**Dianthus gratianopolitanus** Vill.

‘Bath’s Pink’ has a Near-obligate Vernalization Requirement

Sonali R. Padhye and Arthur C. Cameron

Department of Horticulture, Michigan State University, East Lansing, MI 48824

**Abstract.** The flowering response of *Dianthus gratianopolitanus* Vill. ‘Bath’s Pink’ was characterized after varying durations at vernalizing temperatures. Genetically identical clonally propagated plants were treated at 5 °C for 3, 6, 9, 12, or 15 weeks in Expt. I; at 0, 5, or 10 °C for 2, 4, 6, or 8 weeks in Expt. II; and at 0, 5, 10, or 15 °C for 1, 2, 4, 6, or 8 weeks in Expt. III. *Dianthus gratianopolitanus* ‘Bath’s Pink’ exhibited a quantitative vernalization response after treatment at 0 to 10 °C and did not vernalize after 8 weeks at 15 °C, which was the longest duration tested. Complete flowering was achieved after 4 or more weeks at 0 °C, 3 or more weeks at 5 °C, and 8 weeks at 10 °C. Based on time to anthesis and node number at anthesis, the flowering response was saturated after vernalization treatment at 0 °C for 4 or more weeks and 5 °C for 3 or more weeks. However, maximum flowers at anthesis were produced after 8 weeks at 0 °C and 6 or more weeks at 5 °C. Flowering was delayed after the 8-week treatment at 10 °C compared with 6 or more weeks at 0 °C and 4 or more weeks at 5 °C. Based on the minimum vernalization duration required to achieve the maximum flowering response, the order of efficacy of vernalizing temperatures was 5 °C > 0 °C > 10 °C.

Even within a species, the optimum vernalization temperature range may broaden with increasing durations (Lang, 1965). For example, complete flowering of *Campanula* ‘Birch Hybrid’ was achieved after a 5-week vernalization treatment at 2.5 to 7.5 °C; however, all plants flowered after a 9-week treatment at 0 to 12.5 °C (Padhye, 2006). Hence, scheduling herbaceous perennials in flower necessitates empirical studies that investigate the vernalization temperatures and durations most effective for flowering.

*Dianthus gratianopolitanus* is a highly variable herbaceous perennial native to central and central Europe and hardy to U.S. Department of Agriculture cold hardness Zone 3 (Armitage, 1997; Griffiths, 1994). *Dianthus gratianopolitanus* ‘Bath’s Pink’ was originally selected from a seedling population of *D. gratianopolitanus* and since has been clonally propagated from this single plant. *Dianthus gratianopolitanus* ‘Bath’s Pink’ has been popular for its outstanding flower display and garden performance. *Dianthus gratianopolitanus* ‘Bath’s Pink’ flowers profusely in midseason primarily in gardens in Michigan and is then largely vegetative through the summer and autumn (personal observation). Flowers form apically on the primary and axillary stems. In a preliminary experiment, *D. gratianopolitanus* ‘Bath’s Pink’ did not flower without a vernalization treatment when grown in a greenhouse at 20 °C for 15 weeks under 9- or 16-h photoperiods, whereas all plants flowered under 9- and 16-h photoperiods after a 15-week treatment at 5 °C (Cameron et al., unpublished data). Because photoperiod did not influence flowering percentage or flowering time, *D. gratianopolitanus* ‘Bath’s Pink’ was classified as a day-neutral plant. The objectives of this study were to 1) characterize the flowering response of *D. gratianopolitanus* ‘Bath’s Pink’ to a range of thermoinductive temperatures and durations, and 2) determine the effective vernalization temperatures and minimum durations for complete, rapid, and uniform flowering of *D. gratianopolitanus* ‘Bath’s Pink’.

