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FLOWER INDUCTION AND CULTURAL REQUIREMENTS FOR QUICK-CROPPING OF THE HERBACEOUS PERENNIALS VERONICA SPICATA, PHLOX PANICULATA, LEUCANTHEMUM xSUPERBUM, ACHILLEA, GAURA LINDHEIMERI, AND CAMPANULA

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Horticulture

Major professor

Date October 17, 2002

FLOWER INDUCTION AND CULTURAL REQUIREMENTS FOR QUICK-CROPPING OF THE HERBACEOUS PERENNIALS *VERONICA SPICATA*, *PHLOX PANICULATA*, *LEUCANTHEMUM* x*SUPERBUM*, *ACHILLEA*, *GAURA LINDHEIMERI*, AND *CAMPANULA*

By

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A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

2002

ABSTRACT

FLOWER INDUCTION AND CULTURAL REQUIREMENTS FOR QUICK-CROPPING OF THE HERBACEOUS PERENNIALS *VERONICA SPICATA*, *PHLOX PANICULATA*, *LEUCANTHEMUM* x*SUPERBUM*, *ACHILLEA*, *GAURA LINDHEIMERI*, AND *CAMPANULA*

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Optimized production of vegetatively propagated herbaceous perennials requires a proper knowledge of stock plant management, propagation protocols, appropriate vegetative bulking, and flower induction and development requirements. This project was conducted to identify these physiological and cultural elements for six herbaceous perennial species. Stock plant management, propagation, and vegetative bulking for *Veronica spicata* 'Red Fox' and *Campanula* 'Birch Hybrid' required only appropriate light and temperature because both plants were day-neutral following a flowering flush. Plants had an obligate cold requirement for flowering. Photoperiod control during all stages of development was necessary for *Phlox paniculata* 'David', *Leucanthemum* x*superbum* 'Snowcap', *Achillea* 'Moonshine', and *Gaura lindheimeri* 'Whirling Butterflies' because all were long-day plants with facultative cold requirements for flowering. Experiments were conducted to quantify the effects of propagation environment on rooting and subsequent flowering of *P. paniculata* 'David'. Rooting increased as daily light integral increased from 0.8 to 8.6 $mol \cdot m^{-2} \cdot d^{-1}$ but was not affected by auxin concentration or rooting photoperiod. Days to flower decreased up to 17 days as propagation photoperiod increased from 11 to 15 hours, and cold treatment for 5 weeks decreased time to flower by up to 25 days.

ACKNOWLEDGMENTS

I wish to give my sincere thanks to Dr. Royal Heins for offering me the opportunity to complete my degree under his guidance. His patience, support, and insight over the last few years have been invaluable.

The support and valuable insights of my other committee members, Drs. Art Cameron, Will Carlson, and Ken Poff, have also been greatly appreciated.

A special thank-you goes to greenhouse technicians David Joeright and Mike Olrich and their undergraduate employees for keeping the greenhouse running, keeping my plants alive, and helping me collect data or propagate cuttings from time to time. I would also like to mention the faculty, staff, visiting scholars, and students. They made working at MSU very enjoyable and memorable. I would especially like to thank my office mates: Dr. Erik Runkle, Dr. Hyeon-Hye Kim, Kari Robinson, Andrea Beckwith, and Jennifer Dennis for simply putting up with me.

I would also like to thank the greenhouse growers who support MSU floriculture research. Without their support, none of this research would have been possible. A special mention goes to Oro Farms, Center Greenhouse, Ball Seed Company, and Walters Gardens for donation of plant material to my research.

Finally, I would like to thank my family and friends for all their encouragement and support. I never would have made it here without them.

TABLE OF CONTENTS

LIST OF TABLES

SECTION II

SECTION III

APPENDIX A

APPENDIX B

APPENDIX C

APPENDIX D

LIST OF FIGURES

SECTION II

SECTION III

APPENDIX C

APPENDIX D

Thesis Introduction

Ten years ago the floriculture research team at Michigan State University initiated research on the flowering and cultural physiology of herbaceous perennials. Now that the flowering physiology of many herbaceous perennials is understood, growers have the ability to begin programming flowering herbaceous perennials for specific market dates. However, before plants can be uniformly flowered on a specific date, additional research is required because there are several problems associated with current herbaceous perennial production.

The first problem is lack of crop uniformity, which can be caused by a number of factors, including nonuniform propagules attributed to propagation of cuttings from stock plants in various stages of vegetative and reproductive development. Nonuniformity can also be caused by disease, even if propagules are initially uniform. There is a high risk of disease with bare-root material because plants are often stored for extensive periods in cold storage. A second problem is that current production practices do not provide induced and flowering-size plant material for planting during the summer. Although bare-root material is available from winter to spring, availability is generally lacking the rest of the year, and even then plants are often diseased because they have been stored for extended periods. Finally, vernalized plugs are not available after early spring because the temperatures in greenhouses used by growers for vernalization cannot be kept cool enough because of rising outside solar radiation and temperatures. A third problem with current production practices is

that production schedules are generally lengthy. The time from planting the starting material to flower may exceed a year.

This thesis research was directed at developing a production protocol to avoid these problems. The production protocol was termed *quick-cropping*. The primary goal was to optimize the production of herbaceous perennials by determining the proper environmental and cultural requirements for each stage of herbaceous perennial production: stock plant management, propagation, bulking (root establishment and vegetative growth), cold treatment, and forcing to flower. Another goal of quick-crop production was to make distinctions between vegetative and reproductive growth by understanding the cold and photoperiod requirements of an herbaceous perennial species so that uniform flowering crops could be produced. Six species were examined to determine the feasibility of the quick-crop herbaceous perennial production protocol: *Veronica spicata* 'Red Fox', *Phlox paniculata* 'David', *Leucanthemum* x*superbum* 'Snowcap', *Achillea* 'Moonshine', *Gaura lindheimeri* 'Whirling Butterflies' and 'Siskiyou Pink', and *Campanula* 'Birch Hybrid'.

SECTION I

LITERATURE REVIEW

Vernalization

Introduction

In plants, most biological processes proceed more quickly as the temperature rises. An exception is the promotion of flowering by low temperatures, which is known as vernalization. The importance of low temperatures (temperatures below those optimal for growth) in flower induction of certain plants has been known since the 19th century (Bernier et al., 1981).

History

The term *vernalization* was derived in Russia in 1928. Lysenko initially called the process Jaroviation. Jar means god of Spring in Russian, and spring cereals are called Jarovoe (Chouard, 1960). The word was then translated to vernalization. The Latin vernum means Spring.

Before the technique of vernalization had even evolved, research was being conducted on the effects of temperature on plant development and flowering. Klebs, who may be regarded as the initiator of this branch of plant physiology, began research before 1918 (Whyte, 1948). He proposed that it should be possible to control and direct the growth and development processes of a plant by exposing it under experimental conditions to the particular factors that it was exposed to in nature (Whyte, 1948): temperature and light.

Some of the first cold-response experiments were performed in 1918 by Glasner (Chouard, 1960; Whyte, 1948). He wanted to determine the

physiological differences between spring and winter rye. His research showed that spring rye does not need a cold period in order to reach the shooting stage, whereas flowering of winter rye depends on the rye's receiving a cold period either during or after germination. Winter rye that was germinated at 1 to 2 °C reached the shooting stage 9, 21, or 41 d earlier than when germinated at 5 to 6 °C, 12 °C, or 14 °C, respectively (Whyte, 1948). This research showed that in winter rye, low temperature is needed for the "release of flower formation."

A large amount of research concerning low-temperature effects on plant development and flower initiation has been conducted since Klebs and Glasner conducted their research. Two groups studied the physiology of vernalization on an "accurate" experimental basis. In the 1930s and 1940s, Gregory and Purvis performed experiments in London, and in the 1940s and 1950s, Melchers and Lang et al. performed experiments at Tübingen (Chouard, 1960). Vernalization research continues today, but it is often more genetically than physically based.

Effects of Low Temperatures

Plant development is influenced directly and inductively by numerous environmental factors, including photoperiod, light quantity, light quality, nutrient and water availability, and temperature. Direct effects elicit a plant response during exposure to the environmental condition. An inductive effect occurs sometime after the plant has been exposed to the condition but not during exposure. Exposure to low temperatures can evoke direct and inductive responses in plants.

Low temperatures can be used to break dormancy, during which all primordia exist but either do not grow or grow very slowly. Generally, breaking dormancy is thought to involve the removal of growth inhibitors so that active growth can occur as soon as favorable conditions return. Insufficient cold can reduce the flowering response. In most spring-flowering woody plants, dormancy release can be induced only by chilling the buds for a particular length of time (Smith and Kefford, 1964). In some bulbous plants, for example, tulip and narcissus, flower initiation occurs before dormancy. Floral differentiation can take place during the dormant period (Rees, 1985). However, in tulips, floral stalk elongation occurs only after the bulbs have received a low-temperature treatment (Rietveld et al., 2000). The breaking of dormancy by low temperatures can also occur in seed. It is common in seeds of trees and shrubs and in some temperate herbaceous perennials (Hartmann et al., 1997).

Flower initiation and development can also occur under low temperatures. In stock, *Lunaria biennis* L., *Iris* 'Wedgewood', and onion, for example, floral initials differentiate during the exposure to cold (Chouard, 1960; Thomas and Vince-Prue, 1997). In brussels sprouts (*Brassica oleracea gemmifera* L.), flower initiation must take place during slow growth at low temperatures. Plants that have not initiated an inflorescence at transfer to growing temperatures remain vegetative (Friend, 1985).

When exposure to low temperatures is used for the induction and promotion of flowering, it is termed *vernalization*. Without a cold treatment, vernalization-requiring plants show delayed flowering or remain vegetative. In

1960, Chouard defined vernalization as "the acquisition or acceleration of the ability to flower by a chilling treatment." As a rule, initiation of flower primordia does not occur at vernalizing temperatures. It occurs only after plants are moved to warmer temperatures more favorable for growth (Bernier et al., 1981). Numerous plant species require a vernalization treatment in order to flower; for example, *Digitalis purpurea* L. (foxglove), *Althaea rosea* (L.) Cav. (hollyhock), *Beta vulgaris* L. (beet), *Apium graveolens* L. (celery), and *Hyoscyamus niger* L. (black henbane) (Metzger, 1996).

Obligate versus Facultative

Vernalization can be defined as either the acquisition of the ability to flower or the reduction in time to flower. Plants that will not flower without exposure to low temperatures have an obligate vernalization response. Most plants with an obligate vernalization response have embryos within the mature seed that cannot be vernalized (Chouard, 1960). Plants in this category are primarily biennials and herbaceous perennials. For example, seedlings of *Dianthus barbartus* L. have an obligate requirement for vernalization (Cockshull, 1985). Neither chicory (*Cichorium intybus* L.), which can be vernalized as a seed or plant, nor *Raphanus sativus* L., which can be vernalized as a germinating seed, will flower unless it is exposed to a cold treatment (Demeulemeester and De Proft, 1999; Engelen-Eigles and Erwin, 1997).

Plants that will eventually flower without a cold treatment but flower faster after cold have a facultative vernalization response, which generally results in a

reduction in leaf number and fewer days to flower. This response often becomes more pronounced as the length of the vernalization treatment increases. Plants in this category include annuals and herbaceous perennials. *Dianthus allwoodii* and *D. alpinus* L. show a reduction in the number of days to flower and an increase in the total number of flowers in response to cold (Wurr et al., 2000). For certain *Aquilegia* L. species versus uncooled plants, 8 weeks of cooling reduced the time to flower and promoted longer peduncles (Garner and Armitage, 1998). *Cineraria* also shows a facultative response to vernalization. The number of leaves formed before flowering decreases as the chilling duration increases (maximum reduction occurs after 3 to 5 weeks at 6 °C), and all unchilled plants eventually flower when grown at 18 °C (Yeh et al., 1997).

In some cases, vernalization can substitute for long days when plants show a facultative response to vernalization. In these plants, photoperiod is the primary flower induction stimulus, but vernalization can substitute for long days (plants will flower regardless of photoperiod) or hasten flowering under long days. For example, Karlsson et al. (1993) observed 32 ecotypes of *Arabidopsis thaliana* (L.) Heynh. Of the ecotypes that responded to vernalization, two showed an obligate response, while the others showed a facultative response. In most cases, vernalization was able to completely substitute for long days (plants flowered regardless of photoperiod). The substitution of vernalization for long days can also be seen in *Gypsophila paniculata* L. Without a vernalization treatment, plants will flower only under long days; however, after vernalization plants will flower under any photoperiod (Davies et al., 1996; Shilo, 1985).

Requirements of Vernalization

A vernalization treatment is effective only on actively growing plants. Seed of winter annuals respond to vernalization before germination if they have imbibed water and have become metabolically active (Taiz and Zeiger, 1998). Chandler and Dean (1994) showed that late-flowering arabidopsis plants are most sensitive to vernalization at the imbibed seed stage. When cold was applied at that stage, plants produced fewer leaves before flowering. As plant age increased before vernalization, leaf number before flowering also increased.

Most biennials, on the other hand, have to proceed through a juvenile developmental phase in which they are insensitive to vernalizing temperatures. They must reach a minimal size, age, or both before they become sensitive to low temperatures. For example, under continuous light and 18 °C day/15 °C night temperatures, chicory plants younger than 100 d do not react to vernalization but remain rosettes after cold. Once plants reach 112 d or older, they respond to a vernalization treatment (Demeulemeester and De Proft, 1999). In temperate grasses, although seed vernalization is possible in some species, the vernalization rate is higher in seedlings (Heide, 1994). The length of the juvenile period varies widely (usually between 2 and 5 weeks), depending on the grass species. *Geum urbanum* L. cannot respond to vernalization until the four- to five-leaf stage, and then only axillary buds at a certain stage of development are sensitive to low temperatures (4 °C) (Tran Thanh Van, 1985). The terminal

apex of the basal rosette can be vernalized only by 30 to 50 weeks of cold treatment, whereas axillary buds are vernalized in 5 to 15 weeks (Taylor, 1997).

Site of Vernalization

Perception of vernalization occurs mainly in the meristematic zones of the shoot apex. However, all actively dividing cells may be capable of responding to low temperatures (Levy and Dean, 1998). Once vernalization has occurred, it is maintained through mitosis. The vernalization requirement is reset by meiosis or some other aspect of reproductive growth.

In biennials, the overwintering stem apex perceives the stimulus, although there are some reports suggesting that leaves and even isolated roots are responsive in some cases (Hopkins, 1995). A classic case of dividing cells perceiving low temperatures is Wellensiek's (1960) study of *Lunaria annua* L. Cut leaves that were held at 5 °C produced regenerated plants that were able to flower. However, the action of the cold was limited to the base of the petiole, the site of actively dividing cells. If the petiole was removed, the regenerated plants failed to flower. Cell division, however, is not a requirement for vernalization in all cases; for example, winter rye and *Cheiranthus allionii* L. (Thomas and Vince-Prue, 1997).

In some instances, cuttings removed from vernalized stock plants do not require an additional cold treatment in order to flower. The flower induction stimulus is transmitted in the cuttings, which has been shown in *Dianthus*

allwoodii 'Doris' and chicory (Demeulemeester and De Proft, 1999; Wurr et al., 2000).

Effective Temperatures and Durations

Vernalization is a progressive process, and the effect becomes increasingly stable as the duration of cold increases. The optimum temperature generally ranges between 1 and 7 °C (Taiz and Zeiger, 1998). The optimum temperature varies among plant species. For example, in cineraria, the base temperature for vernalization is –0.3 °C, the optimum is 5.9 °C, and the maximum is 15.8 °C (Yeh et al., 1997).

The effect of low temperatures increases with the duration of exposure until the response is saturated. A response usually occurs after at least 4 weeks but varies widely (<10 to >100 d) between species (Metzger, 1996; Thomas and Vince-Prue, 1997). For example, *R. sativus* L. can be vernalized in as few as 40 d (Engelen-Eigles and Erwin, 1997). A saturated vernalization response can be found in certain ecotypes of arabidopsis after 30 to 40 d (Bagnall, 1993). Growing *G. paniculata* 'Bridal Veil' at cool night temperatures (7 °C) results in enhanced flower yield and quality. The cool night temperature could have a vernalizing effect large enough to cause flowering in 'Bridal Veil', which has a low vernalization requirement (Davies et al., 1996).

Molecular and Genetic Response

A great deal is known about the effects of vernalization on growth and development of plants. On the other hand, little is known about the effects of vernalization at the genetic and molecular levels. Vernalization is an epigenetic phenomenon, meaning that the vernalized state is stable through mitosis but not meiosis. Several genes have been identified in *Arabidopsis thaliana* (L.) Heynh that appear to play a role in the vernalization response: *FRIGIDA* (*FRI*), *FLOWERING LOCUS C* (*FLC*), and the *VERNALIZATION* genes (*VRN1* and *VRN2*). It has been proposed that these genes work through several parallel pathways, including the autonomous and vernalization pathway (Koorneef et al., 1998). DNA demethylation may also play an important role in the activation or deactivation of these pathways and genes.

DNA Demethylation

Burns et al. (1993) proposed that vernalization is mediated by the demethylation of promoter genes whose expression is critical for the initiation of flowering. One hypothesis is that exposure to low temperatures decreases methylation, perhaps by uncoupling replication and maintenance methylation (Finnegan et al., 1998a). Work done on arabidopsis by Finnegan et al. (1998b) showed that vernalization for 4 or 8 weeks reduced DNA methylation by 15% compared with that of the control (unvernalized) seedlings.

5-Azacytidine (5-azaC) demethylates DNA. Treating plants with 5-azaC can mimic the vernalization response and thus accelerate flowering. With no

prior vernalization treatment, germinating arabidopsis seeds (vernalization-responsive ecotypes) treated with 5-azaC showed earlier floral initiation and a reduction in the number of rosette leaves formed at flowering (Burns et al., 1993). Furthermore, arabidopsis plants transformed with a methyltransferase (*MET1*) antisense transgene also showed a promotion of flowering in the absence of a cold treatment (Finnegan et al., 1998b).

Current research suggests that demethylation and vernalization may activate the same pathway. However, it is now known that the methylation patterns in plants may not reset between generations (Vongs et al., 1993), which suggests that factors other than DNA methylation may be involved in resetting the vernalization signal.

Vernalization Pathway

Multiple pathways control flowering time in arabidopsis (Alonso-Blanco et al., 1998; Koornneef et al., 1998): the photoperiod pathway, the autonomous pathway, and the vernalization pathway. The vernalization pathway promotes flowering in many late-flowering ecotypes of arabidopsis in response to an extended period of cold temperatures (Simpson et al., 1999). Vernalization is able to overcome or bypass the repressive effects of certain genes (for example, the *FLC* and *FRI* genes). It can also compensate or substitute for the autonomous pathway genes, which suggests that vernalization may operate through a separate pathway parallel to the autonomous pathway (Simpson et al., 1999). However, little else is known about its molecular nature.

VERNALIZATION (*VRN*) Genes

In order to understand the molecular basis of vernalization, *Arabidopsis thaliana* mutants impaired in the vernalization response have been identified and analyzed. Plants with mutations in the *VRN* genes may be defective in either the perception of cold temperatures or the transduction of the cold signal by the vernalization pathway (Levy and Dean, 1998). The *vrn1* and *vrn2* mutants were isolated according to their reduced vernalization response in the late-flowering vernalization-responsive *fca-1* arabidopsis mutant (Simpson et al., 1999). Neither *vrn1* nor *vrn2* is impaired in its ability to acclimate to low temperatures (Chandler et al., 1996, cited in Simpson et al., 1999), which indicates that the defect in these genes is specific to the vernalization pathway and not low-temperature responses in general. Since the *VERNALIZATION* genes have not yet been cloned, their function is unknown. However, *fca/vrn1* and *fca/vrn2* double mutants show *FLC* mRNA accumulation and less reduction in flowering time, suggesting that the *VRN1* and *VRN2* genes may mediate the vernalization-induced down-regulation of the *FLC* gene (Sheldon et al., 1999; Sheldon et al., 2000).

FLOWERING LOCUS C (*FLC*) Gene

FLOWERING LOCUS C encodes a MADS-box type transcription factor (Michaels and Amasino, 1999; Sheldon et al., 1999; Sheldon et al., 2000). It has been identified as a "semidominant repressor of floral induction" and is believed

to be the central regulator of the transition to flowering by vernalization (Sheldon et al., 2000). However, the downstream target genes for *FLC* are unknown.

FLC is expressed most highly in the vegetative shoot apex and in roots (Michaels and Amasino, 1999). *FLC* mRNA accumulates in mutants of the autonomous pathway, which suggests that genes in the autonomous pathway (for example, *FCA*, *FPA*, *LD*, and *FVE*) act to represses *FLC* activity (Simpson et al., 1999).

According to Sheldon et al. (2000), there is a correlation between the level of *FLC* transcript and the response to vernalization. *Arabidopsis* ecotypes with only a slight vernalization response have low levels of *FLC*, even in the absence of vernalization. Ecotypes with minimal to no vernalization response have an undetectable level of *FLC* transcript, whereas ecotypes with a strong vernalization response have a high level of *FLC* transcript.

Vernalization reduces the level of *FLC* protein present in the plant (Michaels and Amasino, 1999; Sheldon et al., 2000). The down-regulation of *FLC* and the subsequent decrease in time to flower is proportional to the duration of the cold treatment (Sheldon et al., 2000). The *FLC* transcript level following vernalization is mitotically stable, just as the vernalized state is mitotically stable. The progeny of vernalized and nonvernalized plants have the same *FLC* transcript level, which means that the *FLC* transcript level, as well as the vernalization requirement, is reestablished to the ground state in the progeny of a vernalized plant (Sheldon et al., 2000).

FRIGIDA (*FRI*) Gene

FRIGIDA gene activity is also associated with late flowering. The *FRI* gene has been recently cloned and found to encode a protein unrelated to any known protein (Johanson et al., 2000). *FRI* acts with *FLC* to inhibit flowering. They act through the autonomous pathway, working antagonistically to the autonomous pathway genes. *FRI* apparently increases the level of *FLC* mRNA (Michaels and Amasino, 1999). Michaels and Amasino (1999) suggested that vernalization suppression of *FLC* expression could be mediated through the effect of the cold treatment on *FRI* activity. However, Sheldon et al. (1999) stated that the repression of *FLC* by the autonomous pathway is mediated by directly targeting *FLC* as opposed to operating indirectly through the inactivation of *FRI*.

Conclusion

Although the physical aspects of vernalization appear to be well researched, the molecular and genetic functions of vernalization remain unclear. Vernalization is a genetically complex physiological process. The *FLOWERING LOCUS C* gene appears to play a key role in the induction of flowering by vernalization. However, it is not yet known whether *FLC* activity is repressed simply by a low-temperature treatment, DNA demethylation, *VRN1* and *VRN2* gene activity, or a combination of these factors.

Herbaceous Perennials

Achillea

Achillea or yarrow has long been valued for its medicinal and even magical purposes. *Achillea millefolium* L., the common variety, is known by several names, including nosebleed, staunchweed, milfoil, and soldier's woundwort (Grieve, 1981). Achillea has astringent and anti-inflammatory properties. It has been used against colds, cramps, fevers, kidney disorders, toothaches, skin irritations, hemorrhages, burns, and bruises.

Achilles, a Trojan War hero, distributed yarrow among his soldiers to stop their wounds from bleeding (Stevens et al., 1993), which is how the plant received its genus name, *Achillea*. For the Navajos, it is a general cure-all, and the British call it allheal (Stevens et al., 1993). It was also consumed by the pioneers in an attempt to cure just about any ailment.

There are between 85 and 100 species of achillea, and they differ in growth habit, flower color, and leaf shape (Stevens et al., 1993; Turner and Wasson, 1997). Most species are native to Europe and north and west Asia. A handful (four or five species) can be found in North America (Turner and Wasson, 1997). There have also been numerous cultivars produced through breeding and selection.

Achillea is a member of Asteraceae. It varies in size from creeping alpine varieties to tall varieties used in border gardens and as cut flowers. The foliage is fernlike, aromatic (spicy fragrance), sometimes gray, and often hairy. It prefers full sun and high light. The gray foliage is indicative of high-light-adapted plants.

It is a hardy perennial that typically has large, flat heads of tiny daisylike flowers. There is a wide variety of flower colors, including shades of white, yellow, orange, pink, and red. Flowering generally occurs from late spring to autumn.

Achillea is a dry-land plant and is not typically tolerant of wet conditions. It does best in soil that is kept moderately moist. According to Stevens et al. (1993), overhead watering of achillea is not recommended because it may damage the flowers, cause spotting on the petals, splash soil onto the foliage, and promote the spread of disease. Constantly moist soil and excess nitrogen can result in tall leggy plants. To reduce plant height in greenhouse production, achillea should be grown with reduced nitrogen (approximately 100 ppm) and water (Nausieda et al., 2000). Achillea is also tolerant of poor soil. Once it is established, it can survive drought and other forms of neglect.

Most achillea species are facultative long-day plants, which means that the plants will flower under all photoperiods. However, they tend to flower more rapidly and consistently under long days, generally longer than 12 h (Nausieda et al., 2000).

Achillea multiplies rapidly by rhizomes. It is easily propagated by division in late winter. Plant clumps are usually pruned during the winter to stimulate strong spring growth. Another form of propagation is by cuttings, which generally occurs in early summer. The most popular species are usually clones and thus must be propagated asexually (Nausieda et al., 2000). Rooting stem cuttings is the common method for plug production.

Good cultural practices are the best insect control. A healthy, actively growing yarrow plant is more resilient against insect attack. Although no insect has been found to be extremely detrimental to achillea, in greenhouse production, the most common insects encountered include aphids, leafhoppers, spider mites, and thrips (Stevens et al., 1993).

Most disease problems arise from overwatering and when plants are under high temperature and humidity. Foliar fungal diseases are the most serious ones associated with achillea. *Botrytis* is common among cultivars derived from 'Taygetea' because they have more leaves at the base of the plant (Nausieda et al., 2000). Other foliar diseases include powdery mildew, downy mildew, and rust. Powdery mildew is distinguished by white spots on both sides of the leaves. Downy mildew is distinguished by yellow spots on the top of the leaves and white mold on the bottom. A mildew problem can be reduced by increasing space between plants, which improves air circulation around the foliage. Rust is characterized by raised spots called pustules, which are found on the underside of leaves and stems. Rust-infected plants should be removed and destroyed. Another disease that affects achillea is stem rot, which is caused by *Rhizoctonia solani*. It results in the decay of the stem base. This soil-borne pathogen is controlled by allowing the soil to thoroughly dry between irrigations.

One popular achillea species is *A.* 'Moonshine', the result of a cross between *A. clypeolata* and *A. aegyptiaca 'Taygetea'* (Armitage, 1989). The plant grows to a height of approximately 18 to 24 inches. The flowers are sulfur

yellow. The foliage is silvery-green and fernlike. It was introduced in the 1950s by Alan Bloom of Bressingham Gardens in England (Armitage, 1989).

