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By

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THE EFFECTS OF PLANT AGE, PHOTOPERIOD, VERNALIZATION, AND TEMPERATURE ON THE GROWTH AND DEVELOPMENT OF *AQUILEGIA* AND *GAURA*

ABSTRACT

By

Leslie M. Finical

Studies were conducted to determine the effects of plant age, vernalization, temperature, and photoperiod on the growth and development of a select group of *Aquilegia* and one cultivar of *Gaura*. *Aquilegia* were cold treated as either plants established in 5" containers, or small seedlings. *Gaura* were cold treated only as small cuttings. Plants were cooled at 5 °C for 0, 3, 6, 9, 12, and 15 weeks then forced in a 20 °C greenhouse. The duration of cold treatment required for flowering varied for the remaining species and cultivars. To determine effects of forcing temperature after cold treatment on flowering. After a 12-week cold treatment, ten plants of each species and cultivar were grown at 14, 17, 20, 23, and 26 °C. Time-to-flower was faster at higher temperatures but plant and flower size was reduced. For plants of *Aquilegia,* leaf unfolding rates were measured at 17, 20, 23 and 26 °C, 2) plant age and number of leaves required before cold treatment for flowering, and 3) the effects of short and long photoperiods before and after cold treatment. Leaf unfolding rates were similar at all temperatures for each cultivar. Age, leaf number, temperature and photoperiod affected subsequent flowering. Plants of *Gaura* were also studied to determine response to photoperiod. After 0 and 15 weeks of cold, plants were grown under 10, 12, 13, 14, 16, 24 and NI lighting.

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CHAPTER I.
LITERATURE REVIEW

Introduction

Environmental signals such as photoperiod and temperature can strongly influence flowering patterns and induction of flowers or inflorescences in plants. Plant response to these stimuli depends upon the species and genotype of the plant. For some plants, particularly those growing in northern latitudes, a period of exposure to low temperatures below 7 °C, which is referred to as vernalization is required to induce flowering (Lang 1965).

Vernalization has been defined as a cold treatment that promotes subsequent flowering when it is given to imbibed seeds, bulbs, or whole plants (Vince-Prue, 1975). The word vernalization, named by Russian scientist Lysenko, originates from the Latin word "vernum" meaning spring (Chouard, 1960). Interest in the concept of vernalization originated from early agricultural experience with winter and spring cereals. Winter cereals needed to be planted before the end of winter in order to fruit within 12 months of sowing, whereas spring cereals flowered soon after spring sowing (Chouard, 1960). Early research concentrated on the chilling of germinated seeds of cereal grains. Treating seeds with thermoinductive temperatures was practiced frequently in Russia during the early 1900s to quicken the development of grain crops. Modern day uses of vernalization treatments include forcing of herbaceous perennials for use in the garden along with potted flowering plant production such as Easter lilies.

History of Vernalization

Many early vernalization experiments dealt with chilling germinating seeds of cereal crops such as spring and winter wheat. Much of the early work in the area of seed vernalization is credited to Klebs and Gassner in the early 1900's. One of the earliest references of the chilling of seed is that of Klippart in 1857 and even earlier records go back to the 1830s. Klippart experimented with spring and winter wheat and stated that "To convert winter into spring wheat, nothing more is necessary than that the winter wheat should be allowed to germinate slightly in the fall or winter, but kept from vegetation by a low temperature or freezing, until it can be sown in the spring...". Klippart explained his procedure for soaking and freezing the seeds, and presented the results of his experiment. Lysenko, however, is credited for reviving the interest in temperature and for defining the technique known as vernalization. The basic principles of seed vernalization have not changed since these early experiments, although new methods have been developed for the sterilization and handling of many different types of seeds.

The vernalization requirement for wheat and other cereal grains may be fulfilled while the kernels are still developing in the ear, although, in the early stages of embryo development, the vernalization response seems to be non-existent or very low. Hoogendoorn (1984) found vernalization of kernels while still on the ear advanced flower initiation faster than vernalization of kernels with a fully developed endosperm. (Hoogendoorn, 1884, cited in Krekule, 1987). For example, wheat plants with developing ears were treated with thermoinductive tempe

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temperatures at different stages following anthesis. Seeds from these treated ears were planted and flowering was observed. The developing seeds were most responsive to treatment 10-12 days after fertilization, before the embryo was mature (Krekule, 1987).

Vernalization Versus Breaking of Dormancy

Although the terms "vernalization" and "cold treatment" are frequently used interchangeably, two different phenomenons may be taking place. Vernalization is a cold treatment, but a cold treatment isn't necessarily vernalization. Vernalization, as defined above, is a cold treatment that promotes or induces subsequent flowering when given to imbibed seeds, bulbs, or whole plants. The return to warmer temperatures after the requirement for cold is fulfilled is also a necessary step in the vernalization process.

Cold temperatures promote flowering in many plants by breaking dormancy of existing buds when reproductive structures are present prior to the onset of low temperatures. Many woody perennials and flowering bulbs such as azaleas, hydrangeas, and tulips for example, form their flower buds in the summer or fall. These plants then remain dormant throughout the winter. The floral primordia of *Paeonia* are initiated soon after anthesis of the current year's flowers and require a minimum of four weeks at 5.6 °C to break dormancy (Byrne and Halevy, 1986). Full reproductive development, or anthesis, will not occur without a subsequent cold treatment. This would be considered a breaking of dormancy, not vernalization (Thomas and Vince-Prue, 1984).

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In some plants, such as *Brassica oleracea* (Brussels sprouts) and *Dicentra spectabilis*, flower differentiation occurs during the cold, or cool temperature treatment (Thomas, Vince-Prue, 1984). In *Allium cepa* (onion), and *Iris* cv. Wedgewood, low temperatures are also required during floral initiation. In *Matthiola incana*, low temperatures must continue until the buds are visible in order for development to continue upon return to warmer temperatures (Kohl, 1957). For some plants, a cold treatment will speed the process of flowering but is not necessary to initiate flowering. This is termed a facultative, or quantitative response to cold. Exposure of some species to low temperatures will induce subsequent initiation and development of floral primordia. For plants that require a vernalization treatment, flower buds will not form until the requirement for cold is fulfilled and the plants are returned to warmer temperatures.

Requirements of the Vernalization Process

For plants that absolutely require a vernalization period, no flowering will occur until the qualitative or obligate requirement for a cold treatment as been fulfilled (Vince-Prue, 1975). Cold treatments may be administered to seeds, seedlings, or older plants, depending on the species. When treating seeds, there must be sufficient imbibition of water to allow vernalization to proceed.

Lang (1965) claimed that the most effective temperatures for vernalization range from 1 to 7 °C. Duration of the cold treatment in conjunction with temperature is also a factor in the achievement of vernalization. Some species will achieve a stable vernalized state with only two or three weeks of cold treatment (Jedel et al, 1986). The vernalization requirement for some cultivars such as spring wheat is four weeks (Jedel et al, 1986), whereas the requirement for some cultivars of Astilbe is 10 or more weeks (Beattie, 1983). For plants with a vernalization requirement, the lack of an adequate period of cold results in delayed, or complete absence of flowering (Thomas and Vince-Prue, 1984).

Response to vernalization treatments also varies between different cultivars of the same species. This may be due to the differentiation of cultivars as ecotypes which have evolved under different climatic conditions. Thus, some cultivars may require lower or higher temperatures to satisfy their vernalization requirement. In a study performed by Doi, Takeda and Asahira (1984), four cultivars of *Gypsophila paniculata* L. were examined for their response to thermoinductive temperatures. 'Perfecta' was sufficiently vernalized at 10°C or below, 'Diamond','Flamingo' and 'Red Sea' all required temperatures below 5 °C.

Juvenility

For many herbaceous and woody perennial plants, a certain size or age must be reached before any treatment with thermoinductive temperatures is effective. The period of time during which plants are not responsive to external environmental stimuli is called the juvenile phase. Juvenility may be defined as the early phase of growth during which flowering cannot be induced by any treatment (Thomas and Vince-Prue, 1984). Thus, cold treatments will not induce flowerin

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flowering until the plant has made the transition to its mature phase. The duration of the juvenile phase varies from species to species. The juvenile phase may be quite short in some herbaceous perennials, lasting only days or weeks (Thomas and Vince-Prue, 1984), whereas for woody plants, the juvenile phase may last years. Apples, for instance, remain juvenile for five to seven years before becoming reproductive.

Some plants, such as *Hedera helix* L., have several distinct differences between juvenile and mature phases;

Juvenile characters	Adult characters
Three or five lobed palmate leaves	Entire, ovate leaves
Alternate phyllotaxy	Spiral phyllotaxy
Anthocyanin pigmentation of you leaves and stems	No Anthocyanin
Stems pubescent	Stem glabrous
Climbing and plagiotropic growth habit	Orthotropic growth habit
Shoots show unlimited growth and lack terminal buds	Shoots show limited growth terminated by buds with scales
Absence of flowering	Presence of flowers

(Thomas and Vince-Prue, 1984-adapted from Wareing and Frydman,1976). In winter rye, a vernalization treatment is only effective after approximately 22 leaves have developed (Purvis,1961).

Vernalization and Photoperiod

Some plants respond more strongly to a vernalization treatment if a specific photoperiod is provided in conjunction with the cold (Thomas and Vince-Prue, 1997). For example, some plants prefer long days or 24-hour lighting such as *Coreopsis verticillata*, whereas some prefer short-days, such as Chrysanthemum, before a cold treatment (Napp-Zinn, 1984).

The requirement for vernalization is commonly, but not only, found in long day plants (Thomas and Vince-Prue, 1997). Many plants that have a cold requirement also require subsequent treatment with long days, such as *Coreopsis* 'Sunray'. In some cold-requiring plants, such as *Campanula medium,* short days may substitute for, enhance, or be required instead of cool temperatures in order to achieve anthesis (Roberts et al.,1988). Flower bud initiation for young plants of *Campanula medium* to was one month faster when plants were exposed to short-days than when exposed to cool temperatures (Wellensiek, 1960). This phenomenon is sometimes referred to as short-day vernalization. The low temperature requirement for flowering in Japanese winter wheat varieties Norinn 27 and 8 is completely replaced by short-day treatments (Krekule, 1987). Providing certain photoperiods in conjunction with vernalization can also influence the effectiveness of the cold treatment. For some species, providing short-days plus night-interruption lighting, long days, or 24- hour lighting during the cold treatment, can increase the effectiveness of the vernalization (Napp-Zinn,1984).

Conversely, In *Poa pratensis* and celery plants, long-days during vernalization inhibited flower induction (Vince-Prue,1975:Thomas and Vince-Prue, 1984).

The process by which short-days induce flowering seems to be different than that of cold induction. Short days seem to be additive to sub-optimal vernalization but are usually inhibitory to fully vernalized material. An experiment that displays this inhibitory phenomenon was conducted on *Triticum aestivum* L. (Krekule, 1964). Sprouted seeds of three varieties of wheat were chilled at 2-3 ° C for 10, 20, or 30 days. After treatment, the seedlings were placed under long days (14.5-16 hr) or short days (8 hr). In all three varieties, the days to ear emergence (30 days under short day conditions) increased and dry weight decreased with increased number of short days. In two of the varieties, growth remained prostrate for the plants treated with short days. Days to ear emergence for the San Pastore variety increased from 95 days under long day conditions to 115 days under short day conditions (both treatments received 20 days of chilling) (Krekule, 1987).

De-Vernalization

De-vernalization is the reversal, by environmental conditions, of the vernalized state of a plant. The primary cause of de-vernalization seems to be high temperatures, at or above 30 °C, but short days may also substitute for high temperatures (Wellensiek, 1965). Conditions such as high temperatures and short days are most effective in causing de-vernalization when provided immediately after vernalization has occurred. Once a plant has received its' cold requirement, the vernalized condition is usually stable. *Hyoscyamus* plants which had been vernalized and subsequently kept under short days for more th

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than 190 days flowered readily once placed back into long days (Lang,1986). However, if the plant has received a less than optimal period of cold treatment, subsequent high temperatures or short days may revert the plant to a non-vernalized state (Thomas and Vince-Prue, 1984). The stabilization of wild lettuce *Lactuca serriola* is dependent upon or associated with growth during normal temperatures and light during the chilling period. The same can be said for *Arabidopsis* (Napp-Zinn,1960).

The Perennial Plant

If the vernalized condition can be transferred to all parts of the plant including buds, how does a perennial plant ensure its' perennial habit? Would not a vernalized bud go on to produce flowers and the plant remain monocarpic? This would seem logical, but perennial plants have developed mechanisms to ensure that their seeds and subsequent seedlings must be exposed to a vernalization period before flowering (Thomas and Vince-Prue,1984). In *Chrysanthemum morifolium*, some of the vernalized buds become de-vernalized during the summer. The shoots which arise from the base of the plant at the end of summer are also non-vernalized (Thomas and Vince-Prue, 1984). In some perennial grasses, the vernalized condition is not perpetuated through all cell divisions. This enables new tillers which are produced in the summer to be non-vernalized (Purvis, 1961).

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Site of vernalization perception

There are two established theories for the perception of vernalization (Lang, 1965; Purvis, 1966): the original theory was that vernalization affects leaves and results in a hormonal balance in favor of flowering (Melchers 1936, 1937). The second, more widely accepted theory, states that cold thermoinduces apical cells directly (Schwabe, 1954). Localized cooling treatments applied to certain biennial and perennial plants caused flowering when only the stem apex was chilled independent of the temperature experienced by the rest of the plant (Thomas and Vince-Prue, 1984). For other plants, it is apparent that the shoot apex is not the only site where cold temperatures are perceived. Vernalization perception in both the apex and leaves was observed in *Pisum sativum* L. by Reid and Murfet (1975).

Experiments on potential perception of thermoinductive temperatures by roots of Chinese cabbage were conducted by Reitze and Wiebe (1988). The roots were warmed in order to observe any inhibition or retardation of the vernalization process. Warm roots had no influence on the vernalization effect but soil heating decreased the effect of vernalization by heat transfer into the growing point (Riete and Wiebe, 1988). In this experiment, roots were not shown to be a primary site of cold perception.

Wellensiek (1964) proposed that dividing cells are necessary for the action of low temperatures, no matter where in the plant they occur. Experiments were conducted by Wellensiek on *Lunaria biennis* L., which is a typical cold-requiring plant. The procedure involved taking leaf and root cuttings from the plant and placing them under a range of cold treatments. On some of the leaf cuttings, the lower 0.5 centimeter of the petiole was removed from the cutting. This area of the petiole contains meristematic tissue, thus dividing cells. Flowering was not observed in the regenerated shoots on leaf cuttings without petioles. Flowering was observed in the regenerated shoots of the leaf cuttings with intact petioles. Success also seemed to rely on the age of leaves used as cuttings. The vernalization treatments were more effective for younger leaf cuttings than for fully expanded, more mature leaves (Wellensiek, 1963).

The apex, or shoot tip was found to be the site of perception of thermoinductive temperatures in the species *Thlaspi arvense* L. (Metzger, 1988). However, when untreated leaf cuttings from mature induced mother plants were examined, the leaf cuttings generated flowering shoots. The lower 1 to 2 centimeters of the petiole had been removed in order to remove any meristematic tissue from the leaf. The result of the experiment may suggest that different types of cells other than dividing cells may be capable of perceiving thermoinductive temperatures. The possibility remains, however, that there may have been meristematic tissue elsewhere in the leaf that was not removed before treatment, or that there are basic differences between species.

Mechanisms and Genetics

The term 'vernalization' can have at least two different meanings: on the one hand it is a cold treatment that induces or promotes flowering, and on the other it is the specific biochemical processes that occur during cold temperatures allo

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allowing the development of the so-called "internal conditions" that lead to flowering (Napp-Zinn, 1987). These internal conditions are controlled by both genetic and environmental factors.

Evidence suggests that some substance is formed by plants during exposure to thermoinductive temperatures. This substance can apparently be transmitted within the plant. Vernalized seeds and shoot tip may transport this substance to cells which form after the inductive period has been completed. One theory suggests that the hypothetical substance, 'vernalin', may be a gibberellin (Thomas and Vince-Prue, 1997). There are arguments for and against this theory. In *Arabidopsis*, practically all vernalization-requiring genotypes flower faster when treated with any gibberellin from GA1 to GA9 (Napp-Zinn, 1963). On the other hand, gibberellin treatment of winter wheat and other grasses does not replace their chilling requirement (Napp-Zinn, 1987).

It has been suggested that many factors such as temperature and light can enhance or delay vernalization in plants by effecting gene expression or repression. For instance, photoperiod alters the expression of the FRIGIDA gene, which is contained in certain phenotypes of *Arabidopsis* plants (Lee and Amasino, 1995). *Arabidopsis* is normally considered a quantitative long-day plant. The presence of the FRIGIDA gene causes *Arabidopsis* to behave as an "obligate" LDP, since the primary meristem of most un-vernalized FRIGIDA-containing plants fail to produce a flowering stalk under short days (Lee and Amasino, 1995).

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Vernalization requirements may be caused by dominant alleles, as in *Pisum*, or by recessive alleles, as in *Triticum* and *Arabidopsis* (Stankov, 1972). It is possible that the dominant alleles cause the synthesis of a substance which is not formed by recessive alleles. Dominant alleles which provoke a vernalization requirement may initiate the synthesis of a flower inhibitor (Napp-Zinn, 1987). This process may be reversed in plants where recessive alleles cause a vernalization requirement.

Several genes involved in vernalization have been identified. In both *Secale cereale* (Purvis, 1939), and *Hyoscyamus niger* (Lang, 1986) there is only one gene that is responsible for determining the vernalization requirement. Only one gene separates spring and winter wheat while up to 24 genes are responsible for the vernalization requirement in *Arabidopsis* plants.

Low temperatures may alter the sensitivity of the apex or shoot tip to floral initiation. Low temperatures may also inhibit the expression of certain genes which may allow for the vernalization requirement to be fulfilled. In the case of pea plants, low temperature or light can deactivate a gene that codes for a flowering inhibitor, thus allowing the induction of flowering to occur. rRNA synthesis and concentration in the plumula of winter wheat (*Triticum aestivum* L.) plants were found to be significantly different depending on the stage of vernalization (Paldi and Devay, 1982). Certain rRNA is synthesized at low temperatures but decomposes when temperatures reach 25 °C (Paldi and Devay, 1983). The rRNA is necessary for transcription and transfer of the vernalized condition through meiotic divisions. The raising of temperatures

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before rRNA has coded for the vernalized condition may then lead to incomplete vernalization or de-vernalization (Paldi and Devay, 1983). They proposed that the control of gene expression involves DNA methylation in vernalization responses. Methylation of cytosine in the promoter region of a gene may be associated with a lack of transcription of that gene. In *Arabidopsis* and cell cultures of *Nicotiana plumbaginigolia*, cold treatments resulted in a substantially reduced level of methylation. Treatment with the de-methylation agent 5-azacytidine hastened flowering in *Thlaspi arvense* and late-flowering ecotypes and mutants of *Arabidopsis*, but had no effect on flowering in mutants that do not respond to vernalization (Furn et al., 1993). Gamma rays are a presumed de-methylating agent and, in winter wheat, exposure to 5-azacytidine or to gamma rays partially substituted for the cold treatment and significantly hastened flowering (Brock and Davidson, 1994).

Conclusions

As it became clear that the vernalization responses observed in cereals may also be at work in other types of plants, the interest in treating seedlings and young plants with thermoinductive temperatures expanded to include other commercial crops. The seed experiments concentrated mainly on grain crops for the purpose of increasing crop yield and ensuring that a crop finished in a minimum amount of time. Experimentation with whole plants began after researchers felt that the area of seed vernalization had been fully explored. This opened the door to a much larger variety of plants such as potted and ornamen

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ornamental bedding plants. Over the last thirty to fifty years, many perennial plants have been studied for their responses to cold temperatures. These results can be used by commercial growers to produce plants in flower.

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CHAPTER II.

THE EFFECTS OF PLANT SIZE AND VERNALIZATION ON THE GROWTH AND FLOWERING OF *AQUILEGIA FLABELLATA* 'CAMEO', *AQUILEGIA FLABELLATA* 'MINI STAR', *AQUILEGIA XHYBRIDA* 'BLUEBIRD', 'CRIMSON STAR', 'MCKANA'S GIANT', AND 'MUSIK-WHITE'.

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Introduction

The genus *Aquilegia* is made up of about 70 species, commonly known as columbine, that are native to the temperate and mountain regions of the northern hemisphere and are (Bailey, 1976). *Aquilegia* are herbaceous perennials that bloom in spring and early summer and are best known for their dramatic spurred flowers. *Aquilegia caerulea, A. longissima*, *A. chrysantha*, *A. canadensis*, and *A. vulgaris* have been interbred resulting in numerous popular hybrids of many shapes, sizes and colors (Shedron and Weiler, 1982). The diversity of the genus provides commercial producers with many opportunities to adapt *Aquilegia* to pot-plant culture.

Vernalization has been defined as a cold treatment that promotes subsequent flowering when given to imbibed seeds, bulbs, or whole plants (Vince-Prue, 1975). For plants that require a vernalization treatment, flower buds will not form until the cold requirement is fulfilled and the plants are returned to warmer temperatures. For some plants, a cold treatment will speed the process of flowering but is not necessary to initiate flowering. This response is termed a facultative, or quantitative response to cold. Lang (1965) suggests that the most effective temperature range for vernalization of most plants is 1 to 7 °C.

It is well known that most *Aquilegia* species have an obligate vernalization requirement in order to produce flowers (Shedron and Weiler, 1982). As a result, most *Aquilegia* species and cultivars will not flower during the first season from seeds when planted in summer and fall. The vernalization

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requirement has only been studied for a few species and cultivars of *Aquilegia*, and the duration required for each species and cultivar may differ. Shedron and Weiler (1982) found that 50 to 100% of 'McKana's Giant' plants, averaging 17 leaves, flowered after being given eight and ten weeks of cold, respectively (4.5 °C) . Apparently, three weeks at 5 °C was adequate to induce flowering in *A.* atrata, *A. chrysantha*, and *A. vulgaris*, although plants were grown in an unheated greenhouse and it is unclear if additional chilling may have occurred before or after the 5 °C treatment (Masvidal et al., 1989). Merritt et al. (1997) found that two selections from the Musik series (Ernst Benary, Hanover F.R.G.), *A. xhybrida* 'Musik-Blue White' and 'Musik-Red White' did not flower without a cold treatment.

The Weddle's Songbird series was released by Weddle Seed in Palisade Colorado a number of years ago, and has since become very popular. Hybrids from the series, such as 'Bluebird', 'Blue Jay', 'Dove', 'Cardinal', and 'Robin', have large showy flowers, some of which are fragrant. In some reports, selections from the Weddle's Songbird series flowered without a cold treatment (White et al., 1990; Zhang et al., 1991). However, these studies suggest that seedlings were grown outdoors in Colorado until mid-October, possibly receiving some natural cooling before the scheduled cold treatments. One hundred percent of 'Bluebird', 'Cardinal', 'Dove', and 'Robin' plants flowered when first grown under long days from sowing until they reached 15 to 18 leaves, then cooled for six weeks (0°C) (White et al., 1989). However, only 85% and 72% of these cultivars flowered after five and four weeks of cold treatment, respectively

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(White et al., 1989). ‘Blue Jay’ and ‘Dove’ did not flower without a cold treatment when grown under any of four different day/night temperature regimens (Merritt et al., 1997).

For *Aquilegia,* cold treatments typically have not induced flowering until plants have made the transition from the juvenile to mature phase (Shedron and Weiler, 1982). Juvenility has been defined as the early phase of growth during which flowering cannot be induced by any treatment (Thomas and Vince-Prue, 1984). Plant age, or maturity, has typically been defined by the number of leaves on a given plant (Bernier et al., 1981). Crown fresh weight may also be a factor in determining when the transition from juvenile to adult occurs (White et al., 1990b). The duration of the juvenile phase varies from species to species and may be quite short in some herbaceous perennials, lasting only days or weeks (Thomas and Vince-Prue, 1984) but usually longer for *Aquilegia*. Eight-week-old plants of *A. xhybrida* ‘McKana’s Giant’, with an average of 7 leaves, never reached 100% flowering after cold treatment. However, 12-week-old plants, averaging 12 leaves, reached 100% flowering after ten weeks of cold treatment at 4.5 °C (Shedron and Weiler, 1982). Twelve-week-old plants of *A. xhybrida* ‘Fairyland’ averaging 12 leaves per plant never reached 100% flowering , but sixteen-week-old plants averaging 15 leaves reached 100% flowering after only four weeks of cold treatment (Shedron and Weiler, 1982).