**Materials and Methods**

Propagation and propague culture. To create stockplants, shoot-tip cuttings were taken from a single nonvernalized *D. gratianopolitanus* ‘Bath’s Pink’ plant, rooted, and transplanted into 13-cm-square pots (1.1 L) filled with a commercial peat-perlite media (Sure-Mix; Michigan Grower Products, Galesburg, MI). Stockplants were maintained in a glass greenhouse set at 20 °C under a 16-h photoperiod from 0600 to 2200 hr provided by a combination of sunlight and high-pressure sodium (HPS) lamps. HPS lamps turned on when the ambient light was below 200 μmol·m⁻²·s⁻¹ and turned off when ambient light was above 400 μmol·m⁻²·s⁻¹ and provided an additional 150 μmol·m⁻²·s⁻¹ photosynthetic photon flux (PPF). Plants were hand-watered when necessary with reverse osmosis water containing nutrients.

Received for publication 27 Sept. 2007. Accepted for publication 31 Oct. 2007.

We gratefully acknowledge private companies supportive of Floriculture Research Program at Michigan State University and Michigan Agricultural Experiment Station for providing financial support. We also thank Michael Olrich and undergraduate students in Floriculture Research Program at Michigan State University for providing plant care.

To whom reprint requests should be addressed; e-mail cameron@msu.edu

**Additional index words.** flowering, herbaceous perennial, quantitative vernalization

The wholesale value of herbaceous perennials produced in the United States has grown by 63% in the last 5 years, reaching over $708 million in 2005 (U.S. Department of Agriculture, 2006). This increase in sales of herbaceous perennials can at least partly be attributed to the increasing ability of producers to force and market perennials in flower for scheduled market dates. Forcing perennials to flower on specific dates requires an understanding of the regulation of flowering and subsequent manipulation of environmental factors, including photoperiod and temperature, to promote flower induction. Flowering of many winter annuals, biennials, and perennials is promoted after exposure to low temperatures. This phenomenon, known as vernalization, has been defined as “the acquisition or acceleration of the ability to flower by a chilling treatment” (Chouard, 1960), and a low-temperature treatment that induces or accelerates flowering has been referred to as vernalization treatment (Napp-Zinn, 1987). An important aspect of vernalization is the temporal separation of the thermoinductive treatment and the flowering response. Therefore, at the end of thermoinductive treatment, the only visible indicator of completion of vernalization requirement is the subsequent flowering after exposure to higher temperatures. Insufficient vernalization can result in incomplete or delayed flowering, which can cause considerable monetary losses to commercial producers of herbaceous perennials.

Vernalization has been extensively studied in many winter annuals and biennials and select perennials and the physiology of vernalization was comprehensively reviewed (Chouard, 1960; Lang, 1965; Thomas and Vince-Prue, 1984; Vince-Prue, 1975). Vernalization responses of plants can be categorized as qualitative (obligate) or quantitative (facultative; Lang, 1965). Plants exhibiting a qualitative vernalization response require a cold treatment to acquire the ability to flower, whereas a cold treatment accelerates flowering or improves flowering characteristics of plants with a quantitative vernalization response. Regardless of vernalization response category, vernalization is a quantitative process; the flowering response increases with vernalization duration until saturation (Lang, 1965). The effective range of thermoinductive temperature and duration that elicits a maximum vernalization response is species-specific. For example, the thermoinductive temperatures producing the greatest flowering response of *Veronica spicata* L. ‘Red Fox’ and *Laurentia axillaris* (Lindl.) E. Wimm. were –2.5 °C and 5 to 10 °C, respectively (Fausey and Cameron, 2007). Therefore, selection of appropriate thermoinductive temperatures is important for commercial production of herbaceous perennials.

**HortScience** Vol. 43(2) April 2008
(125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe, 0.5 Mn, 1.0 Cu, 0.3 B, 0.1 Mo mg L⁻¹; MSU Special, Greencare Fertilizers, Chicago).