Campanula

Campanula is distributed throughout temperate zones of the Northern Hemisphere, particularly in southern Europe and Turkey. They grow in diverse habitats, including high alpine rock crevices, meadows, and woodlands. More than 600 species of annuals, biennials, and perennials make up Campanulaceae (Finical et al., 2000b).

All campanula species are long-day plants, some being facultative and others obligate. Some species require a vernalization treatment in order to flower; others simply require a specific photoperiod. They prefer full sun, and in some species, flower number can be decreased if the plants are grown under low light (Whitman et al., 2000).

Pollination mechanisms vary widely among campanula species. Some species are predominantly self-pollinated, while others are cross-pollinated. According to Nyman (1992), pollen germinability plays a large role in the mechanism used by each species. For example, temporal overlap of high pollen germinability and stigma receptivity is associated with self-pollinating species, while temporal separation of high pollen germinability and stigma receptivity is associated with cross-pollinating species.

For most campanula species, the soil pH should be maintained around 6.0 and soil fertility should be kept at moderate levels (Finical et al., 2000b; Whitman

et al., 2000). Campanula prefers well-drained soil. Most species are drought tolerant, and in some cases, drought stress can delay flowering.

Campanulas are easily grown from seed. Seed may be sown in late winter or early spring. It should be sown thinly on the surface and covered with a very fine layer of sharp sand or fine grit (Lewis and Lynch, 1998). Light is beneficial for germination. Established campanula plants can be divided in the fall or spring when new growth has just started. Campanula can also be propagated by cuttings. In the greenhouse industry, plants are propagated primarily from stem cuttings.

Campanulas are generally trouble-free in cultivation. In the garden, the primary pest of campanula is the slug, but it is only troublesome with some of the more rare, succulent, smaller species. In greenhouse production, spider mite can be a problem with certain species (Whitman et al., 2000).

Rust is probably the most troublesome disease in campanula, but again, it is species specific. However, damping-off root rot caused by *Pythium* and *Rhizoctonia* can sometimes be problematic. Damping-off can be prevented by maintaining well-drained soil. *Botrytis cinerea* on leaves can also be problematic in some species (Whitman et al., 2000). However, if the foliage is kept dry, the disease should not be a problem.

One particularly interesting species is *Campanula* 'Birch Hybrid'. It was introduced by Walter Ingwersen and is the result of a cross between *C. portenschlagiana* and *C. poscharskyana*. 'Birch Hybrid' was initially introduced as *C. xportenscharskyana*. In 1945, it was given an Award of Merit along with a

recommendation that its name be changed. The name 'Birch Hybrid' came from the nursery where it was first propagated, Birch Farm (Lewis and Lynch, 1998).

Campanula 'Birch Hybrid' is a miniature campanula that grows up to 6 inches (15 cm) high and spreads up to 12 inches (30 cm) (Finical et al., 2000b). The branching stems bear numerous open star-shaped blooms of light blue or mauve. New flowers continue to open from June through September. It is an evergreen perennial with underground runners and small, ovate, heart-shaped, toothed, bright green leaves. It is a rock-garden/alpine species that requires moist but well-drained soil in sun or partial shade (Brickell and Zuk, 1997). 'Birch Hybrid' requires a vernalization treatment in order to flower (Finical et al., 2000b). Without a cold treatment, plants remain vegetative. It is a facultative long-day plant following vernalization and is also fairly free of disease and insect problems.

Gaillardia

According to Koning (1986), gaillardia was first noted in 1783. Within *Gaillardia,* there are approximately 30 species ranging from annuals to biennials and perennials, and all are members of Asteraceae. All species are native to primarily the southwestern United States, with the exception of two South American species. The common name *blanket flower* arose because the colors in the flowers resembled the blankets traditionally worn by Native Americans.

Gaillardia has been used in pharmaceutical research (Koning, 1986). The plant produces the sesquiterpene lactones: spathulins, pulchellins, and

gaillardins. Spathulins and pulchellins are antibiotics used for *Staphylococcus* and *Streptococcus*. Spathulin and gaillardin, in cell cultures, inhibit human nasopharynx carcinomas.

The plant's flowers have a long blooming season, from summer until the first frost. The flowers are daisylike, either single or double, and can be as much as 6 inches wide. Flower colors range from bright yellow to oranges and reds.

Gaillardia tolerates extreme heat, cold, dryness, strong winds, and poor soil. The plants prefer full sun and well-drained, moderately fertile soil. It is considered a quantitative long-day plant (Koning, 1986). Under short days the plant forms a rosette.

The annual varieties are usually propagated from seed in spring or early summer. Seed propagation is used widely for commercial production. Seeds germinate in the light at 21 to 24 °C and under high humidity (90 to 95%) (Yuan et al., 2000). The perennial species can also be divided in the spring or propagated from stem cuttings. Division is used most commonly by gardeners to control plant size and form.

Gaillardia, as a whole, is not particularly susceptible to many diseases or insect pests. In fact, gaillardia plants can inhibit population expansion of *Protylenchus penetrans* and *Ditylenchus dipsaci* nematodes in garden soil (Koning, 1986). In the greenhouse, *Gaillardia xgrandiflora* Van Houtte is susceptible to aphids (Yuan et al., 2000). Also, plants in the greenhouse are more susceptible to aster yellows and powdery mildew.

Gaillardia xgrandiflora is a hybrid of *G. aristata* and *G. pulchella* and is the most commonly grown blanket flower (Turner and Wasson, 1997). The plants form mounds up to three feet high.

Some cultivars of *G. xgrandiflora* have a distinct juvenile phase. Most of the plant population reaches maturity when 16 nodes per plants have formed (Yuan et al., 2000; Yuan et al., 1998a). According to research conducted by Yuan et al. (1998a), periods of cold exposure (10 to 15 weeks) enhanced the flowering percentage and greatly accelerated flowering of *G. xgrandiflora* 'Goblin'. In production, increasing the temperature from 15 to 26 °C reduced the number of days to flower by approximately 25 d for the same cultivar (Yuan et al., 1998b). Gaillardia requires long days after cold in order to flower. Evans and Lyons (1988) showed that applications of GA_{4+7} could substitute for long days and promote flowering under short days in the same amount of time required by untreated, photoperiodically induced plants.

Koning has done much work on flower formation and development in *G. xgrandiflora*. He described five distinct stages of disk flower development (Koning, 1983a, 1983b, 1984). During stage 1, flowers are relatively unpigmented and tightly closed. In stage 2, the tip of the corolla is pigmented. In stage 3, the corolla begins to unroll and the filaments elongate. During stage 4, the corolla unrolls completely and the style and stigma elongate. And in stage 5, the corolla expands completely, the stigmatic surface between the branches is exposed, and the flower is pollinated. Filament elongation is controlled by auxin (Koning, 1983a). Corolla elongation is controlled by the level of endogenous

gibberellin activity (Koning, 1984). Style and stigma development is controlled by at least three hormones. Their growth is inhibited by high levels of gibberellins during stages 1 through 3. During stages 3 and 4, high auxin triggers elongation. Finally, at the end of stage 5, ethylene production increases and promotes stigma unfolding (Koning, 1983b).

Gaura

The genus name *Gaura* translates as gorgeous (Turner and Wasson, 1997). Gaura has about 20 species of annuals, biennials, perennials, and subshrubs. All species, which belong to Onagraceae, are native to North America (primarily Texas and Mexico). Despite their showy flowers, they are apt to be weedy. They have simple, narrow leaves. The flowers are flat, star-shaped, and pink or white and are borne on either panicles or racemes (Brickell and Zuk, 1997).

In its native range (Texas to Louisiana) and other warm areas, gaura can grow to four feet tall and wide. In Seattle, Washington, and Vancouver, British Columbia, it tends to be shorter and more compact, reaching a height and width of only about 2 feet (Farmer, 1993). Different species of gaura are broadly interfertile, but different species in nature are isolated by a number of mechanisms that minimize the occurrence of natural hybrids (Carr et al., 1990).

Gaura prefers full sun. It does not require excessive fertilizer or water because many species have long fleshy roots that store water. Because of this,

gaura is well adapted to dry areas and tolerates extended periods of drought and heat stress.

Gaura can be propagated by using several techniques. It can be propagated in spring or fall by division, which is used primarily to rejuvenate the plant and control overall plant size. Gaura can also be propagated by seed, which generally germinate in five to 11 days at 21 °C (Finical et al., 2000a). Vegetative stem cuttings can also be propagated, and this method generally is used in the summer. Commercially, all three means of propagation are used.

The gaura commonly sold commercially is *G. lindheimeri*. It is native to the United States/Mexico border region (Turner and Wasson, 1997). It has loosely branched stems with tiny hairs. Flowering lasts from late spring to midfall (Farmer, 1993). It produces long sprays of pink buds that open into white flowers, reaches a height of 4 feet, and has a spread of 3 feet (Turner and Wasson, 1997).

In *G. lindheimeri*, juvenility does not appear to affect flowering. Vegetatively propagated plants with as few as six leaves will flower (Finical et al., 2000a). It also does not require a vernalization treatment in order to successfully flower. It is considered a facultative long-day plant, flowering faster under longer daylengths. *Gaura lindheimeri* also grows in a wide variety of soil types, ranging from dry clay to sand. It appears to have no problematic diseases or insect pests. However, from greenhouse observations, spider mites could be a problem.

Research performed by Carr et al. (1990) divided gaura into eight species according to trends in floral symmetry, fruit morphology, and plant growth habits. It was concluded from this work that the placement of *G. lindheimeri* within the section *Gaura* "depends heavily on the assumptions that perennial life-cycle and loosely clumped growth habit are secondarily derived in this distinctive species."

Leucanthemum

Leucanthemum, part of Asteraceae, is composed of about 25 species of annuals and perennials (Turner and Wasson, 1997). All species are native to Europe and temperature Asia. Many botanists included these plants in *Chrysanthemum* (Turner and Wasson, 1997). Leucanthemum is a clump-forming plant with variable leaves ranging from toothed to lobed.

The most common garden leucanthemum is *L.* x*superbum* Bergmans ex J. Ingram, which is better known as the shasta daisy. *Leucanthemum* x*superbum* translates as *superior* (or superb) *white flower* (Coombes, 1994). It has also been classified as *Chrysanthemum maximum* Ramond and *Chrysanthemum* x*superbum* (Turner and Wasson, 1997).

Shasta daisies were once thought to be *L. maximum*, a native of the Pyrenees, but now they are believed to be the result of a cross between *maximum* and *L. lacustre* Brot. from Portugal. Luther Burbank, a plant breeder, first noticed them naturalized on the slopes of Mount Shasta in Washington (Turner and Wasson, 1997).

Most cultivars reach a height and spread of 2 to 3 feet. Flowers can be up to 3 inches across and range from doubles and singles to fringed petals (Turner and Wasson, 1997). Most plants produce flowers from summer through early fall. *Leucanthemum xsuperbum* requires full sun to partial shade. They prefer moderate to high light levels (Runkle et al., 2000a). Plants also prefer moist, rich, well-drained soil.

Some shasta daisies are extremely sensitive to many insecticides, which cause moderate to severe phytotoxicity, including leaf and flower burn, chlorosis, and widespread plant death (Runkle et al., 2000a). Common methods of propagation include tip cuttings, tissue culture, and bare-root divisions.

Kessler and Keever (2000) determined that shasta daisy cultivars show a varied response to photoperiod and vernalization time. The cultivar Becky is an obligate long-day plant regardless of vernalization. On the other hand, the cultivars Snowcap and Snow Lady show a facultative long-day response. In all three cultivars tested, shoot height, flower shoot number, and market quality increased, while time to flower decreased with increasing vernalization up to 6 weeks under long days.

The most desirable and attractive short cultivar of *L. xsuperbum* is most likely 'Snowcap'. It was introduced by Adrian Bloom of Blooms of Bressingham, United Kingdom (Runkle et al., 2000a).

Because of its thick fleshy leaves, it requires relatively frequent irrigation, especially under high light levels. Plants recover well from short periods of

drought stress, but prolonged drought stress can cause leaf margins to become necrotic (Runkle et al., 2000a).

'Snowcap' has a unique flowering habit in response to a cold treatment (vernalization). Without a cold treatment, it is a qualitative long-day plant, flowering only under photoperiods longer than or equal to 16 h. With a cold treatment, it is a quantitative long-day plant, flowering faster under photoperiods longer than or equal to 16 h (Runkle et al., 1998a). Flowering characteristics such as increased flowering percentage, improved crop uniformity, reduced time to flower, and increased flower number are also enhanced by exposure to cold temperatures.

'Snowcap', as well as most shasta daisies, is relatively disease and insect-pest free. In young transplants, *Pythium* can be a problem if plants are overwatered (Runkle et al., 2000a). In greenhouse production, whiteflies, aphids, and thrips can be problematic as well.

Phlox

When translated, the word *phlox* means flame (Turner and Wasson, 1997). Phlox, which is a member of Polemoniaceae, contains 61 species from North America and Siberia (Grant, 1959). Plants range from evergreen to semievergreen annuals and perennials. Most phlox are grown for their profuse, fragrant flowers. Fossil fruits of a phlox probably close to *P. sibirica* L. or *P. borealis* Wherry have been found in a Pleistocene deposit from Fairbanks, Alaska (Grant, 1959).

Tall perennial phloxes grow easily in any temperate climate but can require a lot of water. Annual species will grow in almost any climate, ranging from the tropics to the coldest region. Phlox prefers sunny or partly sunny growing areas. It thrives in moist but well-drained soil in full sun or, in drier soils, light shade. Phlox has few disease and insect pests. The most common include spider mites and powdery mildew.

Phlox paniculata Lyon ex Pursh, or tall garden phlox, is a commonly grown herbaceous perennial native to the eastern United States. The terminal flower heads are produced on long stems and are composed of many small five-lobed flowers, and plants can reach a height of more than 3 feet (Turner and Wasson, 1997). Flower color ranges from various shades of violet and red to salmon and white. *Phlox paniculata* produces long-stemmed cut flowers in mid to late summer when grown under field conditions, but demand and prices for these stems is best during winter and spring (Garner and Armitage, 2000).

The base photoperiod for *P. paniculata* is approximately 13 h for uncooled plants and less than 10 h for cooled plants (Runkle et al., 1998b). Vernalization is not required for flowering; however, cooled plants have shown increased stem length and accelerated flowering (Garner and Armitage, 2000). Providing plants with a cold treatment and photoperiods of less than 10 h should produce stock plants for vigorous vegetative cuttings (Runkle et al., 1998b).

Phlox paniculata can be propagated several ways: crown division, tissue culture, and terminal and root cuttings. Terminal cuttings from vegetative shoots can be easily rooted in about 3 weeks (Garner and Armitage, 2000). According

to Schnabelrauch and Sink (1979), clonal multiplication of *P. paniculata* by conventional methods of crown division of dormant stock plants and root cuttings has led to disease and nematode infestations during field culture that can be traced back to the stock material used for propagation.

Veronica

Legend tells that Saint Veronica was the woman who wiped the face of Christ with her veil. She was rewarded with having his image imprinted on it. Her connection to this flower is that the savants of the Middle Ages thought the markings on the flowers of some species resembled the markings on the veil (Armitage, 1989; Turner and Wasson, 1997).

Veronica's common name is speedwell and it is a member of Scrophulariaceae, or the snapdragon family. Within veronica, there are between 200 and 250 species of herbaceous annuals and perennials (Runkle et al., 2000b; Turner and Wasson, 1997). They range from creeping plants suitable for rock gardens to 6-foot-tall giants. One species, *V. officinalis* L., was substituted for tea in Europe until the 19th century (Armitage, 1989).

Veronica flowers are small. The largest flower is about ½ inch wide (Turner and Wasson, 1997). Blue is the predominant flower color. However, white and pink flowers are also common.

Veronica are fully to moderately frost hardy. They are easy to grow in any temperate climate. They tolerate any soil condition and will grow in full sun as well as full shade. Veronica can be propagated many ways, including from seed

in the fall or spring, from cuttings in the summer, and by division in early spring or early fall.

Veronica longifolia L., or long-leaf speedwell, is native to northern and central Europe and Asia (Turner and Wasson, 1997). It has been naturalized in North America (Runkle et al., 2000b). It generally grows to a height of 3 feet. The leaves are narrow and taper, are arranged in whorls, and are toothed on the edges. The flowers are ¼ inch wide and closely packed on 12-inch-long racemes (Armitage, 1989). The inflorescences are generally lilac blue.

Veronica longifolia is a day-neutral plant following vernalization (Runkle et al., 2000b). Plants will flower regardless of photoperiod. It prefers rich soil and warm climates. Some cultivars have thick leaves and require frequent irrigation to prevent wilting. Powdery mildew has been known to cause trouble, and *Botrytis* can be problematic on lower leaves.

The most common method of propagation is by tip cutting. Unchilled stock plants produce cuttings with an obligate cold requirement (Runkle et al., 2000b). Some rooted cuttings may flower without a cold treatment if they were taken from cold-treated stock plants.

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SECTION II

THE FLOWERING RESPONSE OF *VERONICA SPICATA* 'RED FOX' TO COLD AND BULKING TREATMENTS

The Flowering Response of *Veronica spicata* 'Red Fox' to Cold and Bulking Treatments

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Additional index words: vernalization, pinching

Received for publication _____. We appreciate the greenhouse growers providing support for Michigan State University floriculture research.
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Abstract

Veronica spicata L. 'Red Fox' plants were treated at 5 °C for 0 through 5 weeks and subsequently forced to flower under a 16-h photoperiod to determine minimum cold requirements for flowering. Plants had an obligate cold requirement for flowering. As cold duration increased through 5 weeks, flowering percentage increased from 0% to 100%. In a separate experiment, *V. spicata* 'Red Fox' plants were pinched and grown (bulked) in 13-cm pots or 4.4- or 4.1-cm plugs for 0 through 3 weeks. Plants were then cold treated for 5 weeks at 5 °C and subsequently forced to flower under a 16-h photoperiod to determine the effects of pinching, bulking duration, and bulking container size on the number of flowering shoots per plant. Flowering percentage was greatest for unpinched plants, irrespective of bulking container size. As bulking container size increased, the number of flowering shoots per plant increased. Unpinched plants flowered sooner than pinched plants. Time to flower decreased as bulking duration increased. Unpinched plants bulked in a 13-cm pot had more flowering shoots per plant than pinched plants bulked in a 13-cm pot. In contrast, for both plug sizes, pinched plants had more flowering shoots than unpinched plants.

Introduction

Understanding the flowering physiology of herbaceous perennials is important for producing a uniform flowering crop. Since a flowering plant is more marketable than a vegetative plant, predictable flowering of herbaceous perennials is becoming a priority for many greenhouse growers. Minimizing production time is also important for profitability. Therefore, identifying desirable herbaceous perennials for container production and understanding their flowering physiology is critical for the success of and implementation of profitable production on specific market dates. Speedwell (*Veronica* sp.) is one such herbaceous perennial. *Veronica* is available in a variety of colors, pink, white, and blue, and can be grown from seed or vegetatively propagated material (Nau, 1998). *Veronica spicata* L. 'Red Fox' is one particular cultivar of interest. It is grown from vegetatively propagated material and produces spikes of rose-pink flowers. It has a compact growth habit that makes it ideal for container production.

For *V. spicata* L. 'Blue' (Engle, 1994; Runkle 1996) and *V. longifolia* L. 'Icicle' (Frane, unpublished data), flowering percentage increases and days to flower decreases with cold treatment. *Veronica longifolia* 'Sunny Border Blue' (Runkle, 1996) and *V. spicata* 'Red Fox' (Frane, unpublished data), on the other hand, have an obligate cold requirement. Without cold, plants will not flower. However, only 0 and 15 weeks at 5 °C have been tested for *V. spicata* 'Red Fox'. Following a cold treatment, all *Veronica* cultivars tested have been reported as day-neutral plants, flowering regardless of photoperiod.

When forced to flower in containers, *V. spicata* cultivars tend to produce a single flowering shoot, whereas *V. longifolia* cultivars tend to produce multiple flowering shoots. Many floriculture crops such as cut flowers, poinsettia (*Euphorbia pulcherrima*), and other herbaceous perennials are pinched to increase flower number. In 2002 preliminary research (Enfield, unpublished data), *V. spicata* 'Red Fox' plants that were pinched immediately before cold treatment flowered, but with only one to two flowering shoots per plant. Plants that were pinched immediately after a 5-week cold treatment failed to flower. Apparently, shoot tips that were induced to flower by the cold treatment were removed when the plants were pinched following cold treatment. Thus, this data suggest that in order to increase the number of flowering shoots per plant, pinching needs to occur before cold treatment, and a period of growth (bulking) is needed after pinching and before cold treatment to allow lateral shoots to develop sufficiently to perceive cold. A period of growth before flower induction is also needed for poinsettia so that lateral branches are the appropriate size for the finish pot (Ecke et al., 1990).

Perception of low temperatures occurs mainly in the meristematic zones of the shoot apex. However, Levy and Dean (1998) propose that all actively dividing cells may be capable of responding to low temperatures. Vernalization, or the promotion of flowering by a cold treatment, has been achieved by applying localized cooling temperatures to the stem apex of plants, and the effect seems to be largely independent of temperatures experienced by the rest of the plant

(Thomas and Vince-Prue, 1997). This suggests that the apex must be present to perceive cold.

The first objective of this research was to identify the minimum cold duration required for rapid and uniform flowering of *V. spicata* 'Red Fox'. The second objective was to determine the effects of bulking container size, pinching, and subsequent bulking duration following pinch on the number of flowering shoots of cold-treated *V. spicata* 'Red Fox' plants.

Materials and Methods

Cold-duration experiment. *Veronica spicata* 'Red Fox' stock plants were received from Center Greenhouse (Denver, Colo.; Sept. 1999) and potted in 13-cm square plastic containers (1.1 L) filled with a commercial soilless medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened course perlite, along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.). Plants were grown under a 12-h photoperiod provided by supplementing natural daylengths with lighting from high-pressure sodium lamps from 0800 to 2000 HR. Stock plants were pinched at three- to four-week intervals to ensure continued branching and cutting production. Harvested cuttings were propagated in 72-cell (0.03-L) plug trays (Landmark Plastic Corporation, Akron, Ohio).

After propagation, plants were grown (bulked) in the plug trays for an additional 3 weeks in a greenhouse at 20 °C to establish root systems and increase vegetative growth before cold treatment. Photoperiod was maintained

at 12 h as with the stock plants. Plugs then received no cold treatment or were placed in a controlled-environment chamber for 1, 2, 3, 4, or 5 weeks at 5 °C.

Bulking experiment. *Veronica spicata* 'Red Fox' stock plants were potted (Oct. 2001) in 13-cm square plastic containers (1.1 L) filled with a commercial soilless medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened course perlite, along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.).

The experiment was replicated in time. On Nov. 28, 2002, and again on Jan. 8, 2002, harvested cuttings were propagated in 72-cell (0.04-L) and 50-cell (0.08-L) plug trays (Landmark Plastic Corporation, Akron, Ohio). Immediately following propagation, half of the 50-cell and half of the 72-cell plugs were transplanted to 13-cm square plastic containers (1.1 L). All plants were maintained at 20 °C under a 16-h photoperiod, natural daylengths supplemented with high-pressure sodium lamps from 0530 to 2130 HR. Plants were grown (bulked) for 2 weeks, and then half of the 50-cell plugs, 72-cell plugs, and 13-cm plants were pinched (two apical nodes removed). All plants were then bulked an additional 0, 1, 2, or 3 weeks. After the appropriate bulking duration, plants were cold treated for 5 weeks at 5 °C.

Propagation. Plug trays contained a mixture of 50% commercial medium (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.) and 50% screened course perlite (Therm-O-Rock, East, Inc.; New Eagle, Pa.). Basal portions of each cutting were dipped in a 1500-ppm solution of liquid auxin (DIP

'N GROW; Astoria-Pacific, Clackamas, Ore.) before stick. Cuttings were propagated under natural photoperiods. Propagation air temperatures were maintained at 23 °C and bottom heat (soil temperature) was maintained at 25 °C. A vapor pressure deficit of 0.3 kPa was maintained by injecting water vapor as needed. Cuttings were rooted and weaned from propagation 3 weeks after sticking.

Cold treatments. Plants were maintained at 5 °C in a cooler lighted from 0800 to 1700 HR by cool-white fluorescent lamps (F96T12/CW/VHO, Philips, Somerset, N.J.) The photosynthetic photon flux (*PPF*) from the lamps was approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. While in the cooler, plants were watered as needed with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter.

General plant culture. Following all cold treatments, plugs were potted in 13-cm square plastic containers (1.1 L) containing the same commercial medium used for the stock plants. All plants were forced to flower under a 16-h photoperiod, natural days supplemented with high-pressure sodium lamps from 0530 to 2130 HR. Plants were top-watered as necessary with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and containing water-soluble fertilizer providing 125 N, 12 P, 125 K, 13 Ca ($mg \cdot L^{-1}$; 30% ammoniacal N) plus ($mg \cdot L^{-1}$) 1.0 Fe, 0.5 Mn, 0.5 Zn, 0.5 Cu, 0.1 B, 0.1 Mo (MSU Special; Greencare Fertilizers, Chicago, Ill.). *Veronica spicata* 'Red Fox' plants in the bulking duration experiment received an additional 0.5 ppm Cu and 0.1 ppm B at every watering.

Greenhouse temperature control. Plants were grown in glass greenhouses set at 20 °C. Greenhouse temperatures were controlled by a greenhouse climate-control computer (Model CD750; Priva, De Lier, The Netherlands). Average daily temperature and daily light integral were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge (0.013 mm in diameter) type E thermocouples and a quantum sensor (Model LI-189; LI-COR, Inc., Lincoln, Neb.), respectively. The datalogger collected data every 10 seconds and recorded the hourly average. Actual average daily temperatures and daily light integrals (cold duration experiment only) for the beginning of forcing to the average date of flowering were calculated and are presented in Table 1.

Data collection and analysis. Dates of visible bud and first flower as well as the number of flowering shoots and lateral inflorescences per flowering shoot were recorded. For the cold duration experiment, plant height and number of nodes formed below the flower shoot were also recorded. A completely randomized design with 10 observations for each treatment was used for the cold duration experiment. A randomized complete block design was used for the bulking duration experiment. Data were analyzed with SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA), generalized linear model (GENMOD), and general linear model (GLM) procedures.

Results

Cold-duration experiment. Veronica spicata 'Red Fox' had an obligate vernalization requirement for flowering. All plants remained vegetative until they

received at least a 3-week cold treatment (Table 2). Percentage of flowering plants increased to one-hundred percent as cold duration increased from 3 to 5 weeks at 5 °C. There was no statistical improvement in flowering characteristics (decrease in time to flower, plant height, and number of flowering shoots) of flowering *V. spicata* 'Red Fox' plants with increasing cold duration, although the single plant that flowered following a 3-week cold treatment took about 1 week longer to flower than plants cooled for longer periods.

Bulking experiment. Bulking container size did not significantly affect days to visible bud, days from visible bud to flower, or days to flower (data not shown). Therefore, bulking containers were pooled for statistical analysis of time to flower.