There are two established theories for the perception of vernalization : The first being that vernalization affects leaves and results in a hormonal balance in favor of flowering (Melchers et al., 1936, 1937), secondly, that cold the

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thermoinduces apical cells directly (Schwabe et al., 1954). Localized cooling treatments applied to certain biennial and perennial plants caused flowering when only the stem apex was chilled independent of the temperature experienced by the rest of the plant (Thomas and Vince-Prue, 1984). For other plants, it is apparent that the shoot apex is not the only site where cold temperatures are perceived. Vernalization perception in both the apex and leaves was observed in *Pisum sativum* L. (Reid and Murfet, 1975). For *Aquilegia*, however, leaves are not necessary. In *Aquilegia,* the central growing point, not the leaf tissue, appears to perceive the cold temperatures (Shedron and Weiler, 1982). Plants of 'McKana's Giant' were cooled both with leaves under light and without leaves in darkness (Shedron and Weiler, 1982). The percent of flowering plants was not significantly different between the plants cooled with or without leaves (Shedron and Weiler, 1982).

The ability to cool small plugs of *Aquilegia*, as opposed to overwintering plants in pots, is an attractive alternative for many commercial producers. For crops that experience a long juvenile phase, such as *Aquilegia*, commercial producers must increase the vegetative growth (bulking) of the plants before they can administer cold treatments. In some cases, *Aquilegia* species are planted from plug trays to larger containers in the fall season and over wintered in the container. In cold climates, the plants in containers can be grown in unheated or minimally heated greenhouses where they receive natural cooling in the container. In mild climates, however, the average winter temperature may not be low enough to provide adequate cooling to induce flowering for many herbaceo

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herbaceous perennials and it may be necessary to cool plants in a controlled-environment chamber or facility. The ability to cool plants in a controlled-environment chamber allows the producer to provide more accurate and constant cooling and to cool plants at different times of year for staggered production schedules. A grower could shorten production time, reduce labor, and maximize space utilization if the plants could be cooled as young seedlings in plug trays as opposed to larger containers. However, the cold-temperature response of plants in plug trays versus that of plants in containers has not been well documented, nor has a direct comparison been made to overwintering the plants in containers.

The main objectives of this experiment were to 1) measure the influence of differing cold durations on flower timing, flower number, final leaf number, and plant height, 2) establish and compare cold-duration requirements for different *Aquilegia* selections and establish the scope of species and cultivar responses 3) compare the influence of differing cold durations on plants cooled in pots versus plants cooled in plug trays, and 4) determine the cold duration that induce the most complete and rapid flowering for each selection. The species and cultivars were chosen to provide a comparison between a wide range of species and popular hybridized *Aquilegia* cultivars.

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Materials and Methods

Plant material.

Seedlings of *Aquilegia alpina* L., *A. caerulea* James. , *A. canadensis* L., *A. chrysantha* A. Gray. , *A. flabellata* Siebold & Zucc. 'Cameo Blue and White' and 'Mini star', *A. xhybrida* Sims 'Bluebird' (Weddle Seed, Palisade, Colo.), *A. xhybrida* Sims 'Crimson Star', *A. xhybrida* Sims 'McKana's Giant, and *A. xhybrida* Sims 'Musik White' (Ernst Benary, Hanover F.R.G) (Bailey, 1976) were grown from seed in 72-cell plug trays (52-ml volume) during the summer of 1997, and grown outdoors by a single commercial producer (Table 1). All seedlings were shipped to MSU in plug trays on September 16, 1997. Upon arrival, half of the seedlings of *A. alpina, A. caerulea,* and 'McKana's Giant' were transplanted into 1.1-L (5" U.S.) square pots. These three were the only species older than 16 weeks upon arrival. The rest of the species and cultivars were grown to 14 weeks of age. On the transplant dates listed in Table 1, half of the plugs from each remaining species were transplanted into 1.1-L (5" U.S.) square pots. All seedlings and potted plants were grown at 20 °C under 16-hour days provided with HPS lamps before cold treatments.

Two weeks after transplanting, the plants in 1.1-L pots and the remaining seedlings in plug trays were transferred to a controlled environment chamber set at 5 °C (Table 1). Because plants were cut back before shipping, the initial leaf count on each seedling was not recorded. Instead, crown diameter for each seedling was measured before they were transferred to the cooler.

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Cold treatments.

Plants of each species or cultivar were cold-treated in a controlled-environment chamber at 5 °C for 0, 3, 6, 9, 12, or 15 weeks in the 72-cell plug trays in which they were received or in 1.1-L pots. The chamber was lit from 0800 to 1700 HR at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from cool-white fluorescent lamps (VHOF96T12; Philips, Bloomfield, N.J.), as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE). While in the cooler, plants were watered approximately two to three times per week as needed with well water acidified (H_2SO_4) to an approximate pH of 6.0. After each cold treatment, seedlings that were cooled in the plug tray were transplanted into 1.1-L pots, then all potted plants were transferred immediately to a 20 °C greenhouse. There were 10 plants of each species or cultivar per treatment.

Greenhouse environment.

Temperature control. All plants were grown in glass greenhouses set at 20 °C. Air temperatures on each bench were monitored with 36-gauge (0.013-mm-diameter) type E thermocouples connected to a CR10 datalogger (Campbell Scientific, Logan, UT). The datalogger collected temperature data every 10 seconds and recorded the hourly average. Actual average daily air temperatures from the beginning of forcing to the average date of flowering were calculated for each species and the average temperature varied from 19.9 to 21.5 °C, with most averaging 20.5 $\pm$ 0.4 °C.

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Lighting. All plants were grown under natural light plus light from high-pressure sodium lamps to extend the photoperiod to 16 hours. The greenhouse was lit from 0700 to 0800 HR and from 1700 to 2300 HR at approximately $50\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE), yielding a 16-h photoperiod. From 0800 to 1700 HR, high-pressure sodium lamps provided a photosynthetic photon flux (PPF) of approximately 50 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ at plant level when the ambient greenhouse PPF dropped to 200 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$,and discontinued when PPF reached 400 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$.

Media and watering

Plants were grown in a commercial soilless medium composed of composted pine bark, horticultural vermiculite, Canadian sphagnum peat moss, processed bark ash, and washed sand (MetroMix 510, Scotts-Sierra Horticultural Products Company, Marysville, Ohio). Plants were top-watered daily as needed with well water acidified (two parts H_3PO_4 plus one part H_2SO_4, which provided $\approx$2.5 mol $P{\cdot}m^3$) to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and fertilized with 14N-0P-6K_2O ($mol{\cdot}m^{-3}$) from potassium nitrate (14-0p-55K_20) (Vicksburg Chemical Co., Vicksburg, MS) and ammonium nitrate (34N-0P-0K_2O) (Cargill, Lexington, KY). Fertilization and acidification rates were adjusted in response to weekly soil test results, so regimens varied during experiments. The target range for soil pH was 5.8 to 6.2 and, and 0.5 to 1.2 for soil EC.

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Data collection and analysis.

Crown diameter was measured with calipers when the plants were transferred to the cooler. For each plant that flowered, the date of the first visible bud and date of the first fully-expanded flower were recorded. Plants that did not have visible buds after 15 weeks of forcing were discarded and considered nonflowering. At flowering, the visible flower bud number, the leaf number on the main stem below the first flower, and total plant height were measured. Days to visible bud, days from visible bud to flower, and days to flower were calculated. Data were analyzed using SAS's (SAS Institute, Cary, NC) analysis of variance and general linear models procedures.

Results and Discussion

A. caerulea, 'Crimson Star', 'McKana's Giant', and 'Musik White' did not flower under any treatment. Only 10% of *A. alpina* pot-cooled plants flowered after each cold treatment, while no plug-cooled plants of *A. alpina* flowered. Therefore, no data for these five selections are presented. Based on the ages of these seedlings and information from previously cited studies, we expected these plants to flower and we have no explanation for why they did not. It may be possible that the sowing dates received from the commercial producer were erroneous.

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Flowering Percentage

'Cameo', was the only cultivar tested that attained 100% flowering without a cold treatment (Table 2, Figure 1). Thirty percent of 'Mini star' plants flowered without cold. We presumed that some of the un-cooled plugs would flower as well and have no explanation for why they did not (Table 3, Figure 1). No plants of 'Bluebird', *A. canadensis*, or *A. chrysantha*, flowered without a cold treatment (Tables 4, 5, 6, Figure 1). With increased cold treatment duration, flowering percentage increased for all species studied whether cold was given to plants in pots or in plugs.

Flowering percentage for all species was higher for pot-cooled than for plug-cooled plants. The difference in flowering percentage between the pot-cooled plants and plug-cooled plants was greatest for plants of 'Bluebird' (Figure 1). Pot-cooled plants of 'Bluebird' reached 100% flowering after only three weeks of cold treatment, while plug-cooled plants never reached 100% flowering. Flowering percentage for both pot-cooled and plug-cooled plants of *A. canadensis* increased significantly as the duration of cold treatment increased, but never reached 100% (Table 5, Figure 1). Flowering percentage was significantly higher for the pot-cooled than for the plug-cooled plants of *A. chrysantha*. Pot-cooled plants of *A. chrysantha* reached 40% after six weeks of cold, and 80% after nine weeks of cold, whereas the plug-cooled plants reached only 60% after fifteen weeks of cold (Table 6, Figure 1).

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Flower Timing

Days to visible bud and days to flower significantly decreased as cold treatment duration increased from 0 to 15 weeks for both pot-cooled and plug-cooled plants for all species. In all cases, the linear contrasts for the decrease were highly significant. The most dramatic change in time to visible bud and flowering was for plants of *A. canadensis*. For *A. canadensis,* the days to visible bud and days to flower decreased by more than four weeks for both pot-cooled and plug-cooled plants (Table 5, Figure 1). However, in some cases the data points were based on only one or a small number of flowering plants. For example, only 10% of plug-cooled plants flowered after 6 and 12 weeks of cold (Figure 1). For plants of 'Cameo', 'Mini star', and 'Bluebird', there was a 50 to 70% reduction in days to visible bud, and a 30 to 50% reduction in days to flower as the duration of cold treatment increased from 0 to 15 weeks (Tables 2, 3, 4, Figure 1). Days to visible bud were reduced by about 17 to 21 days, and days to flower were reduced by about 14 to 21 days for 'Cameo', 'Mini star', and 'Bluebird' (Tables 2, 3, 4, Figure 1). Time-to-flower for plants of *A. chrysantha* decreased by only two weeks with increasing cold treatment duration for pot-cooled plants and less than one week for plug-cooled plants with increasing cold-treatment duration (Table 6, Figure 1).

Days to visible bud and days to flower were generally lower for plants transplanted and grown in pots for two weeks prior to the beginning of the cold treatment (Tables 2, 3, 4, 5, 6, Figure 1). Pot-cooled plants of *A. canadensis* consistently bloomed about 17 days earlier than the plug-cooled plants, independent of cold duration (Table 5, Figure 1). Pot-cooled plants of both 'Bluebird' and 'Cameo' consistently flowered about 7 days faster, while pot-cooled plants of *A. chrysantha* bloomed only a few days faster than plug-cooled plants. There was little, if any, difference in timing for plants of 'Mini star', particularly after long cold treatment durations (Table 4).

Days from visible bud to flower

There were statistically significant differences between days fromvisible bud to flower for 'Mini star', 'Bluebird', *A. canadensis*, and *A. chrysantha* in response to cold treatment, although the actual numerical decreases were small and no trends were observed (Tables 3-6). Time from visible bud to flower ranged from 10 to 16 days for plants of 'Cameo' and 'Mini star' to 15 to 20 days for *A. canadensis* and *A. chrysantha*. In the case of 'Cameo', days from visible bud to flower increased with increased cold duration and there were significant linear and quadratic contrasts (Table 2). For un-cooled plants of 'Cameo' the first flower bud opened at the base of the leaf axis, whereas with increasing cold there was increased peduncle elongation prior to flower opening. This physiological response to cold may have accounted for the increase in days from visible bud to flower for 'Cameo'.

There were no significant differences in days from visible bud to flower between the pot-cooled and plug-cooled plants of 'Cameo', 'Mini star', *A. canadensis*, and *A. chrysantha*.

There was a highly significant effect on days from visible bud to flower due to interaction between plant size (pot or plug) and weeks of cold for plants of 'Mini star' and 'Bluebird', but actual numerical differences were slight and no trends were observed (Tables 3 and 4).

Flower Number

In general, cold increased flower number for all species and cultivars studied (Figure 2). The total flower number for plants of 'Cameo' increased 2 to 3 times for both pot-cooled and plug-cooled plants as the duration of cold treatment increased from 0 to 15 weeks (Table 2, Figure 2). There was no significant increase in flower number for plants of 'Mini star' (Table 3). The flower number for 'Bluebird' and *A. chrysantha* increased approximately 160-180% for pot-cooled plants, and 120-130% for plug-cooled plants as the duration of cold increased from 3 to 15 weeks (Table 4 and 6, Figure 2). Flower number of the plug-cooled plants of *A. canadensis* increased by 320% (Table 5, Figure 2). Conversely, flower number for the pot-cooled plants of *A. canadensis* decreased by 25% as cold treatment increased from 3 to 15 weeks (Table 5 and Figure 2). However, this decrease is complicated the low number of flowering plants, particularly at shorter cold treatment durations (Figure 2).

The effects of plant size and cold on flower number varied between the species. Overall, pot-cooled plants had higher flower numbers. The most dramatic difference between pot- and plug-cooled plants was observed for 'Cameo', 'Mini star', and *A. chrysantha*.

For plants of *A. canadensis* and 'Bluebird', there were no significant differences in flower number between the pot-cooled and plug-cooled plants (Tables 4 and 5).

Final leaf number

Cold had a significant effect on final leaf number for all selections tested (Tables 2-6). In some cases, however, the actual differences were small. Final leaf number for plants of 'Cameo' and 'Bluebird' decreased by about one to two leaves for both the pot-cooled and plug-cooled plants (Tables 2 and 4, Figure 2). For plants of 'Mini star', the final leaf number for pot-cooled plants decreased by three leaves and by seven leaves for plug-cooled plants as cold increased from 0 to 15 weeks (Table 3, Figure 2). Final leaf number for pot-cooled plants of *A. chrysantha* and *A. canadensis* decreased by eight and ten leaves, respectively. Final leaf number for the plug-cooled plants of *A. canadensis* decreased by two or three leaves but did not change at all for plug-cooled plants of *A. chrysantha* as cold treatment increased from 0 to 15 weeks (Tables 5 and 6, Figure 2).

Leaf number at first flower was often lower for the plug-cooled plants, but the actual differences between plug- and pot- cooled plants were small. For plants of 'Cameo' and 'Bluebird', the pot-cooled plants consistently had one to two more leaves at flowering than the plug-cooled plants (Tables 2 and 4). Pot-cooled plants of *A.* canadensis had a significantly higher leaf number than plug-cooled plants, although some of the data points are based on a low number of flowering plants (Table 5). The decrease in final leaf number of *A. chrysantha* foll

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followed a quadratic pattern as the cold duration increased (Table 6). The biggest difference between pot-cooled and plug-cooled plants was observed for 'Mini star' (Table 3 and Figure 2). After 12 and 15 weeks of cold, pot-cooled 'Mini star' plants had almost 50% more leaves than the plug-cooled plants (Table 3, Figure 2).

Plant height

Cold treatment duration had a significant but moderate effect on plant height at first flower. Height tended to decrease as the duration of cold increased, but the differences were generally small. Increased cold treatment reduced plant height by about two to three centimeters for 'Cameo' and 'Mini star' plants (Tables 2 and 3, Figure 2). For plants of 'Bluebird' and *A. chrysantha*, increased cold treatment reduced height for pot-cooled plants by 6 and 10 centimeters, respectively. Cold reduced the height of plug-cooled plants of 'Bluebird' and *A. chrysantha* by 3 or 4 centimeters (Tables 4 and 6, Figure 20). For plants of *A. canadensis*, a longer cold treatment increased plant height (Table 5).

On average, the pot-cooled plants of 'Cameo' and 'Mini star' were two to three centimeters taller than the plug-cooled plants (Tables 2 and 3, Figure 2). There was no significant difference in height between the pot-cooled and plug-cooled plants of *A. xhybrida* 'Bluebird' and *A. chrysantha* and there was no significant interaction between plant size (pot or plug) and weeks of cold for either species (Table 4 and 5, Figure 2). Statistically, there was a difference

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between the pot-cooled and plug-cooled plants of *A. chrysantha*, although the difference was only noted after the 12-week cold treatment. For plants of *A. canadensis*, there were variations in height between cold treatments.
When only the 9-, 12-, and 15 week cold treatments are compared, the pot-cooled plants were consistently taller than the plug-cooled plants.

Crown diameter

No trends were observed for crown diameter in relationship to flowering (averages in Table 7). Some plants with seemingly small crown diameters flowered, whereas some with larger crown diameters did not flower. No other information on crown diameter of *Aquilegia* was found in the literature. Crown diameters were measured because the plugs had been cut back before shipping by the grower, thus making it impossible to count the leaves.

Conclusion

Not only is there great diversity in plant and flower morphology between species of the genus *Aquilegia*, but there is also extensive diversity in the types of habitats that they occupy, including warm-temperate forests, extremely high altitude alpine zones, and desert springs (Hodges and Arnold, 1994a). Thus, it is not surprising that there should be differences in how different species respond to environmental signals such as cold temperatures. A vernalization treatment is not required for flowering of 'Cameo' or 'Mini', but is horticulturally beneficial for both cultivars. Both 'Mini star' and the 'Cameo' series (released

during 1998 by Benary Seed), are cultivars of the species *Aquilegia flabellata*, native to the alpine regions of Japan (Bailey, 1976). It was unexpected that an alpine species such as *A. flabellata* would flower without vernalization, whereas *A. chrysantha*, a species native to much warmer areas such as Arizona and New Mexico, required a lengthy cold treatment in order to flower. *A. canadensis*, a species that has a broad habitat range from Nova Scotia to Texas, also had an obligate cold requirement.

Aquilegia species remain largely inter-fertile, which suggests that, overall, they may be genetically very similar (Hodges and Arnold, 1994a). The name *A. xhybrida* was originally given to hybrids of *A. canadensis* and *A. vulgaris*, but is more often used for long-spurred garden hybrids with a parentage involving *A. caerulea*, *A. chrysantha*, and possibly others (Bailey, 1976). In this study we were not able to determine cold requirements of the alpine species *A. caerulea*, except that it must require cold because no plants flowered in our experiment. *A. chrysantha* also had an obligate requirement for cold and needed at least six weeks of cold for flowering. One would expect that the traits for vernalization response would be heritable to offspring. *A. xhybrida* 'Bluebird', a hybrid with parentage involving *A. caerulea* and *A. chrysantha*, required only three weeks of cold for 100% flowering.

According to previous studies by Shedron and Weiler (1982), White, et al. (1990), and Zhang et al. (1991), and based on the presumed ages of our seedlings, we felt confident that plants of all species in the study would flower. However, five of the species flowered poorly or did not flower at all. According to

Shedron and Weiler (1982), 100% of 'McKana's Giant' plants flowered after ten weeks of cold with an average of 12 leaves before cold, and 100% of 'Crimson Star' plants flowered after eight weeks of cold with an average of 15 leaves before cold. Both cultivars were 16 weeks of age when cooled in this study. Based on the literature, some plants of 'McKana's Giant' and 'Crimson Star' in the present study should have flowered based on the age of the plants, 24 and 16 weeks, respectively. No plants of the cultivar 'Musik-White' flowered under any cold treatment, and there is little information published on selections from the Musik series (Benary, Hann. Muenden, F.R., Germany). Merritt et al. (1997) chilled seeds of 'Musik- Blue White' and 'Musik- Red White', then forced the seedlings under four different day/ low-night temperature regimens. No plants of either cultivar flowered in their experiment.

If juvenility is not the cause for poor flowering of selections in this study, one possible hypothesis is that defoliated plugs of *Aquilegia* may not respond similarly to chilling. However, this seems unlikely due to evidence from previously cited work by Shedron and Weiler (1982) where cooling had no deleterious effects on subsequent flowering of defoliated plants. Another possible hypothesis is that high temperatures while plugs were grown outdoors may have been detrimental to the seedlings. High temperatures before cold treatment reduced flowering for plants studied in chapter 3.

Based on studies by White et al. (1989, 1990) and Zhang et al. (1991), we expected plants from the Weddle's Songbird series to flower without a cold treatment. White and co-workers reported in 1990 and 1991 that plants of

'Bluebird', 'Dove', 'Purple', and 'Robin' flowered five months after seeding without exposure to low temperatures. However, in our experiments, no plants of 'Bluebird' flowered without a cold treatment. We also received seedlings of 'Cardinal' and 'Dove' for this experiment but there were only enough plants of each cultivar for one treatment, so the plants were grown without a cold treatment. After 100 days, no plants of either 'Cardinal' or 'Dove' flowered. Our seedlings were sown in the summer months of 1997 and received on September 16, 1997. The seedlings were then placed in our greenhouses set at 20 °C under long-day photoperiods of 16 hours. The seedlings used in the studies by White and Zhang had been grown outdoors and were shipped to Pennsylvania State University in mid-October. It is possible that the plants used in the studies by White and Zhang may have received some natural cooling before forcing

There is little published information on flower timing for species and cultivars of *Aquilegia*. According to Shedron and Weiler (1982), 50% of 'McKana's Giant' plants flowered after an average of 165 days without a cold treatment under a 10-h photoperiod and after an average of 118 days under 18-h of light. After ten weeks of cold, 100% of plants flowered under short days after average of 76 days and after 70 days under long-days. Days from visible bud to flower for plants of 'McKana's Giant' generally decreased as the duration of cold treatment increased, but was variable in all experiments (Shedron and Weiler, 1982). According to White et al. (1989), 'Bluebird' seedlings cooled for a total of six weeks at temperatures from -1.5 °C and 9 °C, then forced at a greenhouse temperature of 15.5 °C under 24 hours of light from HPS lamps, reached visible bud after 29 days and flowered after 45 days. When the cooled plugs were forced at the same temperature under natural short-days in December, plants reached visible bud after 40 days and flowered after 51 days. As the duration of cooling increased, the days from visible bud to flower remained about the same (White et al., 1989). The timing for 'Bluebird' in White's study is similar to the timing of 'Bluebird' seedlings in our experiment that were cooled for six weeks. Our seedlings reached visible bud after 24 days and flowered after 40 days.

White et al. (1990) reported that increased duration of cold actually delayed the days to visible bud for plants of 'Bluebird' and 'Robin'. However, their plants had already initiated flower buds before they were cold treated, thus, the cold temperatures were causing bud abortion or delayed further flower bud development rather than affecting flower initiation. Zhang et al. (1990) reported that there was no effect of cold on days to visible bud or flower for plants of 'Purple' and 'Dove', however, plants had already initiated flower buds before they were cold treated, therefore, no comparative flower timing can be extrapolated from these two studies. Overall, increased cold durations decreased the time to visible bud and first flower for all flowering species in the present study. Cold duration recommendations for each flowering species and cultivar in the present study are listed in Table 8.

There is little information on the effects of vernalization duration on flower number, leaf number, and plant height of *Aquilegia* at the time of flowering. According to White and Zhang, there was a decrease in flower bud number and no effect on plant height of *Aquilegia* plants with increasing durations of cold treatment. However, because flower buds initiated before cold in both of these studies, the results cannot be compared to our results. Our experiments showed an overall increase in flower bud number and an overall decrease in plant height at flowering with increased cold duration, and, to our knowledge our plants had not initiated flower buds before they were cold-treated.