To initiate an experiment, shoot-tip cuttings with six to eight nodes (leaves are opposite) were harvested from stockplants and dipped in a commercial rooting hormone (Dip ‘N Grow, Clackamas, OR) containing 1 g L⁻¹ indole-3-butyric acid and 0.5 g L⁻¹ naphthalene acetic acid and were rooted in 72-cell trays (50-mL cell volume; Landmark Plastic Corporation, Akron, OH) containing 50% commercial peat–perlite media (Sure-Mix; Michigan Grower Products) and 50% coarse perlite (Therm-O-Rock; East Inc., New Eagle, PA). Cuttings were rooted under mist for ≈2 weeks in a propagation facility with air and medium temperature set points of 23 and 26 °C, respectively, and a 0.3 kPa vapor-pressure deficit that was generated by injecting water vapor into the air. After rooting, propagates were grown in 72-cell plug trays (50-mL cell volume per plant) filled with the previously described media and grown under the same environmental conditions and nutritional regimen as stockplants until vernalization experiments were initiated.

Expt. I. Dianthus gratianopolitanus ‘Bath’s Pink’ cuttings were harvested on 14 Dec. 2003, rooted for ≈2 weeks, and grown in 72-cell trays for ≈1 month. The 72-cell trays containing propagules were placed in a growth chamber set at 5 °C on 3 Feb. 2004 for 3, 6, 9, 12, or 15 weeks. Cool-white fluorescent lamps provided ≈10 μmol m⁻² s⁻¹ PPFD from 0800 to 1700 h in the cooler. During vernalization treatments, plants were hand-watered when necessary with acidified well water (H₂SO₄ to a titratable alkalinity of ≈140 mg L⁻¹ CaCO₃) containing nutrients (40 N, 4 P, 40 K, 5 Ca, 0.2 Mn, 0.2 Cu, 0.03 B, 0.03 Mo, 0.2 Zn mg L⁻¹; MSU Special). Ten additional plants were maintained in the greenhouse as nonvernalized controls and were grown under the same conditions as stockplants.

Expts. II and III. Dianthus gratianopolitanus ‘Bath’s Pink’ cuttings were propagated on 25 Apr. 2004, rooted for ≈2 weeks in the propagation house, and grown in 72-cell trays for ≈1 month in Expt. II. On 11 June 2004, 72-cell trays containing the propagules were placed in growth chambers set at 0, 5, or 10 °C for 2, 4, 6, or 8 weeks. In Expt. III, cuttings were harvested on 27 Oct. 2004 and 1, 2, 4, and 6 weeks thereafter. Propagules were rooted for ≈2 weeks, grown for 2 weeks, and placed in growth chambers set at 0, 5, or 10 °C for 2, 4, 6, or 8 weeks. The light conditions and watering and fertility regimens were as described in Expt. I. An additional 10 and 40 plants were maintained as nonvernalized controls in the greenhouse in Expts. II and III, respectively.

Postvernalization conditions and environmental control. In all three experiments, after vernalization treatments, plants were transplanted into 13-cm-square plastic containers (1.1 L) filled with commercial soil-less medium (Sure-Mix) and randomly placed on benches and grown in a glass greenhouse under a 16-h photoperiod at a 20 °C greenhouse temperature set point and were maintained under the same nutritional regimens as stockplants.

The environmental conditions in the greenhouse were regulated by a climate control computer (Model CD750; Priva, De Lier, The Netherlands). Type E thermocouples (TT-E-40; Omega Engineering, Stamford, CT) placed in aspirated tubes measured temperature at plant height on every greenhouse bench and the light intensity at plant height was measured by line quantum sensors that each contained 10 photodiodes (Apogee Instruments, Logan, UT) and were placed at two locations in the greenhouse. Temperature and light sensors were connected to a CR10 datalogger (Campbell Scientific, Logan, UT) and data were collected every 10 s, and hourly means were computed and recorded by a computer. The average daily temperatures (ADT) and mean photosynthetic daily light integrals (DLI) received by each plant were computed from the date of transplant until the day of anthesis for all flowering plants. For vegetative plants, ADT and DLI were computed for 15 weeks from the date of transplant. The ADT and DLI of plants transplanted on the same date are reported in Table 1.

