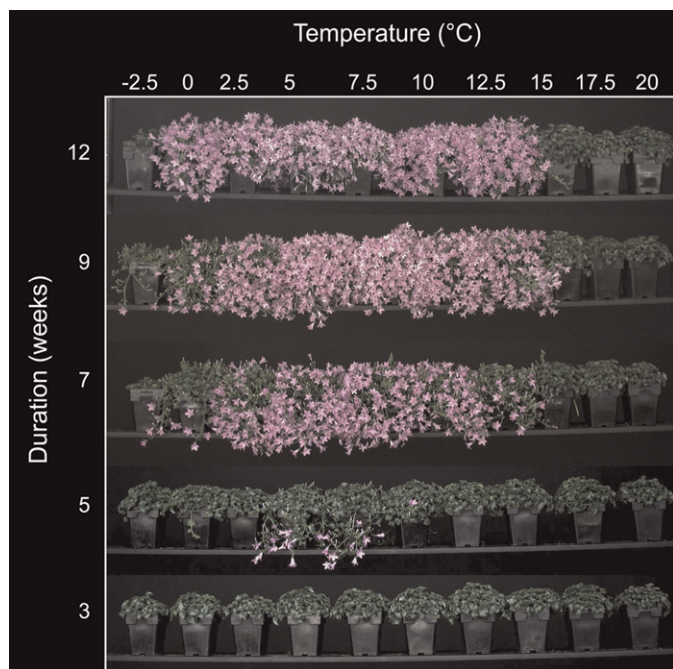
Fig. 2. Flowering response of *Campanula* 'Birch Hybrid' as influenced by temperature treatment and duration. The photograph was taken 63 d after plants were transferred to the greenhouse.

Fig. 3. Flowering percentage plants (**A**) and percentage of reproductive lateral branches (**B**) of *Campanula* 'Birch Hybrid' plants as influenced by temperature treatment and duration. Plants were considered vegetative if no open flowers had formed after 105 d and flowering percentage was computed for 18 plants from two replications. The number of vegetative and reproductive laterals was counted 1 week after the first open flower and percentage was computed for each plant. Reproductive lateral percentage was computed 1 week after the first open flower. Means ± SE of pooled data from two replications (n = 18) are presented in **B**.

treatment, more reproductive buds and open flowers were produced by plants treated at 7.5 to 12.5 °C compared with the other temperatures (Fig. 4, B and F). After 9 and 12 weeks of treatment, the maximum number of flowers was recorded mostly at 7.5 to 12.5 °C in replication 1, whereas in replication 2, plants treated at 5 to 12.5 °C generally produced more flowers (Fig. 4, C and D). In replications 1 and 2, the number of open flowers recorded after treatment at 0 °C did not increase with additional treatment durations after 7 and 5 weeks of treatment, respectively. Number of open flowers after treatment at 2.5 to 7.5 °C increased as the treatment duration increased to 12 weeks in replication 1, while in replication 2, the number of open flowers after treatment at 2.5, 5, and 7.5 °C did not increase after 9, 12, and 7 weeks, respectively. After treatment at 10 °C, the average number of open flowers did not increase significantly after 7 and 9 weeks of treatment in replications 1 and 2, respectively. In both replications, after treatment at 12.5 and 15 °C, maximum open flowers were produced after 9 weeks of treatment, with no further increase after treatment for 12 weeks.

Several plants produced over 1000 reproductive buds (Fig. 4, E–H), which was clearly evident in flower intensity (Fig. 2). The effect of treatment temperatures on the number of reproductive buds was variable, though in general, increasing treatment duration from 5 to 7 weeks increased the number of reproductive buds, while further increases to 9 or 12 weeks had little to no effect on the number of reproductive buds.