All the flowering species performed better when cooled in pots as opposed to plugs in this study. Pot size in conjunction with bulking time could be an important factor in flowering *Aquilegia.* One explanation for why Shedron and Weiler (1982) were able to flower *Aquilegia* cultivars such as 'McKana's Giant' and 'Crimson Star', and we were not, may be pot size. The plug-cooled plants in our experiment were grown to the same age (16 weeks) as those in Shedron and Weiler's study, but our seedlings were grown in 50 ml cells (72-cell plug trays). Our pot-cooled plants were transplanted from plug trays into 1.1-L (5" U.S.) square pots and grown for only two weeks before cold treatment. The plants in Shedron and Weiler's study were transplanted into 7.5 or 10 cm diameter (approx. 350 to 470 ml) pots and grown for 4, 8, and 12 additional weeks before cold treatment. It is possible that not only age or leaf number determines the change from juvenile to mature stage in *Aquilegia*, but also root growth and increased nutrient storage prior to vernalization.

Shedron and Weiler (1982) proposed production of *Aquilegia* in small cell packs (48 cells per 28 x 53-cm flat). Plants would be transplanted directly into the cell pack, grown to maturity, vernalized, forced, and sold in the same pack. Due to the low flowering percentages of the plug-cooled plants of most species tested in the present study, the production of these cultivars in 72-cell trays prior to forcing does not seem feasible, at least not at the ages we tested. The exception would be 'Cameo', which could be cooled in 72-cell trays, then transplanted into pots, and grown to flowering for sale on specific dates. It may be that the other species tested could be produced from cooled plugs as well, perhaps if they were grown in a larger plug size or for a longer period of time before a cold treatment.

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Table 1. Ages, sowing dates, transplant dates, and transfer dates for the *Aquilegia* species used in the experiment. All of the seedlings were grown in 72-cell plug trays (each cell equals 50ml volume). The seedlings were shipped to MSU on September 16, 1997 and grown under a 16-h photoperiod at 20°C. On the transplant dates below, half of the plugs from each species were transplanted into 1.1-L (5" U.S.) square pots. Two weeks later, the remaining seedlings in the 72-cell plug trays and the plants in the 5" pots were transferred to a 5 °C cooler for cold treatment. The plants that were older than 14 weeks upon arrival were transplanted the day after they were received.

Species	Date sown	Date transplanted to pots	Cold treatments started	Age of plants when moved to cooler
Aquilegia alpina	4/9/97	9/17/97	9/30/97	25 wks
Aquilegia caerulea	5/21/97	9/17/97	9/30/97	18 wks
Aquilegia canadensis	6/8/97	9/23/97	10/08/97	16 wks
Aquilegia chrysantha	6/18/97	10/3/97	11/17/97	16 wks
Aquilegia flabellata 'Cameo Blue & White'	8/3/97	11/20/98	12/5 /97	16 wks
Aquilegia flabellata 'Mini star'	6/17/97	10/3/97	11/17/97	16 wks
Aquilegia xhybrida 'Crimson Star'	6/18/97	10/3/97	11/17/97	16 wks
Aquilegia xhybrida 'McKana Giant''s g	4/9/97	9/17/97	9/30/97	25 wks
Aquilegia xhybrida Musik 'White'	6/11/97	9/26/97	10/12/97	16 wks
Aquilegia xhybrida Songbird 'Bluebird'	7/1/97	10/16/97	11/1/97	16 wks

Table 2. Regrowth and flowering responses of *Aquilegia flabellata* 'Cameo Blue and White' following 0 to 15 weeks at 5 °C. Plants were cooled in 72-cell plug trays or in 1.1-L (5" U.S.) square pots. Following cold treatment, plants were grown at 20 °C under 16-h photoperiods. Flower number, final leaf number, and final plant height were measured at first open flower.

	Weeks of 5 °C	Percent flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Final leaf number	Final plant height (cm)
Pots	0	100	29	10	38	4	11	13
	3	100	17	12	29	5	10	9
	6	100	13	11	24	6	10	11
	9	100	11	14	25	8	10	11
	12	100	7	15	23	12	11	11
	15	100	8	15	23	12	9	10
Plugs	0	60	35	10	45	3	9	10
	3	70	23	11	34	5	8	7
	6	90	23	12	35	5	9	9
	9	100	18	14	33	6	9	9
	12	100	15	13	28	7	9	8
	15	100	15	16	31	8	8	9
Significance								
Pot or plug			***	NS	***	***	*	***
Weeks of cold			***	***	***	***	**	***
Pot or plug x Weeks of cold			NS	NS	NS	*	NS	NS
Contrasts								
0, 3, 6, 9, 12, and 15 weeks 5C								
P_{Linear}			***	***	***	***	*	NS
$P_{Quadratic}$			***	***	***	***	**	*

NS, *, **, *** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively
[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 3. Regrowth and flowering responses of *Aquilegia flabellata* 'Mini star' following 0 to 15 weeks at 5 °C. Plants were cooled in 72-cell plug trays or in 1.1-L (5" U.S.) square pots. Following cold treatment, plants were grown at 20 °C under 16-h photoperiods. Flower number, final leaf number, and final plant height were measured at first open flower.

	Weeks of 5 °C	Percent flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Final leaf number	Final plant height (cm)
Pots	0	30	28	14	42	3	18	14
	3	80	29	11	40	5	18	15
	6	100	19	14	33	8	16	15
	9	100	19	13	32	7	12	14
	12	100	18	16	34	9	15	15
	15	100	10	14	24	7	15	15
Plugs	0	0	-	-	-	-		-
	3	60	36	11	51	4	17	15
	6	80	23	15	44	4	15	12
	9	80	22	13	35	5	10	14
	12	90	17	11	28	3	8	11
	15	70	10	13	24	5	9	10
Significance								
Pot or plug			NS	NS	NS	***	***	NS
Weeks of cold			***	**	***	NS	***	***
Pot or plug x Weeks of cold			NS	***	**	NS	**	*
Contrasts								
0, 3, 6, 9, 12, and 15 weeks 5C								
P_{Linear}			***	NS	***	***	**	NS
$P_{Quadratic}$			***	NS	***	*	**	NS

NS, *, **, *** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively
[1] Plants that were not in bud after 100 days were considered nonflowering.

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Table 4. Regrowth and flowering responses of *Aquilegia xhybrida* 'Bluebird' following 0 to 15 weeks at 5 °C. Plants were cooled in 72-cell plug trays or in 1.1-L (5" U.S.) square pots. Following cold treatment, plants were grown at 20 °C under 16-h photoperiods. Flower number, final leaf number, and final plant height were measured at first open flower.

Weeks of 5 °C		Percent flowering[1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Final leaf number	Final plant height (cm)
Pots	0	0	-	-	-	-	-	-
	3	100	30	15	46	10	17	41
	6	100	16	19	35	14	17	37
	9	100	17	16	32	15	15	33
	12	100	12	13	24	15	15	32
	15	100	9	15	25	16	16	35
Plugs	0	0	-	-	-	-	-	-
	3	10	34	17	50	11	15	39
	6	30	24	16	40	15	12	35
	9	40	26	17	43	15	15	31
	12	30	17	16	33	15	14	33
	15	20	17	14	31	11	13	36
Significance								
Pot or plug			***	NS	***	NS	***	NS
Weeks of cold			***	***	***	**	*	***
Pot or plug x Weeks of cold			NS	**	NS	NS	*	NS
Contrasts								
0, 3, 6, 9, 12, and 15 weeks 5C								
P_{Linear}			***	NS	***	NS	NS	NS
$P_{Quadratic}$			***	NS	***	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively
[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 5. Regrowth and flowering responses of *Aquilegia canadensis* following 0 to 15 weeks at 5 °C. Plants were cooled in 72-cell plug trays or in 1.1-L (5" U.S.) square pots. Following cold treatment, plants were grown at 20 °C under 16-h photoperiods. Flower number, final leaf number, and final plant height were measured at first open flower.

Weeks of 5 °C		Percent flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Final leaf number	Final plant height (cm)
Pots	0	0	-	-	-	-	-	-
	3	10	46	17	63	20	24	15
	6	10	38	17	55	17	19	22
	9	40	29	19	48	16	12	49
	12	70	25	17	42	14	13	47
	15	70	17	16	33	15	14	38
Plugs	0	0	-	-	-	-	-	-
	3	20	60	17	77	5	15	41
	6	10	56	17	73	9	16	38
	9	20	47	18	65	16	12	34
	12	10	49	16	65	14	12	34.
	15	40	31	15	45	16	13	31
Significance								
Pot or plug			***	NS	***	*	**	*
Weeks of cold			***	***	***	**	***	*
Pot or plug x Weeks of cold			***	NS	***	***	*	**
Contrasts								
0, 3, 6, 9, 12, and 15 weeks 5C								
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

[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 6. Regrowth and flowering responses of *Aquilegia chrysantha* following 0 to 15 weeks at 5 °C. Plants were cooled in 72-cell plug trays or in 1.1-L (5" U.S) square pots. Following cold treatment, plants were grown at 20 °C under 16-h photoperiods. Flower number, final leaf number, and final plant height were measured at first open flower.

Weeks of 5 °C		Percent flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Final leaf number	Final plant height (cm)
Pots	0	0	-	-	-	-	-	-
	3	0	-	-	-	-	-	-
	6	40	37	20	57	15	28	48
	9	60	27	17	43	15	24	45
	12	80	25	20	45	20	19	39
	15	80	24	15	39	27	20	38
Plugs	0	0	-	-	-	-	-	-
	3	0	-	-	-	-	-	-
	6	0	-	-	-	-	-	-
	9	40	32	19	51	15	19	45
	12	40	29	20	48	17	19	52
	15	60	27	18	44	18	19	41
Significance								
Pot or plug			***	NS	***	***	*	***
Weeks of cold			***	***	***	***	**	***
Pot or plug x Weeks of cold			NS	NS	NS	*	NS	NS
Contrasts								
0, 3, 6, 9, 12, and 15 weeks 5C								
P_{Linear}			***	***	***	***	*	NS
$P_{Quadratic}$			***	***	***	***	**	*

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

[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 7. The average crown diameter measured in millimeters for each species before cold treatment. The diameter of each seedling was measured using calipers before they were moved to the cooler. There were no significant trends in flowering associated crown diameter before cold for any of the species or cultivars studied.

Cold treatments given after measurements were taken		*Aquilegia canadensis*	*Aquilegia chrysantha*	*Aquilegia flabellata* 'Cameo'	*Aquilegia flabellata* 'Mini star'	*Aquilegia xhybrida* 'Bluebird'
Pots	0	9.7	9.8	10.6	7.8	13.4
	3	11.6	8.5	9.9	9.1	11.1
	6	10.4	10.0	9.7	9.1	10.8
	9	8.7	9.6	9.5	8.1	11.4
	12	9.5	9.6	9.3	8.6	11.1
	15	8.8	9.2	9.1	8.1	10.8
Plugs	0	9.7	9.2	8.9	8.2	12.9
	3	10.8	9.5	9.1	8.6	12.5
	6	9.8	8.7	9.0	7.8	10.6
	9	10.6	9.7	8.9	8.5	10.5
	12	9.3	8.9	9.3	8.9	9.4
	15	9.8	9.4	9.2	8.3	10.9

Table 8. The recommended cold treatment durations for all flowering species tested.

Species	Shortest duration of cold required for 100% flowering		Recommendations
	Plug-cooled	Pot-cooled	
Aquilegia flabellata 'Cameo Blue & White'	9 weeks	0 weeks	9-12 weeks at 5 °C in pots or plugs. Cold not required, but will quickened flowering and increased flower number
Aquilegia flabellata 'Mini star'	100% flowering never reached	6 weeks	9 weeks at 5 °C in pots . Cold increased uniformity and quickened time to flower
Aquilegia xhybrida 'Bluebird'	100% flowering never reached	3 weeks	3 to 6 weeks at 5 °C in pots.. 3 weeks of cold was enough for uniform flowering, but a longer duration quickened flowering
Aquilegia canadensis	100% flowering never reached	100% flowering never reached	$\geq$ 12 weeks at 5 °C in pots. Flowering % increased with increased duration of cold treatment, and flowering time decreased
Aquilegia chrysantha	100% flowering never reached	100% flowering never reached	$\geq$ 12 weeks at 5 °C in pots. Flowering % increased with increased duration of cold treatment, and flowering time decreased

Figure 1. Effect of cold duration at 5 °C on percent flowering, days to visible bud, and days to flower on plants of *Aquilegia flabellata* 'Cameo Blue and White', *A. flabellata* 'Mini star', *A. xhybrida* 'Bluebird', *A. canadensis*, and *A. chrysantha*. Plants were cooled either in 1.1-L (5" U.S.) square pots (○) or in 72-cell plugs (△).

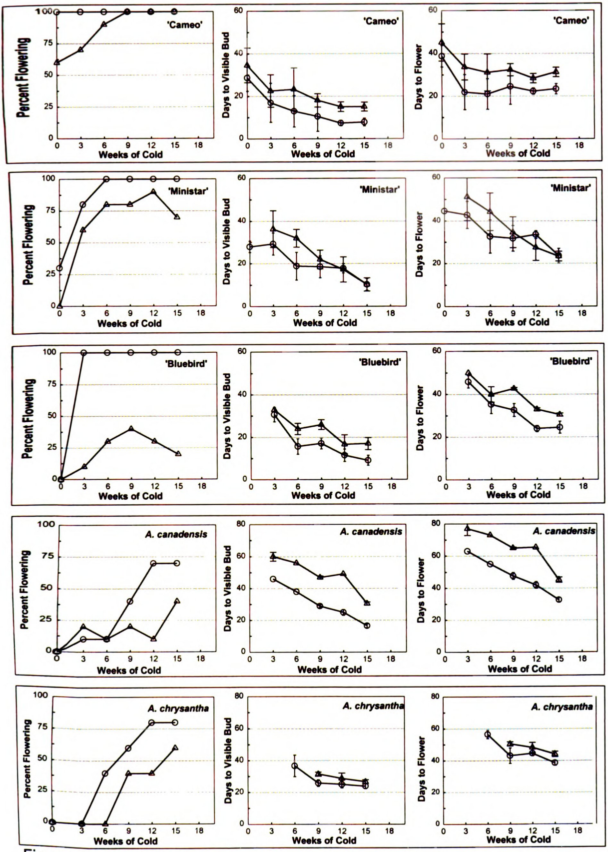
'Cameo'
'Ministar'
'Bluebird'
A. canadensis
A. chrysantha
Percent Flowering
Days to Visible Bud
Days to Flower
Weeks of Cold
Figure 1.

Figure 2. Effect of cold duration at 5 °C on total plant height, final leaf number, and flower number on plants of *Aquilegia flabellata* 'Cameo Blue and White', *A. flabellata* 'Mini star', *A. xhybrida* 'Bluebird', *A. canadensis*, and *A. chrysantha*. Plants were cooled either in 1.1-L (5" U.S.) square pots (○) or in 72-cell plugs (△).

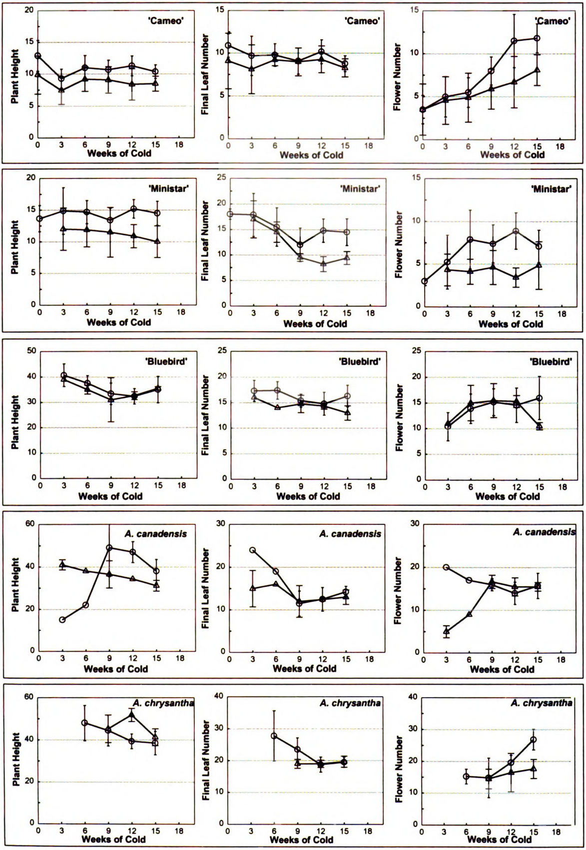
'Cameo'
'Ministar'
'Bluebird'
A. canadensis
A. chrysantha
Plant Height
Final Leaf Number
Flower Number
Weeks of Cold

Figure 2.

CHAPTER III.

THE EFFECTS OF FORCING TEMPERATURE ON THE GROWTH AND FLOWERING OF *AQUILEGIA CANADENSIS, AQUILEGIA FLABELLATA* 'CAMEO' AND 'MINI STAR', AND *AQUILEGIA XHYBRIDA* 'BLUEBIRD'

Introduction

Aquilegia, or columbine, are herbaceous perennials belonging to the Ranunculaceae family. There are about 70 species of *Aquilegia* native to temperature regions around the globe. *Aquilegia* are well know wild flowers, and have also been intensely cultivated for use in the perennial border, as potted flowering plants, and as cut flowers (Ball, 1991). *Aquilegia caerulea, A. longissima*, *A. chrysantha*, *A. canadensis*, and *A. vulgaris* have been interbred resulting in many popular hybrids of various shapes, sizes and colors. The diversity of the genus provides commercial producers with many opportunities and challenges to adapt *Aquilegia* to pot-plant culture.

It is well known that most *Aquilegia* species require some duration of cold in order to produce flowers (Shedron and Weiler, 1982). Most *Aquilegia* species and cultivars will not flower during the first season from seeds planted in the fall. For most species of *Aquilegia*, cold treatments will not induce flowering until the plant has made the transition from its juvenile to mature phase (Shedron and Weiler, 1982). The duration of the juvenile phase varies from species to species and may be quite short in some herbaceous perennials, lasting only days or weeks (Thomas and Vince-Prue, 1984), but can be quite long for *Aquilegia*. For example, seedlings of *A. xhybrida* 'McKana's Giant' required an average of 12 leaves, about 12 weeks of growth at 20 °C, before uniform flowering occurred (Shedron and Weiler, 1982).

For most herbaceous perennials, forcing temperature plays a significant role in the rate of flower induction and production time (Yuan et al., 1998, Whitman et al., 1996). Many physiological processes, such as flowering, are quickened as temperatures rise until an optimum temperature is reached. This optimum temperature may be different for different physiological processes and different species. At temperatures below a species-specific minimum (T_b), the time to flower (*f*) is infinite and at temperatures above a ceiling value (T_{opt}) flowering is delayed (Roberts and Summerfield, 1987). When developmental time is converted to a rate by taking the reciprocal, the relationship between mean temperature and rate of development is often linear in the range between T_b and T_{opt} (Roberts and Summerfield, 1987). Thus, the relationship between the rate of development toward flowering (1/DTF, where DTF is the days to flower) and temperature can be described as follows

$$1/DTF = b_0 + b_1 * T \qquad [1]$$

(Yuan et al., 1998). Using the constants b_0 and b_1, the base temperature, T_b, and degree-days (°days) can be calculated as follows:

$$T_b = -b_0/b_1 \qquad [2]$$

$$°days = 1/b_1 \qquad [3]$$

(Yuan et al., 1998). Below the base temperature, the rate of progress toward flowering is zero. Degree-days represent the thermal time required for flowering (Yuan et al., 1998).

Forcing temperature also has an effect on flowering percentage. When seedlings of *Pharbitis nil* were grown under several different day/night temperature regimens, it was found that the percent of flowering plants progressively increased as the day temperature increased from 12 to 30 °C

(Reese and Erwin, 1997). Percent of flowering and flower bud number of *Pharbitis nil* were greatest when seedlings were induced with a 24 or 30 °C day temperature and a 30 °C night temperature (Reese and Erwin, 1997).

There is limited information on the effect of forcing temperature on growth and flowering of *Aquilegia* species and cultivars. Selections from the Weddle's Songbird Series reached visible bud and flowered faster with night temperatures of 15 °C than with night temperatures of 10 °C, but no data was given for the daytime temperatures (White et al., 1989). Day temperatures of 16-19 °C with average night temperatures between 9 °C and 12 °C induced earlier flowering than day temperatures greater than 24 °C for several selection of *Aquilegia xhybrida* Sims, suggesting heat delay at higher temperatures (Merritt et al., 1997).

The objectives of our study were to 1) establish and compare the effect of forcing temperature on the flowering of several *Aquilegia* species and cultivars and to establish the scope of species and cultivar responses, 2) measure the influence of forcing temperature on flower number, flower diameter, and plant height, and, 3) establish the optimum forcing temperature to induce the most complete and rapid flowering for each selection. The species and cultivars were chosen to provide a comparison between a wide range of species and popular hybridized cultivars.

Materials and Methods

Plant material.

Species and cultivars studied were *Aquilegia alpina* L., *A. caerulea* James. , *A. canadensis* L., *A. chrysantha* A. Gray. , *A. flabellata* Siebold & Zucc. 'Cameo Blue and White' and 'Mini star', *A. xhybrida* Sims 'Bluebird' (Weddle Seed, Palisade, Colo.), *A. xhybrida* Sims 'Crimson Star', *A. xhybrida* Sims 'McKana's Giant, and *A. xhybrida* Sims 'Musik White' (Ernst Benary, Hanover F.R.G) (Bailey Hortorium Staff, 1976). Seeds were sown and seedlings were produced by a single commercial producer and shipped to MSU on September 16, 1997. Upon arrival, the seedlings of *A. alpina* and 'McKana's Giant' were placed directly into a controlled environment chamber set at 5 °C for 12 weeks of cold treatment. The seedlings of the remaining species were grown at 20 °C under 16 hours of extended day light from high-pressure sodium lamps until they reached 16 weeks of age. Upon reaching 16 weeks of age, the seedlings were transferred to a controlled environment chamber set at 5 °C for 12 weeks. Dates that the seedlings were transferred to the greenhouse are included in Table 1. Because plants had been cut back before shipping, we were not able to take accurate initial leaf counts on each seedling. There were 10 plants of each species or cultivar per treatment.

Cold treatments

Plants of each species or cultivar were cold-treated for 12 weeks in the 72-cell plug trays in which they were received in a controlled-environment chamber at 5 °C. The chamber was lit from 0800 to 1700 HR at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from cool-white fluorescent lamps (VHOF96T12; Philips, Bloomfield, N.J.), as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE). While in the cooler, plants were watered approximately two to three times per week as needed with well water acidified (H_2SO_4) to an approximate pH of 6.0.

Greenhouse environment.

Temperature treatments. After cold treatment, the seedlings were transplanted into 1.1-L (5" U.S.) square pots and transferred immediately into glass greenhouses set at 14, 17, 20, 23, and 26 °C. Air temperatures on each bench were monitored with 36-gauge (0.013-mm-diameter) type E thermocouples connected to a CR10 datalogger (Campbell Scientific, Logan, UT). The datalogger collected temperature data every 10 seconds and recorded the hourly average. Actual average daily air temperatures from the beginning of forcing to the average date of flowering were calculated for each species (Table 2).

Lighting. All plants were grown under natural light plus light from high-pressure sodium lamps to extend the photoperiod to 16 hours. The greenhouse was lit from 0700 to 0800 HR and from 1700 to 2300 HR at approximately 50 $\mu mol \cdot m^{-2}\ s^{-1}$ as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE), to deliver the correct photoperiod. From 0800 to 1700 HR, high-pressure sodium lamps provided a photosynthetic photon flux (PPF) of approximately 50 μmol·m^{-2} s^{-1} at plant level when the ambient greenhouse PPF dropped to 200 μmol·m^{-2} s^{-1}, and discontinued when PPF reached 400 μmol·m^{-2} s^{-1}.

