Vernalization and Devernalization of *Campanula* ‘Birch Hybrid’ and *Leucanthemum* $\times$ *superbum* ‘Snow Cap’

Genhua Niu,¹ Royal Heins,² Arthur Cameron, and William Carlson
Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

Additional index words. flowering characteristics, herbaceous perennials, vernalization temperature

Abstract. The influence of vernalization temperature and duration and devernalization treatments on subsequent growth and flower development of *Campanula* ‘Birch Hybrid’ and *Leucanthemum* $\times$ *superbum* Bergman ex J. Ingram ‘Snow Cap’ was determined. In the vernalization experiment, plants of ‘Birch Hybrid’ were vernalized at 0, 2.5, 5, 7.5, or 10 °C for 2, 4, 6, or 8 weeks. ‘Snow Cap’ was vernalized at 0, 2.5, 5, or 10 °C for 2, 4, 6, or 8 weeks. In another devernalization experiment, plants of both species were moved to a high temperature (30/10 °C, day/night) growth chamber for 2 or 4 days at various times during or after the 6-week vernalization period. A 6-week vernalization was necessary to obtain 100% flowering in ‘Birch Hybrid’, and 8 weeks of vernalization decreased time to flower by 7 to 10 days compared with 6-week vernalization. Exposure to high temperature for 2 days during or immediately after vernalization did not devernalize ‘Birch Hybrid’ plants, while a 4-day exposure decreased flowering percentage in some treatments and delayed flowering by 7 to 10 days. There were no significant differences in flowering characteristics of ‘Snow Cap’ plants vernalized at 0 to 5 °C for 4 to 8 weeks. A 2-week vernalization at 0, 2.5, 5, or 10 °C or 4 to 8 week vernalization at 10 °C delayed flowering by 5 to 10 days compared with those vernalized at 0 to 5 °C for 4 to 8 weeks. Exposure to high temperature for 2 d did not devernalize ‘Snow Cap’ plants regardless of exposure times, but a 4-day exposure delayed flowering by 4 to 5 days in some treatments. Combined, the data indicate that ‘Birch Hybrid’ has an obligate 6-week vernalization requirement and ‘Snow Cap’ has a facultative 4-week vernalization requirement that can be fulfilled in the 0 to 10 °C range. Exposure to temperatures of 30 °C (9 h·d⁻¹) for 12 out of 42 days did not devernalize either species but in some cases caused a small delay in time to flower.

Herbaceous perennial sales and demands for vernalized plants throughout the year are expected to increase. Flowering at the time of sale is an important marketing concern, because many consumers prefer to purchase plants in flower. Therefore, producers are interested in understanding flower induction requirements and how to bring herbaceous perennials into flower at scheduled dates.

Many herbaceous perennials require a certain period of cold treatment or vernalization for flowering. Vernalization is defined as a cold treatment that promotes subsequent flowering when given to imbibed seeds, bulbs, or whole plants (Vince-Prue, 1975). The effectiveness of vernalization is governed by both genetic and environmental factors (Napp-Zinn, 1987). Devernalization is a complete or partial reversal of vernalization (Lang, 1965). It results from an interaction between the degree of vernalization and environmental conditions during or immediately after the vernalization. High temperatures (>25 °C) during or immediately after vernalization may promote devernalization. Some

herbaceous perennial producers vernalize their plants in simple growing structures, relying on the naturally cold conditions of winter. In this case, plants may experience warm temperatures (>25 °C) on sunny days, particularly in late winter or early spring. Devernalization by high temperature has been reported in some cold-requiring plants, such as *Daucus carota* L. (Hiller and Kelly, 1979) and *Apium graveolens* L. (Booij and Meurs, 1993). Exposure to high temperature (30 °C) before or after vernalization delayed flowering in a range of genotypes of spring rape (*Brassica napus* var. *nanura* L.) and 4 d of exposure had more effect than that of 2 d (Dahanayake and Galwey, 1998). However, information on devernalization based on experimental data is limited in herbaceous perennials.

The objective of this study was to better understand the processes of vernalization and devernalization in order to ensure uniform flowering of herbaceous perennials. Specifically, we aimed to characterize the effect of a range of vernalization temperatures and durations on subsequent growth and flowering of *Campanula* ‘Birch hybrid’ and *Leucanthemum* $\times$ *superbum* ‘Snow Cap’. We also aimed to examine if these species would be devernalized by exposing the plants to high temperatures for certain periods of time during or immediately after vernalization.