TIME TO FLOWER, TOTAL TIME TO FLOWER, AND RATE TO FLOWER. All parameters relating to flowering time were significantly affected by treatment temperatures, durations, and their interaction. The fastest time to flower from the end of temperature treatments was achieved after 12 weeks at 5 to 10 °C, whereas the fastest flowering from the start of temperature treatments was achieved after 5 weeks at 5 or 7.5 °C (Fig.
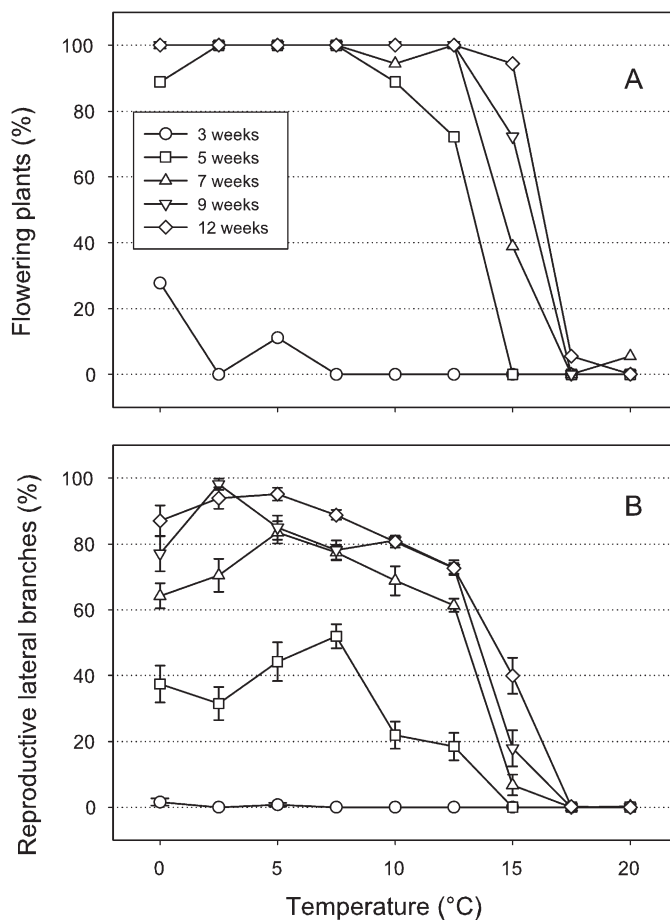
5, A and B). After treatment at 0 to 15 °C, the rate to flower from the end of temperature treatment continued to increase with treatment durations for up to 12 weeks, the longest duration tested. The most rapid rate to flower from the end of temperature treatment was after 12 weeks at 7.5 °C (Fig. 6A). However, the most rapid flowering from the start of temperature treatment was after 5 and 7 weeks at 5 and 7.5 °C (Figs. 5B and 6B). One plant each treated at 17.5 or 20 °C flowered, but flowering was considerably delayed and the rate to flower was close to zero and statistically similar to that of the vegetative plants. Also, rate to flower after a 3-week treatment was significantly less than flowering rates of all treatments that resulted in complete flowering.