Media and watering

Plants were grown in a commercial soilless medium composed of composted pine bark, horticultural vermiculite, Canadian sphagnum peat moss, processed bark ash, and washed sand (MetroMix 510, Scotts-Sierra Horticultural Products Company, Marysville, Ohio, year 1). Plants were top-watered daily with well water acidified (two parts H_3PO_4 plus one part H_2SO_4, which provided ≈2.5 mol P·m^{3}) to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and fertilized with 14N-0P-6K_2O (mol·m^{3}) from potassium nitrate (14-0p-55K_20) (Vicksburg Chemical Co., Vicksburg, MS) and ammonium nitrate (34N-0P-0K_2O) (Cargill, Lexington, KY). Fertilization and acidification rates were adjusted in response to weekly soil test results, so regimens varied during experiments.

Data collection and analysis.

For each plant that flowered, the date of the first visible bud and date of opening of the first flower were recorded. Linear regression analysis was used to calculate rates of development for each event. At flowering, the number of visible flower buds, diameter of the first open flower, number of leaves on the main stem below the first flower, and total plant height were measured. Days to visible bud, days from visible bud to flower, and days to flower were calculated. Data were analyzed using SAS's (SAS Institute, Cary, NC) analysis of variance and general linear models procedures.

Results and Discussion

No plants of *A. caerulea,* 'Crimson Star', 'McKana's Giant', and 'Musik White' flowered under any treatment. Only 10 to 20 % of *A. alpina*, and *A. chrysantha* plants flowered under each temperature treatment. Therefore, no data for these plants are presented. Based on the ages of these seedlings and the results of previously cited studies, we expected these plants to flower and we have no explanation for why they did not. It may be possible that the sowing dates received from the commercial producer were erroneous.

Flowering Percentage

'Cameo' and 'Mini star' were the only two cultivars to reach 100% flowering (Tables 3 and 4, Figure 1). Flowering percentage for both 'Cameo' and 'Mini star' plants decreased by 60% as the forcing temperature increased from 23 to 26 °C (Table 3 and 4, Figure 1). Flowering percentage for plants of *A. canadensis* and 'Bluebird' reached only 40-50% and 80-90%, respectively (Tables 5 and 6, Figure 1).

Flower Timing

Days to visible bud and days to flower generally decreased as temperature increased, but heat-delay (increased time-to-flower at high temperatures) was observed at 26 °C for both *A. canadensis* and 'Bluebird' (Tables 5 and 6, Figures 4 and 5).

The most dramatic change in time to visible bud and flowering in response to increased temperature was observed for plants of *A. canadensis* (Table 5, Figure 4). Days to visible bud and days to flower both decreased by thirty days as the forcing temperature increased from 14 to 23 °C. Flowering of *A. canadensis* was delayed, however, by about eight days as temperatures increased from 23 to 26 °C (Table 5, Figure 4). For plants of 'Bluebird', days to visible bud decreased by 16 days as the temperature increased to 23 °C, and increased by about a day at 26 °C (Table 6, Figure 5). These results suggest that 23 °C is near the optimum temperature, in reference to timing, for *A. canadensis* and 'Bluebird'. For 'Cameo' and 'Mini star', the relationship between temperature and time to visible bud and time to flower followed a linear pattern, with no delay in flowering observed at 26 °C (Tables 3 and 4, Figures 2 and 3). Days to visible bud decreased by 19 and 22 days for 'Cameo' and Mini star' as temperature increased from 14 to 26 °C (Tables 3 and 4, Figures 2 and 3). Days to flower were reduced by 22 days for both 'Cameo' and 'Mini star' plants as the forcing temperature increased from 14 to 26 °C (Tables 3 and 4, Figures 2 and 3).

Days from visible bud to flower was also significantly affected by forcing temperature, but the differences were relatively small in most cases. Days from visible bud to flower for *A. canadensis* and 'Mini star' decreased by five and four days, respectively, as the forcing temperature increased from 14 to 23 °C. (Table 4 and 5, Figures 2 and 3). Days from visible bud to flower for plants of 'Cameo' and 'Bluebird' differed by only 3 days and 2 days respectively, over the temperature range tested.

There were significant linear relationships between temperature and rate of progress toward visible bud and flowering for plants of 'Cameo' (Table 7). The rate to visible bud and flowering continued to increase linearly as temperature increased from 14 to 26 °C, therefore, all data for 'Cameo' was included in the regression analysis (Table 7). Since the rate to flowering increases linearly with temperature only at sub-optimal ranges (Roberts and Summerfield, 1987), the data at 26 °C were excluded from regression analysis for *A. canadensis* and 'Bluebird'. Surprisingly, the rate to visible bud for plants of 'Mini star' increased as the temperature increased from 23 to 26 °C. Because this response to temperature is atypical, the data for 26 °C was excluded from regression analysis.

The rate of progress from visible bud to flower followed a statistically significant linear pattern in relation to temperature only for *A. canadensis* (Table 7). The rate of progress from visible bud to flower for 'Cameo' increased with temperature (Table 7). Statistically, there was no relationship between the rate of visible bud to flower and temperature for plants of 'Mini star' and 'Bluebird'.

Base temperatures and degree-days for each developmental stage of each species were determined using equations [2] and [3]. Base temperature and degree-days can be used to predict the flowering date in commercial greenhouse environments in which temperatures fluctuate (Yuan et al., 1998). The base temperature for forcing to visible bud and forcing to flower the selections in the present study ranged from 4.2 to 10.6 and -3.9 to 4.7 respectively (Table 7). Base temperature for the rate of progress from visible bud to flower was estimated to be far below 0 °C for 'Cameo' and *A. canadensis*, suggesting that there may have been bud initiation while plants were still in the cooler. There was no significant linear relationship between rate of progress between days from visible bud to flower for 'Mini star' and 'Bluebird'.

Final leaf number

As forcing temperature increased, final leaf number for each species in this experiment decreased linearly (Tables 3-6). Final leaf number for plants of 'Cameo', 'Mini star', and 'Bluebird' decreased by 50-60% as the forcing temperature increased from 14 to 26 °C (Tables 3 and 4).

Flower diameter and number

For all species studied, there was a dramatic decrease in flower diameter, measured across the open face of the flower, as the forcing temperature increased. Flower diameter decreased linearly for plants of *A. canadensis* and 'Bluebird', and quadratically for plants of 'Cameo' and 'Mini star' with increasing temperature (Tables 3, 4, 5, and 6). For 'Bluebird', which featured the largest flowers of all species studied in this experiment, flower diameter increased two-fold as the forcing temperature decreased from 26 to 14 °C (Table 6, Figure 6). Flowers of 'Bluebird' plants grown at 14 °C measured more than 13 cm across on average. For all species tested, flowers at the highest temperature were also reduced in color, and overall quality, although no data to support these observations were recorded. No gross flower deformities or doubling were observed at 26 °C, though flowers at this temperature were much smaller than those at lower temperatures.

Reducing temperature from 26 to 14 °C promoted a three-fold increase in flower number for 'Cameo and 'Bluebird' plants (Tables 3 and 6, Figure 6). There were no significant trends in flower number in relation to temperature for plants of *A. canadensis* and 'Mini star' (Tables 4 and 5, Figure 6).

Plant height

The effect of forcing temperature on plant height at first flower was species dependent. Height for *A. canadensis* plants was independent of forcing temperature (Table 5, Figure 6). Final plant height for plants of 'Cameo' decreased by 25% as the forcing temperature increased from 14 to 26 °C (Table 3, Figure 6). Plant height for both 'Mini star' and 'Bluebird' decreased by nearly half as the forcing temperature increased from 14 to 26 °C (Table 4 and 6, Figure 6).

Conclusion

Based on the presumed ages of our seedlings, we felt confident that plants of all species in this study would flower. However, three species and three hybrids flowered poorly or did not flower at all. According to Shedron and Weiler (1982), 100% of 'McKana's Giant' plants with an average of 12 leaves flowered after ten weeks of cold, and 100% of 'Crimson Star' plants with an average of 15 leaves flowered after eight weeks of cold. In these experiments, both cultivars were 16 weeks of age when cooled. Based on this information, some plants of 'McKana's Giant' and 'Crimson Star' should have flowered based on the age of the plants, 24 and 16 weeks, respectively. In our experiment, no plants of the cultivar 'Musik-White' flowered under any cold treatment, and there is little information published on selections from the Musik series (Benary, Hann. Muenden, F.R., Germany). Merritt et al. (1997) chilled seeds, but not whole seedlings, of 'Musik- Blue White' and 'Musik- Red White', then forced the seedlings under four different day/ low-night temperature regimens. No plants of either cultivar flowered in their experiment.

Despite the striking diversity amongst *Aquilegia* species, there is still a great deal of inter-fertility which suggests that, overall, species of *Aquilegia* may be genetically very similar (Hodges and Arnold, 1994). The name *A. xhybrida* was originally given to hybrids of *A. canadensis* and *A. vulgaris*, but is more often used for long-spurred garden hybrids with a parentage involving *A. caerulea*, *A. chrysantha*, and possibly others (Bailey, 1976). We included parental species in this study for the expressed purpose of comparing the temperature to those of the hybrids. In this study we were not able to determine the response to temperature of the alpine species *A. caerulea*, *A. alpina*, or *A. chrysantha* because no plants , or very few plants, flowered.

Fewer plants of the *Aquilegia flabellata* selections flowered when forced at 26 °C than when forced at lower temperatures, suggesting that 23 °C is near the optimum for this species. Alternatively, temperatures $\geq$ 23 °C may have de-vernalized the plants that did not flower. Flowering plants of the *A. flabellata* selections at 26 °C did not exhibit heat delay, in fact, flowering accelerated for plants of 'Mini star'. High temperatures did not delay the plants that did flower, but reduced the number of plants that flowered.

For both 'Cameo' and 'Mini star', higher forcing temperatures greatly reduced leaf number at the time of first flower and the decrease was strongly correlated with faster flowering times for both cultivars.

Flower timing of *A. canadensis*, a species with a native range from Nova Scotia to Texas, and *A. xhybrida* 'Bluebird', were most sensitive to temperature compared to *A. flabellata* selections, but 100% flowering was never reached for either species and no trends were associated with temperature (Figure 1).

Temperature had a dramatic effect on flower size of all species tested and flower number for two species tested. Flower size, for a given species, can be affected by the rate of plant growth (Pearson et al., 1995). A higher plant growth rate, thus a shorter period of growth, is most likely responsible for a smaller final flower size. Pearson et al. (1995) showed that flower size was reduced at higher temperatures, and further, that the flowers were sensitive to high temperature from the time of induction to anthesis. In two varieties of tomato, heat-sensitive Pusa Ruby and heat-tolerant CL-1311, many floral anomalies such as stigma exertion without anthesis, empty flowers, and persistent flowers without fruit-set were observed in when grown under a 35 / 30 °C day/night temperature regimen (Lohar and Peat, 1998). Abnormalities such as flower doubling occurred for plants of *Campanula* 'Birch Hybrid' when grown at constant day night temperatures of 29 °C (Frane, unpublished data). No flower abnormalities were observed in this study for plants of *Aquilegia* grown at 26 °C, but flower size and color were both reduced.

Overall, plants grown at lower temperatures had more and larger flowers and were taller, but took longer to reach anthesis than plants grown at lower temperatures. Plants of all species grown at the highest temperature were too weak and diminutive in size for 1.1-L (5" U.S.) pots, whereas the plants of *A. canadensis* and 'Bluebird' grown at the coolest temperature may have been too large for them. Settings of 14 to 20 °C are recommended to force *A. canadensis*, *A. flabellata* 'Cameo' and 'Mini star', and *A. xhybrida* 'Bluebird'. Plants flowered slightly slower at temperatures between 14 and 20 °C than those grown at higher temperatures, but they were much more attractive.

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Table 1. Ages, sowing dates, and transfer dates for the Aquilegia species used in the experiment. All of the seedlings were sown and grown commercially in 72-cell plug trays (each cell equals 50 ml volume). The seedlings were shipped to MSU on September 16, 1997. All seedlings were grown under a 16-h photoperiod at 20 °C until they reached the age of 16 weeks before they were transferred to a 5 °C cooler. The seedlings that were older than 16 weeks upon arrival were transferred to a 5 °C cooler the day after they were received.

Species	Date sown	Cold treatments started	Age of plants when moved to cooler
Aquilegia alpina	4/9/97	9/17/97	23 wks
Aquilegia caerulea	5/21/97	9/30/97	16 wks
Aquilegia canadensis	6/8/97	10/08/97	16 wks
Aquilegia chrysantha	6/18/97	11/17/97	16 wks
Aquilegia flabellata 'Cameo Blue & White'	8/3/97	12/5 /97	16 wks
Aquilegia flabellata 'Mini star'	6/17/97	11/17/97	16 wks
Aquilegia xhybrida 'Crimson Star'	6/18/97	11/17/97	16 wks
Aquilegia xhybrida 'McKana Giant'	4/9/97	9/17/97	23 wks
Aquilegia xhybrida Musik 'White'	6/11/97	10/12/97	16 wks
Aquilegia xhybrida Songbird 'Bluebird'	7/1/97	11/1/97	16 wks

Table 2. Dates of forcing and the average temperature during forcing.

Species	Greenhouse temperature set point during forcing (°C)	Date of forcing	Average temperature during forcing (°C), Photoperiod (hours) 16-hr (HID): Force to VB	VB to Flower
Aquilegia flabellata 'Cameo Blue & White'	14	2/27/98	14.2	14.2
	17		16.9	17.2
	20		19.9	20.4
	23		23.2	23.4
	26		26.1	26.2
Aquilegia flabellata 'Mini star'	14	2/11/98	14.3	14.4
	17		17.2	17.7
	20		20.4	19.7
	23		23.9	23.1
	26		26.4	26.3
Aquilegia canadensis	14	12/31/97	14.3	14.2
	17		17.2	17.6
	20		20.3	20.1
	23		23.7	23.9
	26		26.4	26.2
Aquilegia xhybrida 'Bluebird'	14	1/24/98	14.2	14.6
	17		17.2	17.5
	20		20.4	20.0
	23		23.9	23.3
	26		26.4	26.3

Table 3. Regrowth and flowering responses of *Aquilegia flabellata* 'Cameo Blue and White' at five different forcing temperatures. Plants were cooled in 72-cell plug trays for 12 weeks. Following cold treatment, plants were grown under a 16-h photoperiod at temperatures of 14, 17, 20, 23, and 26 °C. Flower number, flower diameter, final leaf number, and final plant height were measured at first open flower.

Forcing Temperature (°C)	Percentage flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Flower diameter (cm)	Final plant height (cm)	Final leaf number
14	100	27***	15***	42***	7***	4***	8***	8***
17	100	23	15	38	8	3.8	8	8
20	100	16	12	29	8	3.3	8	7
23	100	12	12	24	6	2.4	5	7
26	40	8	12	20	5	1.7	6	4
Contrasts								
14, 17, 20, 23, and 26 °C								
P_{Linear}		***	***	***	***	***	***	***
$P_{quadratic}$		NS	NS	NS	***	***	***	***

NS, *, **, *** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively
[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 4. Regrowth and flowering responses of *Aquilegia flabellata* 'Mini star' at five different forcing temperatures. Plants were cooled in 72-cell plug trays for 12 weeks. Following cold treatment, plants were grown under a 16-h photoperiod at temperatures of 14, 17, 20, 23, and 26 °C. Flower number, flower diameter, final leaf number, and final plant height were measured at first open flower.

Forcing Temperature (°C)	Percentage flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Flower diameter (cm)	Final plant height (cm)	Final leaf number
14	100	29***	14***	43***	8***	3.9***	9***	10***
17	100	22	15	37	8	3.7	9	10
20	100	17	10	27	7	3.5	10	9
23	100	12	10	23	7	2.8	5	6
26	100	7	13	20	4	1.6	5	5
Contrasts								
14, 17, 20, 23, and 26 °C								
P_{Linear}		***	**	***	***	***	***	***
$P_{quadratic}$		NS	***	**	NS	***	**	NS

NS, *, **, *** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively
[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 5. Regrowth and flowering responses of *Aquilegia canadensis* at five different forcing temperatures. Plants were cooled in 72-cell plug trays for 12 weeks. Following cold treatment, plants were grown under a 16-h photoperiod at temperatures of 14, 17, 20, 23, and 26 °C. Flower number, flower diameter, final leaf number, and final plant height were measured at first open flower.

Forcing Temperature (°C)	Percentage flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Flower diameter (cm)	Final plant height (cm)	Final leaf number
14	40	50***	20***	70***	10^NS	3.6***	36^NS	17***
17	50	45	19	64	11	3.3	37	16
20	50	34	17	51	12	3.0	36	15
23	50	25	15	40	10	2.7	34	9
26	40	32	17	48	9	2.1	35	14
Contrasts								
14, 17, 20, 23, and 26 °C								
P_{Linear}		***	***	***	*	***	NS	***
$P_{quadratic}$		***	NS	***	NS	*	*	**

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively
[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 6. Regrowth and flowering responses of *Aquilegia xhybrida* 'Bluebird' at five different forcing temperatures. Plants were cooled in 72-cell plug trays for 12 weeks. Following cold treatment, plants were grown under a 16-h photoperiod at temperatures of 14, 17, 20, 23, and 26 °C. Flower number, flower diameter, final leaf number, and final plant height were measured at first open flower.

Forcing Temperature (°C)	Percentage flowering [1]	Days to visible bud	Days from visible bud to flower	Days to Flower	Flower number	Flower diameter (cm)	Final plant height (cm)	Final leaf number
14	80	33***	14**	47***	17***	13.3***	46***	15***
17	80	28	13	42	16	11.3	41	13
20	90	22	12	34	13	9.1	34	14
23	80	18	14	32	8	7.6	34	10
26	80	18	13	31	10	5.7	26	6
Contrasts								
14, 17, 20, 23, and 26 °C								
P_{Linear}		***	NS	***	NS	***	***	***
$P_{quadratic}$		***	NS	***	***	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively
[1] Plants that were not in bud after 100 days were considered nonflowering.

Table 7. Parameters of linear regression analysis relating forcing temperature to rate of progress to visible bud (VB), to first open flower (FLW), and from VB to FLW in *A. flabellata* 'Cameo' and 'Mini star', *A. canadensis*, and *A. xhybrida* 'Bluebird'. Intercept and slope were used to calculate base temperature ($T_{b)}$ and degree-days (°days).

Developmental stage (d)	Intercept (b_0) 1/d	Slope (b_0) (1/d)/C	T_b (°C)	°days	r^2
	A. flabellata 'Cameo'				
Forcing to VB	-0.08035 $\pm$ 0.01135[z]	0.00756 $\pm$ 0.00119	10.6	132	0.93
VB to FLW	-0.00907 $\pm$ 0.00224	0.0022 $\pm$ 0.00023	-48.0	882	0.67
Forcing to FLW	0.05456 $\pm$ 0.00436	0.00113 $\pm$ 0.00046	4.0	453	0.97
	A. flabellata 'Mini star'				
Forcing to VB	-0.01305 $\pm$ 0.00395	0.0049 $\pm$ 0.00055	7.7	204	0.98
VB to FLW	-0.01097 $\pm$ 0.00154	0.00232 $\pm$ 0.00016	-67.0	1056	0.08
Forcing to FLW	0.01985 $\pm$ 0.01338	0.00347 $\pm$ 0.00213	4.7	430	0.98
	A. canadensis				
Forcing to VB	-0.01305 $\pm$ 0.00248	0.00217 $\pm$ 0.00038	6.0	460	0.95
VB to FLW	-0.00302 $\pm$ 0.00125	0.00114 $\pm$ 0.00018	-15.0	606	0.95
Forcing to FLW	0.02521 $\pm$ 0.00182	0.00165 $\pm$ 0.00026	2.6	874	0.95
	A. xhybrida 'Bluebird'				
Forcing to VB	-0.01204 $\pm$ 0.00164	0.00286 $\pm$ 0.00023	4.2	349	0.99
VB to FLW	0.00457 $\pm$ 0.00135	0.00117 $\pm$ 0.0002	-24.5	541	0.88
Forcing to FLW	0.04524 $\pm$ 0.00258	0.00185 $\pm$ 0.00067	-3.9	855	0.95

[z] Standard error.

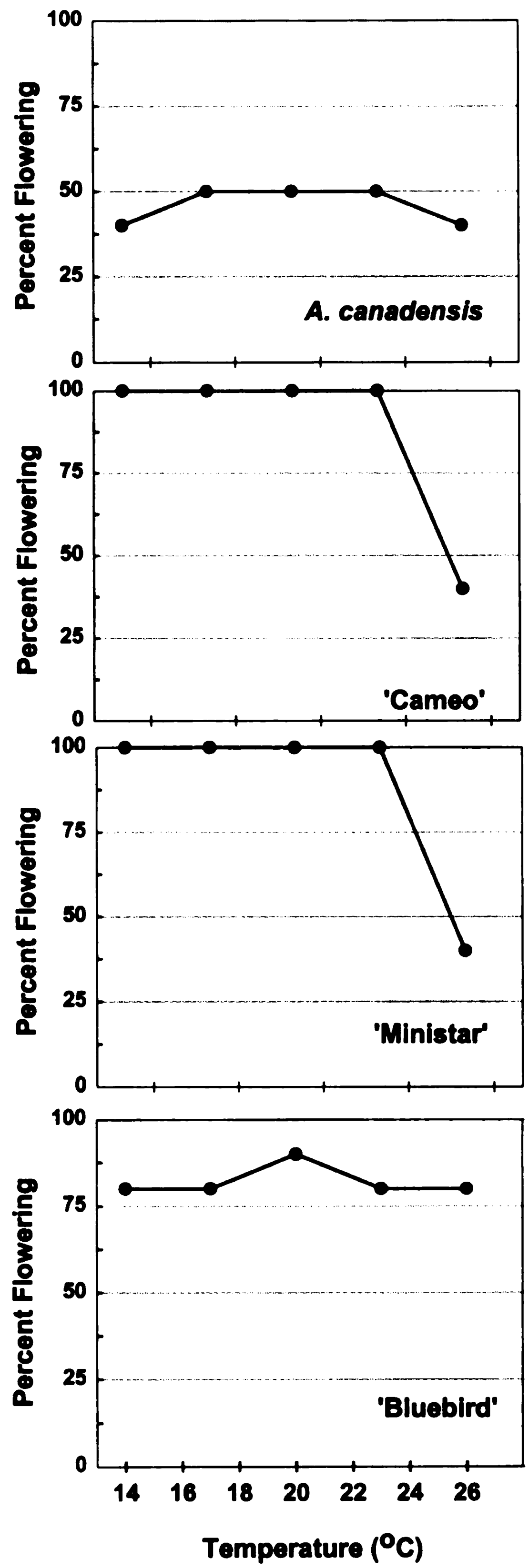

Figure 1. The effects of forcing temperature on the flowering percentage of *A. canadensis*, 'Cameo', 'Mini star', and 'Bluebird'. Plants that did not flower within 100 days were considered non-flowering.