**Results and Discussion**

Flowering response. In Expt. I, one of the 10 nonvernalized control plants flowered, and in subsequent experiments, a mean of 21% of nonvernalized control plants flowered (Figs. 1A and 2A). Because some plants flowered without exposure to low temperatures, *D. gratianopolitanus* ‘Bath’s Pink’ technically exhibited a facultative vernalization response. However, 100% flowering was achieved only after vernalization at 0 °C for 4 or more weeks, 5 °C for 3 or more weeks, and 10 °C for 8 weeks. Only one of the 40 plants flowered after vernalization treatment at 15 °C for 8 or less weeks. The order of the most effective vernalization temperatures based on the shortest duration of vernalization treatment required for achieving 100% flowering was 5 °C > 0 °C > 10 °C.

The effective temperature and duration of vernalization for promotion of flowering is species-specific (Lang, 1965). For example, *Lavandula angustifolia* Mill. ‘ Munstead’ required a 10-week vernalization treatment at 5 °C for best flowering (Whitman et al., 1996), whereas radish can be vernalized in 4 to 8 d (Erwin et al., 2002). Many ornamental herbaceous perennials, including *Agulegia × hybridra* hort., *Campanula ‘Birch Hybrid’, Isotoma axillaris* Lindl., *Lavandula angustifolia* Mill. ‘ Munstead’, *Thalictrum delavayi* Franch. ‘ Hewitt’s Double’, and *Veronica spicata* L. ‘Red Fox’, require relatively long (4 to 10 weeks) exposures to vernalizing temperatures for 100% flowering (Fausey and Cameron, 2007; Huang et al., 1999; Padhye, 2006; Shendon and Weiler, 1982; Whitman et al., 1996). In this study, *D. gratianopolitanus* ‘Bath’s Pink’ flowered after comparatively short exposures to vernalizing temperatures; flowering percent-ages after 1, 2, and 3 or more weeks at 5 °C were 70%, 95%, and 100%, respectively (Figs. 1A and 2A). Similarly, 100% flowering was observed when *Oenothera fruticosa* L.

| Table 1. Planting date, mean daily temperature (ADT), and mean photosynthetic daily light integral (DLI) of *Dianthus gratianopolitanus* ‘Bath’s Pink’ vernalized for different durations.* |
|-----------------|-----------------|-----------------|-----------------|
| Expt. | Vernalization duration (weeks) | Planting date | ADT (°C) | DLI (mol m⁻² d⁻¹) |
| --- | --- | --- | --- | --- |
| I | 0 | 3 Feb. 2004 | 20.9 ± 0.0 | 13.3 ± 0.0 |
| | 3 | 24 Feb. 2004 | 20.7 ± 0.0 | 13.6 ± 0.2 |
| | 6 | 16 Mar. 2004 | 20.2 ± 0.0 | 17.5 ± 0.0 |
| | 9 | 4 Apr. 2004 | 20.8 ± 0.1 | 14.4 ± 0.5 |
| | 12 | 27 Apr. 2004 | 21.8 ± 0.0 | 9.7 ± 0.0 |
| | 15 | 18 May 2005 | 22.4 ± 0.0 | 10.5 ± 0.0 |
| II | 0 | 11 June 2004 | 23.0 ± 0.0 | 11.6 ± 0.1 |
| | 2 | 25 June 2004 | 23.0 ± 0.1 | 12.0 ± 0.1 |
| | 4 | 9 July 2004 | 23.4 ± 0.1 | 11.6 ± 0.1 |
| | 6 | 23 July 2004 | 22.3 ± 0.0 | 10.7 ± 0.0 |
| | 8 | 6 Aug. 2004 | 22.6 ± 0.0 | 10.4 ± 0.0 |
| III | 0 to 8 | 5 Jan. 2005 | 20.5 ± 0.1 | 11.7 ± 0.4 |

*ADT and DLI were calculated for each flowering plant from day of transplant to anthesis, and for vegetative plants, 105 d after transplant. Mean ± se of ADT and DLI of 10, 30, and 240 plants are reported for Expts. I, II, and III, respectively.**
‘Youngii-lapsley’ plants were vernalized for 3 weeks at 5 °C (Clough et al., 2001).