THERMAL TIME TO FLOWER. The thermal time to flower from the start of temperature treatment varied between 1200 and 3600 °C d, depending on treatment temperature and duration, and generally increased from 0 to 20 °C (Fig. 7A). Based on thermal time from the start of temperature treatment, the vernalization response was saturated by 7 weeks, and the most rapid flowering occurred after exposure to the lowest temperatures tested. Plants flowering after treatment at 17.5 and 20 °C
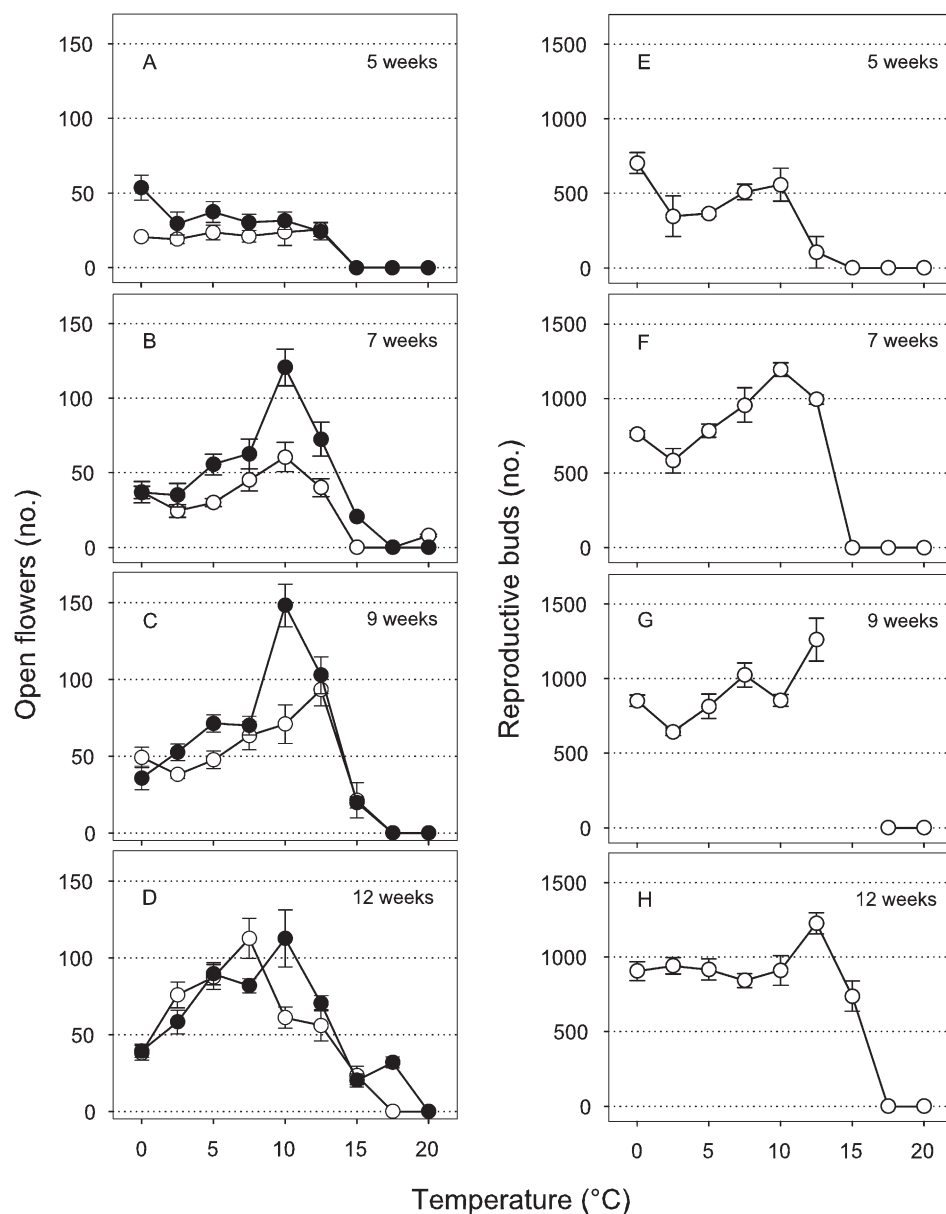
Fig. 4. The number of open flowers (**A–D**) and reproductive buds (**E–H**) on *Campanula* 'Birch Hybrid' plants as influenced by temperature treatment and duration. Durations were 5 weeks (**A** and **E**), 7 weeks (**B** and **F**), 9 weeks (**C** and **G**), and 12 weeks (**D** and **H**). One week after first open flower, the number of open flowers was counted on all plants and reproductive buds were counted on 4th, 5th, and 6th plant to flower in each treatment combination, and treatments resulting in <4 flowering plants were considered to have 0 buds and flowers. Open and closed symbols represent data from replications 1 and 2, respectively. Reproductive buds were only counted in replication 1. Means ± SE of data from each replication (n = 9) are presented in **A** through **D** and means ± SE of data from replication 1 (n = 3) are presented in **E** through **H**.

two flowering plants had low percentage of reproductive lateral branches and few open flowers. These findings indicate that *Campanula* 'Birch Hybrid' has a near-obligate vernalization requirement. From a practical perspective, *Campanula* 'Birch Hybrid' has an obligate vernalization requirement for complete, uniform, rapid, and profuse flowering (Runkle et al., 2001). A similar vernalization response has been reported in *Oenothera fruticosa* 'Youngii-lapsley' where only one of 180 plants flowered in four different experiments, with considerably delayed flowering (Clough et al., 2001).