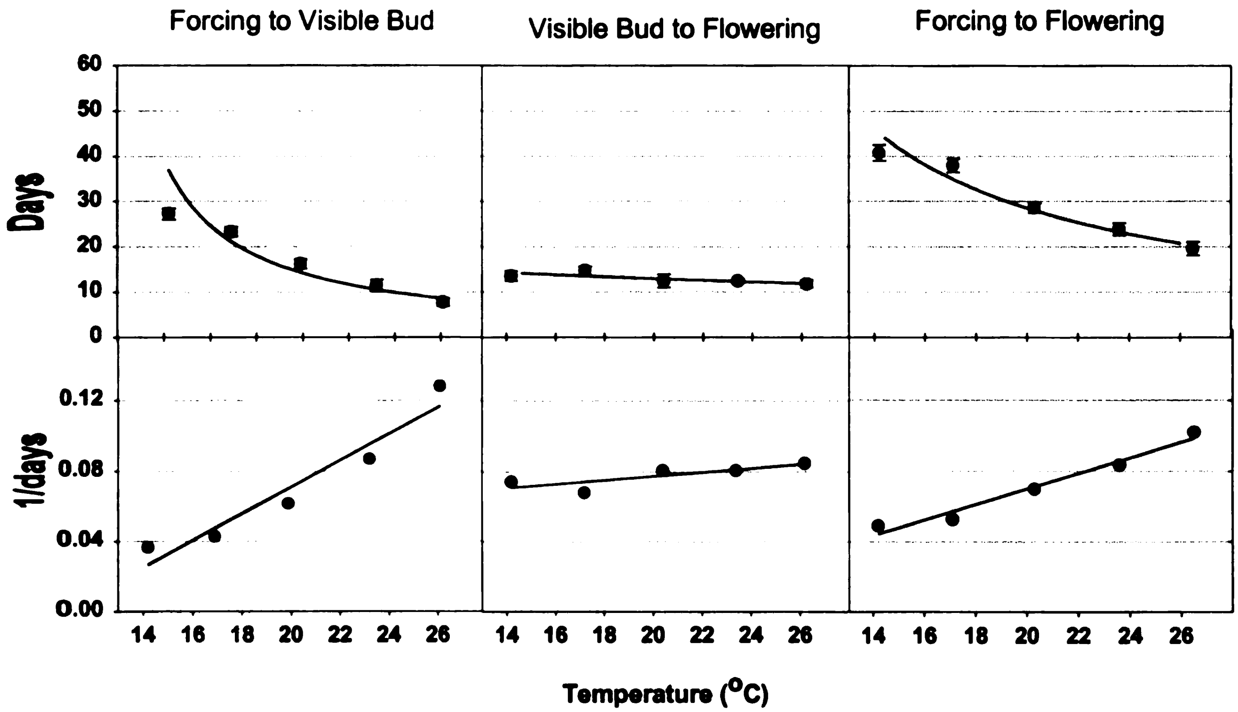

Figure 2. The effects of forcing temperature on the days to visible bud, days from visible bud to flower, and days to flower, and the rates ot progress to each event for *A. flabellata* 'Cameo'. The points represent the actual data points and the curves and straight lines were calculated from regression analysis.

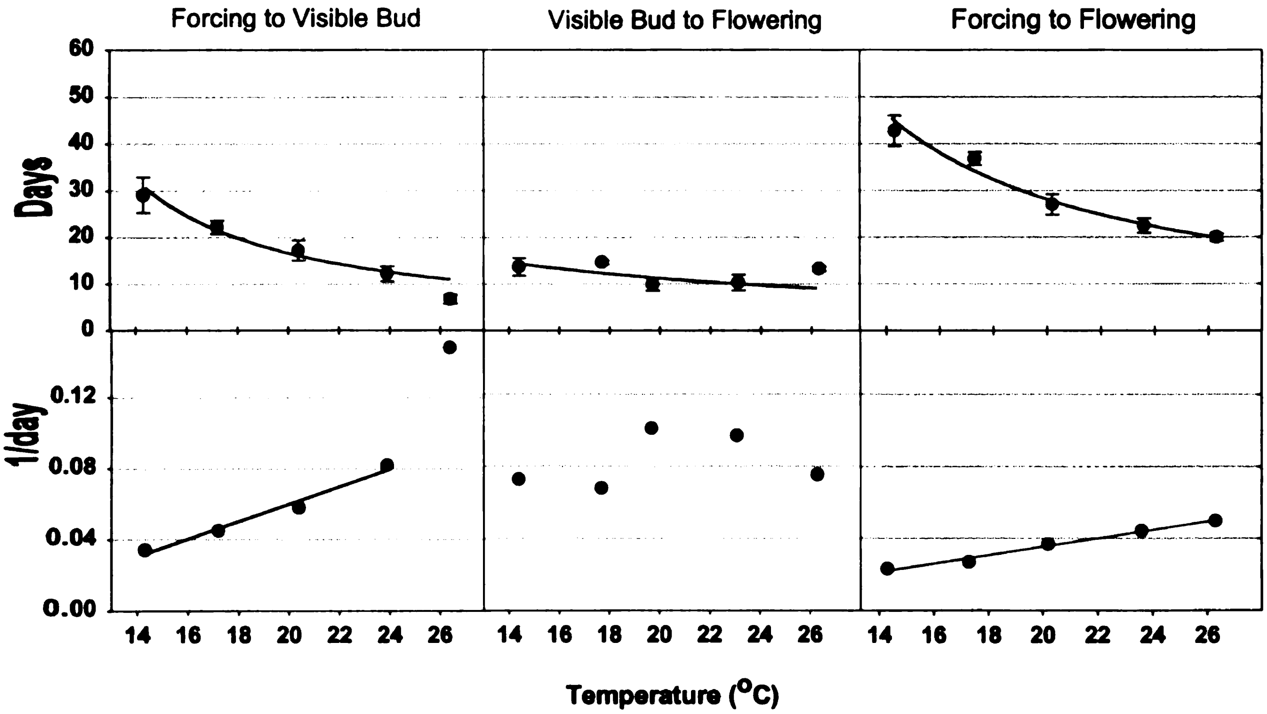

Figure 3. The effects of forcing temperature on the days to visible bud, days from visible bud to flower, and days to flower, and the rates of progress to each event for *A. flabellata* 'Mini star". The points represent the actual data points and the curves and straight lines were calculated from regression analysis.

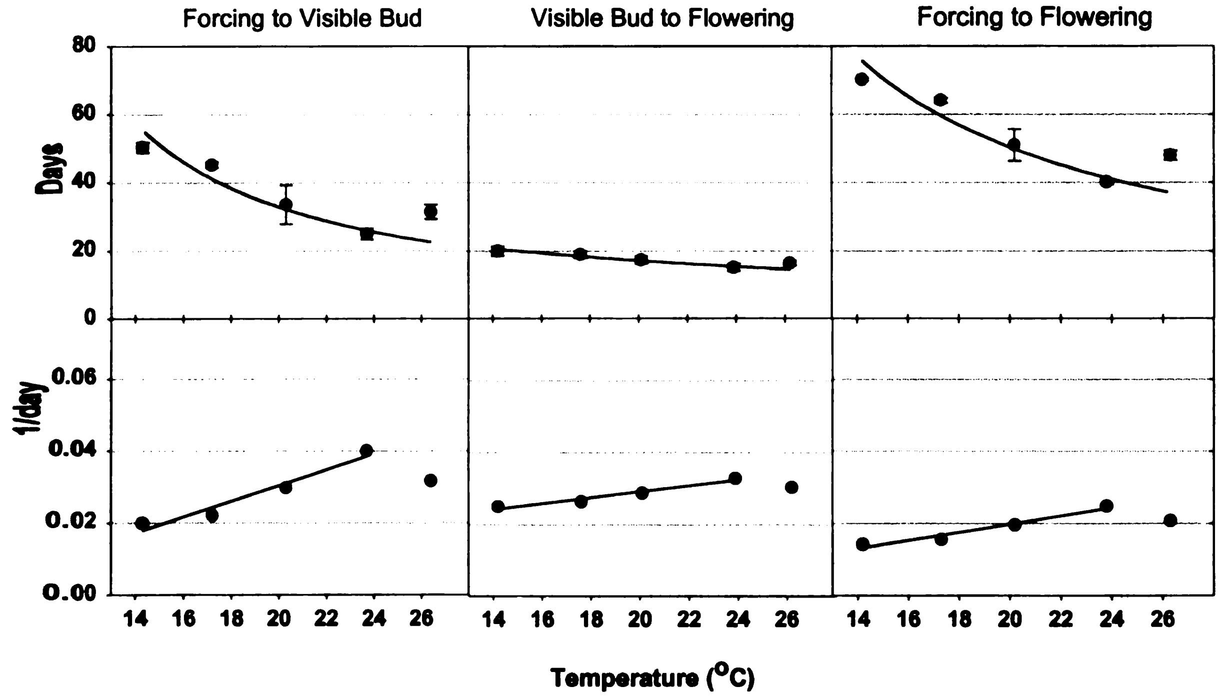

Figure 4. The effects of forcing temperature on the days to visible bud, days from visible bud to flower, and days to flower, and the rates ot progress to each event for *A. canadensis.* The points represent the actual data points and the curves and straight lines were calculated from regression analysis.

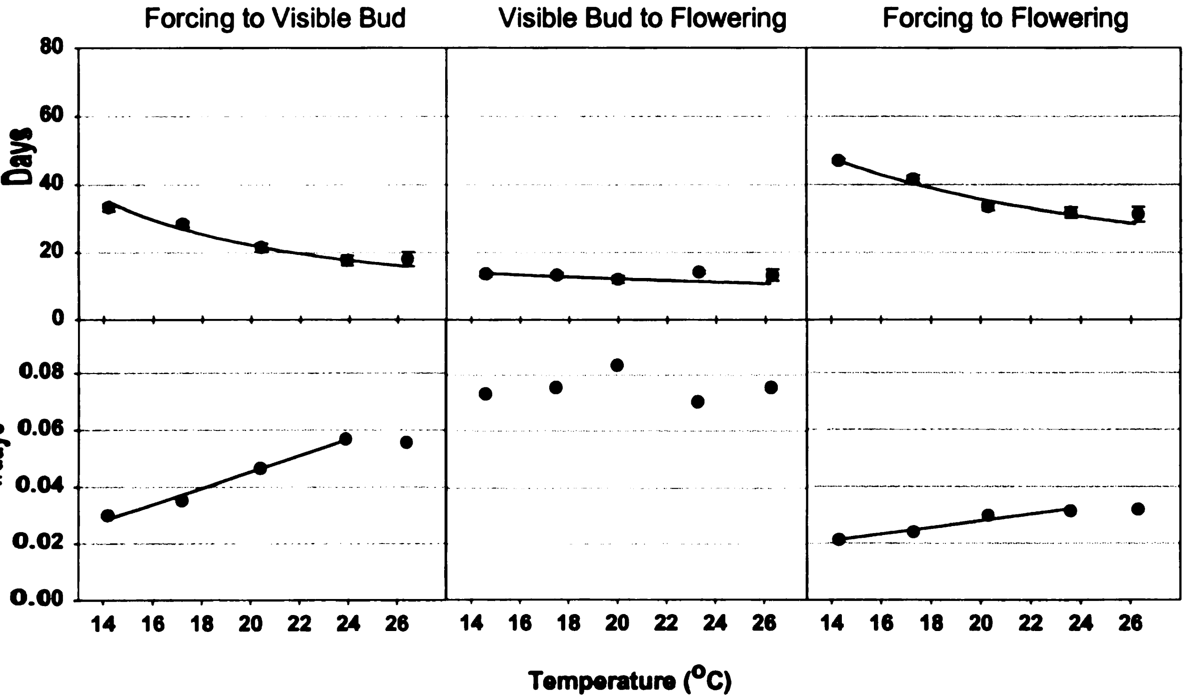

Figure 5. The effects of forcing temperature on the days to visible bud, days fromvisible bud to flower, and days to flower, and the rates of progress to each event for *A. xhybrida* 'Bluebird'. The points represent the actual data points and the curves and straight lines were calculated from regression analysis.

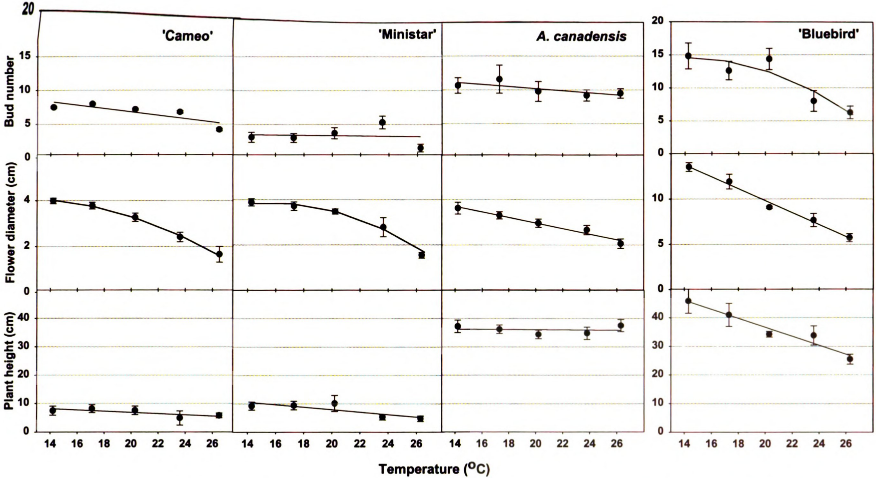

Figure 6. Influence of forcing temperature on number of flower buds, flower diameter, and plant height measured at first flower. Error bars show standard deviation.

CHAPTER IV

THE EFFECTS OF TEMPERATURE, PLANT AGE, COLD TREATMENT, AND PHOTOPERIOD ON THE GROWTH AND FLOWERING OF *AQUILEGIA FLABELLATA* 'CAMEO', *AQUILEGIA XHYBRIDA* 'BLUEJAY', 'CRIMSON STAR', 'MCKANA'S GIANT' AND 'MUSIK-WHITE'.

Introduction

One of the most distinctive group of plants in the Ranunculaceae family is that of the genus *Aquilegia*, commonly known as columbine. There are about 70 *Aquilegia* species that are native to the temperate and mountain regions of the northern hemisphere (Bailey Hortorium Staff, 1976). Due to the wide geographical distribution of the genus *Aquilegia,* there are many environmental factors that influence the physiology of each species.

Leaf unfolding rate increases with increasing temperature to a maximum and then decreases with continued increased temperature (Karlsson, 1992). The ability to follow progression of leaf development would facilitate scheduling and timing of *Aquilegia* crops. Average daily temperature controls rate of leaf development in Easter lily, and leaf counting is used successfully to monitor and schedule the crop (Friend et al, 1962; Karlsson et al., 1982; Tollenaar et al, 1979). Determining leaf unfolding rates for *Aquilegia* species and cultivars at a range of daily average temperatures would promote proper scheduling.

Aquilegia typically do not flower during the first season of growth due to juvenility and a requirement for vernalization. Therefore, it is necessary to determine an appropriate measurement of maturity, such as leaf number. Plant age, or maturity, has typically been defined by leaf number for many plants (White, 1990). Cold treatments will not induce flowering until the plant has made the transition to its' mature phase.

Published information provides only general recommendations for determining plant age or maturity of *Aquilegia*. Shedron and Weiler (1982) studied the juvenile stage of *Aquilegia xhybrida* 'McKana's Giant', 'Fairyland', and 'Crimson Star'. Plants were chilled at three different ages; 8, 16, and 20 weeks. 'McKana's Giant' plants with an average of 6.7 leaves did not flower, nor did 'Fairyland' plants with and average of 7.8 leaves or 'Crimson Star' plants with an average of 7.6 leaves (Shedron and Weiler, 1982). After 16 weeks of age and an average number of 12 to 15 leaves, all three cultivars flowered with varying percentages following 6 different cold treatments; 0, 4, 8, 10, and 12 weeks of cold treatment at 4.5 °C. One hundred percent of 'McKana's Giant' plants flowered at the 12-leaf stage after ten weeks of cold, and all 'Fairyland' and 'Crimson Star' plants flowered at the 15-leaf stage after eight weeks of cold treatment (Shedron and Weiler, 1982). However, because only average leaf numbers were given for each age group, it cannot be determined exactly what plants with a specific leaf number flowered or did not flower.

The information from Shedron and Weiler's study has served as the basis for much of the subsequent research on *Aquilegia*. Latter publications cite Shedron and Weiler (1982) and have used plants with 12 to 15 leaves before administering treatments. However, this leaf number range may not to be valid for every species or cultivar. White (1990) investigated floral initiation and development in *Aquilegia* by scanning electron microscopy using selections from the Weddle Songbird series. Plants with a minimum of 12 to 14 leaves usually produced an average of only one flower (White, 1990) but it is not clear from the data presented if 100% of plants with 12 leaves initiated flower buds.

A cold requirement for flowering is often linked with a requirement for a long photoperiod after cold, and the requirement for vernalization is most commonly, but not only, found in long day plants (Thomas and Vince-Prue, 1997). *Aquilegia* is considered a day-neutral plant after a cold treatment, and will flower under both long days and short days. However, *Aquilegia* could also be considered a facultative long-day plant because plants will flower faster under long-days (Shedron and Weiler, 1982). After a 12 week cold treatment, cultivars of *Aquilegia xhybrida* reached visible bud approximately 20% faster when grown under 18-hour long days (7 to 18 μmol m^{-2} sec^{-1} incandescent lighting) as compared to those grown under short 9-hour days (Shedron and Weiler, 1982). Days from visible bud to flower, however were not significantly affected by photoperiod.

There are morphological differences in plants grown under long days that may be considered undesirable. Leaf petioles were highly elongated compared to those of plants grown under short days and total plant height at flowering was also greater for plants grown under long days (Shedron and Weiler, 1982). Plants grown under 10-hour days after 12 weeks of cold had an average height of 43 cm whereas plants grown under 18-hour days after 12 weeks of cold had an average height of 52cm (Shedron and Weiler, 1982).

The objectives of the present study were to 1) measure leaf unfolding development of five cultivars of *Aquilegia* and calculate rates of leaf development at four average daily temperatures; 17, 20, 23, and 26 °C , 2) determine the age or leaf number at which the plants make their transition from the juvenile to mature stage of development, 3) determine if different temperatures could hasten the transition from the juvenile to mature stage of development, and 4) determine the effects of temperature during plug growth, and of photoperiod before and after a cold treatment on the growth and flowering of each cultivar.

Materials and Methods

General. Seedlings of *A. flabellata* Siebold & Zucc. 'Cameo Blue and White', *A. xhybrida* Sims 'Bluejay' (Weddle Seed, Palisade, Colo.), *A. xhybrida* Sims 'Crimson Star', *A. xhybrida* Sims 'McKana's Giant, and *A. xhybrida* Sims 'Musik White' (Ernst Benary, Hanover F.R.G) (Bailey, 1976) were sown on the same day and grown for four weeks in 128-cell plug trays (35-ml volume) by a single commercial grower, then shipped to MSU on October 13, 1997. Upon arrival, the seedlings were placed in a 20 °C greenhouse under natural days. On October 15, 1997, the seedlings were placed into Expt 1.

Plants were grown in a commercial soilless medium composed of composted pine bark, horticultural vermiculite, Canadian sphagnum peat moss, processed bark ash, and washed sand (MetroMix 510, Scotts-Sierra Horticultural Products Company, Marysville, Ohio, year 1). Plants were top-watered with well water acidified (two parts H_3PO_4 plus one part H_2SO_4, which provided ≈2.5 mol $P{\cdot}m^{-3}$) to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and fertilized with 14N-0P-6K_2O (mol·m^{-3}) from potassium nitrate (14-0p-55K_20) (Vicksburg Chemical o., Vicksburg, MS) and ammonium nitrate (34N-0P-0K_2O) (Cargill, Lexington, KY). Fertilization and acidification rates were adjusted in response to weekly soil test results, so regimens varied during experiments. Our target range for soil pH was 5.8 to 6.2 and, and 0.5 to 1.2 for soil EC.

All cold treatments were delivered in a 5 $\pm$ 0.5 °C controlled environment chamber lit for 9 $h \bullet d^{-1}$ at approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with cool-white fluorescent lamps (VHOF96T12; Philips, Bloomfield, N.J.), as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE). Plants were cold-treated in the 128-cell plug trays in which they were received or the 1.1-L (5" U.S.) square pots they were transplanted into. While in the cooler, plants were watered two to three times a week as needed with well water acidified (H_2SO_4) to an approximate pH of 6.0.

Leaf unfolding rates (Expt. 1). On October 15, 1997, 128 plant of each cultivar were grown in plug trays at each of four different temperatures; 17, 20, 23, and 26 °C, to three ages; 12 (group1), 16 (group 2), and 20 (group3) weeks. New, fully expanded leaves were counted at 5, 6, 7, 8 and 12 weeks of age for each individual plug in group 1 at each temperature. Leaf counts were taken at 5, 6, 7, 8, 12, and 15 weeks of age for group 2, and 5, 6, 7, 8, 12, 16, and 20 weeks for group 3. New leaves were marked with a dot of liquid paper correction fluid. Leaf un-folding rates were calculated for each temperature. Linear regression analysis was used to determine and describe the rate of leaf unfolding within the range of temperatures studied. Linear regression functions were developed for the rate of leaf unfolding per day for each cultivar studied.

All plugs were grown under natural light plus light from high-pressure sodium lamps at PPF levels of 50 $\mu mol \cdot m^{-2} s^{-1}$ to extend the photoperiod to 16 hours. From 0800 to 1700 HR, high-pressure sodium lamps provided a photosynthetic photon flux (PPF) of approximately 50 $\mu mol \cdot m^{-2} s^{-1}$ at plant level when the ambient greenhouse PPF dropped to 200 $\mu mol \cdot m^{-2} s^{-1}$ and discontinued when PPF reached 400 $\mu mol \cdot m^{-2} s^{-1}$ as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE).

Effects of temperature, plant age, and leaf number before cold, and the effects of photoperiod before and after cold. (Expt. 2).

For Expt. 2, a third of the plugs of each cultivar from groups 1, 2, and 3 in Expt. 1 were removed from the greenhouse at 12, 16, and 20 weeks of age. One-half of these plugs were placed directly into a 5 °C cooler for 12 weeks. The other half were transplanted directly into 1.1-L (5" U.S.) square pots, then grown to the ages of 20 (group 4), 24 (group 5), and 28 (group 6) weeks. Half of these potted plants were grown for the eight week period under a 4-hour night interruption (NI) from 2200 to 0200 HR and the other half under 9 hours of continuous natural light before cold treatment. Potted-plants were then transferred to a 5 °C cooler for 12 weeks.

After the cold treatment, plug-cooled seedlings from groups 1, 2, and 3 were transplanted into 1.1-L (5"U.S.) square pots. Half of the plugs from each group were grown under NI from 2200 to 0200 HR and the other half under 9 hours of continuous natural light in a greenhouse set at 20 °C. The NI photoperiod was completed with incandescent lamps at 1 to 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. High-pressure sodium lamps provided a photosynthetic photon flux (PPF) of approximately 50 $\mu mol \cdot m^{-2}\ s^{-1}$ at plant level when the ambient greenhouse PPF dropped to 200 $\mu mol \cdot m^{-2}\ s^{-1}$ and discontinued when PPF reached 400 $\mu mol \cdot m^{-2}\ s^{-1}$ as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE).

After cold treatment, the pot-cooled plants from groups 4, 5, and 6 were grown in a greenhouse set at 20 °C, under 9 hours of natural light plus light from high-pressure sodium lamps at a PPF level of 50 $\mu mol \cdot m^{-2}\ s^{-1}$ to extend the photoperiod to 16 hours. From 0800 to 1700 HR, high-pressure sodium lamps provided a photosynthetic photon flux (PPF) of approximately 50 $\mu mol \cdot m^{-2}\ s^{-1}$ at plant level when the ambient greenhouse PPF dropped to 200 $\mu mol \cdot m^{-2}\ s^{-1}$ and discontinued when PPF reached 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE). Due to a lighting problem in the greenhouse, data for the 12-week-old plugs grown under SD are not presented for the cultivars studied.

Counts of new, fully-expanded leaves were conducted for the potted plants at 15 and 20 weeks of age for group 4 , weeks 20 and 24 for group 5, and weeks 24 and 28 for group 6 in order to establish leaf number before cold treatment. The procedure for leaf-counting is as described in experiment 1. Air temperatures on each bench were monitored with 36-gauge (0.013-mm-diameter) type E thermocouples connected to a CR10 datalogger (Campbell Scientific, Logan, UT). The datalogger collected temperature data every 10 seconds and recorded the hourly average. Actual average daily air temperatures from the beginning of forcing to the average date of flowering were calculated for each species. 480 plugs per cultivar were used to provide 10 plants per treatment.

Data collection and analysis

The initial leaf number was recorded for each plant before the cold treatment. Date of the first visible bud and date of opening of the first flower were recorded for each plant. At flowering, the number of visible flower buds, and total plant height were determined. Days to visible bud, days from visible bud to flower, and days to flower were calculated.

Results and Discussion

Leaf unfolding rates (Expt 1). All cultivars studied had similar rates of leaf unfolding. Increasing temperature from 17 to 26 °C slightly increased total leaf number (Table 3, Figure 1). A peak leaf unfolding value was observed for 'Bluejay' and 'Crimson Star' at 23 °C (Table 3, Figure 1). For 'Cameo', 'McKana's Giant', and 'Musik-White', the rate of leaf unfolding rate continued to increase linearly as temperature increased from 17 to 26 °C (Table 3). Maximum rates for all cultivars were between 0.14 and 0.13 leaves/day (Table 3). Thus, 7.3 days and 7.9 days are expected to be required to unfold one leaf for each cultivar.