Pinching and bulking duration significantly affected days to visible bud and days to flower (Figure 1, Table 3), but the two-way interaction was not significant. Both days to visible bud and days to flower decreased linearly as bulking duration increased. The slopes of the lines for pinched versus unpinched plants were not significantly different, but the intercepts of the two lines were. Unpinched plants reached visible bud and flowered sooner than pinched plants.

Days from visible bud to flower were significantly affected by pinching, bulking duration, and their interaction (Figure 1). Days from visible bud to flower had significant linear and quadratic trends. Both the slopes and intercepts of the lines for pinched versus unpinched plants were significantly different. However, although the data were statistically significant, the difference in the number of days from visible bud to flower between pinching treatments and across bulking

duration averaged one day or less and horticulturally would not be considered significant.

The percentage of flowering plants, irrespective of bulking container size and bulking duration, was higher when plants were not pinched (Figure 2A and B). Pinched plants, irrespective of bulking container size, did not obtain flowering percentages comparable to that of unpinched plants until they had been bulked for 3 weeks. Flowering probability (a measure of the likelihood that a plant will flower under given treatment conditions) was strongly affected by bulking container size, pinching, and bulking duration (Table 3). The larger the bulking container, the higher the flowering probability. The probability of plants flowering also increased as bulking duration increased. Plants that were pinched and bulked for 3 weeks had the same likelihood of flowering as unpinched plants that were not bulked.

The number of flowering shoots per plant was significantly affected by bulking container size, pinching, and bulking duration, and all two-way interactions were also significant (Table 3, Figure 2). As bulking duration increased, regardless of pinching, the number of flowering shoots per plant increased (Figure 2C and D) although differences among treatments were large. For unpinched plants bulked in a 13-cm pot, the number of flowering shoots increased from about one to more than five as bulking duration increased (Figure 2C). Unpinched plants bulked in a 13-cm pot had more flowering shoots than pinched plants. In contrast, for both plug sizes, pinched plants had more flowering shoots than unpinched plants.

Bulking container size, pinching, bulking duration, and the two-way interaction of bulking container size and pinching significantly affected the number of lateral inflorescences per flowering shoot (Table 3). As the number of flowering shoots per plant increased, the number of lateral inflorescences per flowering shoot decreased (Figure 3). Plants appeared to compensate for the lack of flowering shoots by producing more lateral inflorescences.

Discussion

Veronica spicata 'Red Fox' has an obligate cold requirement for flowering (Table 2). *Veronica spicata* 'Blue', on the other hand, did not have an obligate cold requirement; plants flowered poorly without cold, but flowering percentage increased from 43 to 100 following 5 weeks at 5 °C (Engle, 1994). These results may be misleading because plants were obtained from commercial growers and may have accumulated some cold before initiation of the experiment (personal communication. Royal Heins). In *V. spicata* 'Red Fox', only shoots that were visible before cold treatment flowered, which supports Metzger's (1988) research with *Thlaspi arvense* L. that found that vernalization was perceived by shoot tips and not just the stem apex, as stated by Thomas and Vince-Prue (1997).

Removal of the shoot apex by decapitation or pinching out the growing tip removes the source of apical dominance and induces growth of lateral buds (Cline, 1991). Plants are pinched to increase lateral branching and ultimately flower number. The timing of pinch before flower induction is important in several potted crops, including poinsettia and chrysanthemum. Ecke et al. (1990) state

that the shoot tip of poinsettia should be removed early enough to provide sufficient growing time to produce the length of stem required for the pot size. Timing of pinch for chrysanthemums is in accordance with their natural flowering height. Short varieties are induced to flower 1 to 2 weeks after pinch; medium varieties, the day of pinch; and tall varieties, 1 to 2 weeks before pinch (Williams and Bearce, 1964). In this experiment, unpinched *V. spicata* 'Red Fox' plants in 13-cm pots produced more flowering shoots than pinched plants in 13-cm pots. For *Kalanchoe tomentosa*, pinched plants produced more lateral branches, while unpinched plants of *Columnea microphylla* produced more branches than pinched plants (Lyons and Hale, 1987).

Chrysathemum morifolium Ramat. has no flower induction-insensitive phase following pinch. Plants can be induced to flower by short-day photoperiods immediately following pinch (Adams et al., 1998). *Veronica spicata* 'Red Fox', in contrast, has a flower induction-insensitive phase. Plants require a period of growth following pinch before they are able to respond to flower induction by vernalization.

Pinched *V. spicata* 'Red Fox' plants took a few days longer to flower than unpinched plants. Garner et al. (1997) showed that the time from planting to harvest was longer in pinched plants of *Delphinium* than in unpinched plants. Pinched *Phlox paniculata* Lyon ex Pursh plants require approximately 11 days longer to flower than unpinched plants (Enfield, unpublished data). However, in both examples, pinching occurred after plants were already induced to flower. In the case of *V. spicata* 'Red Fox', pinching occurred before flower induction.

Lateral shoots of pinched plants may be large enough to perceive cold but may not be as developed as unpinched plants before cold treatment. Therefore, a longer growing time following cold was required for plants to flower.

Differences in time to flower during an experiment can be caused by differences in temperature between treatments. As bulking duration increased from 0 to 3 weeks, the average forcing temperature increased for replicate one by 0.7 °C, but there was no change for replicate two. Average increase in temperature as bulking duration increased averaged over both replicates was 0.3 °C. By applying a model developed to determine the number of days to flower for *V. longifolia* 'Sunny Border Blue' (Runkle, unpublished data) to *V. spicata* 'Red Fox', approximately one day can be accounted for by increased temperatures as bulking duration increased from 0 to 3 weeks. However, there was a four-day difference in days to flower; therefore, the decrease in time to flower as bulking duration increased was a significant treatment effect.

The number of flowering shoots increased as bulking duration increased, regardless of bulking container size. As bulking duration increased, there was more vegetative growth present before cold treatment. Dybing and Grady (1994) found that the length of the vegetative growth period in flax was positively correlated with flower production. As the duration of vegetative growth increased, flower production increased. Many *V. spicata* 'Red Fox' plants in the cold-duration experiment had only one flowering shoot. Plants were not pinched and were bulked in 72-cell plug trays for 3 weeks. Three weeks in the cold-duration experiment was equivalent to 1 week of bulking in the second

experiment. Unpinched plants bulked in 72-cell plug trays for 1 week in the second experiment averaged one flowering shoot per plant.

For unpinched plants, the decrease in the number of flowering shoots per plant as bulking container size decreased may be the result of a shade avoidance response caused by a change in the red to far-red (R/FR) ratio. A low R/FR, found in a dense plant canopy, results in reduced branching, while a high R/FR results in increased branching (Smith, 1994). Plants bulked in a 72-cell plug tray are grown at a higher plant density (approximately 19 cm^2 per plant) than plants bulked in the 13-cm pot (169 cm^2 per plant) and would be expected to have fewer flowering shoots because of reduced plant branching caused by a change in the R/FR rates an total intercepted light. In 13-cm pots, as bulking duration increased, more lateral shoots became large enough to perceive cold; thus, the number of flowering stems increased. When plants are pinched, apical dominance is removed and lateral shoots develop. For plants bulked in plug trays, pinching increased the number of flowering shoots per plant. For plants bulked in 13-cm pots, potential lateral shoots are actually removed with the pinch and the overall number of flowering shoots is reduced compared with that of unpinched plants.

Conclusions

Veronica spicata 'Red Fox' has an obligate 5-week cold requirement for rapid and uniform flowering. Plants should not be pinched after cold because it eliminates flowering. Unpinched plants had a higher probability of flowering than

pinched plants, at least if pinched plants are bulked 3 or fewer weeks. The larger the bulking container, the greater the number of flowering shoots. The longer the bulking duration, at least through 3 weeks, the greater the number of flowering shoots. When *V. spicata* 'Red Fox' was bulked in a 13-cm pot, the greatest number of shoots occurred when plants were not pinched and were bulked for 3 weeks. When *V. spicata* 'Red Fox' was bulked in a 50- or 72-cell plug tray, pinched plants bulked for 3 weeks had the highest number of flowering shoots per plant and the highest flowering percentage.

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Table 1. Dates of forcing following cold treatment, average air temperatures, and average daily light integral (DLI) from date of forcing to average date of flowering for *Veronica spicata* 'Red Fox'.

Date of forcing following cold	Weeks of bulking	Weeks of 5 °C	Forcing Avg. temp. (° C)	Forcing Avg. DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
Cold duration experiment				
2/1/00	3	0	--[z]	--
2/8/00	3	1	--	--
2/15/00	3	2	--	--
2/22/00	3	3	21.4	14.8
2/29/00	3	4	21.3	15.5
3/10/00	3	5	21.1	15.7
Bulking duration experiment				
Replicate 1				
2/13/02	0	5	21.7	10.2
2/20/02	1	5	21.7	10.6
2/27/02	2	5	22.1	12.1
3/6/02	3	5	22.4	12.9
Replicate 2				
3/22/02	0	5	22.7	13.4
3/29/02	1	5	23.0	13.2
4/8/02	2	5	22.8	13.8
4/16/02	3	5	22.6	14.4

[z]Dashes indicate no plants flowered.

Table 2. The effects of 5 °C cold treatment on flowering of *Veronica spicata* 'Red Fox'.

Weeks of 5 °C	Flowering percentage	Days to visible bud	Days from visible bud to flower	Days to flower	Final node number[z]	Final plant height (cm)	Number of lateral Inflor.[y]
0	0	--[x]	--	--	--	--	--
1	0	--	--	--	--	--	--
2	0	--	--	--	--	--	--
3[w]	10	37	20	57	8	53.5	5
4	50	28	22	50	7	57.9	5
5	100	26	22	48	7	53.5	7
Significance							
Weeks at 5C[v]		NS	NS	NS	NS	NS	NS

[z]Number of nodes below flowers.
[y]Number of lateral inflorescences off main flowering shoot.
[x]Dashes indicate no plants flowered.
[w]Data based on one flowering plant.
[v]Analysis of 4- and 5-week cold treatments only.

Table 3. Significance of bulking container size, pinch, and bulking duration on flowering of *Veronica spicata* 'Red Fox'.

Treatment	Flowering probability	Days to visible bud	Days from visible bud to flower	Days to flower	Flowering shoots	Lateral inflorescences per flowering shoot
Bulking container (BC)	**	--[z]	--	--	***	***
Pinch (P)	***	***	**	***	**	***
Bulking duration (BD)	***	***	***	***	***	***
P_{Linear}	--	***	***	***	***	NS
$P_{Quadratic}$	--	NS	**	NS	NS	**
BC X P	*	--	--	--	***	**
BC X BD	NS	--	--	--	***	NS
P X BD	*	NS	***	*	NS	NS
BC X P X BD	--	--	--	--	NS	NS

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

[z]Dashes indicate data not presented/not tested.

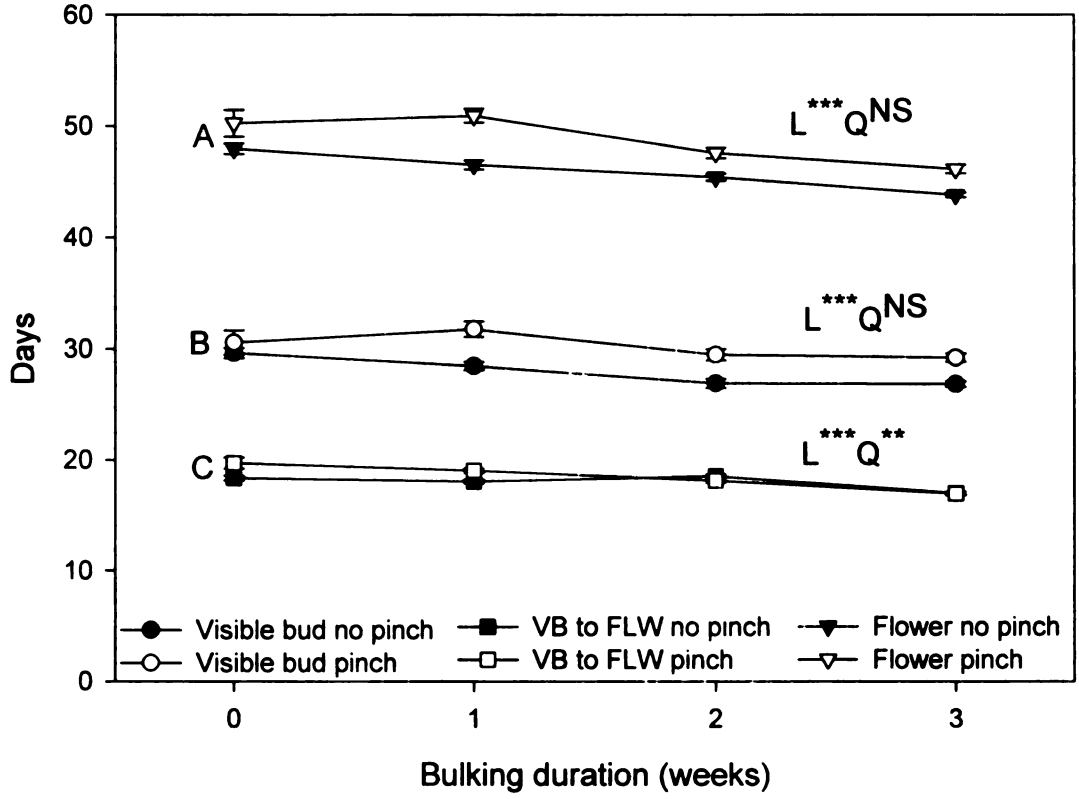

Figure 1. The effects of bulking duration and pinching on days to visible bud (●,○, no pinch and pinch, respectively), days from visible bud to flower (■,□, no pinch and pinch, respectively), and days to flower (▼,▽, no pinch and pinch, respectively). Error bars represent the standard error of the mean. Probability of linear and quadratic relationships indicated by L and Q, respectively, ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

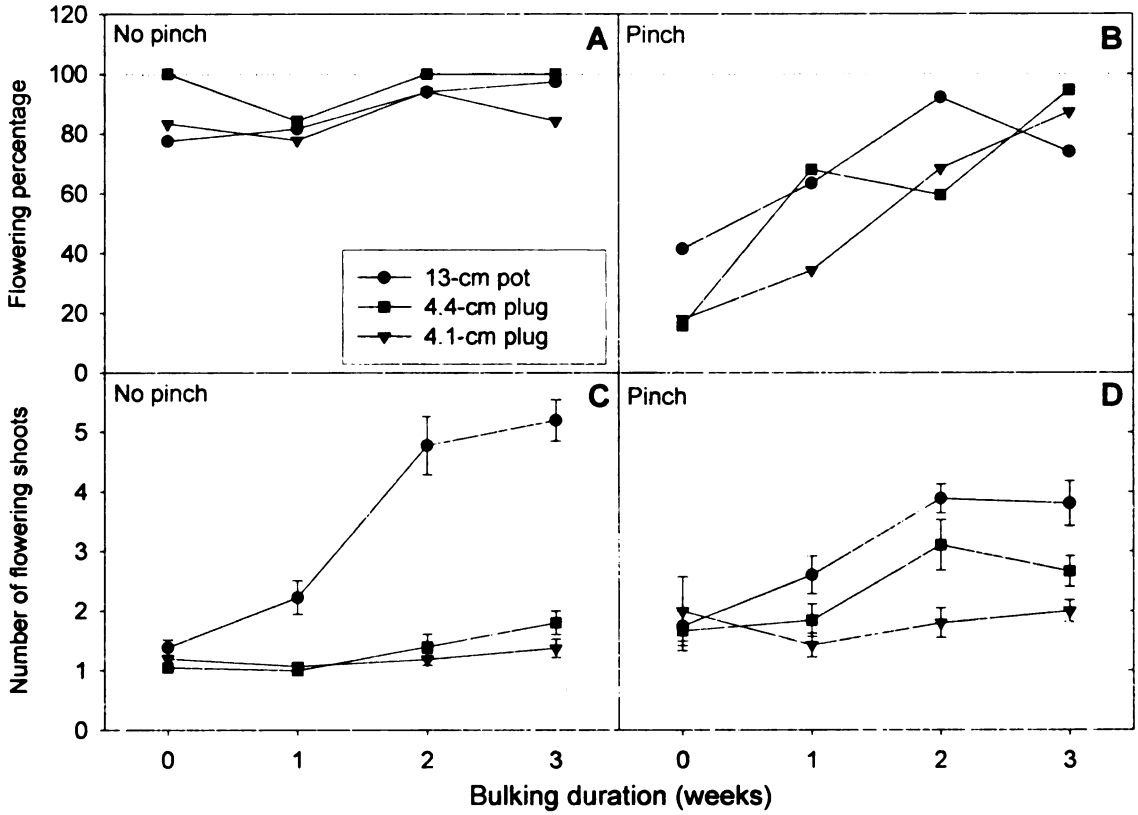

Figure 2. The effects of bulking duration, bulking container size (●, ■, ▼ = 13-cm pot, 4.4-cm plug, and 4.1-cm plug, respectively), and pinching (A and C = no pinch, B and D = pinch) on flowering percentage (A and B) and the number of flowering shoots per plant (C and D). Error bars on graphs C and D represent the standard error of the mean.

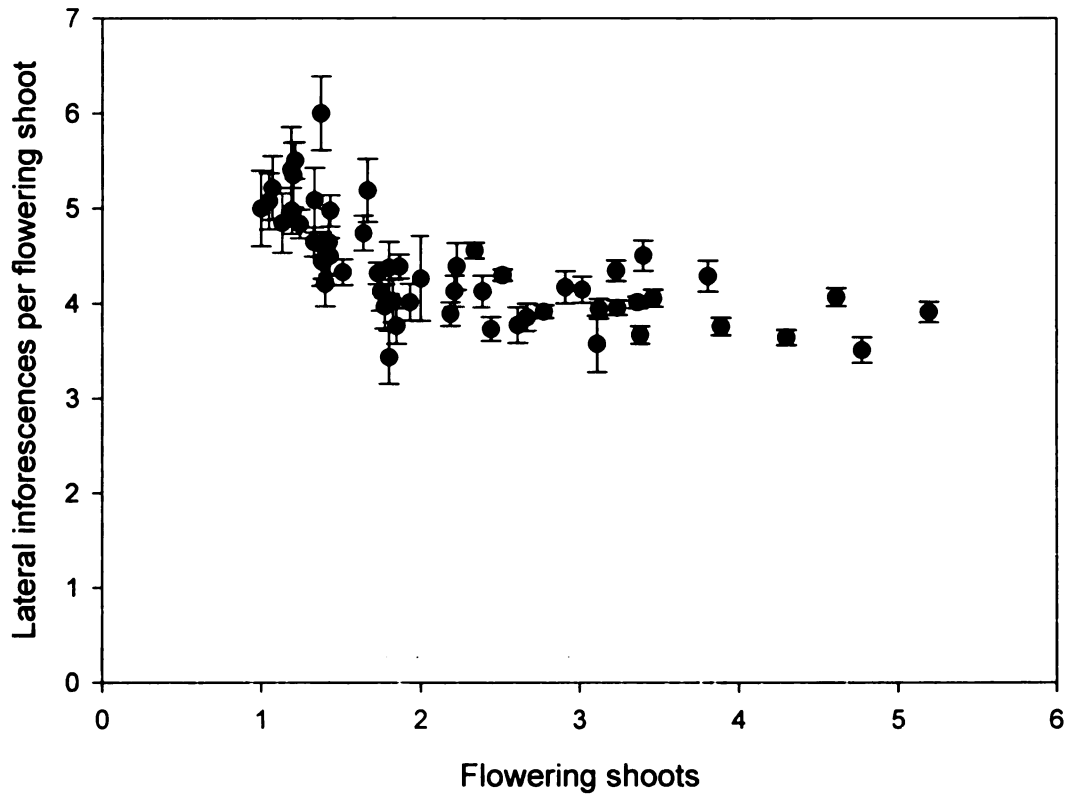

Figure 3. The relationship between the number of flowering shoots per plant and the number of lateral inflorescences produced per flowering shoot for *Veronica spicata* 'Red Fox'. Data points represent the means of all possible treatment effects. Error bars represent the standard error of the mean.

SECTION III

THE EFFECT OF DAILY LIGHT INTEGRAL, AUXIN CONCENTRATION, AND PROPAGATION PHOTOPERIOD ON THE ROOTING AND FLOWERING OF *PHLOX PANICULATA* 'DAVID'

The Effect of Daily Light Integral, Auxin Concentration, and Propagation Photoperiod on the Rooting and Flowering of *Phlox paniculata* 'David'

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Additional index words: vernalization

Received for publication _____. We appreciate the greenhouse growers providing support for Michigan State University floriculture research.
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Abstract

Experiments were performed on *Phlox paniculata* Lyon ex Pursh 'David' to determine the effects of daily light integral (DLI) of 0.8 through 11.3 $mol \cdot m^{-2} \cdot d^{-1}$: auxin concentrations of 0, 1500, 3000, and 6000 ppm; and photoperiods of 11 through 15 h and 9 h with a 4-h night interruption (NI) on root formation during propagation. Cuttings were evaluated at 1-week intervals for 4 weeks. Root number, root mass, and rooting percentage increased as DLI increased from 0.8 to 8.6 $mol \cdot m^{-2} \cdot d^{-1}$. A DLI of 5.1 $mol \cdot m^{-2} \cdot d^{-1}$ produced cuttings with the most uniform root development 21 days after propagation. An auxin concentration of 1500 ppm produced cuttings with the most uniform root development 21 days after propagation. Propagation photoperiod did not significantly affect rooting. A separate experiment was performed to determine the effects of the same propagation photoperiods and subsequent cold treatments of 0, 2, and 5 weeks on time to flower. Although plants flowered regardless of propagation photoperiod and cold treatment, days to flower decreased by approximately 15 to 25 days as propagation photoperiod decreased. Time to flower decreased by as many 15 for propagation photoperiods of 13 h or less with increased cold duration. Plant height increased with increasing cold duration, but the number of nodes at flower was not significantly affected.

Introduction

Phlox paniculata Lyon ex Pursh is a member of Polemoniaceae and is native from New York to Georgia and west to Arkansas and Illinois. *Phlox paniculata* 'David' (a perennial garden phlox) was awarded the title Perennial Plant of the Year 2002 by the Perennial Plant Association (Perennial Plant Association, 2002). 'David' has large, fragrant, white flower panicles with 2.5-cm-diameter florets, making it an attractive candidate for flowering herbaceous perennial potted plant production. Understanding the propagation requirements for successful rooting and the physiological requirements for uniform flowering are two important production issues.

Adventitious rooting is an essential step in the propagation of vegetative cuttings. Daily light integral (DLI) or light quantity during propagation can significantly affect rooting. In most cases there is an optimum irradiance for maximum rooting, and light intensities in excess of this value can sometimes reduce root formation (Lovell et al., 1972). The effects of light intensity and DLI during propagation on rooting percentage, root number, and root mass have been examined in several species, including *Acer palmatum* Thunb. (Behrens, 1988), *Quercus* sp. (Zaczek et al., 1999), *Rhododendron* (Davis and Potter, 1987), and petunia microshoots (Cabaleiro and Economou, 1992). In most cases, there was an optimal light intensity or DLI for each species, while levels above or below the optimum range reduced or inhibited rooting.

Adventitious rooting of vegetative cuttings can also be influenced by the presence or absence of rooting hormones. Rooting hormones, or synthetic

auxins, are not essential for adventitious rooting of all vegetatively propagated plants but for crops such as poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) can speed up the rate of rooting and improve crop uniformity (Ecke et al., 1990). Indole-3-butyric acid (IBA) and 1-napthaleneacetic acid (NAA) have been shown to affect rooting (root number and rooting percentage) in many woody perennials including, *Clematis xjackmanii* T. Moore 'Jackmanii' (Erwin et al., 1997), neem (Kamaluddin and Ali, 1996), Douglas fir (Copes and Mandel, 2000), and *Callinandra calothyrsus* Meissn. (Tchigio and Duguma, 1998).

Phlox paniculata is a long-day plant that flowers when photoperiods are greater than or equal to 13 h (Runkle, 1996). Photoperiod during propagation should theoretically influence time to flower. One would predict earlier flowering for cuttings rooted under long days compared with those rooted under short days. Smith and Wareing (1972) addressed the effects of storage photoperiod on the subsequent rooting of *Populus xrobusta* C. K. Schneid. The researchers showed that cuttings have a higher rooting percentage and root number when rooted under long days (18-h photoperiod) for 28 days than short days (9-h photoperiod). Economou and Read (1986) found that longer and higher-quality azalea shoot cuttings were harvested from tissue cultures under 16-h light durations than from 24-h light durations.

Thomas and Vince-Prue (1997) define vernalization as a cold-temperature treatment that is given to an imbibed seed or young plant and promotes flowering at subsequent higher temperatures. The botanical definition of vernalization ignores other flowering characteristics of horticultural importance. A cold-

temperature treatment may not be required for flowering but could enhance flowering by increasing flowering percentage, hastening flowering, and improving flowering uniformity. There is contradicting research about cold requirements for flowering of *P. paniculata*. Runkle et al. (1998) showed that without cold treatment, a *P. paniculata* population never reached 100% flowering. On the other hand, Garner and Armitage (2000) found that plants flowered under photoperiods longer than 10 h, and cooling of plants was not required for flowering, although it accelerated flowering.

The first objective of this research was to identify the environmental conditions (photoperiod, daily light integral, and synthetic auxin concentrations) during propagation that optimized rooting of *P. paniculata* 'David' cuttings. The second objective was to determine the effects of propagation photoperiod on subsequent time to flower. The final objective was to determine the minimum cold duration required for rapid and uniform flowering of *P. paniculata* 'David'.

Materials and Methods

Plant culture. Vegetative *Phlox paniculata* 'David' cuttings were received from Guatemala in Dec. 2001 and Feb., Mar., Apr., May, June, and July 2002. Cuttings were propagated in 72-cell (0.04-L for propagation photoperiod and auxin concentration experiments; 0.03-L for DLI experiment) plug trays (Landmark Plastic Corporation, Akron, Ohio) containing a mixture of 50% commercial medium (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.) and 50% screened course perlite (Therm-O-Rock; East, Inc., New Eagle, Pa.).

Propagation environmental control. Plants were propagated in a glass greenhouse. Propagation air temperatures were maintained at 23 °C and bottom heat (soil temperature) was maintained at 25 °C. A vapor pressure deficit of 0.3 kPa was maintained by injecting water vapor as needed. Average daily temperature and DLI were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge (0.013 mm in diameter) type E thermocouples and line quantum sensor (Apogee Instruments, Inc., Logan, Utah), respectively. The datalogger collected data every 10 seconds and recorded the hourly average.

Daily light integral experiment. Beginning on June 7 and July 4, 2002, basal portions of each cutting were dipped in a 1500-ppm solution of liquid auxin (DIP 'N GROW; Astoria-Pacific, Clackamas, Ore.) before stick. Plug trays were propagated under one of four levels of shade: zero, one, two, or three layers of a 50% shade curtain (Ludvig Svensson, Kinna, Sweden). Light levels under each shade treatment were monitored with a line quantum sensor (model LQSV-SUN; Apogee Instruments, Inc., Logan, Utah). Average DLIs for both replications are presented in Figure 1.