In this study, it was established that based on flowering percentage, T_{min} for vernalizing *Campanula* 'Birch Hybrid' was <0 °C and T_{max} was between 15 and 17.5 °C. T_{min} ranged between –5 and 8 °C for *S. cereale* 'Petkus' and *Miltoniopsis* 'Trinity', respectively (Lang, 1965; Lopez and Runkle, 2006), and T_{max} ranged between 12.5 and 17 °C for *V. spicata* 'Red Fox' and *Hyoscyamus niger*, respectively (Fausey and Cameron, 2007; Lang, 1965). Thus, T_{min} and T_{max} of *Campanula* 'Birch Hybrid' were within the reported range for other species. Due to plant mortality and evidence of freezing injury, data for *Campanula* 'Birch Hybrid' plants treated at –2.5 °C were not presented in this study, though plants that survived exposure to –2.5 °C flowered. Based on this observation, it is expected that the T_{min} of *Campanula* 'Birch Hybrid' is less than –2.5 °C. The USDA cold hardiness zones of the parents of *Campanula* 'Birch Hybrid', *C. portenschlagiana* and *C. poscharskyana*, are 4 and 3, respectively (Griffiths, 1994), and *Campanula* 'Birch Hybrid' is hardy at least up to zone 5 (personal observation). Lange and Heins (1990) reported death of *L. longiflorum* 'Nellie White' at –2.5 °C and Fausey and Cameron (2007) reported death of some *Laurentia axillaris* at –2.5 °C and 0 °C. It is likely that acclimation at 0 or 5 °C for 2 weeks before treatment at –2.5 °C would have improved the survival of *Campanula* 'Birch Hybrid' as reported by Engle (1994) for 20 species of herbaceous perennials, including *Campanula carpatica*.

The T_{opt} for vernalization is also species-specific and can differ depending on the duration of temperature treatment and the flowering response being assessed (Lang, 1965). Additionally, increased duration of low temperature exposure typically
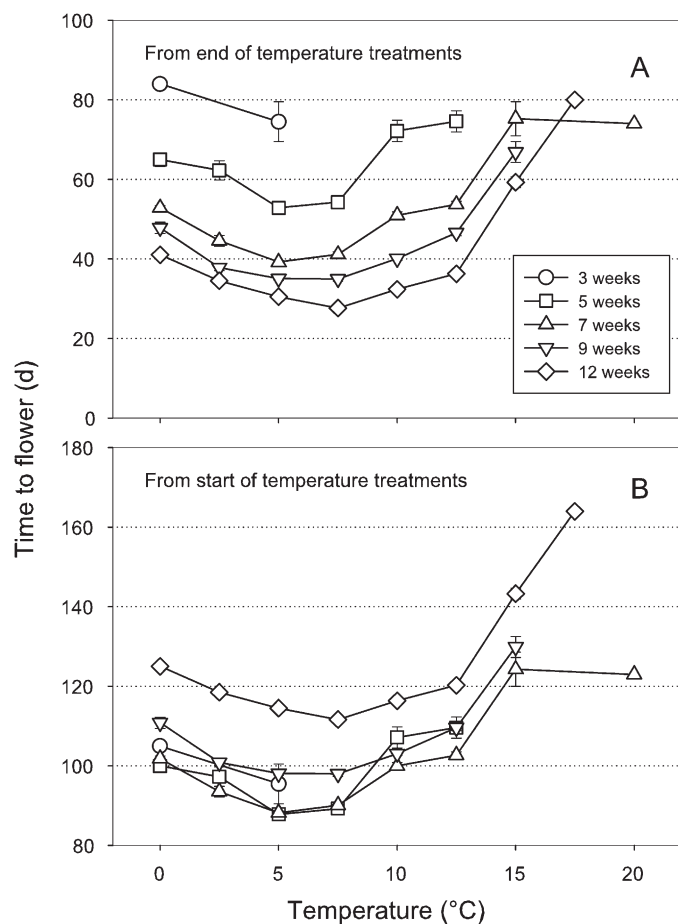
required significantly greater thermal time to flower compared with plants treated at 0 to 15 °C at the respective durations.

Discussion

Campanula 'Birch Hybrid' has been previously reported to have an obligate vernalization requirement (Enfield, 2002; Niu et al., 2004). In the current study, two of 180 plants flowered after exposure to 17.5 and 20 °C. However, flowering of these plants was delayed and their average rate to flower approached zero and was similar to vegetative plants. Additionally, these

Fig. 5. Time to flower from the end (**A**) and start (**B**) of temperature treatments as influenced by treatment temperature and duration in *Campanula* 'Birch Hybrid'. Means ± SE of pooled data from two replications (n = 18) are presented.