The overall effect of temperature on the rate of leaf unfolding was small for all cultivars studied. The difference in rate of leaf unfolding per day from the lowest temperature to the temperature that promoted maximum leaf unfolding

rate was 0.004 leaves/ day for 'Crimson Star', 0.025 leaves/ day for 'McKana's Giant', and 0.03 leaves/ day for 'Bluejay' and 'Musik-White' (Table 3). The largest difference observed was for plants of 'Cameo', which ranged from 0.06 leaves unfolded/ day at 17 °C, to 0.14 leaves/day at 26 °C (Table 3). The temperatures that promoted the highest rate of leaf development in *Aquilegia* cultivars, 23 to 26 °C, were similar to other plants such as *Saintpaulia ionantha* (African Violet) (Faust and Heins, 1993).

Effects of temperature, plant age, and leaf number before cold, and the effects of photoperiod before and after cold. (Expt. 2).

Initial leaf number. Increasing temperature before cold treatment increased total leaf number (Table 9). The temperature during plug growth had a lasting effect on seedlings from groups 4, 5, and 6 that were transplanted and grown for eight additional weeks at 20 °C prior to cold treatment. The plants that were previously grown at higher temperatures consistently maintained a slightly higher leaf number than those previously grown at lower temperatures (Tables 4-8, Figure 2). As expected, older seedlings had more leaves than younger seedlings (Tables 4-10, Figure 2).

There were some statistically significant effects of photoperiod on leaf number for the pot-cooled plants of all cultivars (Tables 4-8). Plants grown under NI lighting before cold treatment almost always had a higher leaf number than those grown under SD lighting (Tables 4-8, Figure 2). The greatest differences in leaf number in relation to photoperiod were observed for plants of 'Bluejay' and 'Cameo'. For example, the 20 week-old potted plants of 'Bluejay' averaged 13.2

to 14.7 under SD, and 15.1 to 18.8 leaves under NI before cold treatment (Table 5, Figure 2). The 20 week-old potted plants of 'Cameo' averaged 11.9 to 12.1 leaves under SD, and 12.5 to 17.6 leaves under NI lighting (Table 5, Figure 2). Differences were less significant for plants of "Crimson Star', 'McKana's Giant', and 'Musik-White' (Tables 6-8).

There was no effect of photoperiod on the initial leaf number of the plug-cooled plants because different photoperiods were not delivered to these plants until after cold treatment (Tables 6-8, Figure 2).

Flowering percentage. Initial leaf number before cold treatment had a significant effect on percentage flowering for all cultivars studied. All pot-cooled plants of 'Cameo', 'Crimson Star', and 'McKana's Giant', had $\geq$ 10 leaves before cold treatment. One hundred percent of these plants flowered with the exception of the 10-leaf 'McKana's Giant' plants grown under NI lighting before cold (Tables 4,5, and 7, Figure 3).

All pot-cooled plants of 'Bluebird' or 'Musik-White had $\geq$ 11 or 14 leaves, respectively, before cold treatment (Tables 4 and 8, Figure 3). No pot-cooled plants of 'Bluejay' or 'Crimson Star' flowered with an initial leaf number $\leq$ 12 (Tables 4 and 6, Figure 3). All plants of both cultivars with an initial leaf number >12 flowered, with the exception of the 13-leaf plants of 'Bluejay' grown under SD before cold (Tables 4 and 6, Figure 3).

Flowering percentage for the plug-cooled plants of all cultivars was far lower than for pot-cooled plants (Tables 4-8, Figure 3). According to the results of the present study, leaf number before cold is not the only factor determining ripeness to flower. In most cases, pot-cooled plants reached a much higher flowering percentage than plug-cooled plants with the same initial leaf number (Tables 4-8, Figure 3). For example, no plug-cooled plants of 'Musik-White' with 14 initial leaves flowered, whereas all 14-leaf pot-cooled plants flowered (Table 8, Figure 3). In fact, 100% flowering was never reached in any treatment for plug-cooled plants of 'Musik-White' (Table 8, Figure 3). Only several treatments of plug-cooled plants of 'Bluejay', 'Crimson Star', and 'McKana's Giant' reached 100% flowering (Tables 4-7, Figure 3). 'Cameo' was the only cultivar in which the plug-cooled plants flowered similarly to the pot-cooled plants (Table 5, Figure 7). All plug-cooled plants of 'Cameo' with an initial leaf number ≥ 5 flowered (Table 5, Figure 7). Only plug-cooled plants of 'Cameo' with an initial leaf number < 5 did not flower or did not reach 100% flowering (Table 5, Figure 3).

Temperature before cold treatment greatly affected flowering percentage for plug-cooled plants (Tables 4-8). Flowering percentages for 'McKana's Giant', 'Crimson Star', 'Bluejay', and 'Musik-White' were higher for plugs that were grown at 17 and 20 °C as opposed to 23 and 26 °C (Tables 4-8). Photoperiod also affected flowering percentage for plug-cooled plants, overall, more plants flowered when grown under SD after cold than under NI lighting (Table 4-8, Figure 3)

Flower timing. Overall, the temperature at which the plugs were grown before cold treatment, and the photoperiod plants were grown under before and after cold, both had an effect on days to visible bud after cold treatment for all cultivars studied (Tables 4-8, Figure 8). Plants grown under SD before cold flowered slightly faster than plants grown under NI before cold. Conversely, plants forced under SD after cold flowered slightly slower than plants forced under NI, although, the actual differences were small and no trends were observed (Tables 4-8).

Total plant age had a significant effect on days to visible bud for all cultivars tested (Tables 4-10). For example, days to visible bud for pot-cooled and plug-cooled plants of 'Bluejay' decreased by and average of 6 and 14 days as age increased from 20 to 28 and 12 to 20 weeks, respectively (Table 4, Figure 5). Days to visible bud for pot-cooled plants of 'Cameo' decreased by an average of 6 days, and 9 days for plug-cooled plants of 'Cameo' (Table 5, Figure 5). Days to visible bud for pot-cooled plants of 'Crimson Star' decreased by an average of 14 days, but decreased by only four days for plug-cooled plants (Table 6, Figure 5). Highly significant linear and quadratic trends were observed for 'Bluejay', 'Crimson Star', and 'Cameo' (Tables 4-6). Significant quadratic trends were observed for plants of 'McKana's Giant' and 'Musik-White' (Tables 7 and 5). Days to visible bud for pot-cooled plants decreased by an average of 14 days for both 'McKana's Giant' and 'Musik-White' as age increased from 20 to 28 weeks, but varied with age for the plug-cooled plants (Tables 7 and 8, Figure 5).

As leaf number increased with age, there were statistically significant decreases in days to flower for all cultivars studied (Tables 4-8). However, no linear or quadratic trends were observed for plants of 'McKana's Giant' or 'Musik-

White' (Tables 7 and 8). Significant linear and quadratic trends were observed for plants of 'Bluejay', 'Cameo', and 'Crimson Star' and days to flower decreased with increased leaf number (Tables 4, 5, and 6, Figure 6). For plants of 'Bluejay' and 'McKana's Giant', the time from visible bud was affected by total plant age as initial leaf number increased (Table 4 and 6, Figure 6). As leaf number increased, the days from visible bud to flower decreased slightly and significant linear and quadratic trends were observed (Tables 4 and 6). There were no significant trends for days from visible bud to flower for plants of 'Cameo', 'Crimson Star', or 'Musik-White' (Tables 5, 6, and 8). Overall, days to visible bud and flower were significantly lower for pot-cooled plants than plug-cooled plants (Tables 5-9).

Flower number. Cooling plants in pots had a significant effect on flower number for all cultivars (Tables 4-8). Pot-cooled plants of all cultivars tested consistently had a higher flower number than plug-cooled plants of the same age (Tables 4-8, Figure 7). For example, 20 week-old pot-cooled plants of 'Bluejay' had an average of 22 flowers at the time of first flower, whereas 20 week-old plug-cooled plants had an average of only 9 flowers (Table 4, Figure 7). This trend was similar for all other cultivars tested (Tables 4-8).

Plant age in weeks had a significant effect on flower number and significant linear and quadratic trends were observed for all cultivars tested (Tables 4-8). Flower number of plug-cooled plants increased as age increased from 12 to 20 weeks, however, flower number for the pot-cooled plants actually decreased as age increased from 20 to 28 weeks (Tables 4-8, Figure 7). For example, flower number for the plug-cooled plants of 'Musik-White' increased by 9 as age increased, but decreased by 7 for the pot-cooled plants as age increased from 20 to 28 weeks (Table 8, Figure 7). Flower number or the pot-cooled plants may have decreased because they flowered earlier than plug-cooled plants. Some flower buds may develop after the first flower opens, and some may not yet be visible, therefore, the number of visible flower buds at first flower may not be an accurate estimation of total flower number.

Plant height. Plant size (pot-cooled versus plug-cooled), age, photoperiod, and temperature before cold, all affected plant height (Tables 4-8). Plant height was generally greater for older plants cooled in pots, and there were significant linear and quadratic trends for 'Bluejay', 'Cameo', Crimson Star', and 'McKana's Giant' (Tables 4-7). There was no significant difference in plant height at flower between the pot-and plug-cooled plants of 'Musik-White' (Table 8, Figure 8). Overall, plants grown under NI lighting before or after cold were significantly taller than those grown under SD (Tables 4-8, Figure 8). Temperature before cold treatment significantly affected plant height for plants of 'Bluejay' and 'Crimson Star' with linear and quadratic trends, respectively (Tables 4 and 6). Plant height tended to decrease as the pre-cold temperature increased. There were no significant effects of temperature before cold for 'Cameo', 'McKana's Giant', or 'Musik-White' (Tables 5, 7, and 8, Figure 8).

Conclusion

The results of the present study indicate that faster leaf unfolding and vegetative development may be achieved at high temperatures for the *Aquilegia* cultivars studied. In the temperature range of 17 to 26 °C, the leaf unfolding rate showed minor variations and remained above 0.13 leaves/day for all cultivars tested as the predicted peak leaf unfolding rate was approached at 23 °C for two cultivars and 26 °C for three cultivars. Overall, the differences in leaf unfolding rates between temperature treatments were small. Because vapor pressure deficit (VPD) was not monitored in the greenhouse, it is possible that the difference in shoot tip temperature was smaller than the range of air temperatures. VPD increases at higher temperatures and causes evaporation from the soil surface, which may result in a lower shoot tip temperature. *Aquilegia* remain in a rosette until bolting and leaves unfold close to the soil surface. Combined, these factors may account for the small difference in leaf unfolding rates in relation to air temperature. In most practical applications, this small difference in leaf unfolding rate is unimportant, but the rates of leaf unfolding can be used to monitor the vegetative plant development in the cultivars of *Aquilegia* studied. The rates can be used to predict how many weeks are necessary at specific temperatures for these cultivars of *Aquilegia* to develop enough leaves for maturity and subsequent flowering.

The higher temperatures may have promoted a higher rate of leaf development, however, flowering percentage for plants grown at higher temperatures before cold treatment decreased. It was expected that the seedlings grown at the higher temperatures would have a higher flowering percentage because they had more leaves before cold treatment. However, it seems that the higher temperatures caused deleterious effects on subsequent flowering. It is possible that soil temperature increased sufficiently to impede root growth. Because the differences in leaf unfolding rates/day are so small in relation to temperature for each cultivars studied, it is advantageous to grow plugs at 20 °C as opposed to temperatures $\geq$ 23 °C to prevent deleterious effects on flowering.

The purpose of the second phase of the experiment was to accurately determine the specific leaf number required by each cultivar for flowering. However, leaf number alone could not be used to predict maturity. Plant size affected flowering percentage as well as leaf number. Chilling plants that were allowed to grow and establish in pots before cooling increased flowering percentage in comparison to plug-cooled plants, regardless of leaf number. Results from the present study suggest that root growth, in addition to leaf development, may be an important factor in reaching maturity for *Aquilegia* plants. When root growth is restricted in small containers, it is possible that plants are unable to store enough nutrients for subsequent flowering even if the vernalization requirement has been met. The results correspond to those reported in chapter 2. No studies were found in the literature which provided similar information on *Aquilegia*.

The *Aquilegia* cultivars studied flowered as day-neutral plants, but flowered slightly faster under long days, Leaf petioles elongated excessively under NI compared to those of plants grown under SD, and total plant height at flowering was also greater for plants grown under long days. Because the difference in time-to-flower in relation to photoperiod is small, growing plants under natural days or SD after cold treatment is recommended to avoid undesirable elongation and height.

The ability to cool small plugs of *Aquilegia*, as opposed to overwintering plants in large pots, is an attractive alternative for many commercial producers. For crops with a long juvenile phase, such as *Aquilegia*, commercial producers increase vegetative growth (bulking) of the plants before they administer cold treatments. The ability to cool seedlings in a controlled-environment chamber would allow the producer to provide more accurate and constant cooling and to cool plants at different times of year for staggered production schedules. Production time and labor could be reduced, and space utilization maximized if plants could be cooled in small plugs instead of large containers. However, based on the results of the present study and those presented in chapter 2, it is difficult to achieve proper plant size when seedlings are grown and cooled in the plug tray. Container volume in addition to container depth must be considered when forcing *Aquilegia*. Aquilegia plants need to be grown in containers that do not restrict root growth prior to cold treatment. Growing and forcing recommendations for 'Cameo', 'Bluejay', 'Crimson Star', 'McKana's Giant', and 'Musik-White' are listed in Table 11.

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Table 1. All seedlings were four weeks of age when they were received at MSU on October 13, 1997. 128 Seedlings of each cultivar were placed at 17, 20, 23, and 26 °C under 16-h photoperiods with HPS lamps. Half of the seedlings from each cultivar were moved directly into a 5 °C cooler on the dates listed below and cooled in the plug tray. The treatments for the remaining seedlings are listed in Table 2.

All plugs were received on 10/13/97 at four weeks of age. Plugs were placed under each temperature on 10/15/97.			Average greenhouse air temperature during plug growth (°C)			
Weeks grown under each temperature	Total age when moved to cooler	Date moved to cooler	Temperature			
			17°C	20°C	23°C	26°C
8	12	12/8/98	17.3	20.3	23.2	26.4
12	16	1/5/98	17.5	20.4	22.9	26.5
16	20	2/2/98	17.7	20.8	23.4	26.1

Table 2. Average greenhouse temperatures for all cultivars during forcing, after cold treatment.

Total age when moved to greenhouse after cold under SD or NI at 20 °C	Date moved to SD or NI	Average greenhouse temperature (°C)	
		20 °C SD	20 °C NI
12	3/8/98	20.5	20.6
16	4/6/98	21.1	21.2
20	5/3/98	21.3	21.3
Total age when moved greenhouse after cold to 16-hour photoperiod at 20 °C	**Date moved to 16-hr photoperiod**	**Average greenhouse temperature (°C)**	
		20 °C SD	
12	5/7/98	21.1	
16	6/6/98	22.2	
20	7/4/98	23.4	

Table 3. Leaf unfolding rates/day at each growing temperature before cold treatment.

Temperature (°C)	Leaf unfolding rate /day	r^2
	A. xhybrida 'Bluejay'	
17	0.108	0.99
20	0.122	0.99
23	0.138	0.99
26	0.135	0.99
	A. flabellata 'Cameo'	
17	0.068	0.98
20	0.100	0.97
23	0.122	0.98
26	0.135	0.99
	A. . xhybrida 'Crimson Star'	
17	0.121	0.99
20	0.125	0.99
23	0.125	0.99
26	0.105	0.97
	A. xhybrida 'McKana's Giant'	
17	0.100	0.98
20	0.104	0.99
23	0.114	0.99
26	0.125	1.00
	A. xhybrida 'Musik-White'	
17	0.108	0.98
20	0.127	0.99
23	0.124	0.98
26	0.138	0.99

Table 4. Plants of *Aquilegia xhybrida* 'Bluejay' were cooled in either pots (20, 24, and 28 weeks old) or in plug trays (12, 16, and 20 weeks old). Initial leaf counts for each plug were taken before they were placed into the 5°C cooler. Flower number and final plant height were measured at the time of first open flower.

Table 4.

	Photo-period *before cold **after cold	Age wks	Plug growing temperature before cold (° C)	Percentage flowering	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Pots	*9	20	17	100	13.2	15	15	30	22	41
	*9	20	20	100	14.1	20	16	36	22	40
	*9	20	23	100	14.9	21	17	38	18	36
	*9	20	26	90	14.7	27	15	42	23	39
	*NI	20	17	70	15.1	18	15	33	21	42
	*NI	20	20	100	16.1	17	15	32	25	47
	*NI	20	23	100	17.2	23	17	40	23	41
	*NI	20	26	100	18.8	21	16	37	26	47
	*9	24	17	100	16.3	20	18	38	27	39
	*9	24	20	100	16.5	20	18	38	26	43
	*9	24	23	100	16.9	19	17	36	21	36
	0	24	26	100	16.8	15	18	33	26	40
	*NI	24	17	100	18.3	20	16	36	24	45
	*NI	24	20	100	18.6	19	17	36	25	44
	*NI	24	23	100	20.6	20	15	35	24	45
	*NI	24	26	100	21.2	19	17	36	28	38
	*9	28	17	100	19.7	14	11	25	13	34
	*9	28	20	100	20.9	14	14	28	19	36
	*9	28	23	100	22	12	16	28	16	41
	*9	28	26	100	23.8	11	15	26	20	43
	*NI	28	17	100	22.9	17	15	32	16	35
	*NI	28	20	100	23.1	18	14	32	18	41
	*NI	28	23	100	24	19	17	36	18	39
	*NI	28	26	100	25.1	17	15	32	14	44
Plugs	**NI	12	17	0	7.1	.	.	.	.	.
	**NI	12	20	0	7.3	.	.	.	.	.
	**NI	12	23	0	7.3	.	.	.	.	.
	**NI	12	26	0	8.2	.	.	.	.	.
	**9	16	17	30	10	32	17	49	8	29
	**9	16	20	50	11.7	33	16	49	7	31
	**9	16	23	50	12.2	35	16	51	8	28
	**9	16	26	40	12.6	33	14	47	11	30
	**NI	16	17	60	10.1	31	18	49	9	32
	**NI	16	20	40	10.8	29	16	45	10	31
	**NI	16	23	80	12.6	30	16	46	14	31
	**NI	16	26	60	12.9	31	19	49	9	24
	**9	20	17	20	12.8	33	13	45	9	29
	**9	20	20	60	11.6	31	13	44	9	30
	**9	20	23	50	16.1	28	16	44	8	31
	**9	20	26	40	16.1	30	14	44	9	27
	**NI	20	17	50	13.2	32	14	46	10	27
	**NI	20	20	20	13.4	50	14	64	11	30
	**NI	20	23	60	16.6	30	15	45	11	27
	**NI	20	26	50	16.6	34	16	50	12	37

Table 4 (con't)

	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Significance						
Pot or Plug (Potplug)	***	***	NS	***	***	***
Age	***	***	***	***	***	**
Potplug x Age	-	-	-	-	-	-
Photoperiod	***	NS	NS	NS	NS	***
Potplug x Photoperiod	***	**	NS	NS	NS	*
Age x Photoperiod	***	NS	*	***	NS	NS
Potplug x Age x Photoperiod	-	-	-	-	-	-
Temperature	***	NS	NS	**	*	*
Potplug x Temperature	*	*	***	NS	NS	*
Age x Temperature	***	***	***	***	NS	***
Potplug x Age x Temperature	-	-	-	-	-	-
Photoperiod x Temperature	***	NS	NS	NS	NS	NS
Potplug x Photoperiod x Temperature	*	*	***	NS	NS	**
Age x Photoperiod x Temperature	***	*	NS	NS	NS	*
Potplug x Age x Photoperiod x Temperature	-	-	-	-	-	-
Contrasts						
9 hours vs. 4-hr Night Interruption (NI)	NS	NS	NS	*	NS	*
Age: 12, 16, 20, *20, 24, 28 wks						
P_{Linear}	***	***	NS	***	***	***
$P_{quadratic}$	*	**	NS	***	***	***
Temperature: 17C, 20C, 23C, and 26C						
P_{Linear}	NS	NS	NS	NS	NS	**
$P_{quadratic}$	NS	NS	*	NS	NS	NS

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

Table 5. Plants of *Aquilegia flabellata* 'Cameo' were cooled as either pots (20, 24, and 28 weeks old) or as plugs (12, 16, and 20 weeks old). Initial leaf counts for each plug were taken before they were placed into the 5°C cooler. Flower number and final plant height were measured at the time of first open flower.

Table 5.

	Photo-period *before cold **after cold	Age (wks)	Plug growing temperature before cold (° C)	Percentage flowering	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Pots	*9	20	17	100	11.9	16	13	29	9	12
	*9	20	20	100	12.4	16	6	29	8	12
	*9	20	23	100	13.3	18	8	32	8	11
	*9	20	26	100	12.1	9	9	23	11	13
	*NI	20	17	100	12.5	18	10	28	10	10
	*NI	20	20	100	15.0	13	12	25	13	11
	*NI	20	23	100	16.5	18	9	27	10	11
	*NI	20	26	100	17.6	18	8	26	10	11
	*9	24	17	100	14.7	13	5	18	6	11
	*9	24	20	100	16.4	14	5	19	6	11
	*9	24	23	100	19.0	13	9	23	6	12
	*9	24	26	100	22.2	12	8	20	8	14
	*NI	24	17	100	12.5	21	5	30	8	13
	*NI	24	20	100	13.6	17	9	26	8	12
	*NI	24	23	100	12.9	17	9	26	8	12
	*NI	24	26	100	11.8	19	10	29	10	14
	*9	28	17	100	14.1	19	5	24	14	12
	*9	28	20	100	15.0	20	7	27	14	15
	*9	28	23	100	17.6	22	9	31	12	13
	*9	28	26	100	19.2	23	11	34	12	12
	*NI	28	17	100	16.3	22	8	30	8	13
	*NI	28	20	100	18.2	21	8	29	9	10
	*NI	28	23	100	20.3	23	10	33	7	13
	*NI	28	26	100	23.7	22	8	30	8	14
Plugs	**NI	12	17	100	4.7	28	10	37	6	9
	**NI	12	20	100	6.9	22	10	32	7	9
	**NI	12	23	100	8.0	27	10	37	7	7
	**NI	12	26	100	6.5	29	16	45	5	7
	**9	16	17	100	7.2	23	14	37	5	8
	**9	16	20	100	9.5	23	14	36	6	10
	**9	16	23	100	11.1	22	14	36	4	8
	**9	16	26	100	14.6	22	14	36	6	8
	**NI	16	17	100	7.7	19	14	33	7	9
	**NI	16	20	100	8.7	22	11	32	7	10
	**NI	16	23	100	11.9	17	13	30	6	8
	**NI	16	26	100	12.6	18	14	32	6	8
	**9	20	17	100	10.1	18	15	33	6	8
	**9	20	20	100	12.3	18	14	24	7	9
	**9	20	23	100	14.6	19	15	34	8	9
	**9	20	26	100	15.8	18	15	35	7	8
	**NI	20	17	100	9.4	15	16	31	7	10
	**NI	20	20	100	11.0	15	16	31	7	9
	**NI	20	23	100	15	16	14	30	7	8
	**NI	20	26	100	16.2	17	18	35	8	9

Table 5 (con't)

	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Significance						
Pot or Plug (Potplug)	***	NS	NS	***	***	***
Age	***	NS	NS	***	***	*
Potplug x Age	-	-	-	-	-	-
Photoperiod	***	NS	NS	*	***	***
Potplug x Photoperiod	***	NS	NS	NS	NS	*
Age x Photoperiod	**	NS	NS	*	*	*
Potplug x Age x Photoperiod	-	-	-	-	-	-
Temperature	***	NS	NS	***	NS	NS
Potplug x Temperature	***	NS	NS	NS	***	***
Age x Temperature	***	NS	NS	***	**	***
Potplug x Age x Temperature	-	-	-	-	-	-
Photoperiod x Temperature	NS	NS	NS	NS	NS	*
Potplug x Photoperiod x Temperature	*	NS	NS	NS	NS	NS
Age x Photoperiod x Temperature	**	NS	NS	NS	NS	NS
Potplug x Age x Photoperiod x Temperature	-	-	-	-	-	-
Contrasts						
9 hours vs. 4-hr Night Interruption (NI)	*	NS	NS	NS	NS	*
Age: 12, 16, 20, *20, 24, 28 wks						
P_{Linear}	***	NS	NS	***	***	***
$P_{quadratic}$	NS	NS	NS	***	***	NS
Temperature: 17C, 20C, 23C, and 26C						
P_{Linear}	***	NS	NS	NS	NS	NS
$P_{quadratic}$	NS	NS	NS	NS	NS	NS

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

Table 6. Plants of *Aquilegia xhybrida* 'Crimson Star' were cooled as either pots (20, 24, and 28 weeks old) or as plugs (12, 16, and 20 weeks old). Initial leaf counts for each plug were taken before they were placed into the 5°C cooler. Flower number and final plant height were measured at the time of first open flower.