Auxin experiment. The experiment was replicated in time beginning on Mar. 20, Apr. 25, and May 30, 2002. Basal portions of each cutting were dipped in one of four solutions of liquid auxin (DIP 'N GROW; Astoria-Pacific, Clackamas, Ore.) before stick: 0, 1500, 3000, or 6000 ppm. Cuttings were propagated under natural daylengths.

Propagation photoperiod experiment (rooting). The experiment was replicated in time beginning on Mar. 20, Apr. 25, and May 30, 2002. Basal portions of each cutting were dipped in a 1500-ppm solution of liquid auxin (DIP 'N GROW; Astoria-Pacific, Clackamas, Ore.) before stick. Cuttings were propagated under one of six photoperiods: 11, 12, 13, 14, or 15 h of continuous light or 9 h with a 4-h night interruption (NI). Natural photoperiods were extended with incandescent lamps. For continuous photoperiodic treatments, lamps were turned on at 1700 HR and turned off after each photoperiod was completed. The NI was delivered from 2200 to 0200 HR.

Propagation photoperiod experiment (flowering). Cuttings were propagated on Dec. 21, 2001, and again on Feb. 8, 2002. Propagation setup was identical to that of the rooting experiment. Cuttings were rooted and weaned from propagation 3 weeks after sticking.

Immediately following propagation, plugs were cold treated for 0, 2, or 5 (first replicate only) weeks at 5 °C. The chamber was lit from 0800 to 1700 HR by cool-white fluorescent lamps (F96T12/CW/VHO; Philips, Somerset, N.J.) at a photosynthetic photon flux (*PPF*) of approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. While in the cooler, plants were watered as needed with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter.

Following cold treatment, plugs were potted in 13-cm square plastic containers (1.1 L) containing a soilless commercial medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened

course perlite, along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.). All plants were forced to flower under a 16-h photoperiod, natural daylengths supplemented with lighting from high-pressure sodium lamps from 0530 to 2130 HR. Plants were top-watered as necessary with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and containing water-soluble fertilizer providing 125 N, 12 P, 125 K, 13 Ca ($mg \cdot L^{-1}$; 30% ammoniacal N) plus ($mg \cdot L^{-1}$) 1.0 Fe, 0.5 Mn, 0.5 Zn, 1.0 Cu, 0.2 B, 0.1 Mo (MSU Special; Greencare Fertilizers, Chicago, Ill.). Half of the plants in each treatment of the first replicate were soft pinched (two apical nodes removed) 3 weeks after potting. Half of the plants in each treatment of the second replicate were hard pinched (six apical nodes removed) 50 days (0 weeks at 5 °C) and 35 days (2 weeks after 5 °C) after potting.

Plants were grown in glass greenhouses set at 20 °C. Greenhouse temperatures were controlled by a greenhouse climate-control computer (Model CD750; Priva, De Lier, The Netherlands). Average daily temperature and DLI were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge (0.013 mm in diameter) type-E thermocouples and quantum sensors (Model LI-189, LI-COR, Inc., Lincoln, Neb.), respectively. The datalogger collected data every 10 seconds and recorded the hourly average. Actual average daily temperatures and DLIs for the beginning of forcing to the average date of flowering were calculated and are presented in Table 1.

Data Collection and Analysis

Rooting experiments. For the first replication of the propagation photoperiod and auxin concentration experiments, 10 cuttings were randomly selected 10, 15, 20, 25, and 30 days after propagation. For all other experiments and replications, 15 cuttings were randomly selected 1, 2, 3, and 4 weeks after propagation. Data were collected after 1, 2, and 3 weeks in the second replications of the photoperiod and auxin concentration experiments. Root number and fresh root mass were recorded for each cutting. Data were analyzed using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA) and general linear model (GLM) procedures.

Flowering experiment. Dates of visible bud and first flower as well as final node number, height at flowering, and the number of reproductive branches (pinched plants only) were recorded. A completely randomized design was used Data were analyzed using SAS's (SAS Institute, Cary, N.C.) ANOVA and GLM procedures.

Results

Daily light integral experiment. Light levels in replicate two were lower than in replicate one (Figure 1) because whitewash was applied to the propagation greenhouse roof between experimental replicates. Cuttings had a minimum DLI requirement of 1.6 $mol \cdot m^{-2} \cdot d^{-1}$ for rooting to occur. Rooting quality and percentage increased as propagation DLI increased up to 8.6 $mol \cdot m^{-2} \cdot d^{-1}$ and then decreased (Figure 2).

Daily light integral had a significant effect on root number, root mass, and rooting percentage after 14 days in propagation (Table 2). Plants rooted under a DLI of 5.1 mol•m^{-2}•d^{-1} had the highest average root mass and average number of roots, while plants propagated under 0.8 or 1.2 mol•m^{-2}•d^{-1} of light showed no evidence of rooting. After 21 days in propagation, cuttings rooted under 8.6 mol•m^{-2}•d^{-1} had the highest average number of roots per cutting, but the number was not significantly different from that of cuttings propagated under 5.1 mol•m^{-2}•d^{-1} (Figure 2A). Cuttings rooted under 8.6 mol•m^{-2}•d^{-1} had a significantly higher average root mass per cutting than all other treatments did (Figure 2B). Rooting percentage after 21 days in propagation was not significantly different for light quantities between 2.2 and 11.3 mol•m^{-2}•d^{-1} (Table 2, Figure 2C). After 28 days in propagation, there were no significant differences in the average number of roots per cutting, average root mass, and rooting percentage for cuttings rooted at a DLI of 5.1 mol•m^{-2}•d^{-1} or greater.

Auxin concentration experiment. Regardless of treatment, the *P. paniculata* 'David' cuttings rooted, although rooting trends varied with replicate. In the first replicate, there was no significant difference in the average number of roots per cutting for any evaluation time (Table 3, Figure 3A). Average root mass, however, 25 days after propagation, was significantly different for cuttings dipped in a 1500-ppm auxin solution before stick versus 0 ppm and 6000 ppm (Figure 3D), but by day 30, there were no significant differences in root mass, and plants from all auxin concentrations had 100% rooting (Figure 3G). In the second replicate, there was a significant effect of auxin concentration on root

number beginning 12 days after propagation (Figure 3B) and on root mass 22 days after propagation (Figure 3E). Twelve days after propagation, plants in the 1500-ppm treatment had significantly more roots per cutting than those in all other auxin concentrations and a significantly higher rooting percentage than 0 ppm (Figure 3H). Twenty-two days after propagation, plants in the 1500- and 3000-ppm treatments were significantly different in terms of average root mass from 0 ppm. In the third replicate, cuttings treated with 6000 ppm had significantly more roots per cutting after 22 days in propagation than those treated with 0 ppm, which also had significantly fewer roots than plants in all other auxin concentrations (Figure 3C) after 29 days. Cuttings treated with 6000 ppm had significantly more roots per cutting than those treated with 1500 ppm. In terms of average root mass per cutting, 29 days after propagation, 0 ppm was significantly different from 6000 ppm (Figure 3F). Cuttings from all auxin concentrations had 100% rooting after 29 days in propagation (Figure 3I).

Propagation photoperiod experiment (rooting). Regardless of propagation photoperiod, all cuttings rooted. Rooting trends varied with replicate (Table 4). In the first replicate, significant differences in average root number and average root mass per cutting were evident beginning 20 days after propagation. Cuttings rooted under a 14-h photoperiod had the most roots (Figure 4A) and the largest average root mass per cutting (Figure 4D) after 30 days in propagation. There was no significant difference in rooting percentage. After 30 days, all cuttings had rooted completely except for cuttings under the 15-h photoperiod (Table 4, Figure 4G). The second replicate showed a significant difference in

root number 21 days after propagation. Cuttings propagated under a 12-, 13-, or 14-h photoperiod had significantly more roots per cutting than cuttings propagated under a 15-h photoperiod (Figure 4B). There was no significant difference in average root mass per cutting (Figure 4E) and rooting percentage (Figure 4H). The third replicate showed a significant difference in root number 15 days after propagation. Cuttings propagated under a 12-h photoperiod were significantly different from cuttings propagated under an 11-h photoperiod (Figure 4C). Twenty-two days after propagation, there were no significant differences in root number, but cuttings propagated under a 12-h, 14-h, or NI photoperiod had a larger average root mass per cutting than cuttings propagated under a 13-h photoperiod (Figure 4F). Fifteen days after propagation, cuttings under an 11-h photoperiod had a significantly lower rooting percentage than those under all other photoperiods (Figure 4I). After 28 days, all cuttings were rooted completely, regardless of propagation photoperiod.

Propagation photoperiod experiment (flowering). Regardless of propagation photoperiod and cold duration combination, all plants flowered. Flowering trends varied with replicate (Table 5). For replicate one, days to visible bud and days to flower were significantly affected by propagation photoperiod, cold duration, pinching, and the interaction between photoperiod and cold. For replicate two, days to visible bud and days to flower were significantly affected by propagation photoperiod, cold duration, and pinching but not the interaction between propagation photoperiod and cold duration. As propagation photoperiod increased, days to flower deceased by up to 28 d (replicate one) or 15 d

(replicate two) for unpinched plants (Figure 5A and B). For pinched plants in replicate one, there was no obvious trend in time to flower as propagation photoperiod increased (Figure 5C). For cold-treated pinched plants in replicate two, time to flower decreased as propagation photoperiod increased (Figure 5D). Regardless of pinching treatment, days to flower decreased within a propagation photoperiod as cold duration increased.

Final plant height was significantly affected by propagation photoperiod, cold duration, and pinching in the first replicate and by propagation photoperiod and pinching in the second replicate (Figures 6 and 7). Pinched plants were 10 to 20 cm shorter than unpinched plants. Increasing cold duration increased final plant height for unpinched plants in replicate one (Figure 6A), but this trend did not occur in replicate two (Figure 6B). Cold-treated plants were approximately 5 to 7 cm taller than plants without a cold treatment for pinched plants in both replicates. However, as propagation photoperiod increased, differences in height between cold treatments became smaller (Figure 7A and B). Plants in replicate two were about 10 cm shorter than replicate one plants.

The number of nodes present at flowering was significantly affected by cold duration and pinching in replicate one and by propagation photoperiod, cold duration, and pinching in replicate two (Figure 6C and D, Figure 7C and D). Pinched plants had approximately two to four fewer nodes than unpinched plants. There was no obvious trend as propagation photoperiod increased for both final plant height and node number.

The number of flowering stems per plant was significantly affected by propagation photoperiod and cold duration in replicate one and by cold duration in replicate two. As cold duration increased in the first replicate, the number of flowering stems increased (Figure 7E). Cold-treated plants had fewer flowering stems per plant than plants that did not receive a cold treatment in the second replicate (Figure 7F). Plants in replicate two had more flowering stems per plant than plants in replicate one.

Discussion

As propagation DLI increased through 8.6 $mol \cdot m^{-2} \cdot d^{-1}$, rooting percentage, root number, and root mass of *P. paniculata* cuttings also increased. Petunia microshoots also showed an increased rooting percentage as DLI increased from 1 to 3 $mol \cdot m^{-2} \cdot s^{-1}$ (Cabaliero and Economou, 1992). *Acer* sp., *Quercus* sp., and *Cornus kousa* Hance had the highest rooting percentage when *PPF* was approximately 150 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Behrens, 1988; Zaczek et al., 1997).

For *P. paniculata* 'David', when light levels during propagation were decreased (<2 $mol \cdot m^{-2} \cdot d^{-1}$), it is possible that the leaves could not harvest enough light energy for adequate photosynthesis; thus, rooting was not promoted. Leaves of cuttings have several functions: source of assimilates (energy), source of food, source of minerals, source of hormones and rooting co-factors, and transpiration during propagation (Costa, 2002).

Jørgensen (1992) found that light intensities of 134 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during a 24-h period (11.6 $mol \cdot m^{-2} \cdot d^{-1}$) in the winter were needed for rapid root initiation of

Rosa hybrids. Davis and Potter (1987) found that a 20-fold light level difference did not affect rhododendron rooting percentage despite significant differences in leaf water potential, net photosynthesis, and sucrose and starch concentrations in the cutting bases. Under low light levels (<2 $mol \cdot m^{-2} \cdot d^{-1}$), most *P. paniculata* 'David' cuttings did not even form callus tissue. *Botrytis* rot was also a problem during propagation under the low light intensities.

DLI affects not only rooting of cuttings but also other flowering characteristics, including flower number, flower size, intensity of flower color, and branching. Niu et al. (2001) showed that increasing DLI from 5.7 to 17 $mol \cdot m^{-2} \cdot d^{-1}$ after visible bud increased flower size and number for *Campanula carpatica* Jacq. 'Deep Blue Clips'. The researchers also showed that plants were more compact under high than under low DLI. The *P. paniculata* 'David' plants forced to flower were shorter in the second replicate than in the first. Average DLI in the second replicate was higher than in the first replicate (11 versus 17 $mol \cdot m^{-2} \cdot d^{-1}$) and may account for differences in plant height.

Rates of synthetic auxins effective for rooting vary with the type of cutting propagated: microshoots for tissue culture, herbaceous, softwood, semihardwood, or hardwood. Auxins were not required for rooting of *P. paniculata* 'David'. However, IBA/NAA applications did increase the average root mass per cutting. Similar results have been recorded for *Eucalyptus* (Fett-Neto et al., 2001) and neem (Kamaluddin and Ali, 1996). For easily rooted cuttings, including *P. paniculata* 'David', auxin concentration (0 to 100 ppm for tissue culture, 0 to ~6000 ppm for others) does not affect rooting percentage (Fett-Neto

et al., 2001; Griffin et al., 1998; Kamaluddin and Ali, 1996). For hard-to-root cuttings, including many woody species, auxin concentration does affect rooting percentage. Difficult-to-root *Eucalyptus globulus* Labill microcuttings had increased rooting percentages when IBA concentrations were increased to 10 and 100 ppm (Fett-Neto et al., 2001). Semihardwood cuttings of *Thuja* x 'Green Giant' had the most rooting when treated with IBA at 3000 or 6000 ppm (Griffin et al., 1998). Increasing IBA from 2000 to 4000 ppm favored root formation in unwounded basal cuttings of *Leucandendron discolor* (Rodriguez-Perez et al., 1997).

Auxin concentration also affects the number of roots formed for many species. For *P. paniculata* 'David', the number of roots per cutting increased as auxin concentration increased. Similar results have been seen in neem (Kamaluddin and Ali, 1996), *Mussaenda erythrophylla* (St. Hilaire and Fierro-Berwart, 2000), *Helianthus debilis* 'Flora Sun' (Norcini and Aldrich, 2000), and *Thuja* x 'Green Giant' (Griffin et al., 1998).

For commercial production, these data suggest auxin concentrations should not exceed 3000 ppm for *P. paniculata*. The goal of most growers is to remove plants from propagation 3 weeks after sticking, and at 3 weeks (20 to 22 days), rooting percentage, root number, and root mass were greatest when auxin at 1500 or 3000 ppm was used.

Propagation photoperiod had no effect on root development for *P. paniculata* 'David'. Research on petunia microshoots by Cabaleiro and Economou (1992) showed that after 10 to 15 days in propagation, cuttings under

a 24-h photoperiod did not differ from those under a 14-h photoperiod in root number, length, and weight. The main site for the perception of daylength is the leaf, but the observed responses usually take place elsewhere in the plant (Thomas and Vince-Prue, 1997). A photoperiodic stimulus is then exported from the leaves to the shoot meristem, which leads to the formation of floral primordia. However, Smith and Wareing (1972) showed that the state of the apex in a leafy *Populus xrobusta* cutting had no significant effect on root initiation. So even if apical meristems of the *P. paniculata* 'David' cuttings had been induced to flower, it did not affect rooting.

Subsequent time to flower under a 16-h photoperiod decreased as propagation photoperiod increased. Runkle et al. (1998) and Runkle (1996) showed that as forcing photoperiod increased, time to flower decreased. Thus, plants had been induced to flower by photoperiods during propagation, and longer photoperiods reduced time to flower. Wurr et al. (2000) showed a similar phenomenon with *Dianthus allwoodii*, which has an obligate vernalization requirement. The vernalization requirements for the cuttings were satisfied before cuttings were removed from the stock plants. Visible flower buds were produced during the rooting phase of propagation, resulting in earlier flowering.

Pinched plants flowered later than unpinched plants. Garner et al. (1997) found that pinched plants of *Delphinium* sp. took longer to flower than unpinched plants.

Vernalization and pinching appeared to independently negate the effect of propagation photoperiod on time to flower in replicate one, and time to flower

was synchronized by cold treatment. Runkle et al. (1998) found that flowering was more synchronized in cold-treated *P. paniculata* plants, and Suzuki and Metzger (2001) had similar results for *Osteospermum ecklonis*.

Phlox paniculata 'David' plants that received a cold treatment flowered sooner than untreated plants, regardless of propagation photoperiod. However, cold treatment was not required for flowering. Time to flower was reduced further as cold duration increased through 5 weeks. Garner and Armitage (2000) and Runkle et al. (1998) showed that a 15- to 16-week cold treatment accelerated flowering of *P. paniculata* by 50 to 65 d. Cold treatment accelerates flowering in many plants, including wheat (Gonzalez et al., 2002), *Thalictrum delavayi* (Huang et al., 1999), *Aquilegia* L. (Garner and Armitage, 1998), and Cicer milkvetch (Townsend and McGinnies, 1973), among others.

Plant height increased with increasing cold duration but not propagation photoperiod. Garner and Armitage (1998) had similar results with *Aquilegia*: cooling plants resulted in longer flowering stems. Conversely, Suzuki and Metzger (2001) showed that stems of *Osteospermum* were shorter following vernalization. In *P. paniculata* 'David', cold treatment did not affect node number. Therefore, increased plant height was due to longer internodes, which is in contrast to findings by Suzuki and Metzger (2001) in *Osteospermum* and Gonzalez et al. (2002) in wheat; they found that vernalized plants had fewer nodes and leaves at flower.

Plants in the second replicate developed more branches following pinch than plants in the first replicate. Berghage et al. (1989) showed that growth of

lateral shoots of poinsettia was influenced by the pinching technique. Lateral shoots of soft- and medium-pinched plants were shorter than those of hard-pinched plants because of residual apical dominance. In chrysanthemum production, pinching into old tissue severely limits the number of breaks (Crater, 1992). In a separate *P. paniculata* 'David' experiment (data not presented), plants were pinched 44 days after the start of long days to different depths (one, two, three, four, or five nodes removed) to examine the effects of pinching depth on lateral branching. The hardness of pinch did not significantly affect the number of branches produced. All plants developed four lateral breaks of similar size. Plants in the second replicate of the propagation photoperiod flowering experiment produced more lateral breaks than plants in the first replicate because they were actively growing at pinch. Plants had been growing 35 to 50 days before pinch. In the first replicate, plants were pinched 21 days after planting, and many plants had not initiated active shoot growth. Thus, many plants produced only one or two lateral branches following pinch.

Conclusions

DLI during propagation is critical for rooting success of *P. paniculata* 'David'. Cuttings should not be rooted below 2 $mol \cdot m^{-2} \cdot d^{-1}$, with an optimal DLI range of about 5 to 10 $mol \cdot m^{-2} \cdot d^{-1}$. Light levels above 11 $mol \cdot m^{-2} \cdot d^{-1}$ did not affect rooting percentage, but root number and mass were decreased. Rooting hormone was not required for successful rooting, but treatments of 1500 to 3000 ppm improved root number and mass. Propagation photoperiod did not affect

rooting of *P. paniculata* 'David' but did affect subsequent time to flower. Longer propagation photoperiods reduced subsequent time to flower, and for the most rapid flowering, a 16-h or NI photoperiod should be used. A 5-week cold treatment reduced time to flower but increased final plant height.

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Table 1. Dates of forcing, average daily temperatures, and average daily light integral from date of forcing to average date of flowering for the *Phlox paniculata* 'David' propagation photoperiod (flowering) experiment.

Date of forcing from end of cold treatment	Weeks at 5 °C	Average daily temperature (°C)	Average daily light integral (mol•m-2•d-1)
Replicate 1			
01/07/02	0	20.1	9.4
01/22/02	2	20.3	10.8
02/13/02	5	20.4	11 9
Replicate 2			
03/14/02	0	22.2	16.7
03/30/02	2	23.0	17.2

Table 2. Effects of daily light integral (DLI) during propagation on rooting of *Phlox paniculata* 'David'.

Evaluation (days)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Average number of roots per cuttings	Average root mass (mg) per cutting	Rooting percentage
7	0.8	0a[z]	0a	0a
	1.2	0a	0a	0a
	1.6	0a	0a	0a
	2.2	0a	0a	0a
	3.6	0a	0a	0a
	5.1	0a	0a	0a
	8.6	0a	0a	0a
	11.3	0a	0a	0a
14	0.8	0b	0b	0c
	1.2	0b	0b	0c
	1.6	1b	4b	13abc
	2.2	0b	1b	7bc
	3.6	1b	5b	33abc
	5.1	6a	32a	53a
	8.6	2ab	8b	47ab
	11.3	2ab	5b	53a
21	0.8	0d	0d	0d
	1.2	1d	1d	7cd
	1.6	3cd	29cd	40bc
	2.2	8bcd	42bcd	73ab
	3.6	14bc	134bc	100a
	5.1	18ab	159b	100a
	8.6	26a	320a	87a
	11.3	13bc	118bcd	93a
28	0.8	0d	0c	7c
	1.2	2cd	9c	40bc
	1.6	2cd	15c	53b
	2.2	9c	102c	93a
	3.6	18b	402b	93a
	5.1	26a	438ab	100a
	8.6	29a	639a	93a
	11.3	22ab	455ab	100a

[z]Mean separation within each evaluation period by Tukey's studentized range test ($P = 0.05$). Numbers within a column and evaluation period with the same letter are not statistically different.

Table 4. Significance of propagation photoperiod on average number of roots per cutting, average root mass per cutting, and rooting percentage of *Phlox paniculata* 'David' cuttings.

Evaluation (days)	Average number of roots per cuttings	Average root mass (mg) per cutting	Rooting percentage
Replicate 1			
10	NS	NS	NS
15	NS	NS	NS
20	*	*	NS
25	*	*	NS
30	*	**	NS
Replicate 2			
7	NS	NS	NS
12	NS	NS	NS
21	**	NS	NS
Replicate 3			
7	NS	NS	NS
15	*	NS	**
22	NS	***	NS
28	NS	NS	–[z]

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

[z]Dash indicates no variance in data.

Table 5. Propagation photoperiod treatment effects on flowering characteristics of *Phlox paniculata* 'David'.

Treatment	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Number of nodes at flower	Number of flowering branches
Replicate 1						
Photoperiod (Ph)	***	NS	***	*	NS	**
Cold duration (C)	***	***	***	***	***	**
Pinch (Pi)	***	NS	***	***	***	***
Ph X C	**	NS	**	NS	NS	NS
Ph X Pi	NS	NS	NS	NS	*	**
C X Pi	NS	**	NS	***	**	**
Ph X C X Pi	NS	NS	NS	NS	NS	NS
Phlox paniculata 'David' plants not pinched						
Photoperiod (Ph)	***	NS	***	NS	NS	–[z]
Cold duration (C)	NS	***	NS	***	***	–
Ph X C	NS	NS	NS	NS	NS	–
Phlox paniculata 'David' plants pinched						
Photoperiod (Ph)	***	**	**	NS	NS	**
Cold duration (C)	***	***	***	NS	*	**
Ph X C	*	**	NS	NS	NS	NS
Replicate 2						
Photoperiod (Ph)	***	NS	***	*	**	NS
Cold duration (C)	***	NS	***	NS	**	***
Pinch (Pi)	***	NS	***	***	***	***
Ph X C	NS	NS	NS	**	NS	NS
Ph X Pi	NS	NS	NS	NS	NS	NS
C X Pi	**	NS	*	***	**	***
Ph X C X Pi	NS	NS	NS	NS	NS	NS
Phlox paniculata 'David' plants not pinched						
Photoperiod (Ph)	***	NS	***	NS	NS	–
Cold duration (C)	*	*	*	*	NS	–
Ph X C	NS	NS	NS	**	NS	–
Phlox paniculata 'David' plants pinched						
Photoperiod (Ph)	NS	NS	NS	NS	*	NS
Cold duration (C)	***	NS	***	***	**	**
Ph X C	NS	NS	NS	NS	NS	NS

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

[z]Dashes indicate branching data not collected.

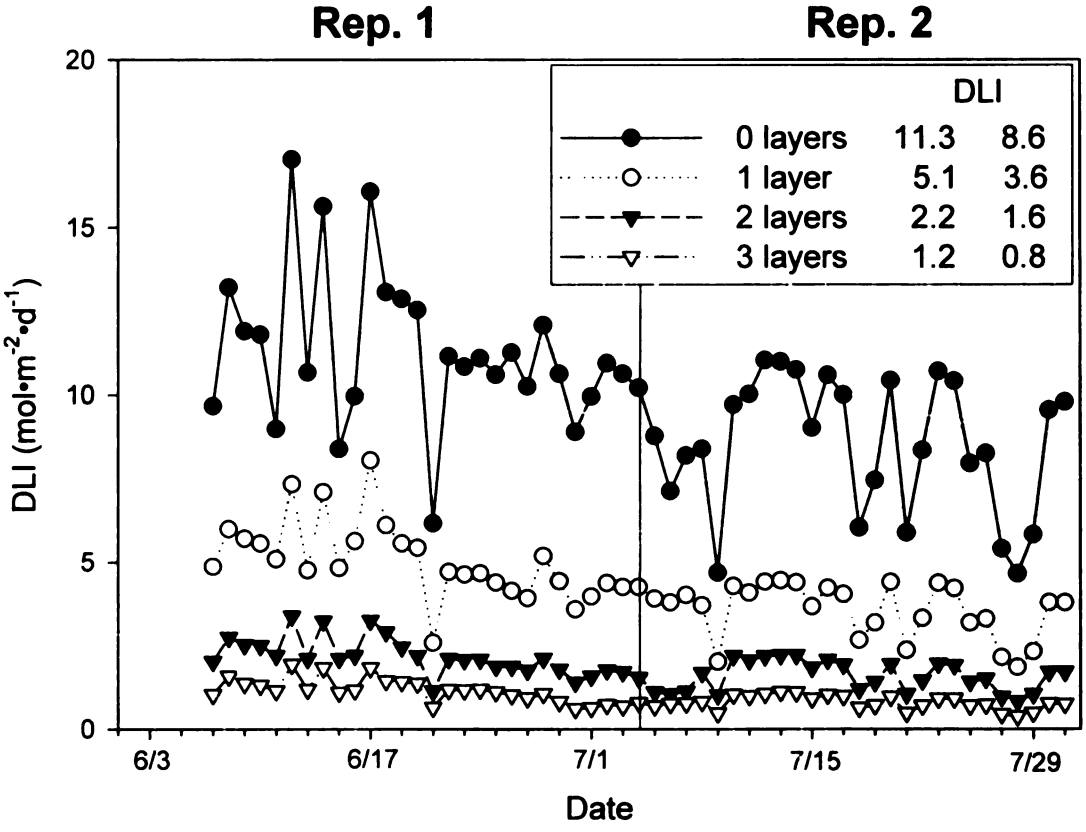

Figure 1. Daily light integrals (DLI, mol•m^{-2}•d^{-1}) during *Phlox paniculata* 'David' propagation for each level of shading.