Plant materials and culture. Vegetative cuttings were taken from stock plants grown under short days (9 h·d⁻¹) of C. ‘Birch Hybrid’ and *L. $\times$ superbum* ‘Snow Cap’ and were rooted in 72-cell trays (50-mL cell volume) for 3 weeks in a propagation house at air and medium temperatures of $\approx$ 23 and 26 °C. Rooted cuttings were then grown in a glass greenhouse under a 12-h photoperiod at 20 $\pm$ 2 °C and a daily light integral of about 5 mol·m⁻²·d⁻¹ (natural sunlight) for 3 weeks from January to early February 2001 before the treatment. Photoperiod was maintained by supplemental lighting from 5:00 to 8:00 PM using incandescent light and by pulling black cloth at 5:00 PM and opening at 8:00 AM. A peat-perlite mix (Sure-Mix, Michigan Grower Products, Galesburg, Mich.) was used for rooting and potting media. Plants were fertilized at every irrigation with a nutrient solution of well water [electrical conductivity (EC) of 0.70 d·Sm⁻¹ and 105, 35, and 85 mg·L⁻¹ Ca, Mg, and S, respectively] acidified with H₂SO₄ to a titratable CaCO₃ alkalinity of 130 mg·L⁻¹ and water soluble fertilizer composed of 125N–12P–125K–13Ca (mg·L⁻¹) plus 1.0Fe–0.5Mn–0.5Zn–0.5Cu–0.1B–0.1Mo (mg·L⁻¹; MSU Special, Greencare Fertilizers, Chicago, Ill.).

Vernalization. Plants of ‘Birch Hybrid’ in 72-cell trays were placed in coolers set at 0, 2.5, 5, 7.5, or 10 °C for 2, 4, 6, or 8 weeks under 5 to 10 μ mol·m⁻²·s⁻¹ photosynthetic photon flux provided by fluorescent lamps for 9 h·d⁻¹. For ‘Snow Cap’, vernalization temperature was 0, 2.5, 5, or 10 °C, since a 7.5 °C cooler was not available when this species was vernalized. The experimental design was a two-way factorial arrangement of treatments in a randomized complete block design.

Devernalization. Plants of both species in 72-cell trays were placed in a cooler set at 5 °C under 5 to 10 μ mol·m⁻²·s⁻¹ provided by fluorescent lamps for 9 h·d⁻¹ for 6 weeks. During the 6-week vernalization, plants were moved to a high-temperature growth chamber at the end of the second, fourth, or sixth week for 2 or 4 d. Therefore, plants were moved to a high temperature growth chamber for 2 or 4 d for once (week 2; week 4; week 6), twice (week 2, 4; week 2, 6; week 4, 6), or three times (week 2, 4, 6). The growth chamber was set at temperature of 30/10 °C (day/night), a photoperiod of 9 h·d⁻¹, and a PPF of 120 μ mol·m⁻²·s⁻¹ provided from cool-white fluorescent lamps during the day. The control plants were vernalized at 5 °C continuously for 6 weeks without being exposed to high temperatures.

While in the coolers or growth chamber in all experiments, plants were irrigated as necessary with well water (CaCO₃ at 340 mg·L⁻¹) acidified (93% H₂SO₄) to a titratable alkalinity of CaCO₃ at 100 mg·L⁻¹. There were 10 plants in each treatment in both experiments. Plants were potted to 13-cm square plastic (1.1-L volume) containers before being placed in greenhouses after vernalization. The temperature in the greenhouse after vernalization was maintained at 20 $\pm$ 2 °C. Photoperiod (16 h·d⁻¹) and supplemental lighting were provided daily

Received for publication 12 Nov. 2003. Accepted for publication 12 Apr. 2004.

¹Current address: Agriculture Research and Extension Center at El Paso, Texas A&M University, 1380 A&M Circle, El Paso, TX 79927.

²Author to whom correspondence should be addressed; e-mail heins@msu.edu.

by HPS lamps between 0600 to 2200 HR at a PPF of about 65 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at plant canopy level when PPF in the greenhouse was lower than 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Data collection and analysis. Dates of visible bud and first flower were recorded for both species in both experiments. For 'Birch Hybrid', number of flower buds per shoot and number of reproductive or flowering shoots per container were recorded when plants first flowered. For 'Snow Cap', number of flower buds per container and height at flower were recorded. Statistical Analysis System's (SAS Institute, Cary, N.C.) PROCMEANS was used to calculate the means and their 95% confidence intervals. PROC general linear model was used to test the significance or contrast among the treatments (control vs. 2- or 4-d exposure and exposure once versus twice).