Fig. 6. Rate to flower from the end (**A**) and start (**B**) of temperature treatments as influenced by treatment temperature and duration in *Campanula* 'Birch Hybrid'. Means ± SE of pooled data from two replications (n = 18) are presented.

improved the vernalization response until saturation (Lang, 1965; Thomas and Vince-Prue, 1984). In the current study, numerous vernalization responses were assessed, including flowering percentage, rate to flower, time to flower from the start and end of temperature treatments, thermal time to flower from the start of temperature treatment, percentage of reproductive lateral branches, and the number of flowers and reproductive buds. When assessed as flowering percentage, the T_{opt} was 2.5 to 10 °C after 5 weeks and 0 to 12.5 °C after ≥7 weeks. Because no treatment duration at 15 °C resulted in complete (100%) flowering, based on flowering percentage, 12 weeks was insufficient to saturate the flowering response at 15 °C.

The quantitative nature of the vernalization response is often illustrated by flowering time or leaf number at flowering. In this study, the influence of temperature treatment on flowering time was evaluated. Time to flower measured from the end of temperature treatments to the first open flower decreased as the treatment durations increased for up to 12 weeks. T_{opt} for fastest time to flower from the end of temperature treatments was 5 to 7.5 °C after 5 weeks, 2.5 to 7.5 °C after 7 weeks, 2.5 to 7.5 °C after 9 weeks, and 2.5 to 12.5 °C after 12 weeks. Hence, similar to flowering percentage, prolonging durations of treatments broadened the T_{opt} of time to flower, although T_{opt} for flowering percentage and time to flower differed. The effect of thermoinductive temperatures and durations
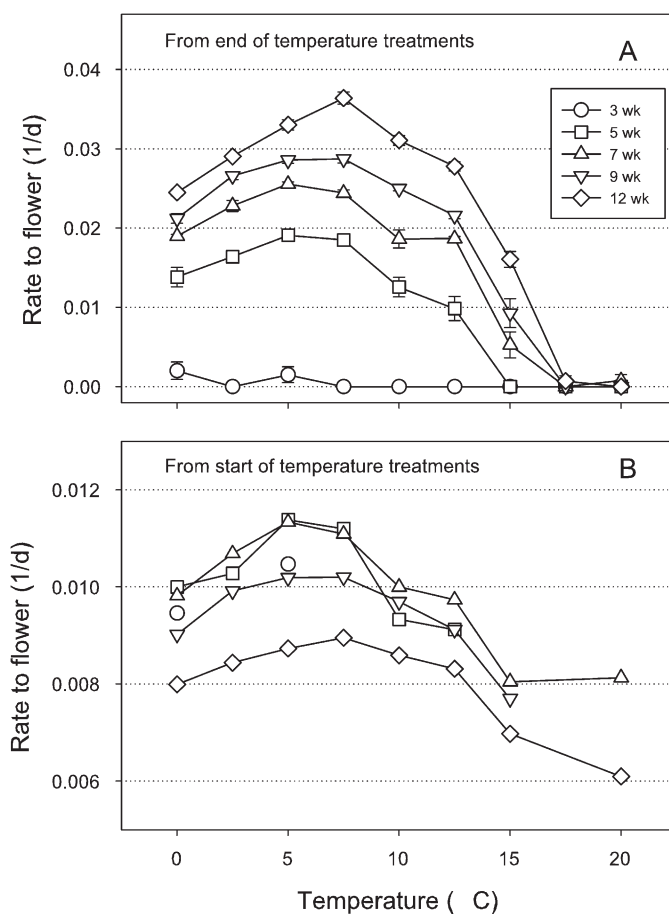
on total flowering time measured from start of temperature treatments to the first open flower was also evaluated to account for plant development during the treatments. The T_{opt} for time to flower from the start and end of temperature treatments was the same but the durations at which the response saturated differed. Time to flower from the start of temperature treatment did not decrease with additional treatment after 3 weeks at 0 °C, 7 weeks at 2.5 °C, 5 weeks at 5 and 7.5 °C, and 7 weeks at 10 to 15 °C. Notably, a 12-week treatment at 0 to 15 °C increased total time to flower significantly compared with 5- to 9-week treatments, however, time to flower measured after treatments decreased at 0 and 5 to 15 °C after 12 weeks. Thus, an additional 3 weeks of treatment decreased time to flower by <3 weeks.