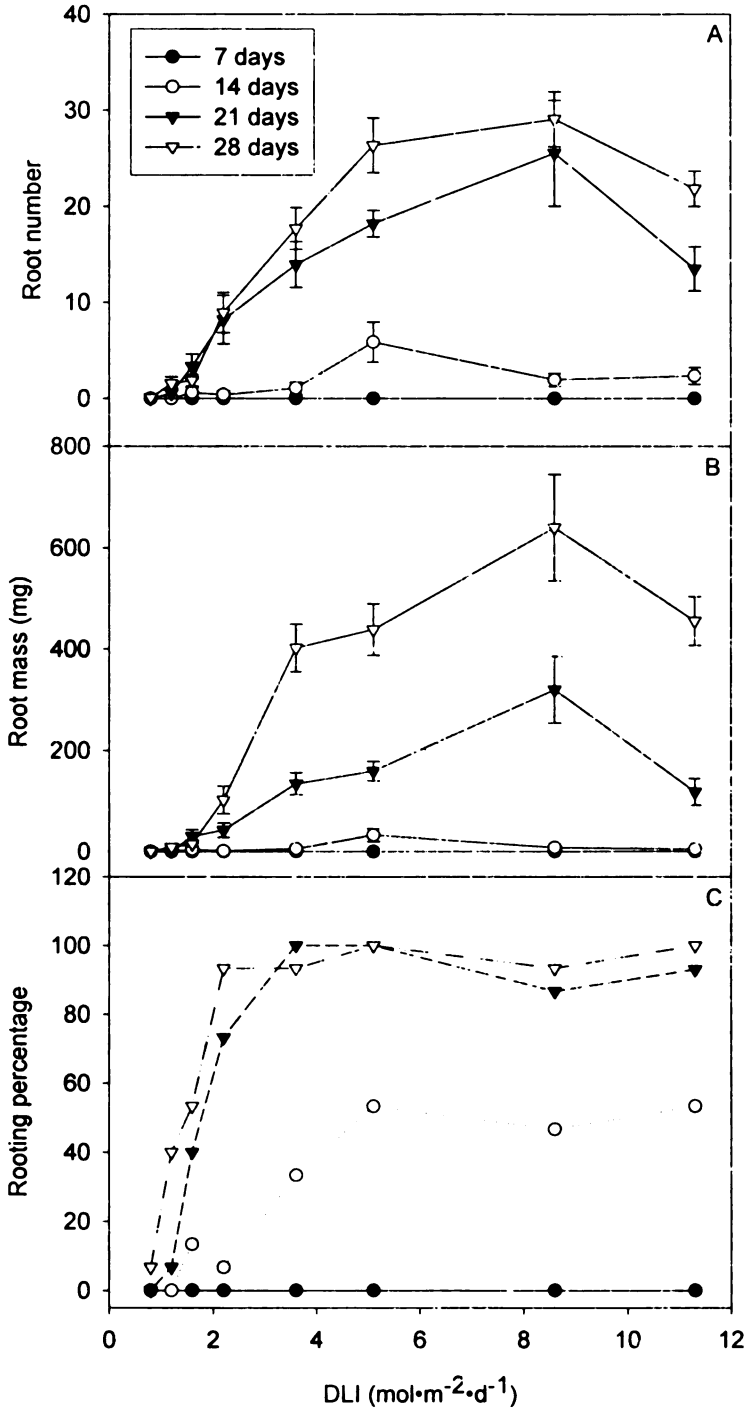

Figure 2. The effects of daily light integral (DLI) during propagation of *Phlox paniculata* 'David' on average root number per cutting (A), average root mass per cutting (B), and rooting percentage (C). Error bars represent standard error of the mean.

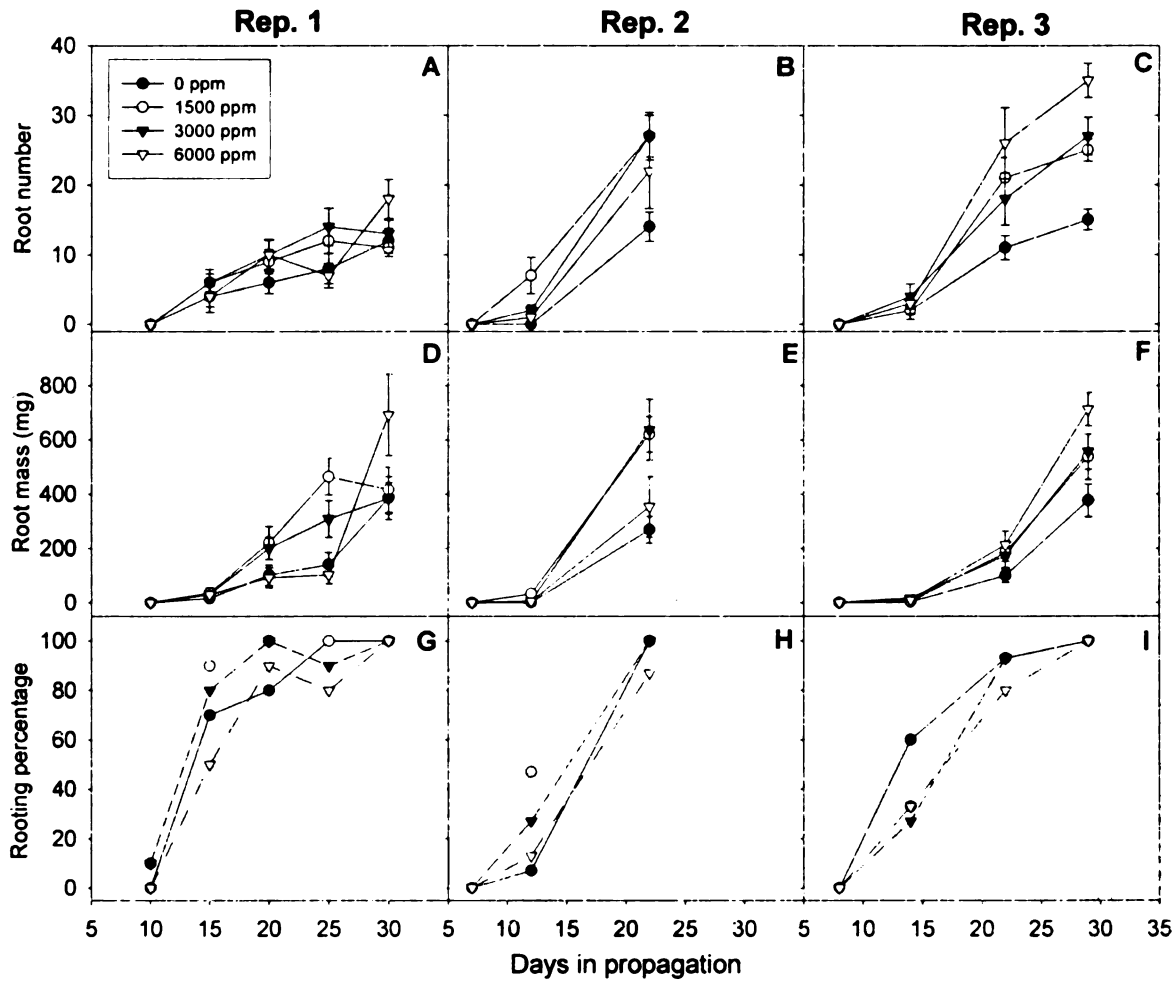

Figure 3. The effects of auxin concentration on root number, root mass, and rooting percentage of *Phlox paniculata* 'David'. Error bars represent standard error of the mean.

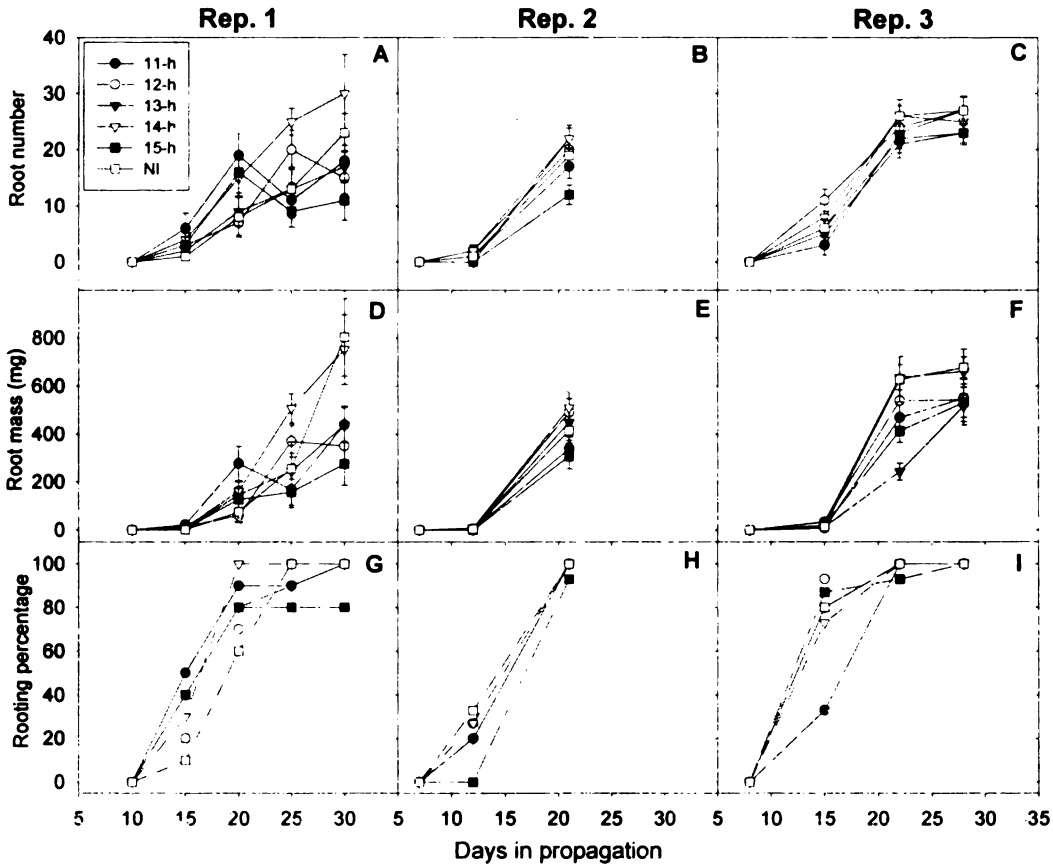

Figure 4. The effects of propagation photoperiod on root number, root mass, and rooting percentage of *Phlox paniculata* 'David'. Error bars represent standard error of the mean.

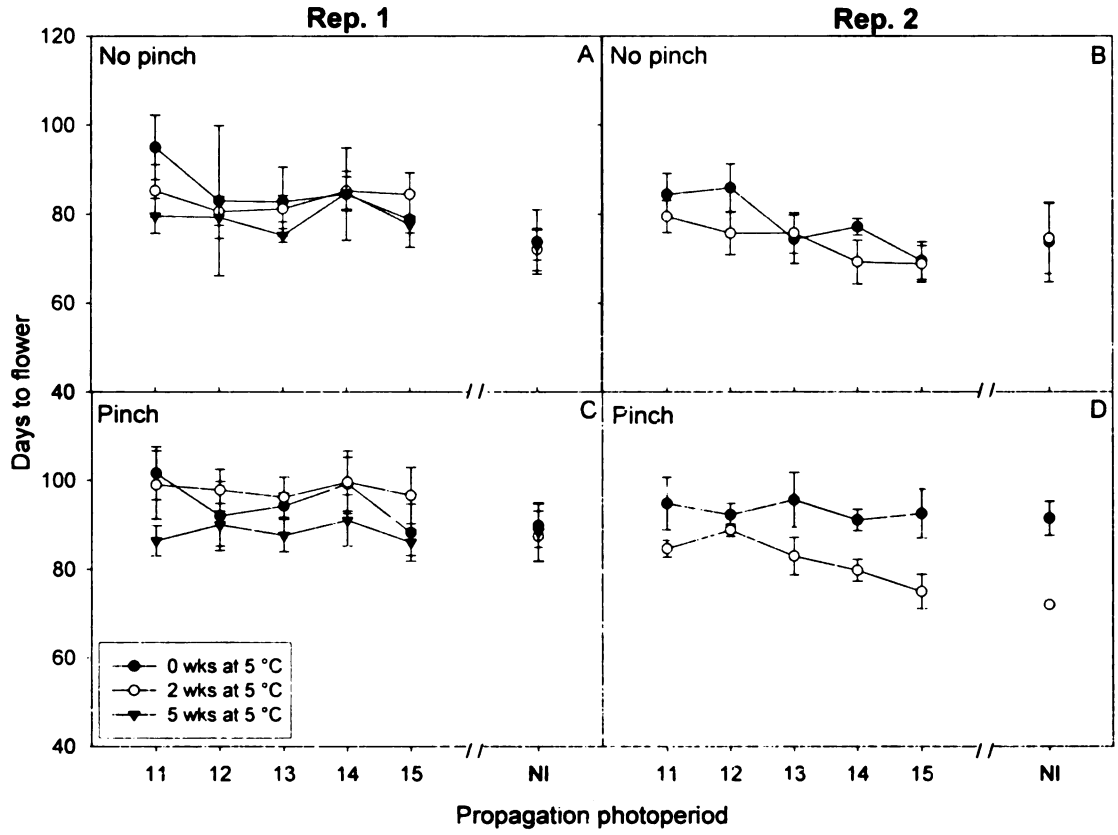

Figure 5. Number of days to flower for *Phlox paniculata* 'David' propagation photoperiod. Error bars represent 95% confidence intervals.

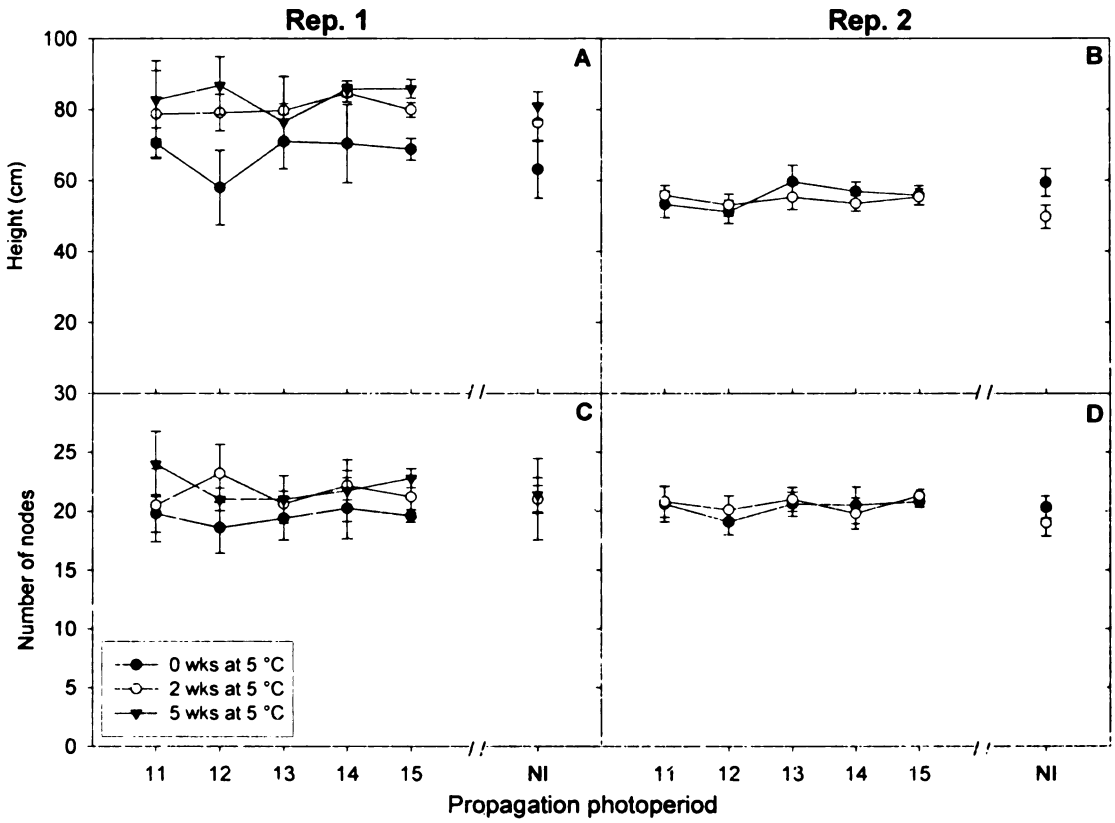

Figure 6. Plant height and number of nodes at flowering for unpinched *Phlox paniculata* 'David' plants. Error bars represent standard error of the mean.

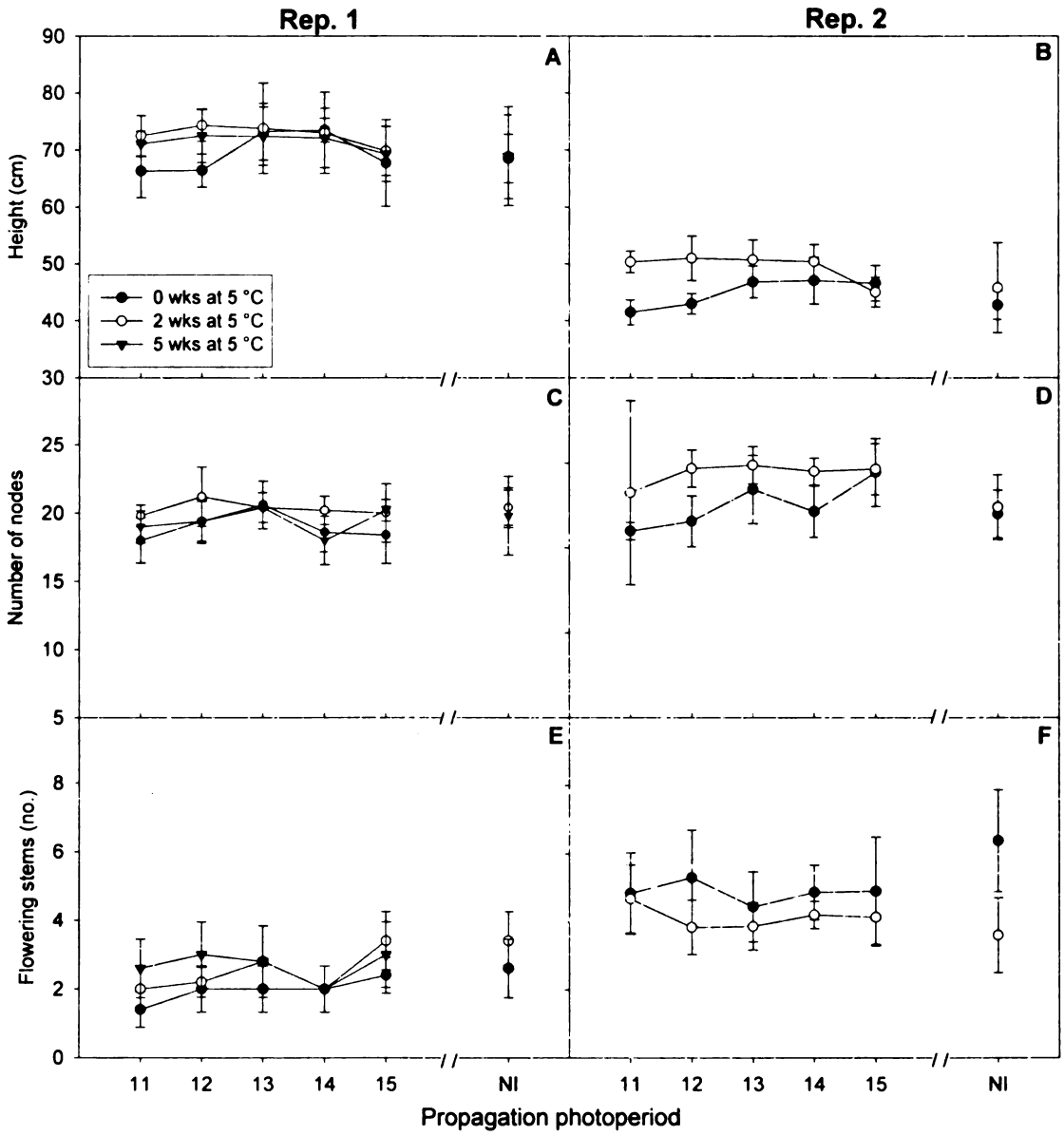

Figure 7. Plant height, node number, and number of flowering stems for pinched *Phlox paniculata* 'David' plants. Error bars represent standard error of the mean.

Thesis Conclusion

Research described in this thesis has successfully quantified the information necessary for the quick-crop production method. This method provides a successful alternative protocol for production of uniform, disease-free, and rapid-flowering herbaceous perennials. Using the quick-crop protocol, growers will be able to produce herbaceous perennials rapidly, uniformly, and efficiently year-round, thus eliminating the problems associated with current herbaceous perennial production. Although more cultural and environmental information is still required for these species, the production of herbaceous perennials for a specific market date is possible with the quick-crop production method.

APPENDIX A

THE FLOWERING RESPONSE OF *LEUCANTHEMUM XSUPERBUM* 'SNOWCAP' TO COLD DURATION

Research Objective

The objective of this research project was to determine the minimum cold duration required for rapid and uniform flowering of *Leucanthemum* x*superbum* 'Snowcap'.

Materials and Methods

Plant material. Leucanthemum x*superbum* 'Snowcap' stock plants were potted (Sept. 1999) in 13-cm square plastic containers (1.1 L) filled with a commercial soilless medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened course perlite, along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.). Plants were grown under a 12-h photoperiod provided by supplementing natural daylength with lighting from high-pressure sodium lamps from 0800 to 2000 HR. Stock plants were pinched at 3- to 4-week intervals to ensure continued branching and cutting production.

Harvested cuttings were propagated in 72-cell (0.03-L) plug trays (Landmark Plastic Corporation, Akron, Ohio) containing a mixture of 50% commercial medium (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.) and 50% screened course perlite (Therm-O-Rock; East, Inc., New Eagle, Pa.). Basal portions of each cutting were dipped in a 1500-ppm solution of liquid auxin (1% indole-3-butryic acid and 0.5% naphthaleneacetic acid; DIP 'N GROW; Astoria-Pacific, Clackamas, Ore.). Propagation air temperatures were maintained at 23 °C and bottom heat (soil temperature) was maintained at 25 °C.

A vapor pressure deficit of 0.3 kPa was maintained by injecting water vapor as needed. Cuttings were rooted and weaned from propagation 3 weeks after sticking.

Plant culture. Plants were grown (bulked) in the plug trays for an additional 3 weeks in the greenhouse at 20 °C in order to establish root systems and increase vegetative growth before cold treatment. Photoperiod was maintained at 12 h as on the stock plants. Following cold treatment, plugs were potted in 13-cm square plastic containers (1.1 L) containing the same commercial medium used for the stock plants. Plants were forced to flower under a 16-h photoperiod (natural days supplemented with high-pressure sodium lamps from 0530 to 2130 HR). Plants were top-watered as necessary with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and containing water-soluble fertilizer providing 125 N, 12 P, 125 K, 13 Ca ($mg \cdot L^{-1}$; 30% ammoniacal N) plus ($mg \cdot L^{-1}$) 1.0 Fe, 0.5 Mn, 0.5 Zn, 0.5 Cu, 0.1 B, 0.1 Mo (MSU Special; Greencare Fertilizers, Chicago, Ill.).

Cold treatments. Plugs received no cold treatment or were placed in a controlled-environment chamber for 1, 2, 3, 4, or 5 weeks at 5 °C. The chamber was lit from 0800 to 1700 HR by cool-white fluorescent lamps (F96T12/CW/VHO; Philips, Somerset, N.J.) at a photosynthetic photon flux (*PPF*) of approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. While in the cooler, plants were watered as needed with well water acidified with sulfuric acid to an approximate pH of 6.0.

Greenhouse temperature control. Plants were grown in glass greenhouses set at 20 °C. Greenhouse temperatures were controlled by a

greenhouse climate-control computer (Model CD750; Priva, De Lier, The Netherlands). Average daily temperature and daily light integral (DLI) were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge (0.013 mm in diameter) type E thermocouples and a quantum sensor (Model LI-189; LI-COR, Inc., Lincoln, Neb.), respectively. The datalogger collected data every 10 seconds and recorded the hourly average. Actual average daily temperatures and DLIs for the beginning of forcing to the average date of flowering were calculated and are presented in Table 1.

Data collection and analysis. Dates of visible bud and first flower were recorded, as well as plant height, node number, and flower bud number at flower. A completely randomized design with 10 observations for each cold treatment was used. Data were analyzed using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA) and general linear models (GLM) procedures. Since the variances for days to visible bud, days from visible bud to flower, and days to flower were not homogeneous, a log transformation was done on the data before ANOVA and GLM were performed.

Results and Discussion

All *L.* x*superbum* 'Snowcap' plants flowered completely under long days with or without a cold treatment (Table 2). However, as cold duration increased from 0 to 5 weeks, time to flower and variability within treatments decreased (Figure 1, Table 2). Plants exposed to a 5-week cold treatment flowered 20 days

sooner and the number of nodes was also reduced by four to five (Figures 1 and 2A).

The number of nodes formed at flowering decreased linearly as cold duration increased (Figure 2A, Table 2). Plant height increased and then decreased quadratically as cold duration increased (Figure 2B). Flower number and days from visible bud to flower were not affected by increasing cold durations (Figure 2C, Table 2).

The *L.* x*superbum* (formerly *Chrysanthemum* x*superbum* or *C. maximum*) cultivars Becky, Snowcap, and Snow Lady flower completely without cold, providing they are forced to flower under long photoperiods (Engle, 1994; Kessler and Keever, 2000; Yuan, 1995). Several experiments have shown that increased cold durations reduce time to flower in *L.* x*superbum* (Kessler and Keever, 2000; Runkle, 1996; Yuan, 1995). Runkle et al. (1998) showed that complete flowering of *L.* x*superbum* 'Snowcap' under a night interruption (NI) photoperiod required no more than 3 weeks of cold (5 °C). In contrast, Shedron and Weiler (1982) showed that 'G. Marconi' (*C.* x*superbum* Bergmans) required between 12 and 16 weeks of cold treatment for 100% flowering. According to Runkle et al. (1998), desirable responses (e.g., increased flowering percentage, improved uniformity, reduced time to flower, and increased flower number) to exposure to 5 °C for *L.* x*superbum* 'Snowcap' were saturated after 6 weeks when plants were subsequently forced under NI.