Table 6.

	Photo-period *before cold **after cold	Age wks	Plug growing temperature before cold (°C)	Percentage flowering	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Pots	*9	20	17	60	12.8	31	16	47	26	39
	*9	20	20	90	14.6	19	25	44	32	35
	*9	20	23	70	14	31	14	45	20	37
	*9	20	26	90	15	30	16	46	22	40
	*NI	20	17	50	13.1	35	15	50	17	41
	*NI	20	20	80	14.9	38	15	53	22	42
	*NI	20	23	80	14.8	37	15	53	24	39
	*NI	20	26	100	15.1	36	15	51	23	41
	*9	24	17	100	16.9	27	13	39	30	31
	*9	24	20	100	17.7	26	17	43	28	39
	*9	24	23	100	17.8	24	17	46	23	35
	*9	24	26	100	18.2	28	20	41	26	36
	*NI	24	17	100	18.2	30	18	48	26	46
	*NI	24	20	100	19.1	31	17	48	27	41
	*NI	24	23	100	18.5	29	18	46	24	43
	*NI	24	26	100	20.8	31	16	46	29	42
	*9	28	17	100	20.9	19	16	35	22	40
	*9	28	20	100	20.5	20	15	35	23	40
	*9	28	23	100	20.8	19	13	32	24	45
	*9	28	26	100	21.6	19	16	35	20	44
	*NI	28	17	100	22	20	15	34	19	43
	*NI	28	20	100	22.6	18	16	34	23	44
	*NI	28	23	100	22.9	19	15	34	22	46
	*NI	28	26	100	23.9	19	16	36	21	43
Plugs	**NI	12	17	50	8.1	40	18	58	8	17
	**NI	12	20	40	8.6	30	16	45	24	31
	**NI	12	23	0	8.9	-	-	-	-	-
	**NI	12	26	0	9.3	-	-	-	-	-
	**9	16	17	30	12.1	43	16	59	13	27
	**9	16	20	40	12.4	41	16	57	17	29
	**9	16	23	30	12.7	42	16	58	16	30
	**9	16	26	20	14.4	40	16	56	18	31
	**NI	16	17	40	13.2	33	16	49	17	39
	**NI	16	20	40	12.4	34	16	50	17	43
	**NI	16	23	40	12.4	33	16	49	17	42
	**NI	16	26	40	12.8	34	17	51	17	40
	**9	20	17	40	14.5	34	18	42	20	29
	**9	20	20	40	15.4	37	18	54	22	32
	**9	20	23	30	15.7	36	15	51	23	30
	**9	20	26	0	16	-	-	-	-	-
	**NI	20	17	30	14.3	34	18	52	20	39
	**NI	20	20	20	15.5	34	17	51	20	37
	**NI	20	23	30	15.3	32	16	48	26	44
	**NI	20	26	0	16.1	-	-	-	-	-

Table 6 (con't).

	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Significance						
Pot or Plug (Potplug)	***	***	*	**	***	***
Age	***	***	NS	***	***	***
Potplug x Age	-	-	-	-	-	-
Photoperiod	**	NS	NS	NS	NS	***
Potplug x Photoperiod	***	NS	*	NS	NS	***
Age x Photoperiod	***	NS	NS	NS	NS	***
Potplug x Age x Photoperiod	-	-	-	-	-	-
Temperature	***	NS	NS	NS	*	***
Potplug x Temperature	*	**	***	**	NS	NS
Age x Temperature	NS	***	NS	***	NS	NS
Potplug x Age x Temperature	-	-	-	-	-	-
Photoperiod x Temperature	NS	NS	NS	NS	NS	NS
Potplug x Photoperiod x Temperature	**	NS	***	NS	NS	NS
Age x Photoperiod x Temperature	NS	NS	NS	NS	NS	NS
Potplug x Age x Photoperiod x Temperature	-	-	-	-	-	-
Contrasts						
9 hours vs. 4-hr Night Interruption (NI)	*	NS	NS	NS	NS	**
Age: 12, 16, 20, 20 [1], 24, 28 wks						
P_{Linear}	***	***	NS	***	***	***
$P_{quadratic}$	NS	***	NS	***	**	**
Temperature: 17C, 20C, 23C, and 26C						
P_{Linear}	NS	NS	NS	*	NS	NS
$P_{quadratic}$	NS	NS	NS	NS	NS	**

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively
[1] 20 week old pots

Table 7. Plants of *Aquilegia xhybrida* 'McKana's Giant' were cooled as either pots (20, 24, and 28 weeks old) or as plugs (12, 16, and 20 weeks old). Initial leaf counts for each plug were taken before they were placed into the 5°C cooler. Flower number and final plant height were measured at the time of first open flower.

Table 7.

	Photo-period *before cold **after cold	Age wks	Plug growing temperature before cold (° C)	Percentage flowering	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Pots	*9	20	17	90	14.3	31	16	47	26	38
	*9	20	20	100	14.2	29	15	44	24	44
	*9	20	23	100	13.9	30	16	46	22	41
	*9	20	26	100	14.7	34	15	49	24	39
	*NI	20	17	100	15.0	37	15	52	26	45
	*NI	20	20	100	14.9	36	15	51	30	39
	*NI	20	23	100	14.9	26	14	50	24	39
	*NI	20	26	100	14.3	33	18	51	26	40
	*9	24	17	100	18.8	29	17	46	28	39
	*9	24	20	100	17.4	32	13	45	20	34
	*9	24	23	100	17.8	31	14	45	25	41
	*9	24	26	100	18.8	33	16	49	21	42
	*NI	24	17	100	19.8	34	14	48	19	40
	*NI	24	20	100	19.0	30	20	50	20	43
	*NI	24	23	100	18.4	27	19	46	19	42
	*NI	24	26	100	19.8	30	19	49	24	35
	*9	28	17	100	21.4	22	12	34	24	39
	*9	28	20	100	19.5	20	15	35	22	38
	*9	28	23	100	20.7	22	15	37	21	43
	*9	28	26	100	21.4	20	15	35	19	35
	*NI	28	17	100	22.1	23	16	39	20	39
	*NI	28	20	100	19.9	22	17	39	20	45
	*NI	28	23	100	21.3	23	17	40	21	47
	*NI	28	26	100	22.1	23	16	39	18	44
Plugs	**NI	12	17	50	9.6	26	24	50	12	30
	**NI	12	20	20	7.2	33	18	51	11	27
	**NI	12	23	0	7.3	.	.	.	.	.
	**NI	12	26	0	7.9	.	.	.	.	.
	**9	16	17	30	10.9	45	17	62	19	32
	**9	16	20	30	10.3	44	17	61	26	30
	**9	16	23	40	10.9	45	18	62	12	22
	**9	16	26	30	11.3	45	16	61	18	26
	**NI	16	17	30	10.4	46	19	65	16	26
	**NI	16	20	40	10.2	50	16	65	17	40
	**NI	16	23	10	11.1	51	16	67	16	45
	**NI	16	26	0	11.3	.	.	.	.	.
	**9	20	17	60	14.1	36	17	52	13	34
	**9	20	20	40	13.3	35	17	52	13	32
	**9	20	23	30	12.1	36	16	52	12	31
	**9	20	26	30	14.7	36	17	43	13	29
	**NI	20	17	50	13.5	31	17	48	15	49
	**NI	20	20	30	13.3	32	16	48	17	47
	**NI	20	23	20	13.4	31	16	47	16	50
	**NI	20	26	40	15.8	30	16	46	19	49

Table 7 (con't)

	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Significance						
Pot or Plug (Potplug)	***	*	NS	*	***	***
Age	***	*	***	*	***	***
Potplug x Age	-	-	-	-	-	
Photoperiod	***	NS	**	NS	NS	***
Potplug x Photoperiod	*	NS	NS	NS	NS	***
Age x Photoperiod	NS	NS	NS	NS	*	*
Potplug x Age x Photoperiod	-	-	-	-	-	-
Temperature	***	NS	NS	NS	NS	NS
Potplug x Temperature	NS	***	**	***	NS	NS
Age x Temperature	***	**	NS	**	NS	NS
Potplug x Age x Temperature	-	-	-	-	-	-
Photoperiod x Temperature	NS	NS	NS	NS	NS	NS
Potplug x Photoperiod x Temperature	NS	NS	*	NS	NS	NS
Age x Photoperiod x Temperature	NS	NS	NS	NS	NS	**
Potplug x Age x Photoperiod x Temperature	-	-	-	-	-	-
Contrasts						
9 hours vs. 4-hr Night Interruption (NI)	*	NS	NS	NS	NS	***
Age: 12, 16, 20, *20, 24, 28 wks						
P_{Linear}	***	NS	***	NS	***	***
$P_{quadratic}$	NS	NS	***	NS	**	**
Temperature: 17C, 20C, 23C, and 26C						
P_{Linear}	NS	NS	NS	NS	NS	NS
$P_{quadratic}$	*	NS	NS	NS	NS	NS

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

Table 8. Plants of *Aquilegia xhybrida* 'Musik White' were cooled as either pots (20, 24, and 28 weeks old) or as plugs (12, 16, and 20 weeks old). Initial leaf counts for each plug were taken before they were placed into the 5°C cooler. Flower number and final plant height were measured at the time of first open flower.

Table 8.

	Photo-period *before cold **after cold	Age wks	Plug growing temperature before cold (° C)	Percentage flowering	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Pots	*9	20	17	90	14.1	34	15	49	29	36
	*9	20	20	100	15.8	29	15	44	33	35
	*9	20	23	100	16.2	29	16	45	24	35
	*9	20	26	100	16.6	31	16	47	31	39
	*NI	20	17	100	15.9	37	17	54	28	36
	*NI	20	20	100	16.4	37	17	53	26	34
	*NI	20	23	100	17.1	37	17	54	21	34
	*NI	20	26	100	17.9	36	18	52	26	38
	*9	24	17	100	16.9	29	15	44	20	29
	*9	24	20	100	18.5	18	17	35	22	31
	*9	24	23	100	19.6	28	18	46	22	32
	*9	24	26	100	20.5	22	19	41	28	35
	*NI	24	17	100	18.6	30	19	49	19	36
	*NI	24	20	100	19.3	30	17	47	22	38
	*NI	24	23	100	20.6	30	16	46	26	42
	*NI	24	26	100	22.3	28	14	42	23	37
	*9	28	17	100	20.2	20	18	38	22	32
	*9	28	20	100	21.5	20	18	38	21	34
	*9	28	23	100	21.5	20	17	37	24	37
	*9	28	26	100	23	21	18	39	20	39
	*NI	28	17	100	21	21	16	37	26	45
	*NI	28	20	100	22.5	20	17	37	23	40
	*NI	28	23	100	22.5	20	14	34	21	43
	*NI	28	26	100	24.4	20	17	37	19	40
Plugs	**NI	12	17	0	7.3	.	.	.	.	.
	**NI	12	20	20	7.2	32	14	46	13	31
	**NI	12	23	0	8.9	.	.	.	.	.
	**NI	12	26	0	9.7	.	.	.	.	.
	**9	16	17	20	11.8	36	17	52	10	28
	**9	16	20	20	12.4	35	16	51	12	30
	**9	16	23	0	12.5	.	.		.	.
	**9	16	26	0	13.1	.	.		.	.
	**NI	16	17	0	11.9	.	.	.	.	.
	**NI	16	20	0	12.3	.	.	.	.	.
	**NI	16	23	0	12.7	.	.	.	.	.
	**NI	16	26	0	13.1	.	.	.	.	.
	**9	20	17	30	13.9	35	20	55	25	34
	**9	20	20	30	14.5	36	19	55	22	29
	**9	20	23	0	15.2	.	.	.	.	.
	**9	20	26	0	15.7	.	.	.	.	.
	**NI	20	17	50	14.4	37	13	51	22	43
	**NI	20	20	40	16.3	36	14	40	22	42
	**NI	20	23	0	15.4	.	.	.	.	.
	**NI	20	26	0	17.4	.	.	.	.	.

Table 8 (con't).

	Initial leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final plant height (cm)
Significance						
Pot or Plug (Potplug)	***	***	**	***	**	NS
Age	***	***	*	***	*	***
Potplug x Age	-	-	-	-	-	-
Photoperiod	***	***	***	**	NS	***
Potplug x Photoperiod	NS	NS	***	**	NS	*
Age x Photoperiod	***	***	*	***	NS	***
Potplug x Age x Photoperiod	-	-	-	-	-	-
Temperature	***	*	*	*	NS	NS
Potplug x Temperature	***	NS	NS	NS	NS	NS
Age x Temperature	***	**	*	NS	**	NS
Potplug x Age x Temperature	-	-	-	-	-	-
Photoperiod x Temperature	NS	***	NS	*	NS	NS
Potplug x Photoperiod x Temperature	NS	NS	NS	*	NS	NS
Age x Photoperiod x Temperature	**	***	NS	NS	NS	NS
Potplug x Age x Photoperiod x Temperature	-	-	-	-	-	-
Contrasts						
9 hours vs. 4-hr Night Interruption (NI)	NS	***	***	NS	***	**
Age: 12, 16, 20, *20, 24, 28 wks						
P_{Linear}	***	NS	NS	NS	*	*
$P_{quadratic}$	NS	**	NS	NS	NS	NS
Temperature: 17C, 20C, 23C, and 26C						
P_{Linear}	**	NS	NS	NS	NS	NS
$P_{quadratic}$	NS	NS	NS	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively

Table 9. The overall average leaf number of both the short-day and NI treatments combined at each growing temperature before cold of each species studied. Also, the overall average leaf number of pot- and plug-cooled plants combined for each growing temperature.

Temperature (°C)	Pot-cooled plants	Plug-cooled plants	Total average leaf number
	A. xhybrida 'Bluejay'		
17	17.6	10.8	14.2
20	18.2	10.6	14.4
23	19.3	13.2	16.2
26	20.1	13.5	16.8
	A. flabellata 'Cameo'		
17	13.7	8.0	10.8
20	15.1	9.9	12.5
23	16.6	12.2	14.4
26	17.8	13.5	15.6
	A. . xhybrida 'Crimson Star'		
17	17.5	12.6	15.0
20	18.1	13.0	15.6
23	18.3	13.2	15.8
26	19.3	14.0	16.6
	A. xhybrida 'McKana's Giant'		
17	17.2	10.6	13.9
20	17.9	11.2	14.5
23	18.4	11.7	15.1
26	19.4	12.8	16.1
	A. xhybrida 'Musik-White'		
17	17.8	12.0	14.9
20	19.0	12.7	15.8
23	19.6	13.1	16.3
26	20.8	13.9	17.3

Table 10. The overall combined average leaf numbers at the ages each species studied was placed into the cooler.

Age (weeks)	Pot-cooled plants	Plug-cooled plants	Age (weeks)
	A. xhybrida 'Bluejay'		
20	15.5	7.5	12
24	18.2	11.6	16
28	22.7	14.3	20
	A. flabellata 'Cameo'		
20	13.9	6.5	12
24	15.4	10.4	16
28	18.1	13.1	20
	A. . xhybrida 'Crimson Star'		
20	14.4	8.7	12
24	18.5	12.8	16
28	21.9	15.4	20
	A. xhybrida 'McKana's Giant'		
20	14.3	7.8	12
24	18.6	10.8	16
28	21.7	14.0	20
	A. xhybrida 'Musik-White'		
20	16.3	8.7	12
24	19.5	12.5	16
28	22.1	15.4	20

Table 11. The requirements for flowering of five cultivars of *Aquilegia*. The following recommendations apply to plants grown in 1.1-L or larger containers prior to cold treatment to achieve 100% flowering. Cold duration recommendations are taken from chapter 2. Force plants at temperatures between 14 and 20 °C (chapter 3).

Species	Leaf # required before cold	Weeks of growing before cold	Photoperiod before cold	Weeks of cold at 5 °C	Photoperiod for forcing
Aquilegia flabellata 'Cameo'	$\geq$ 5	5-6 at 17-20 °C	Natural or SD with supplemental light	9 weeks	Natural or SD with supplemental light
Aquilegia xhybrida 'Bluejay'	$\geq$13	13-14 at 17-20 °C	Natural or SD with supplemental light	9 weeks	Natural or SD with supplemental light
A. xhybrida 'Crimson Star'	$\geq$10	10-11 at 17-20 °C	Natural or SD with supplemental light	3 to 6 weeks	Natural or SD with supplemental light
A. xhybrida 'McKana's Giant'	$\geq$10	10-11 at 17-20 °C	Natural or SD with supplemental light	> 12 weeks	Natural or SD with supplemental light
A. xhybrida 'Musik-White'	$\geq$14	14-15 at 17-20 °C	Natural or SD with supplemental light	> 12 weeks	Natural or SD with supplemental light

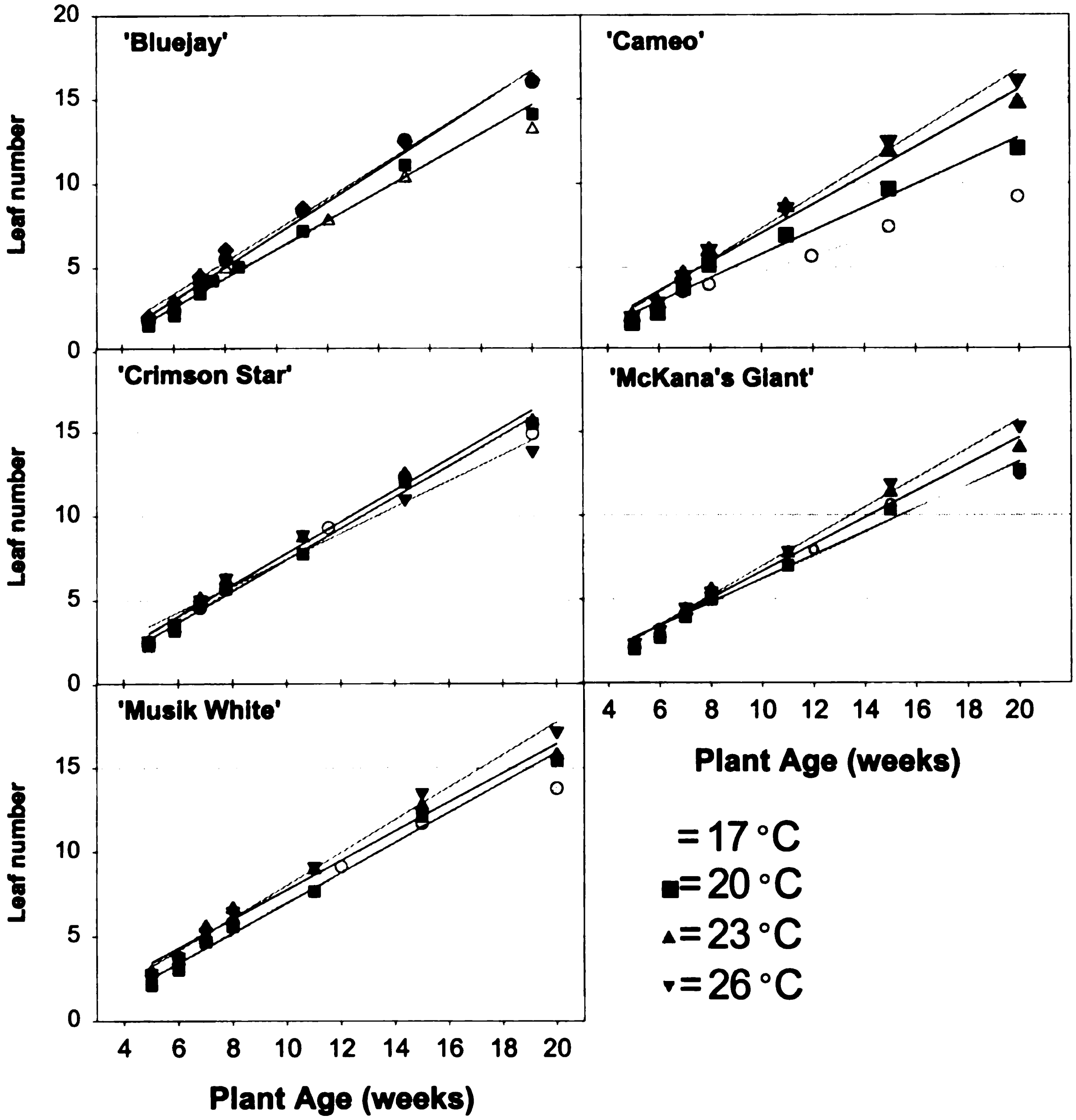


Figure 1. The effects of temperature on leaf development of *Aquilegia xhybrida* 'Bluejay', *A. flabellata* 'Cameo', *A. xhybrida* 'Crimson Star', *A. xhybrida* 'McKana's Giant', and *A. xhybrida* 'Musik-White'. The points indicate the actual number of leaves unfolded at each age. The straight line was calculated from linear regression analysis.

Figure 2. The effects of initial leaf number, temperature before cold treatment, and photoperiod before cold for “Pots”, photoperiod after cold for “Plugs”, on plants of ‘Bluejay’, ‘Cameo’, ‘Crimson Star’, ‘McKana’s Giant’, and ‘Musik-White’. Seedlings were grown at 17, 20, 23, and 26 °C before cold. (○) represents plants grown under SD, (△) represents plants grown under NI.