The flowering times reported in this study are up to 3 weeks longer than those reported by Niu et al. (2004) and up to 3 weeks shorter than those reported by Enfield (2002). Differences in starting material and environmental conditions before and after temperature treatments may have caused these differences. Additionally, Niu et al. (2004) reported that temperature did not affect flowering time after treatment at 0 to 10 °C for 6 and 8 weeks, which is contradictory to the findings of this study. This discrepancy cannot be explained.

The developmental rate of plants is temperature dependant. To account for differences in plant development during
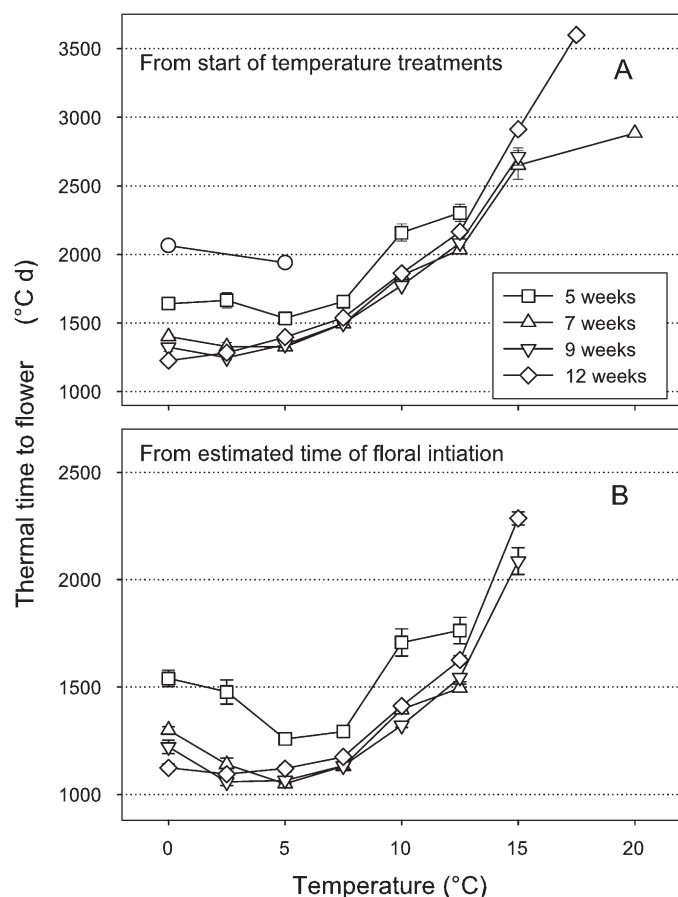
502

Fig. 7. Thermal time to flower of *Campanula* 'Birch Hybrid' calculated from the start of temperature treatments (**A**) and from the estimated time of floral initiation (**B**), which was assumed to be 5 weeks after the beginning of the temperature treatments. Thermal time to flower was calculated as average daily temperature minus estimated leaf unfolding base temperature of –2.9 °C and expressed in °C d. Thermal time to flower was calculated only for treatment combinations resulting in >70% flowering. Means ± SE of pooled data from two replications (n = 18) are presented.

temperature treatments, flowering response was also evaluated based on the thermal time to flower, which was first computed from the start of temperature treatments to the first open flower. Thermal time to flower decreased after treatment at 0 to 15 °C for up to 7 weeks. The T_{opt} based on thermal time to flower was 5 °C after 5 weeks, 0 to 5 °C after 7 and 9 weeks, and 0 and 2.5 °C after 12 weeks. However, theoretically, thermal time to flower should begin when a plant has made a developmental switch to flowering. When calculated from the start of temperature treatments, thermal time to flower would be overestimated. To evaluate the effect of this overestimation on flowering response, 5 weeks was postulated as the time when the switch to flowering was made because most plants flowered in response to a 5-week treatment duration, and the thermal time to flower was recalculated from this point in time (Fig. 7B). After treatment at 2.5 to 12.5 °C, the effect of treatment durations on thermal time to flower after initiation was similar to thermal time to flower from the start of treatments. However, at 0 °C, the minimum thermal time to flower after initiation was achieved after 12 weeks of treatment. T_{opt} for each treatment duration varied slightly and was 5 or 7.5 °C after 5 weeks, 2.5 to 7.5 °C after 7 and 9 weeks, and 0 to 7.5 °C after 12 weeks.