Flowering time. Vernalization promoted flowering of D. gratianopolitanus ‘Bath’s Pink’. The time to anthesis of nonvernalized control plants was highly variable. In Expt. I, the single nonvernalized flowering plant reached anthesis in 114 d (Fig. 1B). In the subsequent experiments, 21% of nonvernalized plants reached anthesis in an average of 58 d (Fig. 2B). Osteospermum ecklonis Norl., another perennial with a quantitative vernalization requirement, flowered occasionally without vernalization treatment and time to flower varied widely (Suzuki and Metzger, 2001). We postulate that D. gratianopolitanus ‘Bath’s Pink’ plants that remained vegetative for 15 weeks (the final duration of our experiments) may have eventually flowered, which would have considerably increased the mean time to anthesis for the entire population.

In Expt. I, 3 weeks at 5 °C accelerated flowering significantly and mean time to anthesis was 41 d. At 5 °C, the standard errors associated with mean time to anthesis were low after 3 or more weeks of vernalization treatment, indicating that vernalization increased uniformity of flowering time. An additional 3 weeks of vernalization treatment hastened flowering time by ≈1 week, but extended periods of vernalization (up to 15 weeks) did not further hasten flowering to the same extent. Nodes that developed on vernalized plants before anthesis did not vary after 3 to 15 weeks of vernalization at 5 °C (Fig. 1C). Therefore, the 1-week decrease in time to flower after 3 and 6 or more weeks of vernalization treatments could not be attributed to a developmental acceleration of flowering. In Expts. II and III, the flowering time of D. gratianopolitanus ‘Bath’s Pink’ decreased as vernalization duration at 5 °C increased from 1 to 2 weeks, but time to flower was not further reduced with extended vernalization treatments (Fig. 2B). The number of nodes at anthesis followed a trend similar to flowering time (Fig. 2C). Overall, the results from all three experiments indicate that 100% flowering of D. gratianopolitanus ‘Bath’s Pink’ was achieved after vernalization treatment at 5 °C for 3 or more weeks.

Time to anthesis and the mean number of nodes at anthesis significantly decreased as the duration of vernalization at 0 °C increased to 4 weeks. All D. gratianopolitanus ‘Bath’s Pink’ flowered and did so more rapidly after 4 weeks at 5 °C compared with 4 weeks at 0 °C. However, 6 or more weeks at 0 °C or 5 °C were equally effective in promoting flowering based on flowering percentage and flowering time.

Vernalization treatment at 10 °C for 6 or less weeks elicited less than 100% flowering after 15 weeks in the greenhouse, when the experiment was ended. It is possible that nonflowering plants in these treatments may have eventually flowered, which would have increased the average flowering time and node number. An 8-week treatment at 10 °C induced 100% flowering; however, plants took significantly longer to reach anthesis and developed more nodes before anthesis compared with plants vernalized at 0 °C for 6 or more weeks or 5 °C for 4 or more weeks. Therefore, 0 and 5 °C were more effective than 10 °C in promoting flowering of D. gratianopolitanus ‘Bath’s Pink’. Based on flowering percentage, flowering time, and node number at anthesis, D. gratianopolitanus ‘Bath’s Pink’ was not completely vernalized at 15 °C after 8 weeks.