Conclusions

Although *L.* x*superbum* 'Snowcap' flowers completely without a cold treatment, time to flower is reduced and crop uniformity is increased following a cold treatment. Therefore, at least a 5-week cold treatment followed by long days (≥16 h) is recommended for flowering of *L.* x*superbum* 'Snowcap'.

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Runkle, E.S., R.D. Heins, A.C. Cameron, and W.H. Carlson. 1998. Flowering of *Leucanthemum* x*superbum* 'Snowcap' in response to photoperiod and cold treatment. HortScience 33:1003–1006.

Shedron, K.G. and T.C. Weiler. 1982. Regulation of growth and flowering in *Chrysanthemum* x*superbum* Bergmans. J. Amer. Soc. Hort. Sci. 107:874–877.

Yuan, M. 1995. The effects of juvenility, temperature, and cultural practices on flowering of *Coreopsis*, *Gaillardia*, *Leucanthemum*, *Heuchera*, and Rudbeckia. MS Thesis, Michigan State Univ., East Lansing.

Table 1. Dates of forcing, average daily temperatures, and average daily light integrals (DLI) from date of forcing to average date of flowering for *Leucanthemum* x*superbum* 'Snowcap'.

Date of forcing	Weeks of 5 °C	Average temperature (° C)	Average DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
2/1/00	0	21.3	13.3
2/8/00	1	21.6	14.6
2/15/00	2	21.3	14.3
2/22/00	3	21.4	14.7
2/29/00	4	21.3	15.3
3/10/00	5	21.2	15.7

Table 2. Significance of cold treatment on flowering of *Leucanthemum xsuperbum* 'Snowcap'.

Weeks of 5 °C	Flowering percentage	Days to visible bud	Days from visible bud to flower	Days to flower	Final node number	Final plant height (cm)	Flower number
0	100	44	23	67	16	28.3	11
1	100	44	24	68	17	30.1	12
2	100	35	24	59	15	28.7	11
3	100	30	25	55	14	29.5	12
4	100	26	25	51	13	28.1	13
5	100	22	25	47	12	26.3	12
Significance							
Weeks of cold		***	NS	***	***	***	NS
95% Confidence intervals (weeks at 5 °C)							
0		5.99	3.54	6.73	2.22	0.82	1.73
1		2.99	2.93	4.03	1.19	1.29	1.73
2		4.85	1.17	4.81	1.90	1.19	2.69
3		2.05	1.25	2.51	0.91	0.77	1 59
4		1.69	1.55	2.94	1.15	0.92	1.43
5		1.10	1.25	1.15	1.05	0.70	1.52
Contrasts							
P_{Linear}		***	NS	***	***	***	NS
$P_{Quadratic}$		NS	NS	NS	NS	***	NS

NS,*** Nonsignificant or significant at $P \leq 0.001$.

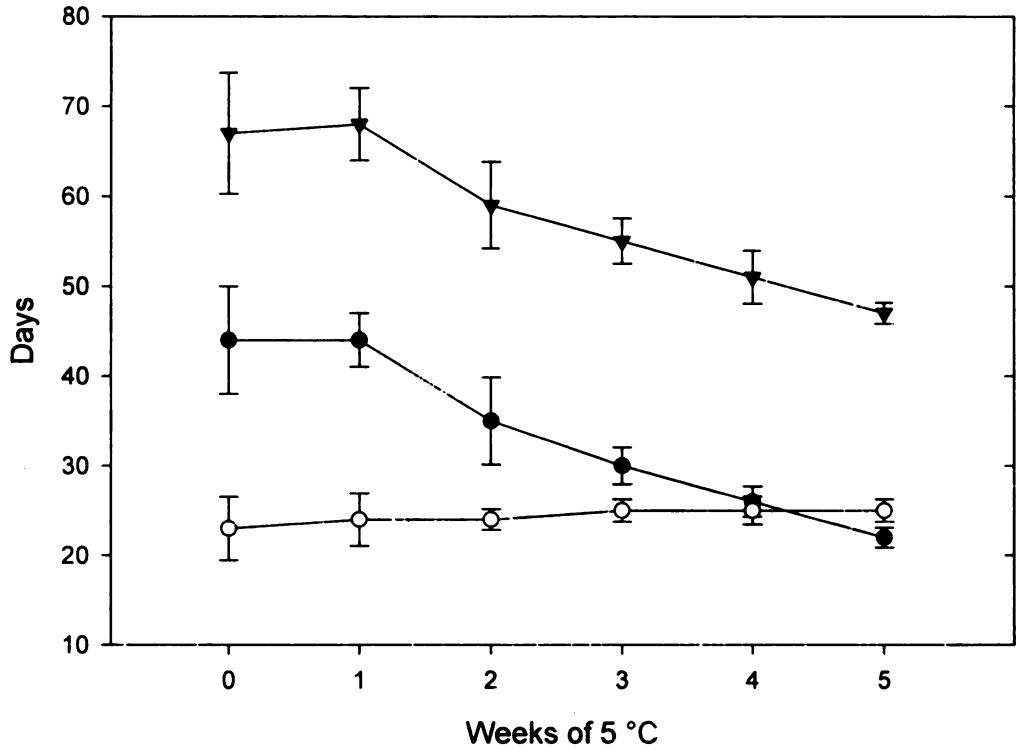

Figure 1. The effects of cold treatment on days to visible bud (●), days from visible bud to flowering (○), and days to flower (▼) for *Leucanthemum* x*superbum* ' Snow Cap'. Error bars represent 95% confidence intervals.

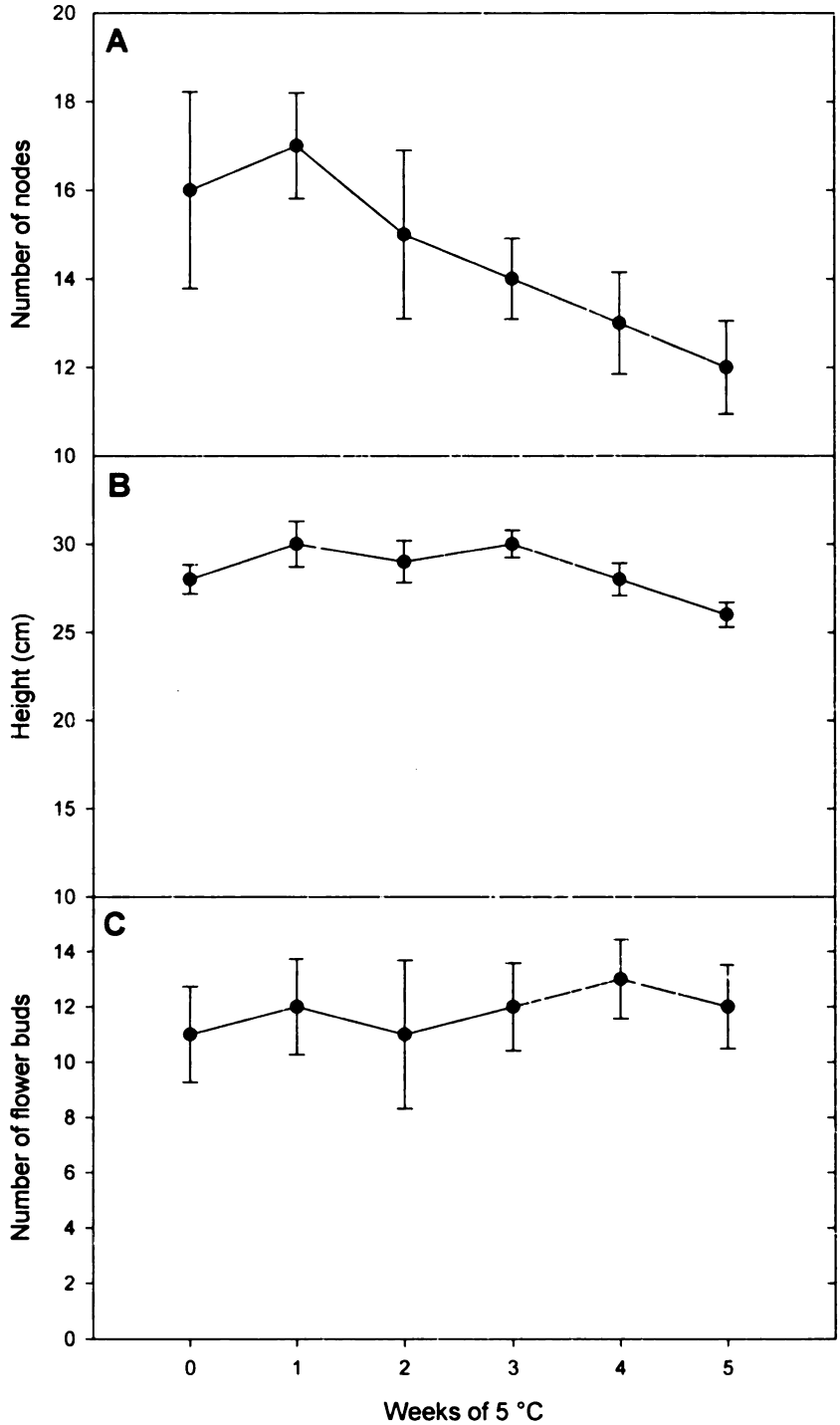

Figure 2. The effects of cold treatment on node number (A), plant height (B), and flower bud number (C) of *Leucanthemum* x*superbum* 'Snow Cap'. Error bars represent 95% confidence intervals.

APPENDIX B

THE FLOWERING RESPONSE OF *ACHILLEA* 'MOONSHINE' TO COLD DURATION, PROPAGATION PHOTOPERIOD, AND BULKING DURATION AND PHOTOPERIOD

Research Objective

The first objective of this research project was to determine the minimum cold duration required for rapid and uniform flowering of *Achillea* 'Moonshine'. The second objective was to determine whether propagation photoperiod affects time to flower of *Achillea* 'Moonshine'. The final objective was to determine whether the duration of and photoperiod during bulking affected time to flower and overall flowering quality of *Achillea* 'Moonshine'.

Materials and Methods

Cold-duration experiment. Achillea 'Moonshine' stock plants were potted (Sept. 1999) in 13-cm square plastic containers (1.1 L) filled with a commercial soilless medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened course perlite, along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.). Plants were grown under a 12-h photoperiod provided by supplementing natural daylengths with lighting from high-pressure sodium lamps from 0800 to 2000 HR. Stock plants were pinched at 3- to 4-week intervals to ensure continued branching and cutting production.

Harvested cuttings were propagated in 72-cell (0.03-L) plug trays (Landmark Plastic Corporation, Akron, Ohio). Following propagation, plants were grown (bulked) in the plug trays for an additional 3 weeks in a greenhouse at 20 °C to establish root systems and increase vegetative growth before cold treatment. Photoperiod was maintained at 12 h as on the stock plants.

Plugs then received no cold treatment or were placed in a controlled-environment chamber for 1, 2, 3, 4, or 5 weeks at 5 °C. The chamber was lighted from 0800 to 1700 HR by cool-white fluorescent lamps (F96T12/CW/VHO, Philips, Somerset, N.J.) The photosynthetic photon flux (*PPF*) from the lamps was approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. While in the cooler, plants were watered as needed with well water acidified with sulfuric acid to an approximate pH of 6.0. Following each cold duration, half of the plants in each treatment were soft pinched (one node removed).

Propagation-photoperiod experiment. Achillea 'Moonshine' cuttings were received from Guatemala on Feb. 15 and again on Mar. 13, 2002. Cuttings were propagated in 72-cell (0.04-L) plug trays (Landmark Plastic Corporation, Akron, Ohio) under one of six photoperiods: 11, 12, 13, 14, or 15 h of continuous light, or 9 h with a 4-h night interruption (NI). Natural photoperiods were extended with incandescent lamps. For continuous photoperiodic treatments, lamps were turned on at 1700 HR and turned off after each photoperiod was completed. The NI was delivered from 2200 to 0200 HR.

Bulking duration and photoperiod experiment. Achillea 'Moonshine' cuttings were received from Guatemala on Dec. 31, 2001. Cuttings were propagated under natural photoperiods in 50-cell (0.08-L) plug trays (Landmark Plastic Corporation, Akron, Ohio).

Plants were grown (bulked) in the plug trays for 2, 4, or 6 weeks in a greenhouse at 20 °C under one of three photoperiods: 10, 12, or 13 h. Natural

photoperiods were extended with incandescent lamps. Lamps were turned on at 1700 HR and turned off after each photoperiod was completed.

Propagation environmental control. Plug trays contained a mixture of 50% commercial medium (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.) and 50% screened course perlite (Therm-O-Rock; East, Inc., New Eagle, Pa.). Basal portions of each cutting were dipped in a 1500-ppm solution of liquid auxin (DIP 'N GROW; Astoria-Pacific, Clackamas, Ore.) before stick. Propagation air temperatures were maintained at 23 °C and bottom heat (soil temperature) was maintained at 25 °C. A vapor pressure deficit of 0.3 kPa was maintained by injecting water vapor as needed. Cuttings were rooted and weaned from propagation 3 weeks after sticking.

General plant culture. Plugs were potted in 13-cm square plastic containers (1.1 L) containing the same commercial medium used for stock plants in the cold-duration experiment. All plants were forced to flower under a 16-h photoperiod, natural daylengths supplemented with lighting from high-pressure sodium lamps from 0530 to 2130 HR. Plants were top-watered as necessary with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and containing water-soluble fertilizer providing 125 N, 12 P, 125 K, 13 Ca ($mg \cdot L^{-1}$; 30% ammoniacal N) plus ($mg \cdot L^{-1}$) 1.0 Fe, 0.5 Mn, 0.5 Zn, 0.5 Cu, 0.1 B, 0.1 Mo (MSU Special; Greencare Fertilizers, Chicago, Ill.). For the bulking photoperiod and duration and propagation photoperiod experiments, plants received additional Cu at 0.5 $mg \cdot L^{-1}$ and B at 0.1 $mg \cdot L^{-1}$ at every watering.

Greenhouse temperature control. Plants were grown in glass greenhouses set at 20 °C. Greenhouse temperatures were controlled by a greenhouse climate-control computer (Model CD750; Priva, De Lier, The Netherlands). Average daily temperature and daily light integral were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge (0.013 mm in diameter) type E thermocouples and a quantum sensor (Model LI-189; LI-COR, Inc., Lincoln, Neb.), respectively. The datalogger collected data every 10 seconds and recorded the hourly average. Actual average daily temperatures and daily light integrals (cold duration experiment only) for the beginning of forcing to the average date of flowering were calculated and are presented in Table 1.

Data collection and analysis. Dates of visible bud and first flower as well as height at flowering were recorded. For all experiments except cold duration, the number of nodes at flower was also recorded. A completely randomized design was used. Data were analyzed using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA) and general linear models (GLM) procedures.

Results and Discussion

Cold-duration experiment. All plants flowered regardless of cold treatment. Days to visible bud and days to flower were not significantly affected by cold treatment. Pinching did significantly affect time to flower (Table 2). Pinched plants flowered three to 10 days later than unpinched plants, depending on duration of cold treatment (Figure 1). In general, as duration of cold increased, the delay in flowering between pinched versus unpinched plants also

increased. Height was not significantly affected by cold duration or pinching. Since plants flowered completely without a cold treatment and increased cold durations did not significantly affect days to flower, all other *Achillea* 'Moonshine' experiments were conducted without a cold treatment.

Propagation photoperiod. The photoperiod during propagation had a small but significant effect on days to flower for replicate one but was not significant for replicate two (Table 3, Figure 2A and B). Height was significantly affected by propagation photoperiod for replicate one, with plants propagated under a 15-h photoperiod being slightly taller (Figure 2C). However, height was not significantly different between treatments in replicate two (Figure 2D). In replicate one, propagation photoperiod did not affect the number of nodes formed at flowering (Figure 2C). All treatments had 13 or 14 nodes at flowering. In replicate two, propagation photoperiod had a significant linear effect on the number of nodes formed, which tended to decrease as propagation photoperiod increased (Figure 2D). *Achillea* 'Moonshine' plants in replicate two took an average of five days longer to flower than plants in replicate one. Temperatures between the two replicates differed only by 0.4 °C, so the difference was not likely due to temperature.

In the first replicate of the experiment, plants from each propagation photoperiod were also forced under short days (9-h photoperiod) to determine whether exposure to potentially reproductive photoperiods in propagation was sufficiently long enough to induce flowering. Since only three plants flowered (one plant from 15 h and two plants from NI, data not presented), we concluded

that the duration of propagation was not long enough to induce flowering. Following 15 weeks of 9-h photoperiods, the plants were moved to a 16-h photoperiod. After 60 days in long days, the plants were still growing vegetatively. It appears that after extended exposure to short photoperiods, *Achillea* 'Moonshine' will not flower under long days and develops an obligate vernalization requirement. Cuttings rooted under an 11-, 12-, or 13-h photoperiod had better root development than cuttings rooted under a 14-h, 15-h, or NI photoperiod, but no data were collected.

Bulking duration and photoperiod. All plants flowered regardless of treatment combination. The duration of bulking, but not bulking photoperiod, significantly affected days to visible bud, days from visible bud to flower, and days to flower from the start of long days (Table 4). As bulking duration increased, days to visible bud increased and days from visible bud to flower decreased (Table 4, Figure 3A and B). As bulking duration increased, time to flower for plants bulked under a 10- or 12-h photoperiod increased linearly. Plant height was significantly affected by bulking photoperiod but not bulking duration (Figure 4A). Bulking duration significantly affected the number of nodes present at flowering. As bulking duration increased, the number of nodes increased linearly, regardless of bulking photoperiod (Figure 4B). Perhaps, as experienced by the *Gaura lindheimeri* 'Whirling Butterflies' plants grown under a 9-h photoperiod in the propagation photoperiod experiment, the bulking photoperiods were short enough to delay flowering as bulking duration increased.

Unresolved Issues Requiring Further Research

The bulking duration and photoperiod requires replication to solidify the validity of the results.

The effects of propagation photoperiod on the rooting of *Achillea* 'Moonshine' cuttings should be examined. Although propagation photoperiod did not affect time to flower, it appeared to affect the rooting quality of cuttings.

It still remains unclear what environmental conditions before forcing to flower produce plants with multiple flowering stems. All unpinched plants had a single flowering stem. For the bulking duration and photoperiod experiment, only one bulking temperature was used. Perhaps temperatures lower than 20 °C during the bulking period would increase the number of flowering shoots per plant without pinching.

Achillea 'Moonshine' plants exposed to prolonged durations of short days (9-h photoperiod for 15 weeks) developed an obligate cold requirement. Regardless of photoperiod following this extended short-day exposure, plants grew vegetatively. However, the number of short days needed before plants require cold treatment is unknown, and the subsequent minimum cold duration required for flowering is also unknown.

Table 1. Dates of forcing, average daily temperatures, and average daily light integral (DLI) from date of forcing to average date of flowering for *Achillea* 'Moonshine'.

Date of forcing	Weeks of bulking	Weeks of 5 °C	Average temperature (° C)	Average DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
Cold-duration experiment				
2/1/00	3	0	19.8	12.1
2/8/00	3	1	19.7	12.0
2/15/00	3	2	19.4	11.8
2/22/00	3	3	19.3	13.3
2/29/00	3	4	19.1	14.4
3/10/00	3	5	19.0	16.0
Propagation photoperiod experiment				
Replicate 1				
3/14/02	0 (16-h force)	0	21.8	16.5
3/14/02	0 (9-h force)	0	20.3	13.5
Replicate 2				
4/16/02	0 (16-h force)	0	22.2	17.8
Bulking duration and photoperiod experiment				
1/29/02	2	0	19.9	--[z]
2/13/02	4	0	20.0	--
2/27/02	6	0	20.5	--

[z]Dashes indicate data not presented.

Table 2. The effects of cold duration on flowering of *Achillea* 'Moonshine'.

Weeks of 5 °C	Pinch	Flowering Percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)
			21	24	45	45
	No		17	25	43	46
	Yes		24	23	47	45
0			21	23	44	45
1			20	26	47	45
2			25	23	48	46
3			19	23	43	46
4			21	24	45	45
5			19	24	43	47
0	No	100	20	25	45	45
	Yes	100	26	21	47	44
1	No	100	19	28	47	44
	Yes	100	22	25	47	46
2	No	100	23	23	46	47
	Yes	100	26	23	49	46
3	No	100	17	23	40	47
	Yes	100	21	24	45	45
4	No	100	18	25	43	46
	Yes	100	24	23	47	43
5	No	100	15	25	40	48
	Yes	100	25	21	46	46
Significance						
	Weeks of cold		NS	*	NS	NS
	Pinch		**	**	**	NS
	Cold X Pinch		NS	NS	NS	NS
Contrasts						
	Pinched plants					
		Cold	NS	NS	NS	NS
		P_{Linear}	NS	NS	NS	NS
		$P_{Quadratic}$	NS	*	NS	NS
	Unpinched plants					
		Cold	NS	NS	NS	NS
		P_{Linear}	NS	NS	NS	NS
		$P_{Quadratic}$	NS	NS	NS	NS

NS,*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 3. The effects of propagation photoperiod on flowering of *Achillea* 'Moonshine'.

Propagation photoperiod	Flowering Percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Nodes at flower
Replicate 1						
		32	15	47	31	14
11-h	100	30	17	47	30	14
12-h	100	33	16	48	32	14
13-h	100	31	14	45	31	14
14-h	100	31	13	44	30	14
15-h	100	37	15	53	34	14
NI	70	29	15	44	30	13
Significance						
	Photoperiod	NS	NS	*	**	NS
Contrasts						
	P_{Linear}	*	NS	NS	*	NS
	$P_{Quadratic}$	NS	*	*	NS	NS
Replicate 2						
		36	17	52	33	15
11-h	100	39	16	55	31	17
12-h	100	37	16	53	33	15
13-h	100	38	15	54	32	15
14-h	100	35	17	51	33	15
15-h	100	32	18	50	33	13
NI	100	34	17	50	34	15
Significance						
	Photoperiod	NS	*	NS	NS	*
Contrasts						
	P_{Linear}	*	NS	*	NS	**
	$P_{Quadratic}$	NS	**	NS	NS	NS

NS,*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 4. The effects of bulking duration and photoperiod on flowering of *Achillea* 'Moonshine'.

Weeks of bulking at 20 °C	Bulking photoperiod	Flowering Percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Nodes at flower
		100	31	19	50	40	18
	10-h	100	30	20	50	41	18
	12-h	100	31	19	50	39	18
	13-h	100	31	20	51	41	17
2		100	27	21	48	40	15
4		100	31	21	52	41	17
6		100	34	17	51	40	21
2	10-h	100	25	22	47	40	15
	12-h	100	28	20	48	40	16
	13-h	100	29	21	50	40	14
4	10-h	100	32	21	53	43	17
	12-h	100	30	21	51	38	16
	13-h	100	31	21	53	41	18
6	10-h	100	34	17	51	41	21
	12-h	100	36	16	52	39	22
	13-h	100	33	17	50	40	20
Significance							
	Bulk duration (BD)		***	***	***	NS	***
	Bulk photoperiod (BP)		NS	NS	NS	**	NS
	BD X BP		*	NS	NS	NS	*
10-h photoperiod							
	Bulk duration		***	***	***	NS	***
	P_{Linear}		***	***	**	NS	***
	$P_{Quadratic}$		NS	***	**	*	NS
12-h photoperiod							
	Bulk duration		***	***	***	NS	***
	P_{Linear}		***	***	***	NS	***
	$P_{Quadratic}$		NS	***	NS	NS	*
13-h photoperiod							
	Bulk duration		*	***	NS	NS	***
	P_{Linear}		**	***	NS	NS	***
	$P_{Quadratic}$		NS	**	*	NS	NS

NS,*,**,*** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively

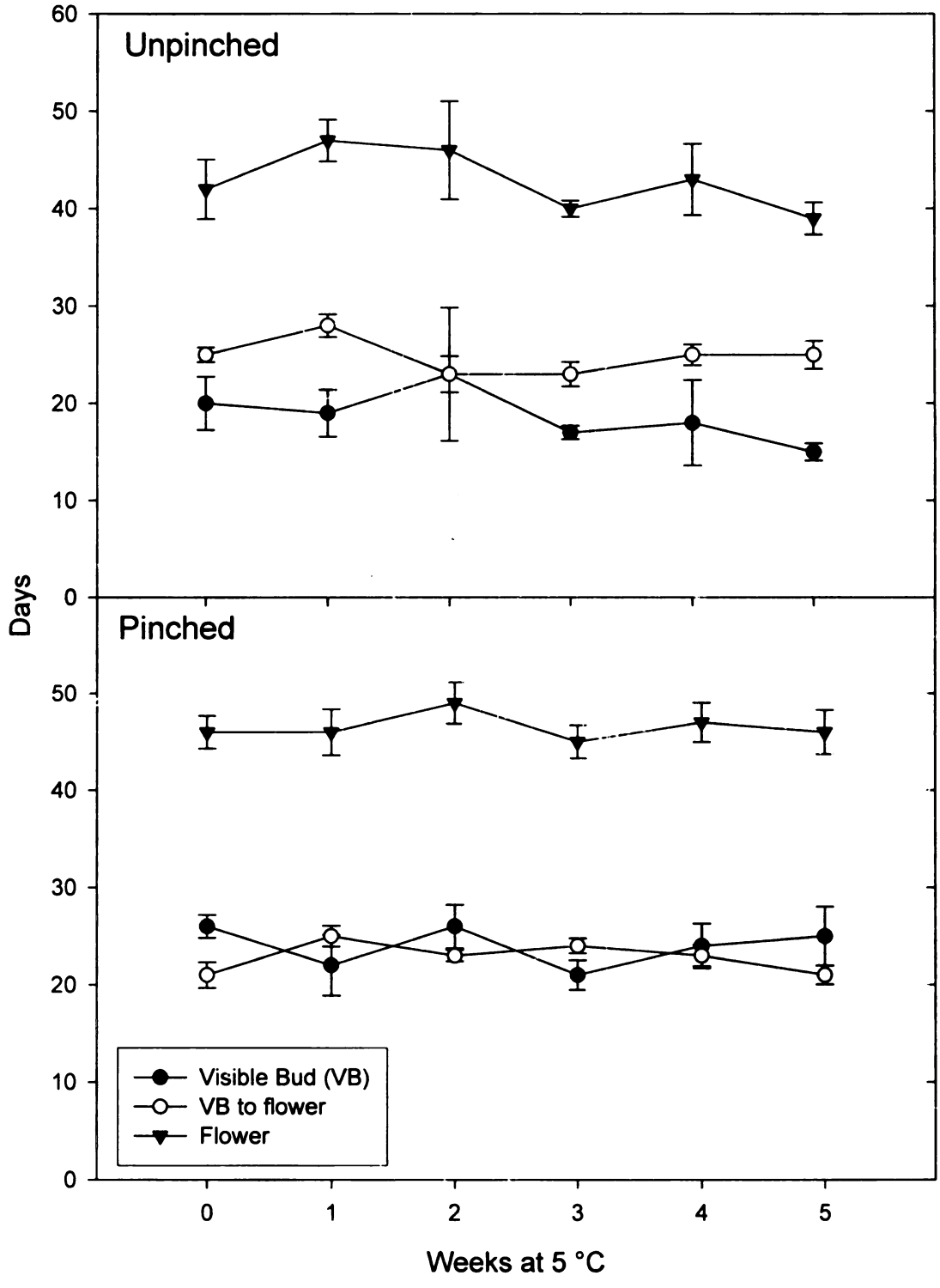

Figure 1. Time to flower for unpinched versus pinched plants of *Achillea* 'Moonshine'. Error bars represent standard error of the mean.