The effect of temperature treatments and durations on flowering is often evaluated based on flowering time, and flowering time has been used to develop vernalization models in crops, including wheat and easter lily (Lange and Heins, 1990; Rawson et al., 1998; Streck and Schuh, 2005). Changes in vernalization optima based on the response variable being assessed were reported in wheat cultivars by Rawson et al. (1998). The response being assessed altered the temperature optima of *Campanula* 'Birch Hybrid' in this study and, therefore, is significant in developing and interpreting vernalization models.

The effect of temperature treatments on percentage of reproductive lateral branches, flower number, and number of reproductive buds was also evaluated. All data were collected 1 week after the first open flower, which was chosen to account for gradual flower opening. Overall, T_{opt} for percentage of reproductive lateral branches was 0 to 7.5 °C after 5 weeks of treatment and 0 to 12.5 °C after 7 to 12 weeks of treatment. Increasing treatment durations for up to 9 weeks typically increased percentage of reproductive lateral branches (Fig. 3). Overall, ≥7 weeks at 0 to 12.5 °C resulted in >60% reproductive lateral branches. It is expected that additional laterals were formed during the treatment at warmer temperatures and, depending on the temperature and duration, only some of these new laterals vernalized during the treatment. However, the specific relationship of treatment temperatures and durations with formation and vernalization of lateral branches is unknown. Also, when flowering was delayed, additional vegetative lateral branches may have developed in the greenhouse, affecting flowering percentage lateral branches reported. However, no correlation was found between percentage of reproductive lateral branches and rate to flower (data not presented), indicating that their relationship is complex.

The number of open flowers varied to even a greater extent, with significant differences in flower number between the two replications of the study (Fig. 4). Flowering response, assessed as the number of open flowers, typically saturated after ≥7 weeks of treatment, depending on the temperature and replication. After 7 weeks of treatment at 0 to 12.5 °C, >25 open flowers were present on all flowering plants. Due to the time constraint imposed by counting the very large number of reproductive buds present, the number of reproductive buds was counted on 4th, 5th, and 6th plants to flower to measure the "average" potential of the flowering treatment group. There was no consistent trend associated with the highest number of reproductive buds at different treatment temperatures and durations. This was at least in part due to high variability amongst the experimental units. It is notable that ≥7 weeks at 0 to 12.5 °C resulted in an average of 583 to 1261 reproductive buds. Unexpectedly, there was no correlation between average number of reproductive buds and average number of open flowers (data not presented). The number of open flowers and reproductive buds may have been affected by percentage of reproductive laterals and development of plants during temperature treatments, especially at warmer temperatures, although these relationships were unclear. The number of open flowers and reproductive buds are important from the standpoint of marketability of potted plants; however, unlike edible crops, higher numbers do not always translate into greater financial returns. Because after ≥7 weeks of treatment at 0 to 12.5 °C, the average number of reproductive buds and open

flowers present were >500 and >25, respectively, these *Campanula* 'Birch Hybrid' plants were certainly floriferous.

The complexity of vernalization is not limited to its physiological aspects, but also extends to the analysis and interpretation of the flowering responses. The effective treatment temperatures and durations for optimum flowering response have been reported based on many responses, including flowering percentage, node development, rate of node development, time to visible bud or flower, thermal time to flower, number of reproductive buds at flowering, and percentage of reproductive lateral branches. The present study conclusively demonstrates that the optimum temperatures and durations for vernalizing *Campanula* 'Birch Hybrid' varied significantly based on the response being assessed. In general, temperatures between 0 and 12.5 °C successfully vernalized *Campanula* 'Birch Hybrid' when given for ≥7 weeks, although extending durations for up to 12 weeks improved some flowering responses.

Literature Cited

Atherton, J.G., J. Craigon, and E.A. Basher. 1990. Flowering and bolting in carrot: I. Juvenility, cardinal temperatures and thermal times for vernalization. J. Hort. Sci. 65:423–429.

Brooking, I.R. 1996. Temperature response of vernalization in wheat: A developmental analysis. Ann. Bot. (Lond.) 78:507–512.

Chouard, P. 1960. Vernalization and its relations to dormancy. Annu. Rev. Plant Physiol. 11:191–237.