Results

Campanula 'Birch Hybrid'. No 'Birch Hybrid' plants flowered with 2 or fewer weeks of vernalization. Following 4-week vernalization, flowering percentages ranged from 20 to 90; plants averaged only three flowering shoots per container, and most shoots were vegetative. These results indicate that vernalization was incomplete with 4 weeks or less vernalization. All plants flowered and no significant differences in time to visible bud or to flower were observed among various vernalization temperatures when plants were vernalized for 6 weeks (Fig. 1A and B). Following 8-week vernalization at 2.5 to 7.5 °C, time to visible buds or to flower decreased by 7 to 10 d, number of flowering shoots per pot increased by ≈ 5 , but number of flower buds per shoot decreased by ≈ 20 , compared to plants with 6-week vernalization (Fig. 1A–D). Significant interaction of vernalization temperature and duration was observed on flowering characteristics (time to visible bud or flower, flowering shoot number per container and number of flower bud per shoot) of this species.

There were no significant differences in time to visible bud or to flower among 'Birch Hybrid' plants that were moved to the high-temperature growth chamber at 30/10 °C (day/night) for 2 or 4 d at various times during the 6-week vernalization compared to the plants in the control (Table 1). Flowering was significantly delayed by 2 to 10 d when plants were exposed to high temperatures for two or three times for 4 d compared with 2 d. Flowering percentage was 70 or 90 for plants when they were moved to high temperatures for 4 d for two or three times at the end of the second or fourth week (weeks 2, 4) or at the end of the second, fourth, and sixth week (weeks 2, 4, 6), respectively. The 4-d exposures to high temperature also significantly decreased number of flowering shoots per pot except for those plants that were moved to high temperature at the end of fourth or sixth week (week 4, 6).

Leucanthemum $\times$ superbum 'Snow Cap'. There were no significant differences in flowering characteristics among 'Snow Cap' plants vernalized at 0 to 5 °C for 4 to 8 weeks (Fig.

2A–C). Plants vernalized at 0 to 5 °C for 2 weeks took 5 to 6 d longer compared with longer vernalization durations (Fig. 2B). Flowering was significantly delayed by 5 to 10 d when plants were vernalized at 10 °C for 4 to 8 weeks compared with 0 to 5 °C (Fig. 2B).

All plants flowered, and generally there were no substantial differences in flowering characteristics, regardless of exposure to high temperature for 2 or 4 d (Table 2). Flowering was delayed by 4 to 5 d in the plants that were exposed to high temperatures for 4 d for two

or three times at the end of fourth and sixth week of the 6-week vernalization (weeks 4, 6) or at the end of second, fourth, and sixth weeks (weeks 2, 4, 6). Number of flower buds per pot was not influenced by the exposure to high temperature. Generally, plants were taller when they were exposed to high temperature during vernalization for 4 d for multiple times compared with those exposed to high temperature once for 2 d.

Discussion

Fig. 1. Influence of vernalization temperature and duration on days to visible bud (A), days to flower (B), number of flowering shoots (C), and number of flower buds per shoot (D) of *Campanula* 'Birch Hybrid'. Vertical bars represent 95% confidence intervals.

Table 1. Flowering of *Campanula* 'Birch Hybrid' exposed to high-temperature (30/10 °C, day/night) growth chambers for 2 or 4 d at various times during or at the end of 6-week vernalization. Control plants were vernalized for 6 weeks at 5 °C.

Treatment week	Days to visible bud	Days to flower	Flowers/shoot	Flowering shoots
Control	33.0	60.7	61.4	9.9
2-Day exposure to high temperature				
2	34.7	64.7	65.0	9.5
4	31.6	59.0	58.9	11.0
6	32.5	61.8	51.8	12.5
2, 4	32.5	62.1	62.3	9.5
2, 6	32.0	60.3	54.9	11.9
4, 6	32.0	61.3	55.7	12.3
2, 4, 6	32.6	61.7	52.0	9.2
4-Day exposure to high temperature				
2	36.3	69.0	59.5	7.3
4	34.6	67.4	52.9	9.9
6	30.1	58.8	57.8	10.7
2, 4	44.4	73.9	65.9	4.6
2, 6	36.1	66.9	55.1	6.0
4, 6	31.3	66.1	64.9	7.2
2, 4, 6	36.2	66.8	55.3	5.7
ANOVA—Orthogonal contrasts				
Control vs. 2-d	NS	NS	*	NS
Control vs. 4-d	NS	NS	NS	NS
2-d vs. 4-d	***	***	NS	***
Once vs. twice	***	*	NS	***
Once vs. twice for 2-d	NS	NS	NS	*
Once vs. twice for 4-d	***	*	NS	*

NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Fig. 2. Influence of vernalization temperature and duration on days to visible bud (A), days to flower (B), and number of flower buds (C) of *Leucanthemum x superbum* 'Snow Cap'. Vertical bars represent 95% confidence intervals.