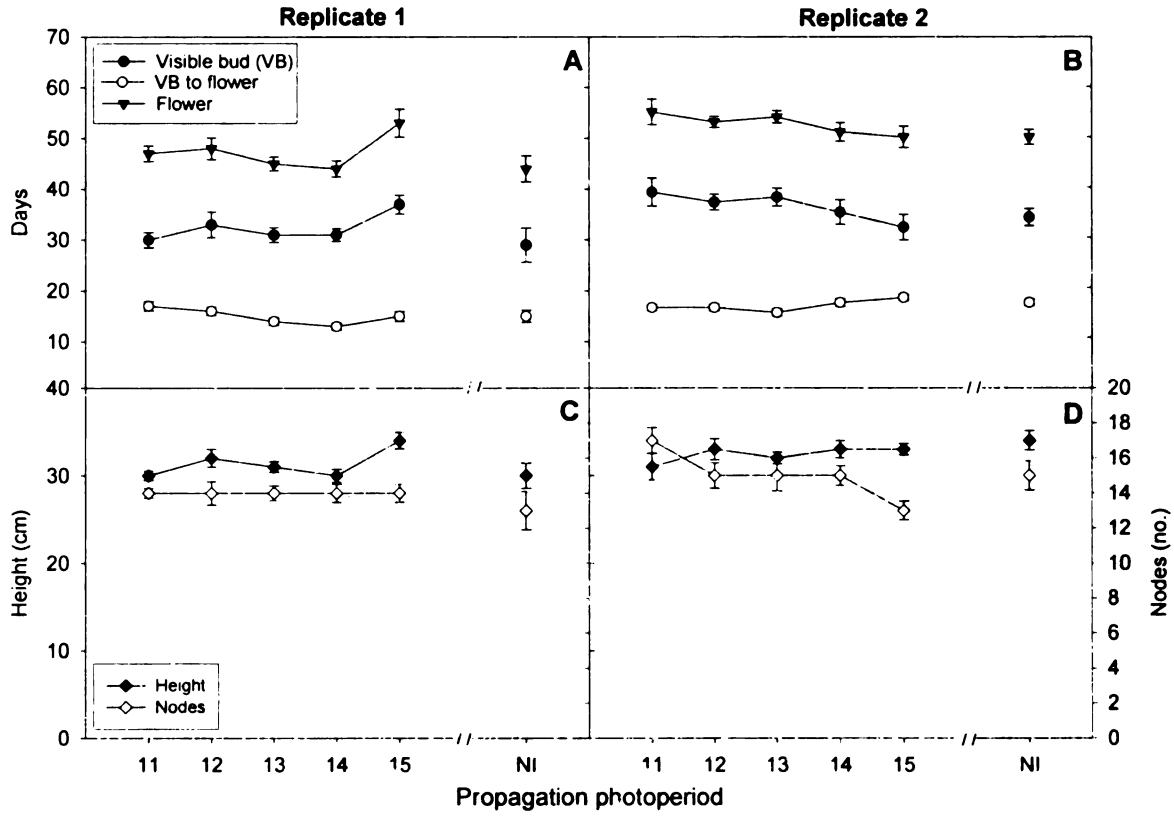

Figure 2. Flowering response of *Achillea* 'Moonshine' to propagation photoperiod. Days to visible bud (●), days from visible bud to flower (○), and days to flower (▼) are presented for replicates 1 (A) and 2 (B). Height (♦) and number of nodes (◊) are presented for replicates 1 (C) and 2 (D). Error bars represent standard error of the mean.

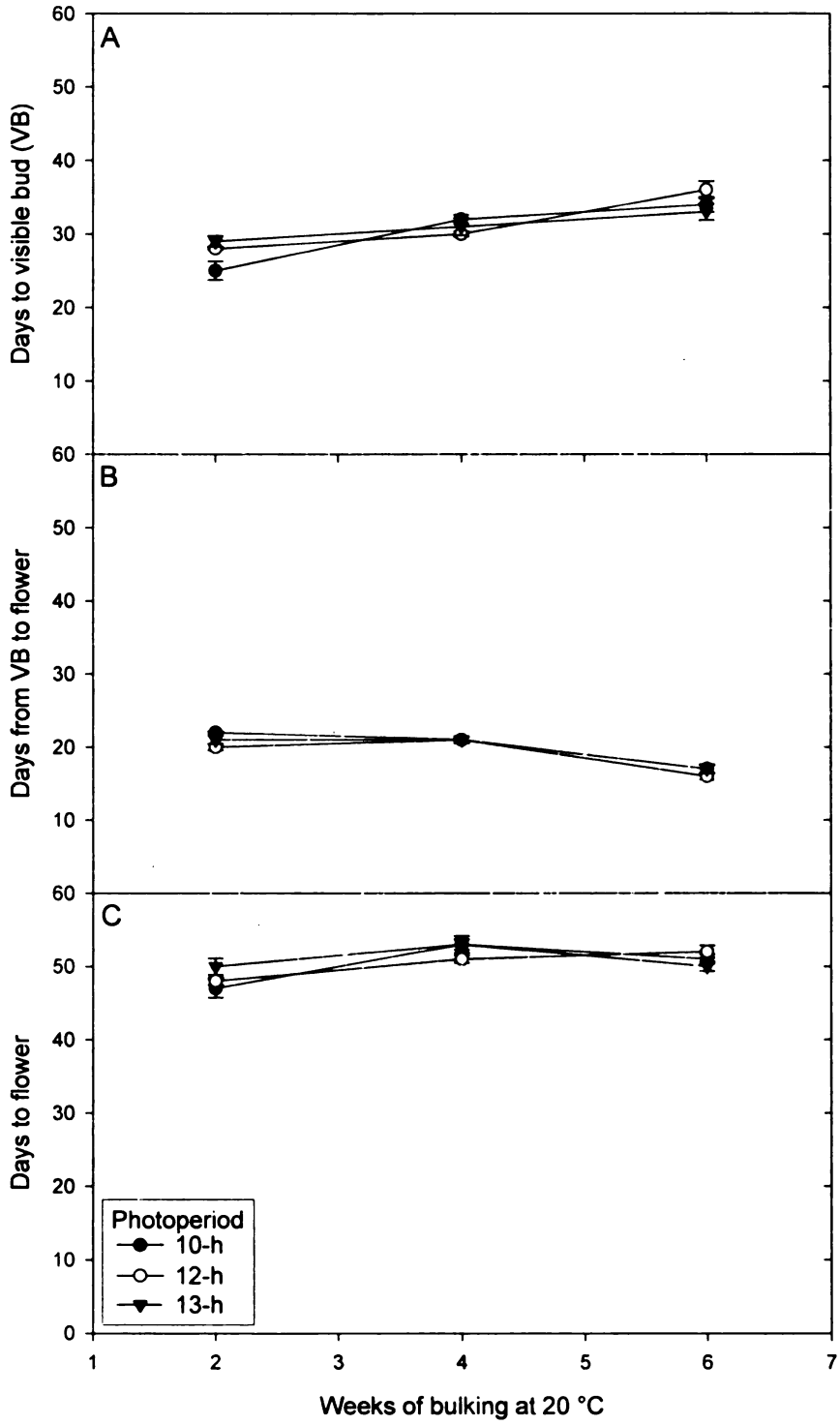

Figure 3. Time to flower, days to visible bud (A), days from visible bud to flower (B), and days to flower (C), for *Achillea* 'Moonshine' bulked under a 10- (●), 12- (○), or 13-h (▼) photoperiod. Error bars represent standard error of the mean.

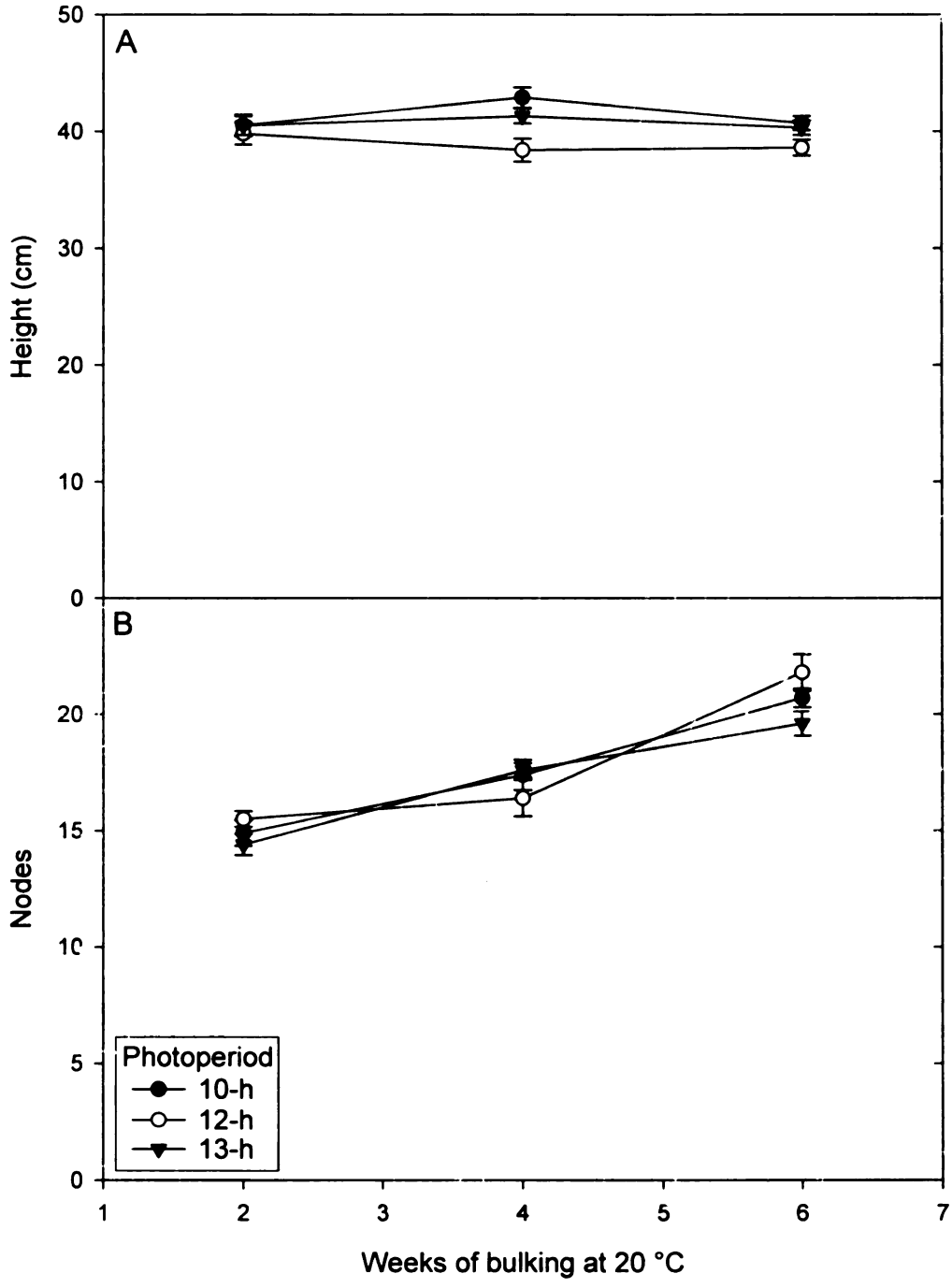

Figure 4. Height (A) and number of nodes (B) at flower for *Achillea* 'Moonshine' bulked under a 10- (●), 12- (○), or 13-h (▼) photoperiod. Error bars represent standard error of the mean.

APPENDIX C

THE FLOWERING RESPONSE OF *GAURA LINDHEIMERI* 'WHIRLING BUTTERFLIES' AND 'SISKIYOU PINK' TO COLD DURATION AND BULKING DURATION AND PHOTOPERIOD

Research Objective

The first objective of this research project was to determine the minimum cold duration required for rapid and uniform flowering of *Gaura lindheimeri* 'Whirling Butterflies'. The second objective was to determine whether the duration of and photoperiod during bulking affected time to flower and overall flowering quality of *G. lindheimeri* 'Whirling Butterflies' and 'Siskiyou Pink'.

Materials and Methods

Cold-duration experiment. *Gaura lindheimeri* 'Whirling Butterflies' stock plants were potted (Sept. 1999) in 13-cm square plastic containers (1.1 L) filled with a commercial soilless medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened course perlite, along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.). Plants were grown under a 12-h photoperiod provided by supplementing natural daylengths with lighting from high-pressure sodium lamps from 0800 to 2000 HR. Stock plants were pinched at 3- to 4-week intervals to ensure continued branching and cutting production.

Harvested cuttings were propagated in 72-cell (0.03-L) plug trays (Landmark Plastic Corporation, Akron, Ohio). Following propagation, plants were grown (bulked) in the plug trays for an additional 3 weeks in a greenhouse at 20 °C to establish root systems and increase vegetative growth before cold treatment. Photoperiod was maintained at 12 h as on the stock plants.

Plugs then received no cold treatment or were placed in a controlled-environment chamber for 1, 2, 3, 4, or 5 weeks at 5 °C. Plugs were pinched

before cold treatment to remove any flowers. The chamber was lit from 0800 to 1700 HR by cool-white fluorescent lamps (F96T12/CW/VHO, Philips, Somerset, N.J.) The photosynthetic photon flux (*PPF*) from the lamps was approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. While in the cooler, plants were watered as needed with well water acidified with sulfuric acid to an approximate pH of 6.0.

Bulking duration and photoperiod experiment. Gaura lindheimeri 'Whirling Butterflies' and 'Siskiyou Pink' cuttings were received from Guatemala on Dec. 31, 2001, Jan. 17, 2002 ('Whirling Butterflies' only) and Feb. 15, 2002. Cuttings were propagated in 50-cell (0.08-L) plug trays (Landmark Plastic Corporation, Akron, Ohio).

After propagation, plants were grown (bulked) in the plug trays for 2, 4, or 6 weeks in a greenhouse at 20 °C under one of three photoperiods: 9, 10, or 12 h. Natural photoperiods were extended with incandescent lamps. Lamps were turned on at 1700 HR and turned off after each photoperiod was completed.

Propagation environmental control. Plug trays contained a mixture of 50% commercial medium (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.) and 50% screened course perlite (Therm-O-Rock; East, Inc., New Eagle, Pa.). Basal portions of each cutting were dipped in a 1500-ppm solution of liquid auxin (DIP 'N GROW; Astoria-Pacific, Clackamas, Ore.) before stick. Propagation air temperatures were maintained at 23 °C and bottom heat (soil temperature) was maintained at 25 °C. A vapor pressure deficit of 0.3 kPa was maintained by injecting water vapor as needed. Cuttings were propagated under

natural photoperiods and were rooted and weaned from propagation 2 weeks after sticking.

General plant culture. Following bulking treatment, plugs were potted in 13-cm square plastic containers (1.1 L) containing the same commercial medium used for the stock plants in the cold-duration experiment. All plants were forced to flower under a 16-h photoperiod, natural daylengths supplemented with lighting from high-pressure sodium lamps from 0530 to 2130 HR. Plants were top-watered as necessary with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and containing water-soluble fertilizer providing 125 N, 12 P, 125 K, 13 Ca ($mg \cdot L^{-1}$; 30% ammoniacal N) plus ($mg \cdot L^{-1}$) 1.0 Fe, 0.5 Mn, 0.5 Zn, 0.5 Cu, 0.1 B, 0.1 Mo (MSU Special; Greencare Fertilizers. Chicago, Ill.). Plants in the bulking duration and photoperiod experiment received additional Cu at 0.5 $mg \cdot L^{-1}$ and B at 0.1 $mg \cdot L^{-1}$ with every watering.

Greenhouse temperature control. Plants were grown in glass greenhouses set at 20 °C. Greenhouse temperatures were controlled by a greenhouse climate-control computer (Model CD750; Priva, De Lier, The Netherlands). Average daily temperature and daily light integral were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge (0.013 mm in diameter) type E thermocouples and a quantum sensor (Model LI-189; LI-COR, Inc., Lincoln, Neb.), respectively. The datalogger collected data every 10 seconds and recorded the hourly average. Actual average daily temperatures and daily light integrals (cold duration experiment only) from the

beginning of forcing to the average date of flowering were calculated and are presented in Table 1.

Data collection and analysis. Dates of visible bud and first flower as well as height at flowering were recorded. For the cold-duration experiment, the number of new leaves formed was also recorded. For the bulking duration and photoperiod experiment, the number of flowering stems for 'Whirling Butterflies' and the number of lateral inflorescences for 'Siskiyou Pink' were recorded. A completely randomized design was used. Data were analyzed using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA) and general linear models (GLM) procedures.

Results and Discussion

Cold-duration experiment. All *G. lindheimeri* 'Whirling Butterflies' plants flowered regardless of cold treatment. The number of weeks at 5 °C had a significant effect on days to visible bud, days to flower, and final plant height (Table 2). As cold duration increased, time to visible bud and flower tended to decrease linearly. However, as cold duration increased, plant height at first open flower tended to increase linearly.

Cuttings were removed from reproductive stock plants. Flowers had to be removed from the plugs before cold treatment. Therefore, time to flower may not be accurate for plants that were completely vegetative at the start of cold. Since there was little difference in time to flower, and since cold-treated plants were

taller, all other *G. lindheimeri* experiments were conducted without a cold treatment.

Bulking duration and photoperiod experiment.

***Gaura lindheimeri* 'Whirling Butterflies'.** All plants, regardless of replicate or treatment combination, eventually flowered. For replicate one, bulking duration had a significant effect on all measured flowering responses (Table 3). Bulking photoperiod had a significant effect on days to visible bud, days from visible bud to flower, and days to flower. Plants bulked under a 12-h photoperiod flowered faster than plants bulked under the other photoperiods (Table 3, Figure 1A). As bulking duration increased, there was little change in time to flower, but the number of flowering stems decreased (Figure 1G). Plants bulked for 4 weeks, irrespective of photoperiod, flowered fastest. Plant height decreased as bulking photoperiod increased, but plant height increased with increasing bulking duration (Figure 1D).

For replicate two, bulking duration had no significant effect on any measured characteristic (Table 4). Bulking photoperiod had a significant effect on days to visible bud, days to flower, and the number of flowering stems. The interaction between bulking duration and photoperiod had a significant effect on plant height. For plants bulked for 2 or 4 weeks, as bulking photoperiod increased, final plant height increased. For plants bulked for 6 weeks, plant height decreased as bulking photoperiod increased (Figure 1E). Days to flower tended to decrease as bulking photoperiod increased, however, plants bulked under a 10-h photoperiod flowered fastest (Figure 1B). Bulking photoperiod

slightly affected the number of flowering stems. In general, plants bulked under a 10-h photoperiod produced more flowering stems (Figure 1H).

For replicate 3, bulking duration and photoperiod significantly affected final plant height (Table 5). As bulking duration increased, plant height decreased. Likewise, as bulking photoperiod increased, plant height decreased (Figure 1F). Neither bulking photoperiod nor bulking duration affected time to flower (Figure 1C) or the number of flowering stems (Figure 1I).

Unlike the cuttings in the cold-duration experiment, the cuttings used in this experiment were vegetative. On average, plants took 10 days longer to flower than predicted by the cold-duration experiment (Table 2). As bulking duration increased, irrespective of bulking photoperiod, an increasing number of plants were delayed in flowering and flowered poorly. The flowering stems failed to elongate and the plants remained rosettes. Although plants did eventually flower, some required 100 or more days from the start of long days to flower. We concluded that vegetative *G lindheimeri* 'Whirling Butterflies' plants, like *Achillea* 'Moonshine', after prolonged exposure to short photoperiods appear to require a cold treatment for rapid, uniform flowering. The plants in the third replicate that were bulked for 6 weeks but not planted were cold treated for 6 weeks at 5 °C. Following the cold treatment, all plants flowered rapidly and uniformly (data not presented).

***Gaura lindheimeri* 'Siskiyou Pink'.** Regardless of replicate or treatment combination, all plants flowered. For replicate one, the interaction between bulking duration and photoperiod had a significant effect on all

measured flowering characteristics (Table 6). Bulking duration significantly affected all measured characteristics except days from visible bud to flower. Bulking photoperiod significantly affected all measured characteristics except the number of lateral inflorescences. As bulking duration increased, regardless of bulking photoperiod, the number of lateral inflorescences decreased linearly (Figure 2E). As bulking photoperiod and duration increased, final plant height (Figure 2C) and the number of days to visible bud and to flower (Figure 2A) decreased.

For replicate two, bulking duration significantly affected all flower characteristics measured (Table 7). Bulking photoperiod affected only final plant height. As bulking duration increased, time to flower (Figure 2B), final plant height (Figure 2D), and the number of lateral inflorescences (Figure 2F) decreased.

Gaura lindheimeri 'Siskiyou Pink' was more sensitive to photoperiod than 'Whirling Butterflies'. 'Siskiyou Pink' did not exhibit the same dormancy problem that 'Whirling Butterflies' did following extended short days. For plants in the second replicate, natural photoperiods were naturally long enough in propagation to induce plants to flower. The 6-week bulking treatment was not planted because all plants had flowered in the plug trays, irrespective of bulking photoperiod.

Unresolved Issues Requiring Further Research

According to data gathered from the bulking duration and photoperiod experiment, minimum cold requirements for *Gaura lindheimeri* 'Whirling Butterflies' should be reevaluated. The initial cold-duration experiment was performed with reproductive plugs, so timing of flowering was not accurate.

The bulking photoperiod and duration experiment should be replicated again for *G. lindheimeri* 'Siskiyou Pink'. Photoperiod control needs to be implemented during propagation to ensure that cuttings are completely vegetative at the start of bulking so that accurate bulking photoperiod effects can be determined.

Table 1. Dates of forcing, average daily temperatures, and average daily light integral (DLI) from date of forcing to average date of flowering for *Gaura lindheimeri*.

Date of forcing	Weeks of bulking	Weeks of 5 °C	Average temperature (° C)	Average DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
Cold duration experiment				
2/1/00	3	0	21.5	12.1
2/8/00	3	1	21.4	12.0
2/15/00	3	2	21.5	11.8
2/22/00	3	3	21.5	13.3
2/29/00	3	4	21.3	14.4
3/10/00	3	5	21.1	16.0
Bulking duration and photoperiod experiment				
Replicate 1				
1/29/02	2	0	20.0	--[z]
2/13/02	4	0	20.3	--
2/27/02	6	0	20.5	--
Replicate 2 ('Whirling Butterflies' only)				
2/28/02	2	0	20.5	--
3/17/02	4	0	20.8	--
3/29/02	6	0	--	--
Replicate 3				
3/13/02	2	0	20.7	--
3/28/02	4	0	--	--

[z]Dashes indicate data not presented.

Table 2. The effects of cold treatment on flowering of *Gaura lindheimeri* 'Whirling Butterflies'.

Weeks of 5 °C	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	New leaves formed (no.)	Final plant height (cm)
0	100	22	18	40	65	40.4
1	100	28	17	44	57	42.0
2	100	22	17	38	62	44.6
3	100	20	16	35	52	45.6
4	100	19	16	34	53	44.6
5	100	18	20	38	59	44.0
Significance						
Weeks of cold		**	NS	**	NS	**
Contrasts						
P_{Linear}		**	NS	**	NS	**
$P_{Quadratic}$		NS	*	NS	NS	**

NS,*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 3. The effects of bulking duration and photoperiod on flowering of *Gaura lindheimeri* 'Whirling Butterflies' (replicate 1).

Bulking duration (weeks)	Bulking photoperiod	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Flowering stems (no.)
		100	37	13	49	33	7
	9-h	100	39	13	52	35	7
	10-h	100	39	13	52	33	7
	12-h	100	33	12	44	32	6
2		100	39	13	52	28	8
4		100	32	13	45	36	8
6		100	39	11	51	37	5
2	9-h	100	41	12	53	26	9
	10-h	100	41	16	57	26	8
	12-h	100	35	13	47	31	8
4	9-h	100	37	14	51	40	8
	10-h	100	36	14	49	35	8
	12-h	100	23	12	36	32	6
6	9-h	100	39	13	51	39	5
	10-h	100	40	11	51	37	6
	12-h	100	40	11	51	34	6
Significance							
	Bulking duration (BD)		*	**	*	**	*
	Bulking photoperiod (BP)		*	*	**	NS	NS
	BD X BP		NS	*	NS	NS	NS
9-h photoperiod							
	Bulking duration		NS	NS	NS	**	*
	P_{Linear}		NS	NS	NS	**	*
	$P_{Quadratic}$		NS	NS	NS	*	NS
10-h photoperiod							
	Bulking duration		NS	**	NS	NS	NS
	P_{Linear}		NS	**	NS	NS	NS
	$P_{Quadratic}$		NS	NS	NS	NS	NS
12-h photoperiod							
	Bulking duration		*	NS	*	NS	NS
	P_{Linear}		NS	NS	NS	NS	NS
	$P_{Quadratic}$		**	NS	*	NS	NS

NS,*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 4. The effects of bulking duration and photoperiod on flowering of *Gaura lindheimeri* 'Whirling Butterflies' (replicate 2).

Bulking duration (weeks)	Bulking photoperiod	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Flowering stems (no.)
		100	46	11	57	25	5
	9-h	100	52	12	62	24	5
	10-h	100	43	11	53	27	6
	12-h	100	45	12	56	25	5
2		100	46	11	58	26	6
4		100	48	12	55	24	5
6		100	46	12	57	25	4
2	9-h	100	49	11	62	22	5
	10-h	100	45	10	55	30	6
	12-h	100	45	11	56	27	5
4	9-h	100	57	11	62	21	4
	10-h	100	43	12	52	24	6
	12-h	100	42	12	52	28	5
6	9-h	100	51	12	61	30	4
	10-h	100	41	11	52	26	5
	12-h	100	47	12	59	19	4
Significance							
	Bulking duration (BD)		NS	NS	NS	NS	NS
	Bulking photoperiod (BP)		**	NS	**	NS	*
	BD X BP		NS	NS	NS	**	NS
9-h photoperiod							
	Bulking duration		NS	NS	NS	NS	NS
	P_{Linear}		NS	NS	NS	NS	NS
	$P_{Quadratic}$		NS	NS	NS	NS	NS
10-h photoperiod							
	Bulking duration		NS	NS	NS	NS	NS
	P_{Linear}		NS	NS	NS	NS	NS
	$P_{Quadratic}$		NS	*	NS	NS	NS
12-h photoperiod							
	Bulking duration		NS	NS	NS	NS	NS
	P_{Linear}		NS	NS	NS	*	*
	$P_{Quadratic}$		NS	NS	NS	NS	NS

NS,*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 5. The effects of bulking duration and photoperiod on flowering of *Gaura lindheimeri* 'Whirling Butterflies' (replicate 3).