Clough, E.A., A.C. Cameron, R.D. Heins, and W.H. Carlson. 2001. Growth and development of *Oenothera fruticosa* is influenced by vernalization duration, photoperiod, forcing temperature, and plant growth regulators. J. Amer. Soc. Hort. Sci. 126:269–274.

Enfield, A.L. 2002. Flower induction and cultural requirements for quick-cropping of the herbaceous perennials *Veronica spicata, Phlox paniculata, Leucanthemum ×superbum, Achillea, Gaura lindheimeri,* and *Campanula*. Michigan State Univ., East Lansing, M.S. thesis.

Engle, B.E. 1994. Use of light and temperature for hardening of herbaceous perennial plugs prior to storage at –2.5 °C. Michigan State Univ., East Lansing, M.S. thesis.

Fausey, B.A. and A.C. Cameron. 2007. Differing vernalization responses of *Veronica spicata* 'Red Fox' and *Laurentia axillaris*. J. Amer. Soc. Hort. Sci. 132:751–757.

Griffiths, M. 1994. Index of garden plants. Timber Press, Portland, OR.

Lang, A. 1965. Physiology of flower initiation, p. 1371–1576. In: W. Ruhland (ed.). Encyclopedia of plant physiology. Springer-Verlag, Berlin.

Lange, N.E. and R.D. Heins. 1990. Modeling temperature and photoperiod inducted flowering in *Lilum longiflorum*. Acta Hort. 272:115–119.

Lopez, R.G. and E.S. Runkle. 2006. Temperature and photoperiod regulate flowering of potted *Miltoniopsis* orchids. HortScience 41:593–597.

Niu, G., R.D. Heins, A.C. Cameron, and W.H. Carlson. 2004. Vernalization and devernalization of *Campanula* 'Birch Hybrid' and *Leucanthemum ×superbum* 'Snowcap'. HortScience 39:1647–1649.

Padhye, S.R. and A.C. Cameron. 2008. *Dianthus gratianopolitanus* Vill. 'Bath's Pink' has a near-obligate vernalization requirement. HortScience 43:346–349.

Rawson, H.M., M. Zajac, and L.D. Penrose. 1998. Effect of seedling temperature and its duration on development of wheat cultivars differing in vernalization response. Field Crops Res. 57:289–300.

Roberts, E. and R. Summerfield. 1987. Measurement and prediction of flowering in annual crops, p. 17–51. In: J.G. Atherton (ed.). Manipulation of flowering. Butterworth, London.

Robertson, M.J., I.R. Brooking, and J.T. Ritchie. 1996. Temperature response of vernalization in wheat: Modeling the effect on the final number of mainstem leaves. Ann. Bot. (Lond.) 78:371–381.

Runkle, E.S., R.D. Heins, A.C. Cameron, and W.H. Carlson. 2001. Minireview of research activity: Horticultural flowering of herbaceous perennials. Flowering Nwsl. 31:34–43.

Streck, N.A. 2003. A vernalization model in onion. Revista Brasileira de Agrociência 10:99–105.

Streck, N.A. and M. Schuh. 2005. Simulating the vernalization response of the "Snow Queen" lily (*Lilium longiflorum* Thunb.). Scientia Agr. 62:117–121.

Streck, N.A., A. Weiss, and P.S. Baezinger. 2003. A generalized vernalization response function for winter wheat. Agron. J. 95:155–159.

Suzuki, A. and J.D. Metzger. 2001. Vernalization in a greenhouse promotes and synchronizes flowering of *Osteospermum ecklonis* Norl. HortScience 36:658–660.

Thomas, B. and D. Vince-Prue. 1984. Juvenility, photoperiodism and vernalization, p. 408–439. In: M.B. Wilkins (ed.). Advanced plant physiology. Pitman, London.

Vince-Prue, D. 1975. Vernalization, p. 263–291. In: Photoperiodism in plants. McGraw Hill, London.

Yan, W. and L.A. Hunt. 1999. Reanalysis of vernalization data of wheat and carrot. Ann. Bot. (Lond.) 84:615–619.

Yeh, D.M., J.G. Atherton, and J. Craigon. 1997. Manipulation of flowering in cineraria. III. Cardinal temperatures and thermal times for vernalization. J. Hort. Sci. 72:379–387.