Table 2. Flowering of *Leucanthemum x superbum* 'Snow Cap' exposed to high-temperature (30/10 °C, day/night) growth chambers for 2 or 4 d at various times during or at the end of 6-week vernalization. Control plants were vernalized for 6 weeks at 5 °C.

Treatment week	Days to visible bud	Days to flower	Flowers/plant	Plant ht (cm)
Control	26.5	52.0	9.3	16.0
2-Day exposure to high temperature				
2	26.5	53.2	12.5	19.2
4	27.4	53.0	10.2	15.7
6	24.1	53.3	13.5	18.3
2, 4	25.0	51.9	11.0	17.9
2, 6	24.6	53.4	12.2	18.3
4, 6	24.2	52.0	12.7	18.7
2, 4, 6	25.4	55.6	10.2	19.2
4-Day exposure to high temperature				
2	27.8	53.2	10.2	14.9
4	28.6	55.3	9.3	16.5
6	28.2	54.3	10.6	19.7
2, 4	27.1	54.0	11.8	19.8
2, 6	24.2	52.7	11.6	20.0
4, 6	29.2	58.1	10.9	19.6
2, 4, 6	28.5	57.3	9.9	19.5
ANOVA—Orthogonal contrasts				
Control vs. 2-d	NS	NS	*	***
Control vs. 4-d	NS	NS	***	NS
2-d vs. 4-d	*	NS	NS	***
Once vs. twice	***	NS	NS	***
Once vs. twice for 2-d	NS	NS	NS	**
Once vs. twice for 4-d	NS	NS	NS	NS

NS, ***, **** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Although optimum vernalization temperatures vary with species or cultivar, vernalization at 1 to 7 °C usually is recommended for most species. In some species the optimum temperature for vernalization varies with the duration of vernalization (Lang, 1965).

'Birch Hybrid' has an obligate vernalization requirement that was equally satisfied with temperatures from 0 to 10 °C when plants were vernalized for at least 6 weeks. 'Snow Cap' plants exhibited a facultative vernalization requirement for flowering (Runkle et al., 1998). Vernalization temperatures from 0 to 10 °C had the same effect on flowering in 'Snow Cap' when plants were vernalized for 2 weeks. However, as the vernalization duration increased, vernalization at 10 °C became slightly less effective compared with other temperatures; plants took longer to flower after vernalization compared with 0 to 5 °C.

Devernalization is a cumulative process that is influenced by temperature and duration and times of exposure. In a previous unpublished study, *Aquilegia hybrida* 'Remembrance' plants were not devernalized by 1 or 2 d exposure to 30/10 °C (day/night) during or immediately after vernalization, regardless of the exposure timing or total times (Niu, unpublished data), which suggested that the 2-d exposure at 30/10 °C was insufficient for devernalization to occur in 'Remembrance'. Therefore, in this study, the exposure time was set for 2 or 4 d. We did not increase the 30 °C duration per day, because high temperatures in a greenhouse are not likely to exceed 9 h·d⁻¹. Flowering was delayed by the 4-d exposure to high temperatures in 'Birch Hybrid', and flowering percentage was decreased in some treatments (weeks 2, 4; weeks 2, 4, 6) of 'Birch Hybrid', while there was almost no negative ef-

fect of high temperature exposure on flowering characteristics in 'Snow Cap'. The effect of 30 °C treatment was more obvious when plants were exposed for 4 d more than once.

Devernalization may be also related to the degree of vernalization. Flowering was not delayed in 'Birch hybrid' plants that were exposed to high temperatures for 2 or 4 d at the end of sixth week vernalization (week 6), and the plants had comparable number of flowering shoots as those of control plants, although there were large variations in the number of flowering shoots. This result may indicate that these plants (week 6) are fully vernalized after the 6-week vernalization and are not devernalized by 30/10 °C for 4 d.

Based on these results, at least 6 weeks vernalization was needed in 'Birch Hybrid' to achieve uniform flowering, and vernalization temperatures from 0 to 10 °C had an equal effect at promoting flowering. In 'Snow Cap', plants vernalized at 0 to 5 °C for 4 weeks or longer had rapid, uniform flowering. Devernalization or incomplete vernalization sometimes occurred in 'Birch Hybrid' when plants were exposed to high temperatures for 4 d during vernalization. Incomplete vernalization may be an appropriate description as plants exposed to high-temperature treatments received fewer total days of cold than plants that did not receive any high-temperature treatment. Overall, 'Snow Cap' and 'Birch Hybrid' are tolerant of high temperatures (30 °C) that greenhouses may experience on bright sunny days during vernalization.

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