Bulking duration (weeks)	Bulking photoperiod	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Flowering stems (no.)
		100	39	12	50	30	6
	9-h	100	39	12	50	32	5
	10-h	100	39	13	51	32	6
	12-h	100	39	11	49	25	5
2		100	41	12	51	33	6
4		100	39	12	49	26	5
2	9-h	100	38	11	50	35	6
2	10-h	100	45	13	54	36	7
2	12-h	100	39	11	49	28	6
4	9-h	100	40	12	51	29	5
4	10-h	100	37	13	48	28	6
4	12-h	100	39	11	49	23	5
Significance							
	Bulking duration (BD)		NS	NS	NS	***	NS
	Bulking photoperiod (BP)		NS	NS	NS	**	NS
	BD X BP		NS	NS	NS	NS	NS
9-h photoperiod							
	Bulking duration		NS	NS	NS	*	NS
10-h photoperiod							
	Bulking duration		NS	NS	NS	*	NS
12-h photoperiod							
	Bulking duration		NS	NS	NS	NS	NS

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

Table 6. The effects of bulking duration and photoperiod on flowering of *Gaura lindheimeri* 'Siskiyou Pink' (replicate 1).

Bulking duration (weeks)	Bulking photoperiod	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Lateral inflorescences (no.)
		100	22	13	35	30	24
	9-h	100	26	14	40	33	23
	10-h	100	25	14	39	31	24
	12-h	100	14	12	26	27	24
2		100	27	13	39	33	31
4		100	22	13	35	29	27
6		100	17	13	30	28	14
2	9-h	100	27	13	40	33	34
	10-h	100	28	13	41	32	30
	12-h	100	24	12	37	33	29
4	9-h	100	27	13	40	31	21
	10-h	100	24	14	38	30	28
	12-h	100	15	12	28	27	32
6	9-h	100	24	15	39	34	14
	10-h	100	23	14	37	30	14
	12-h	100	3	11	14	22	13
Significance							
	Bulking duration (BD)		***	NS	***	*	***
	Bulking photoperiod (BP)		***	***	***	**	NS
	BD X BP		***	*	***	*	**
9-h photoperiod							
	Bulking duration		NS	**	NS	NS	***
	P_{Linear}		*	**	NS	NS	***
	$P_{Quadratic}$		NS	NS	NS	NS	NS
10-h photoperiod							
	Bulking duration		*	NS	NS	NS	***
	P_{Linear}		**	NS	NS	NS	***
	$P_{Quadratic}$		NS	NS	NS	NS	*
12-h photoperiod							
	Bulking duration		***	NS	***	***	***
	P_{Linear}		***	NS	***	***	***
	$P_{Quadratic}$		NS	NS	NS	NS	***

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

Table 7. The effects of bulking duration and photoperiod on flowering of *Gaura lindheimeri* 'Siskiyou Pink' (replicate 2).

Bulking duration (weeks)	Bulking photoperiod	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Final plant height (cm)	Lateral inflorescences (no.)
		100	4	11	15	12	11
	9-h	100	6	10	15	11	10
	10-h	100	4	11	16	11	11
	12-h	100	3	11	14	15	11
2		100	7	13	20	14	13
4		100	1	8	9	11	8
2	9-h	100	10	13	23	14	13
	10-h	100	7	13	20	13	13
	12-h	100	5	14	19	16	12
4	9-h	100	1	7	8	8	8
	10-h	100	1	10	11	8	9
	12-h	100	0	8	9	15	9
Significance							
	Bulking duration (BD)		***	***	***	***	***
	Bulking photoperiod (BP)		NS	NS	NS	***	NS
	BD X BP		NS	NS	NS	*	NS
9-h photoperiod							
	Bulking duration		**	***	***	***	**
10-h photoperiod							
	Bulking duration		***	*	***	**	*
12-h photoperiod							
	Bulking duration		***	***	***	NS	*

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

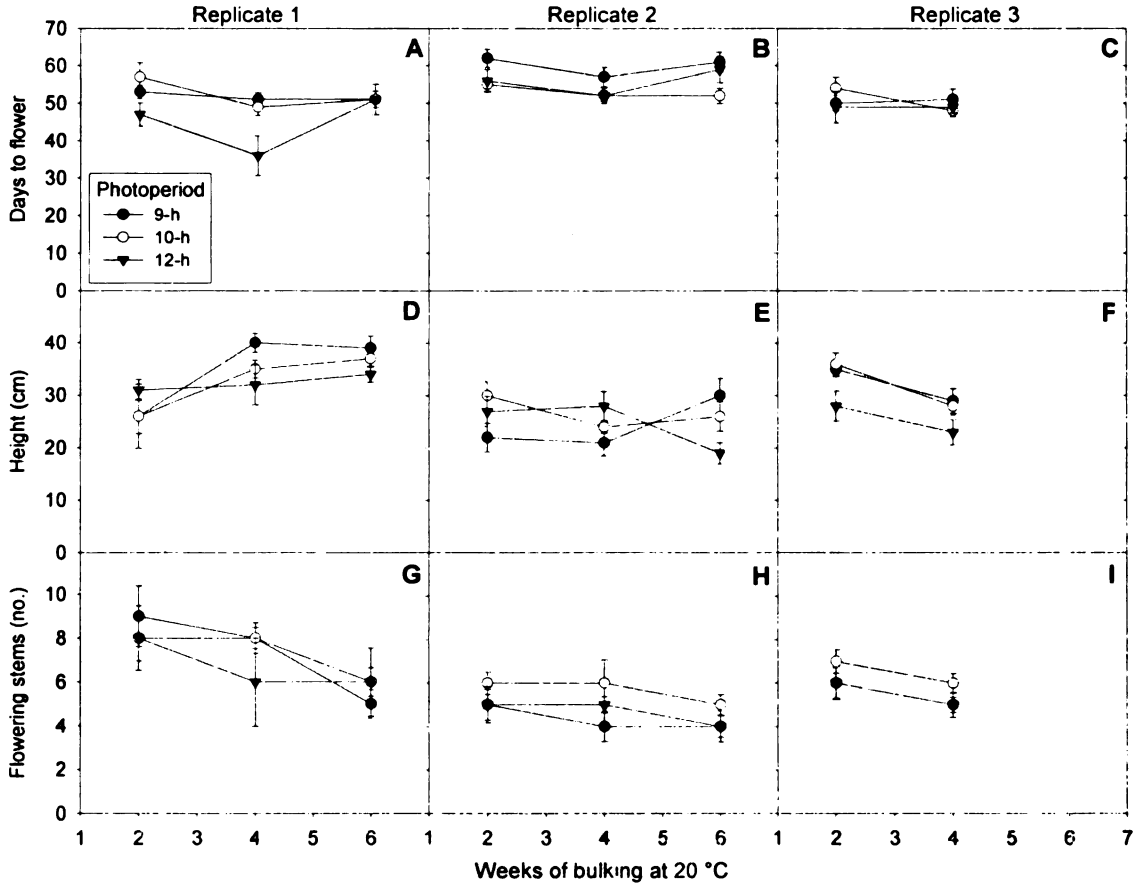

Figure 1. The effects of bulking duration and photoperiod on days to flower (A–C), plant height at flower (D–F), and number of lateral inflorescences at flower (G–I) for *Gaura lindheimeri* 'Whirling Butterflies'. Error bars represent standard error of the mean.

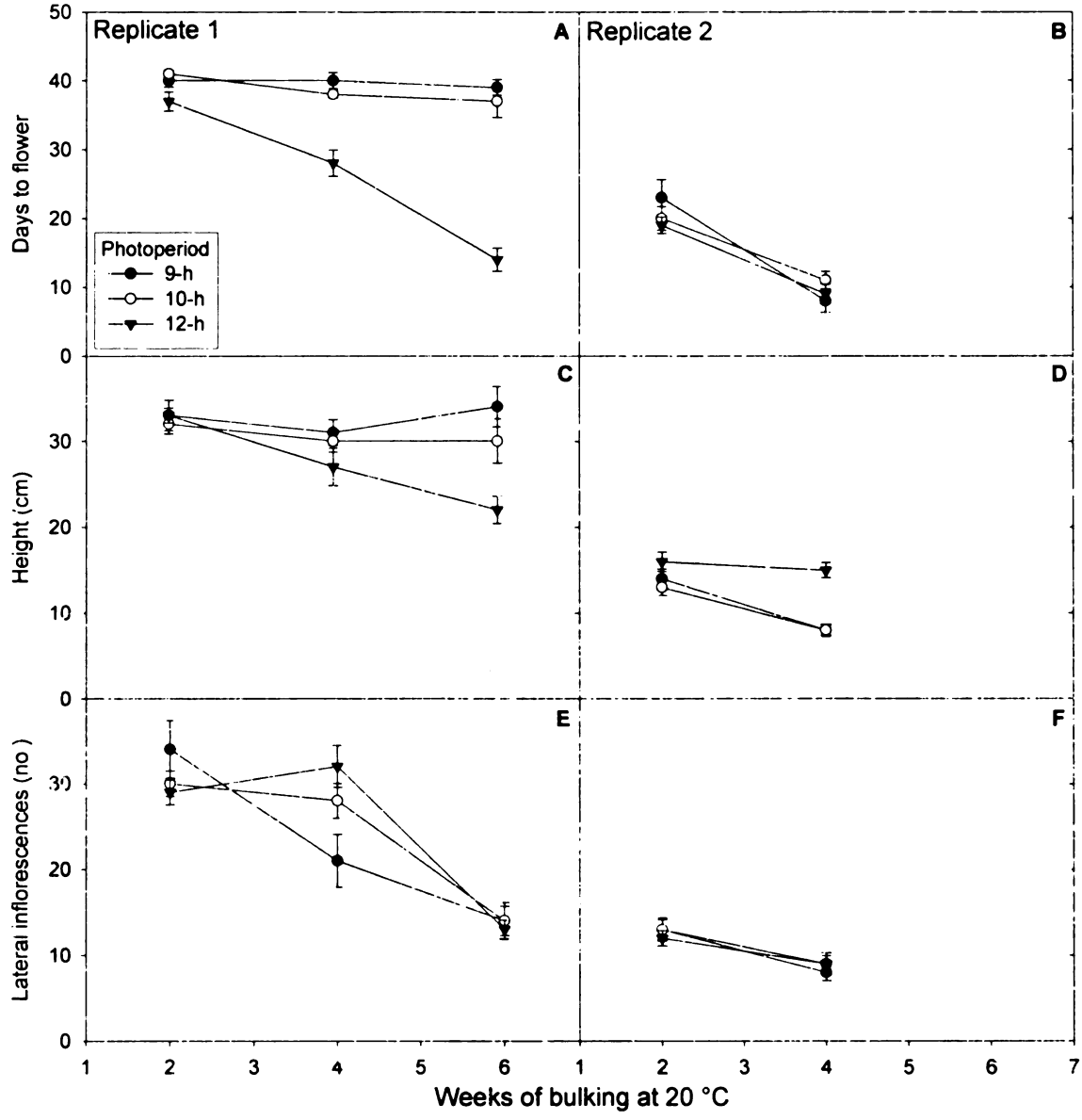

Figure 2. The effects of bulking duration and photoperiod on days to flower (A and B), plant height at flower (C and D), and the number of lateral inflorescences (E and F) for *Gaura lindheimeri* 'Siskiyou Pink'. Error bars represent standard error of the mean.

APPENDIX D

THE FLOWERING RESPONSE OF *CAMPANULA* 'BIRCH HYBRID' TO COLD DURATION AND DURATION OF BULKING

Research Objective

The first objective of this research project was to determine the minimum cold duration required for rapid and uniform flowering of *Campanula* 'Birch Hybrid'. The second objective was to determine whether the duration of bulking and cold affected time to flower and overall flowering quality of *Campanula* 'Birch Hybrid'.

Materials and Methods

Cold-duration experiment. Campanula 'Birch Hybrid' stock plants were potted (Sept. 1999) in 13-cm square plastic containers (1.1 L) filled with a commercial soilless medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened course perlite along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products. Galesburg, Mich.). Plants were grown under a 12-h photoperiod provided by supplementing natural daylengths with lighting from high-pressure sodium lamps from 0800 to 2000 HR. Stock plants were pinched at 3- to 4-week intervals to ensure continued branching and cutting production.

Harvested cuttings were propagated in 72-cell (0.03-L) plug trays (Landmark Plastic Corporation, Akron, Ohio). After propagation, plants were grown (bulked) in the plug trays for an additional 3 weeks in a greenhouse at 20 °C to establish root systems and increase vegetative growth before cold treatment. Photoperiod was maintained at 12 h as on the stock plants. Plugs then received no cold treatment or were placed in a controlled-environment chamber for 1, 2, 3, 4, or 5 weeks at 5 °C.

Bulking-duration experiment. On Oct. 25, 2001, *Campanula* 'Birch Hybrid' stock plants were potted in 13-cm square plastic containers (1.1 L) filled with a commercial soilless medium composed of pine bark, fibrous Canadian sphagnum peat, horticultural vermiculite, and screened course perlite, along with a wetting agent and starter fertilizer charge (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.). Plants were grown under a 16-h photoperiod provided by supplementing natural daylengths with lighting from high-pressure sodium lamps from 0530 to 2130 HR.

Harvested cuttings were propagated in 50-cell (0.08-L) plug trays (Landmark Plastic Corporation, Akron, Ohio). After propagation, plants were grown (bulked) in a greenhouse at 20 °C for 3, 4, 5, or 6 weeks. After the appropriate bulking duration, plants were cold treated for 4, 5, 6, or 7 weeks at 5 °C.

Propagation environmental control. Plug flats contained a mixture of 50% commercial medium (Suremix Perlite; Michigan Grower Products, Galesburg, Mich.) and 50% screened course perlite (Therm-O-Rock; East, Inc., New Eagle, Pa.). Basal portions of each cutting were dipped in a 1500-ppm solution of liquid auxin (DIP 'N GROW; Astoria-Pacific, Clackamas, Ore.) before stick. Propagation air temperatures were maintained at 23 °C and bottom heat (soil temperature) was maintained at 25 °C. A vapor pressure deficit of 0.3 kPa was maintained by injecting water vapor as needed. Cuttings were propagated under natural daylengths and rooted and weaned from propagation 3 weeks after sticking.

Cold treatments. The chamber was lit from 0800 to 1700 HR by cool-white fluorescent lamps (F96T12/CW/VHO; Philips, Somerset, N.J.) The photosynthetic photon flux (*PPF*) from the lamps was approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. While in the cooler, plants were watered as needed with well water acidified with sulfuric acid to an approximate pH of 6.0.

General plant culture. Following all cold treatments, plugs were potted in 13-cm square plastic containers (1.1 L) containing the same commercial medium used for the stock plants. All plants were forced to flower under a 16-h photoperiod (natural days supplemented with high-pressure sodium lamps from 0530 to 2130 HR). Plants were top-watered as necessary with well water acidified with sulfuric acid to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and containing water-soluble fertilizer providing 125 N, 12 P, 125 K, 13 Ca ($mg \cdot L^{-1}$; 30% ammoniacal N) plus ($mg \cdot L^{-1}$) 1.0 Fe, 0.5 Mn, 0.5 Zn, 0.5 Cu, 0.1 B, 0.1 Mo (MSU Special, Greencare Fertilizers, Chicago, Ill.). Plants in the bulking-duration experiment received additional Cu at 0.5 $mg \cdot L^{-1}$ and B at 0.1 $mg \cdot L^{-1}$ at every watering.

Greenhouse temperature control. Plants were grown in glass greenhouses set at 20 °C. Greenhouse temperatures were controlled by a greenhouse climate-control computer (Model CD750; Priva, De Lier, The Netherlands). Average daily temperature and daily light integral were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge (0.013 mm in diameter) type E thermocouples and a quantum sensor (Model LI-189; LI-COR, Inc., Lincoln, Neb.), respectively. The datalogger collected data

every 10 seconds and recorded the hourly average. Actual average daily temperatures and average daily light integrals from the beginning of forcing to the average date of flowering were calculated and are presented in Table 1.

Data collection and analysis. Dates of visible bud and first flower and the number of flowering stems were recorded. For the bulking-duration experiment, the number of nodes present at the end of the cold treatment was also recorded. A completely randomized design with 10 observations for each treatment was used. Data were analyzed using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA) and general linear models (GLM) procedures.

Results and Discussion

Cold-duration experiment *Campanula* 'Birch Hybrid' had an obligate vernalization requirement (Table 2). Only 10% of the plants flowered after 4 weeks at 5 °C. After 5 weeks at 5 °C, all plants flowered, and the average number of flowering stems per plant was six. Even though there was 100% flowering after a 5-week cold treatment, a longer cold duration may reduce time to flower and increase the number of flowering stems.

Bulking-duration experiment. Days to visible bud, days to flower, and the number of flowering stems per plant were significantly affected by bulking duration and cold duration independently, but their interaction was not significant (Table 3). As cold duration increased, days to visible bud and days to flower decreased linearly (Table 4, Figure 1A). The flowering uniformity of the crop also improved with increasing cold, since the standard error for days to flower decreased. The number of nodes present per plant after cold treatment was

significantly affected by bulking duration, cold duration, and their interaction (Table 4). When each cold treatment was evaluated independently, as bulking duration increased, the number of nodes present per plant after cold treatment increased linearly by 3 to 5 nodes (Table 3, Figure 1D). The number of flowering stems per plant increased with increased cold duration (Figure 1C). In general, as bulking duration increased, days to flower decreased and the number of flowering stems increased. As bulking duration increased, a shorter cold treatment was needed for complete flowering (Figure 1B). Plants bulked for 3 weeks never reached 100% flowering, which contradicts findings of the cold-duration experiment. Plants bulked for 7 weeks flowered completely after only 4 weeks at 5 °C.

Campanula 'Birch Hybrid' has characteristics of a plant with a juvenile phase. Younger plants are less likely to flower than older plants, which would explain why plants bulked for 7 weeks had a higher flowering percentage than plants bulked for 3 weeks. However, this does not appear to be correlated with node number, since the bulking treatments, regardless of cold duration, averaged nine nodes per plant. Also, as bulking duration increased, the weeks of cold required for complete flowering decreased, implying that older plants perceive low temperatures faster than younger plants.

The longer *Campanula* 'Birch Hybrid' was bulked, the more flowering stems the plants developed. As bulking duration increased, the number of nodes per plant present after cold treatment also increased. With more nodes, there was a higher probability of producing more flowers, since flower stems develop

from the lateral meristems. The longer the cold treatment, the more flowering stems the plant developed. On average, regardless of bulking duration, the number of nodes did not vary between cold treatments. However, with increased cold duration, there was an increased likelihood that the vernalization requirement of more lateral meristems was saturated, leading to more flowering stems.

Unresolved Issues Requiring Further Research

Minimum cold requirements for *Campanula* 'Birch Hybrid' are still not definite. In the cold-duration experiment, plants bulked for 3 weeks flowered completely after 5 weeks at 5 °C. However, in the bulking-duration experiment, plants bulked for 3 weeks never completely flowered even after 7 weeks at 5 °C. The bulking-duration experiment should be replicated to validate the results of the experiment.

Table 1. Dates of forcing, average daily temperatures, and average daily light integral (DLI) from date of forcing to average date of flowering for *Campanula* 'Birch Hybrid'.

Date of forcing	Weeks of bulking	Weeks of 5 °C	Average temperature (° C)	Average DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
Cold-duration experiment				
2/1/00	3	0	--[z]	--
2/8/00	3	1	--	--
2/15/00	3	2	--	--
2/22/00	3	3	--	--
2/29/00	3	4	21.4	15.2
3/10/00	3	5	21.2	15.4
Bulking-duration experiment				
3/8/00	3	4	22.4	15.8
3/14/00	3	5	22.4	16.1
	4	4	22.4	16.1
3/22/00	3	6	22.4	16.6
	4	5	22.4	16.3
	5	4	22.5	17.7
3/29/00	3	7	22.5	15.5
	4	6	22 5	15.5
	5	5	22.6	16.6
	6	4	22.6	16.7
4/8/00	4	7	22.6	17.1
	5	6	22.8	18.6
	6	5	22.7	18.1
4/16/00	5	7	22.7	18.2
	6	6	22.8	18.7
4/26/00	6	7	23.5	18.9

[z]Dashes indicate no plants flowered.

Table 2. The effects of cold treatment on flowering of *Campanula* 'Birch Hybrid'.

Weeks of 5 °C	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Number of flowering stems
0	0	--[z]	--	--	--
1	0	--	--	--	--
2	0	--	--	--	--
3	0	--	--	--	--
4	10	49	38	87	3
5	100	41	27	68	6

[z]Dashes indicate no plants flowered.

Table 3. Significance of bulking duration and duration of cold on flowering of *Campanula* 'Birch Hybrid'.

	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Number of nodes	Number of flowering stems
Significance						
Bulking duration (BD)	***	**	NS	***	***	***
Weeks of cold (WC)	***	***	NS	***	**	***
BD X WC	*	NS	NS	NS	*	NS
3 weeks of bulking						
Weeks of cold	NS	NS	NS	NS	NS	NS
P_{Linear}	NS	NS	NS	NS	*	*
$P_{Quadratic}$	NS	NS	NS	NS	NS	NS
4 weeks of bulking						
Weeks of cold	NS	***	NS	***	*	***
P_{Linear}	NS	***	NS	***	NS	***
$P_{Quadratic}$	NS	NS	NS	NS	**	**
5 weeks of bulking						
Weeks of c old	***	**	NS	**	NS	**
P_{Linear}	***	*	NS	**	NS	**
$P_{Quadratic}$	***	NS	NS	NS	NS	NS
6 weeks of bulking						
Weeks of cold	-[z]	***	NS	***	**	**
P_{Linear}	-	***	*	***	**	**
$P_{Quadratic}$	-	**	NS	**	NS	*
4 weeks of cold						
Bulking duration	***	NS	NS	NS	***	***
P_{Linear}	**	NS	NS	NS	***	***
$P_{Quadratic}$	NS	NS	NS	NS	NS	NS
5 weeks of cold						
Bulking duration	*	*	NS	**	***	***
P_{Linear}	*	*	NS	**	***	***
$P_{Quadratic}$	NS	NS	NS	NS	NS	NS
6 weeks of cold						
Bulking duration	NS	**	NS	**	***	**
P_{Linear}	NS	*	NS	*	**	**
$P_{Quadratic}$	NS	NS	NS	NS	*	NS
7 weeks of cold						
Bulking duration	*	*	NS	**	*	***
P_{Linear}	*	NS	NS	*	*	***
$P_{Quadratic}$	NS	**	NS	**	NS	NS

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

[z]Dashes indicate no variation within treatments.

Table 4. The effects of bulking duration and duration of cold on flowering of *Campanula* 'Birch Hybrid'.

Bulking duration (weeks)	Weeks at 5 °C	Flowering percentage	Days to visible bud (VB)	Days from VB to flower	Days to flower	Number of nodes	Number of flowering stems
		84	47	14	61	9	9
	4	60	57	15	71	9	4
	5	92	50	14	64	9	9
	6	95	45	14	60	9	11
	7	88	39	14	53	8	11
3		60	53	14	67	7	4
4		95	45	14	59	10	9
5		80	47	15	62	9	8
6		100	44	14	58	10	14
3	4	30	57	14	71	8	1
3	5	70	58	14	72	7	4
3	6	80	52	15	67	7	6
3	7	60	44	15	59	6	5
4	4	80	59	14	73	9	3
4	5	100	47	15	62	11	9
4	6	100	41	15	56	10	13
4	7	100	35	15	50	9	11
5	4	30	53	17	70	9	2
5	5	100	54	15	68	9	8
5	6	100	48	14	62	9	10
5	7	90	38	15	53	8	12
6	4	100	57	15	70	11	8
6	5	100	43	14	57	12	16
6	6	100	40	13	55	10	16
6	7	100	39	13	53	9	17

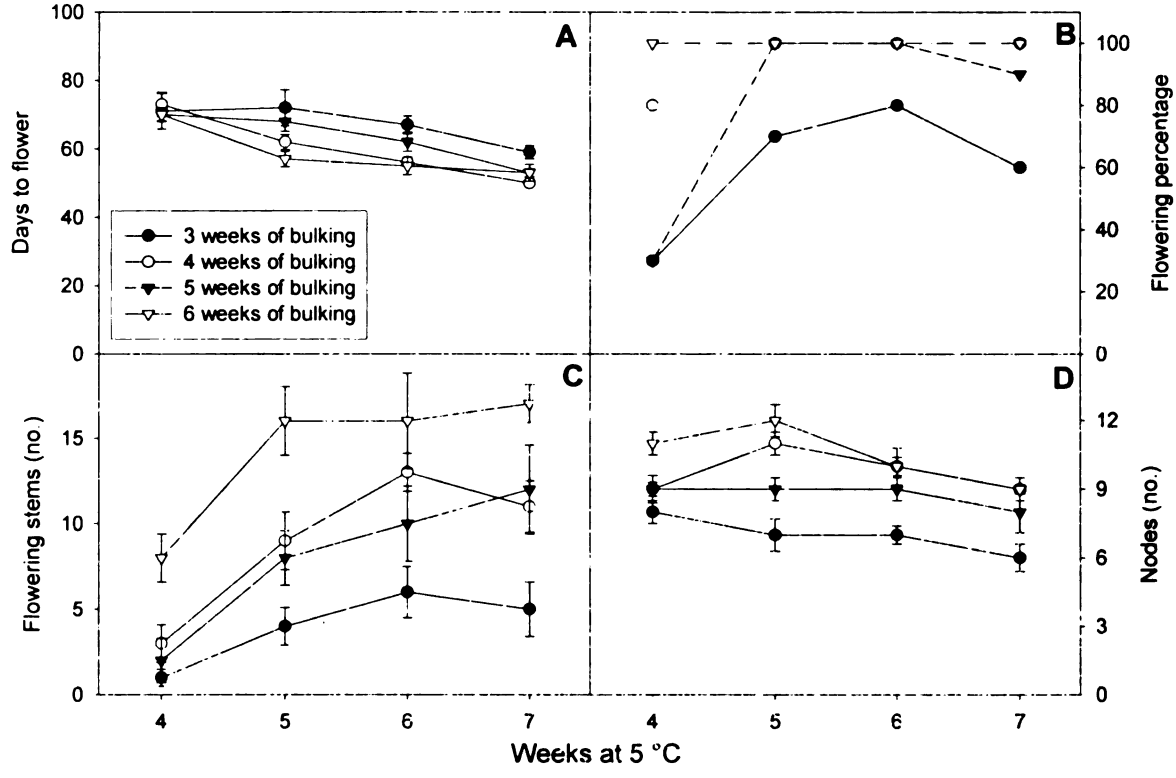

Figure 1 The effects bulking duration and duration of cold on days to flower (A), flowering percentage (B), number of flowering stems per plant (C), and the number of nodes present after cold treatment (D) for *Campanula* 'Birch Hybrid'. Error bars represent standard error of the mean.