Number of flowers and reproductive lateral percentage. All treatments except 8 weeks at 0 °C and 6 or more weeks at 5 °C induced a similar number of flowers at anthesis and averaged between 3 and 9 (Fig. 2D). Plants developed an average of 16 to 21 flowers after 8 weeks at 0 °C and 6 or more weeks at 5 °C. Because DLIs received by plants were similar under all treatments (Table 1), the increased flower number can primarily be attributed to the vernalization
treatment. At anthesis, plants had 18 to 35 average total laterals and the percentage of laterals that were reproductive averaged between six and 33. The highest percentage of reproductive laterals formed after 8 weeks at 0 °C and 6 or more weeks at 5 °C (Fig. 2E). Hence, extended periods of vernalization increased the number of flowers at anthesis and the percentage of reproductive laterals, making plants more visually appealing. Even after 8-week vernalization treatments at 0 or 5 °C, flowering was sparse and plants were of low quality. *D. gratianopolitanus* ‘Bath’s Pink’ usually produces flowers on the terminal and lateral stems, so growing plants for a longer time before the onset of vernalization treatment could promote formation of additional lateral stems before vernalization, which should subsequently increase the number of flowers produced.

In summary, *D. gratianopolitanus* ‘Bath’s Pink’ exhibited a near-obligate vernalization response. The shortest vernalization duration that elicited 100% flowering was 3 weeks at 5 °C, although plants produced fewer flowers at anthesis compared with longer durations. After vernalization for 2 and 4 weeks, 5 °C was the most effective vernalization temperature based on flowering percentage and flowering time; however, after 6 or more weeks, 0 and 5 °C were similarly effective. All *D. gratianopolitanus* ‘Bath’s Pink’ plants flowered after 8-week vernalization at 10 °C, although flowering was delayed and fewer flowers were produced at anthesis compared with 8 weeks at 0 and 5 °C. No plants flowered after 8 weeks at 15 °C and therefore, the maximum temperature for vernalizing *D. gratianopolitanus* ‘Bath’s Pink’ is 10 °C or greater and less than 15 °C. Compared with other treatments, more flowers were produced after 8 weeks at 0 °C and 6 or more weeks at 5 °C.

**Literature Cited**

Armitage, A. 1997. Herbaceous perennial plants: A treatise on their identification, culture, and garden attributes. Varsity Press, Inc. Athens, GA.

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.

Erwin, J.E., R.M. Warner, and A.G. Smith. 2002. Vernalization, photoperiod and GA₃ interact to affect flowering of Japanese radish (*Raphanus sativus* Chinese Radish Jumbo Scarlet). Physiol. Plant. 115:298–302.

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.

Huang, N., K.A. Funnell, and B.R. MacKay. 1999. Vernalization and growing degree-day requirements for flowering of *Thalictrum delavayi* ‘Hewitt’s Double’. HortScience 34:59–61.

Napp-Zinn, K. 1987. Vernalization—Environmental and genetic regulation. p. 123–132. In: Atherton, J.G. (ed.). Manipulation of flowering. Butterworths, London.

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

Padhye, S.R. 2006. The influence of vernalization on flowering of *Campanula ‘Birch Hybrid’* and *Dianthus gratianopolitanus* ‘Bath’s Pink’ and the regulation of flowering of *Coreopsis grandiflora ‘Sunray’* by vernalization, photoperiod and light quantity. Mich. State Univ., East Lansing, PhD Diss.

Shedron, K.G. and T.C. Weiler. 1982. Regulation of growth and flowering in *Aquilegia × hybridra* Sims. J. Amer. Soc. Hort. Sci. 107:878–882.

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: Wilkins, M.B. (ed.). Advanced plant physiology. Pitman Publ., London.

U.S. Department of Agriculture. 2006. Floriculture and nursery crops 2005 summary. Agr. Stat. Board, Washington, DC.

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

Whitman, C.M., R.D. Heins, A.C. Cameron, and W.H. Carlson. 1996. Cold treatments, photoperiod, and forcing temperature influence flowering of *Lavandula angustifolia*. HortScience 31:1150–1153.