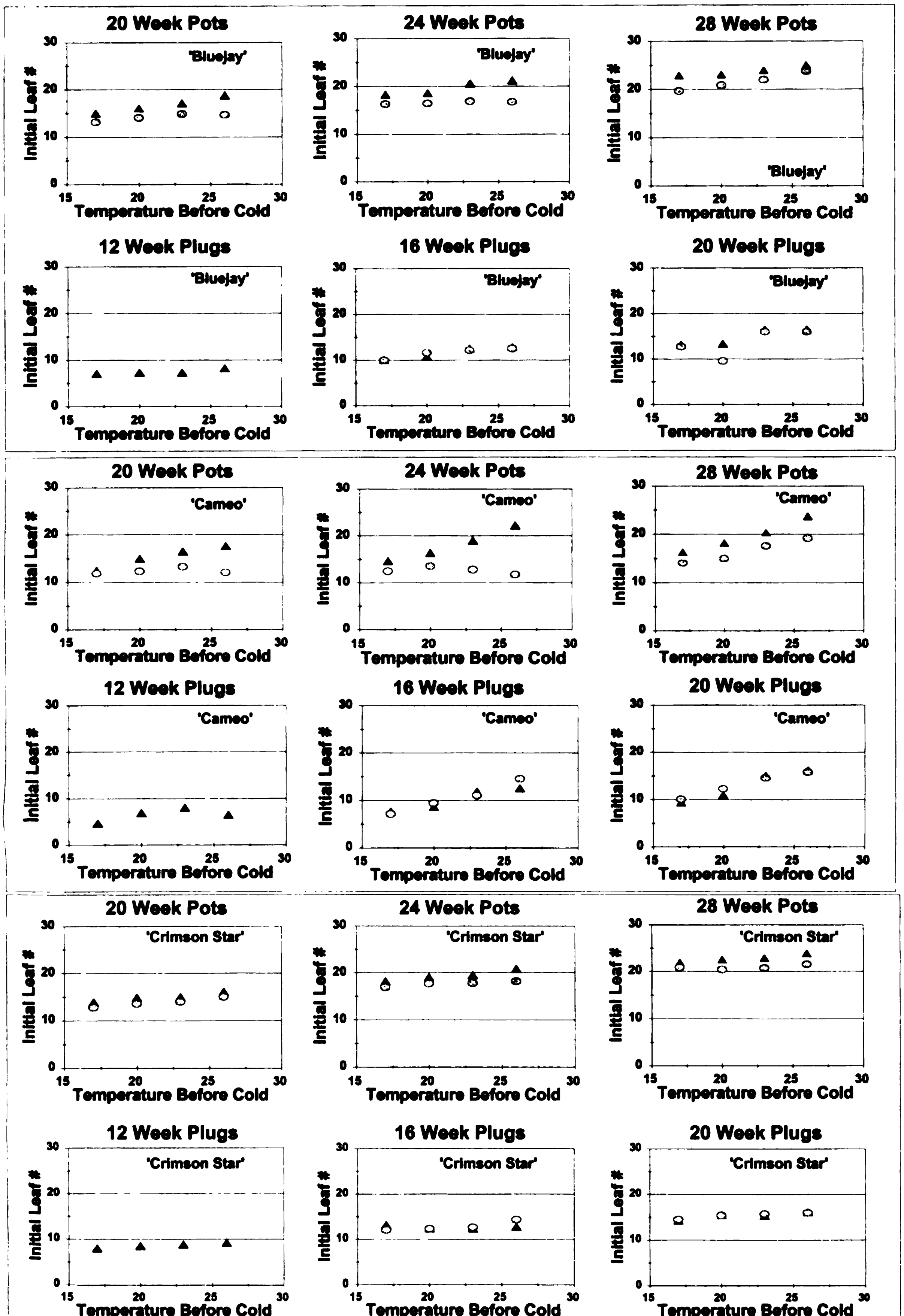

20 Week Pots
'Bluejay'
24 Week Pots
'Bluejay'
28 Week Pots
'Bluejay'
12 Week Plugs
'Bluejay'
16 Week Plugs
'Bluejay'
20 Week Plugs
'Bluejay'
20 Week Pots
'Cameo'
24 Week Pots
'Cameo'
28 Week Pots
'Cameo'
12 Week Plugs
'Cameo'
16 Week Plugs
'Cameo'
20 Week Plugs
'Cameo'
20 Week Pots
'Crimson Star'
24 Week Pots
'Crimson Star'
28 Week Pots
'Crimson Star'
12 Week Plugs
'Crimson Star'
16 Week Plugs
'Crimson Star'
20 Week Plugs
'Crimson Star'
Initial Leaf #
Temperature Before Cold

Figure 2.

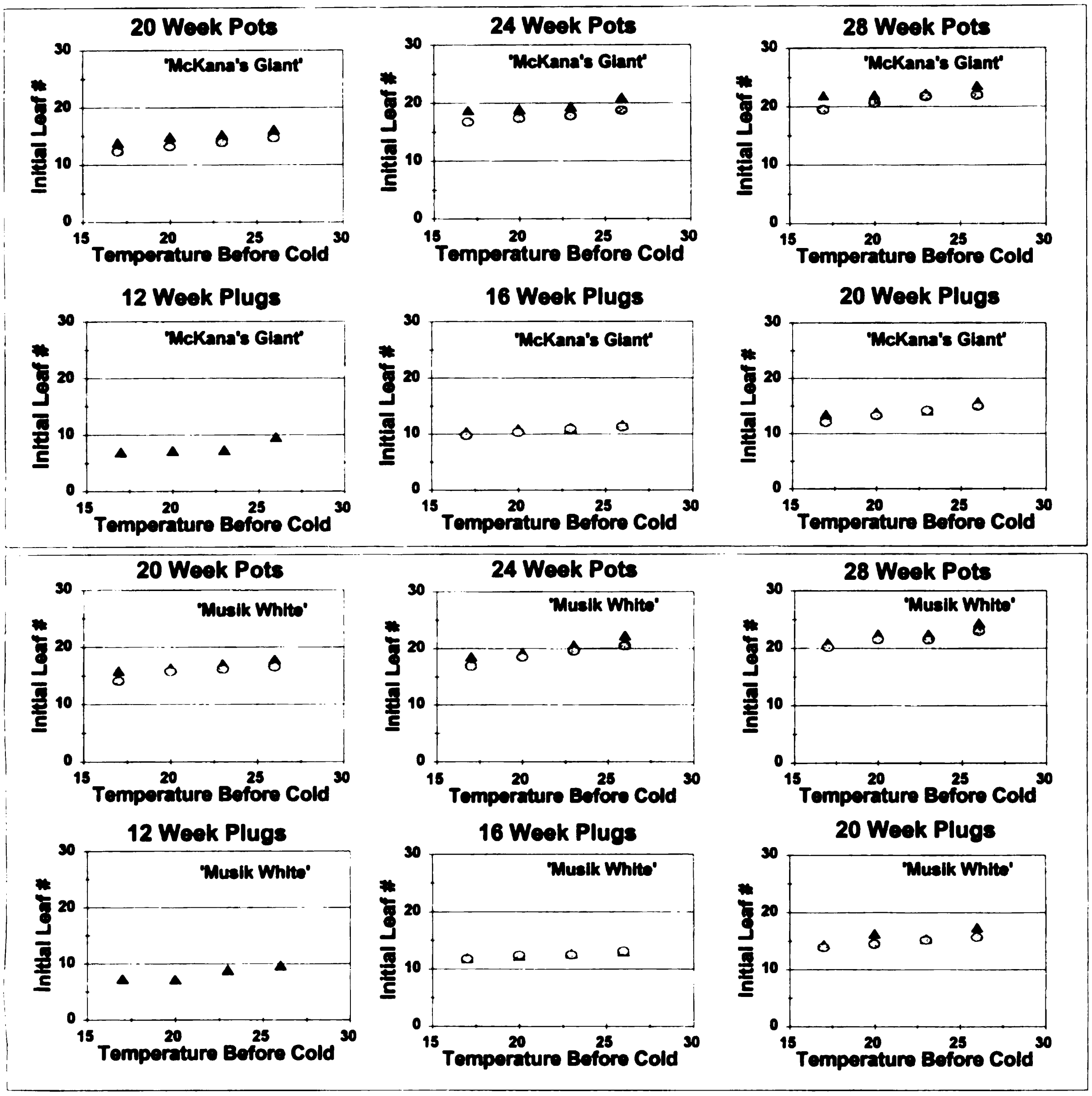


Figure 2, (con't.)

Figure 3. The effects of initial leaf number before cold treatment, and photoperiod before cold for “Pots”, photoperiod after cold for “Plugs”, on flowering percentage of ‘Bluejay’, ‘Cameo’, ‘Crimson Star’, ‘McKana’s Giant’, and ‘Musik-White’. (○) represents plants grown under SD, (△) represents plants grown under NI.

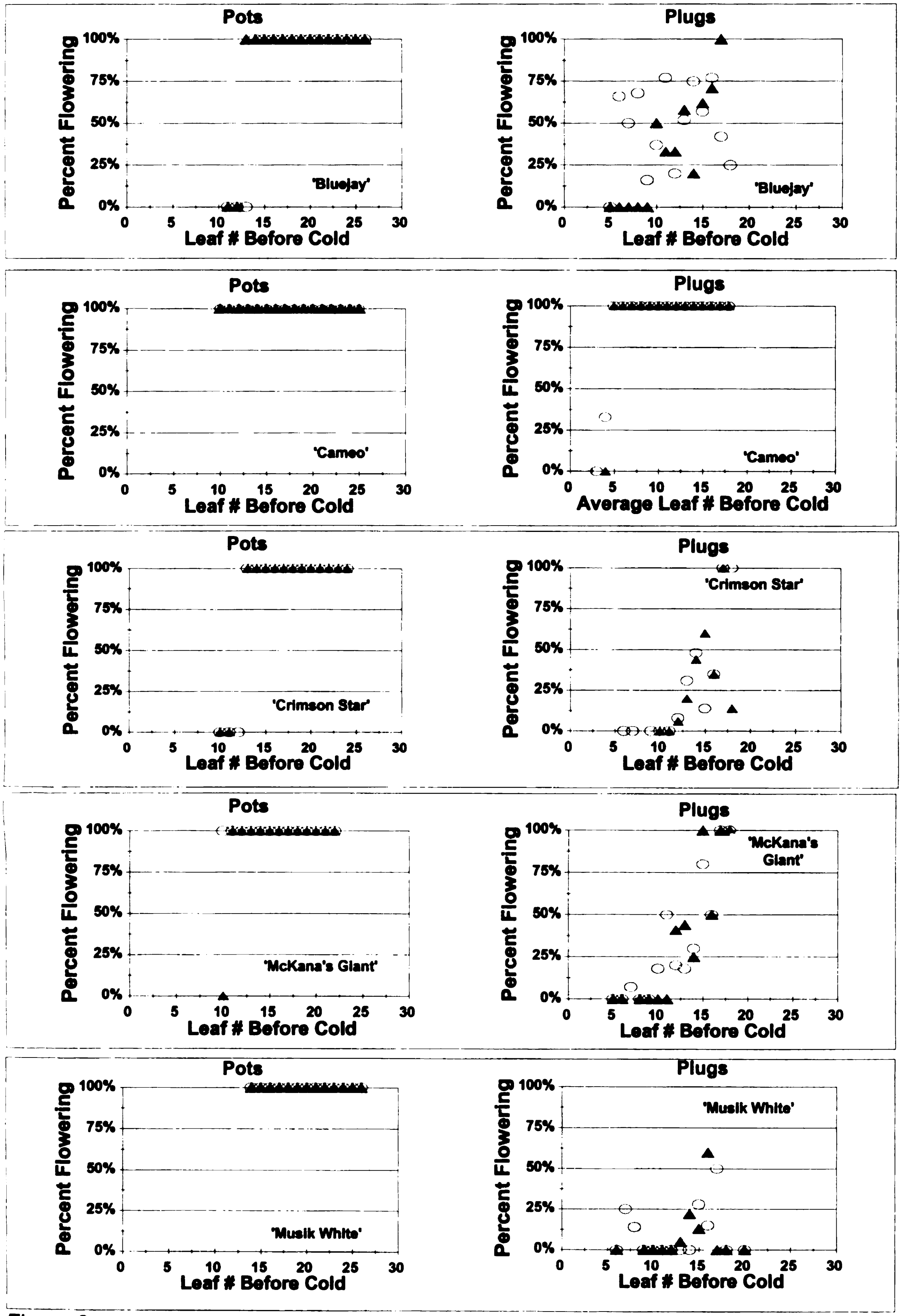

Pots
Plugs
Percent Flowering
100%
75%
50%
25%
0%
0
5
10
15
20
25
30
Leaf # Before Cold
Average Leaf # Before Cold
'Bluejay'
'Cameo'
'Crimson Star'
'McKana's Giant'
'Musik White'

Figure 3.

Figure 4. The effects of temperature before cold treatment, and photoperiod before cold for "Pots", photoperiod after cold for "Plugs", on days to visible bud for plants of 'Bluejay', 'Cameo', 'Crimson Star', 'McKana's Giant', and 'Musik-White'. Seedlings were grown at 17, 20, 23, and 26 °C before cold. (○) represents plants grown under SD, (△) represents plants grown under NI.

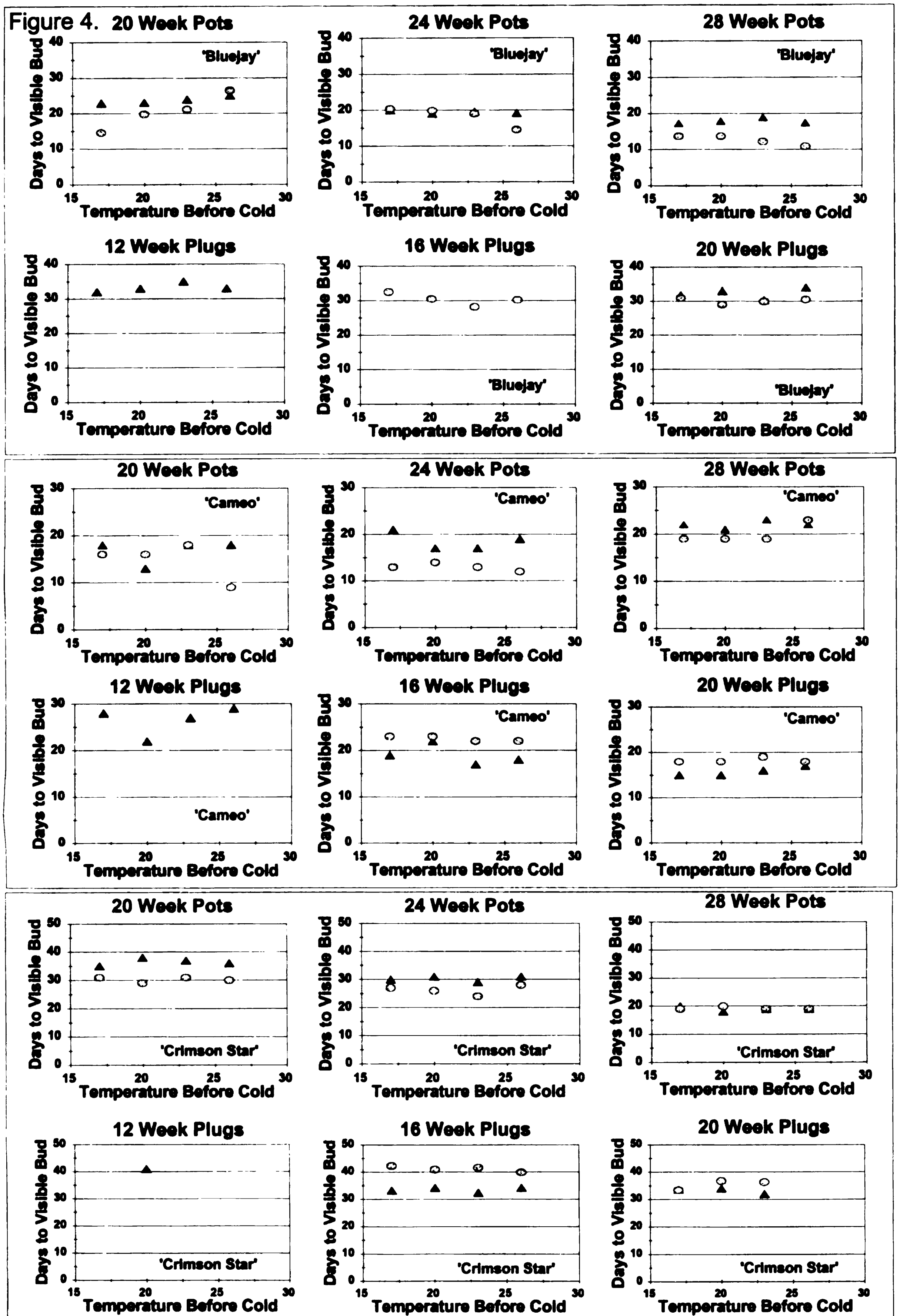


Figure 4.
20 Week Pots
24 Week Pots
28 Week Pots
12 Week Plugs
16 Week Plugs
20 Week Plugs
'Bluejay'
'Cameo'
'Crimson Star'
Days to Visible Bud
Temperature Before Cold

Figure 4.

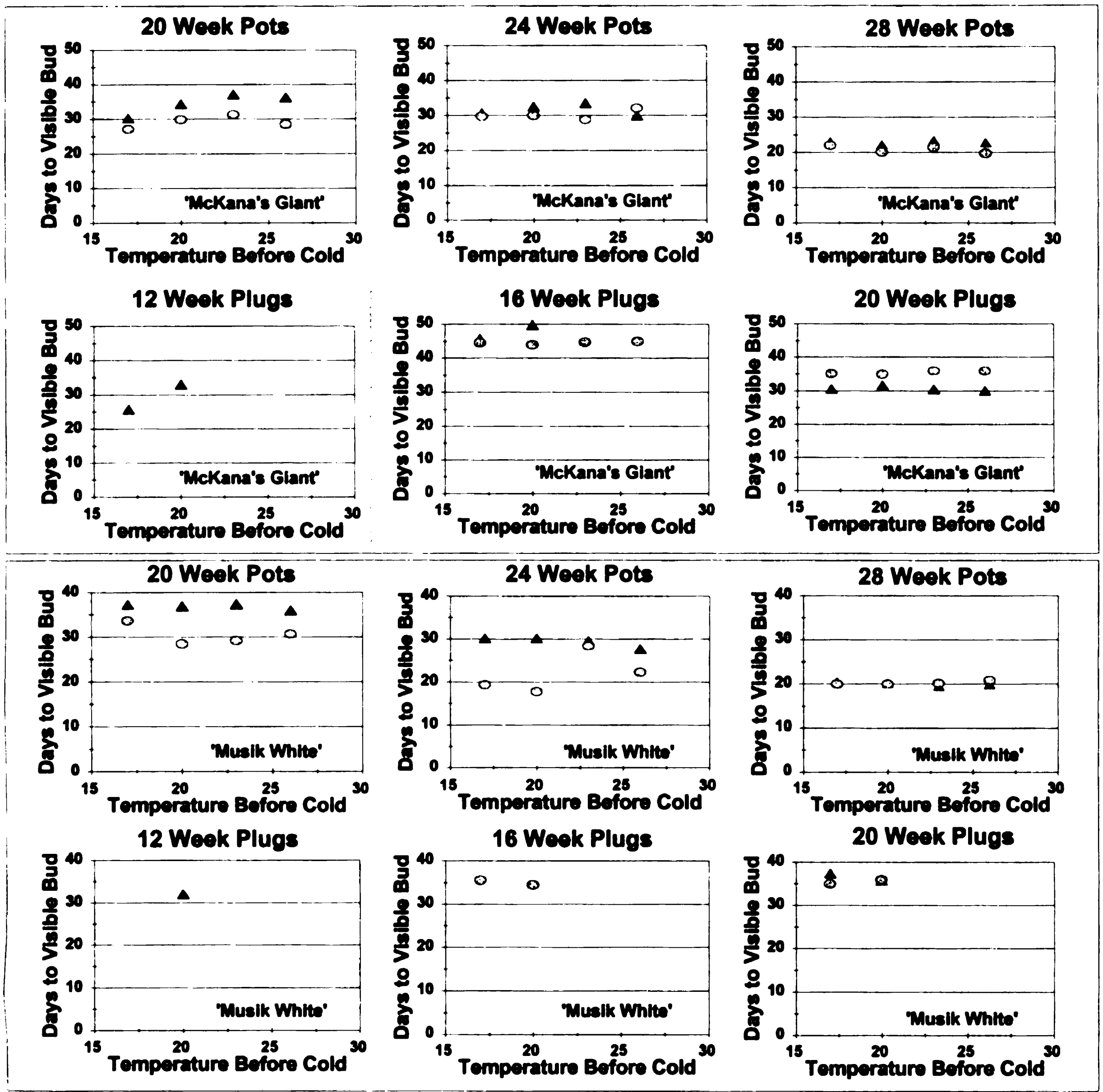


Figure 4, (con't).

Figure 5. The effects of temperature before cold treatment, and overall age of pot- and plug-cooled plants, on the days to visible bud for 'Bluejay', 'Cameo', 'Crimson Star', 'McKana's Giant', and 'Musik-White'. (△) = 17 °C, (○) = 20 °C, (×) = 23 °C, and (□) = 26 °C.

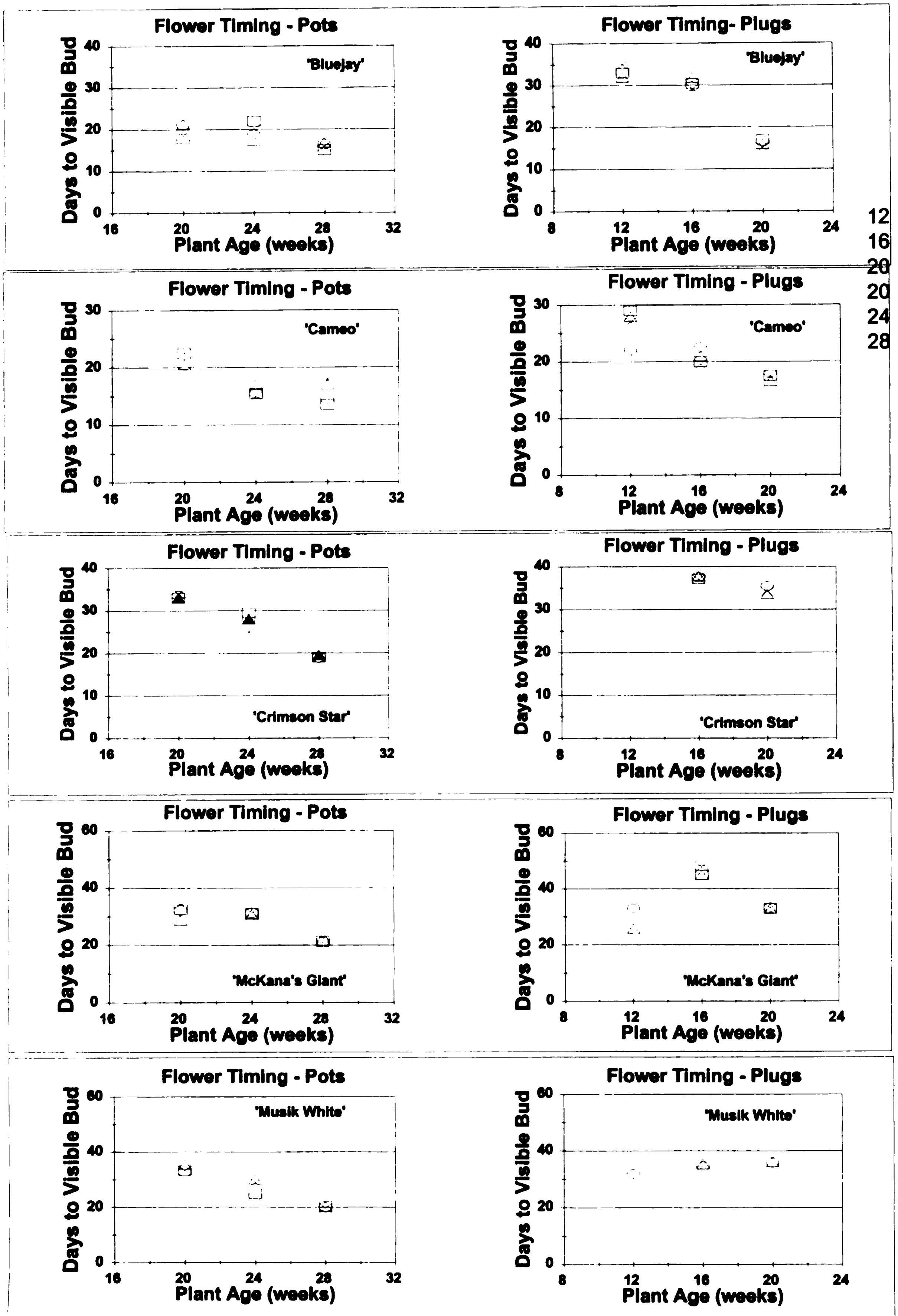

Flower Timing - Pots
'Bluejay'
Flower Timing- Plugs
'Bluejay'
Flower Timing - Pots
'Cameo'
Flower Timing - Plugs
'Cameo'
Flower Timing - Pots
'Crimson Star'
Flower Timing - Plugs
'Crimson Star'
Flower Timing - Pots
'McKana's Giant'
Flower Timing - Plugs
'McKana's Giant'
Flower Timing - Pots
'Musik White'
Flower Timing - Plugs
'Musik White'
Days to Visible Bud
Plant Age (weeks)

Figure 5.

Figure 6. The effects of average leaf number before cold treatment, and photoperiod before cold for "Pots", photoperiod after cold for "Plugs", on days to visible bud, days from visible bud to flower, and days to flower for plants of 'Bluejay', 'Cameo', 'Crimson Star', 'McKana's Giant', and 'Musik-White'. (○) represents days to visible bud, (△) represents days to flower.

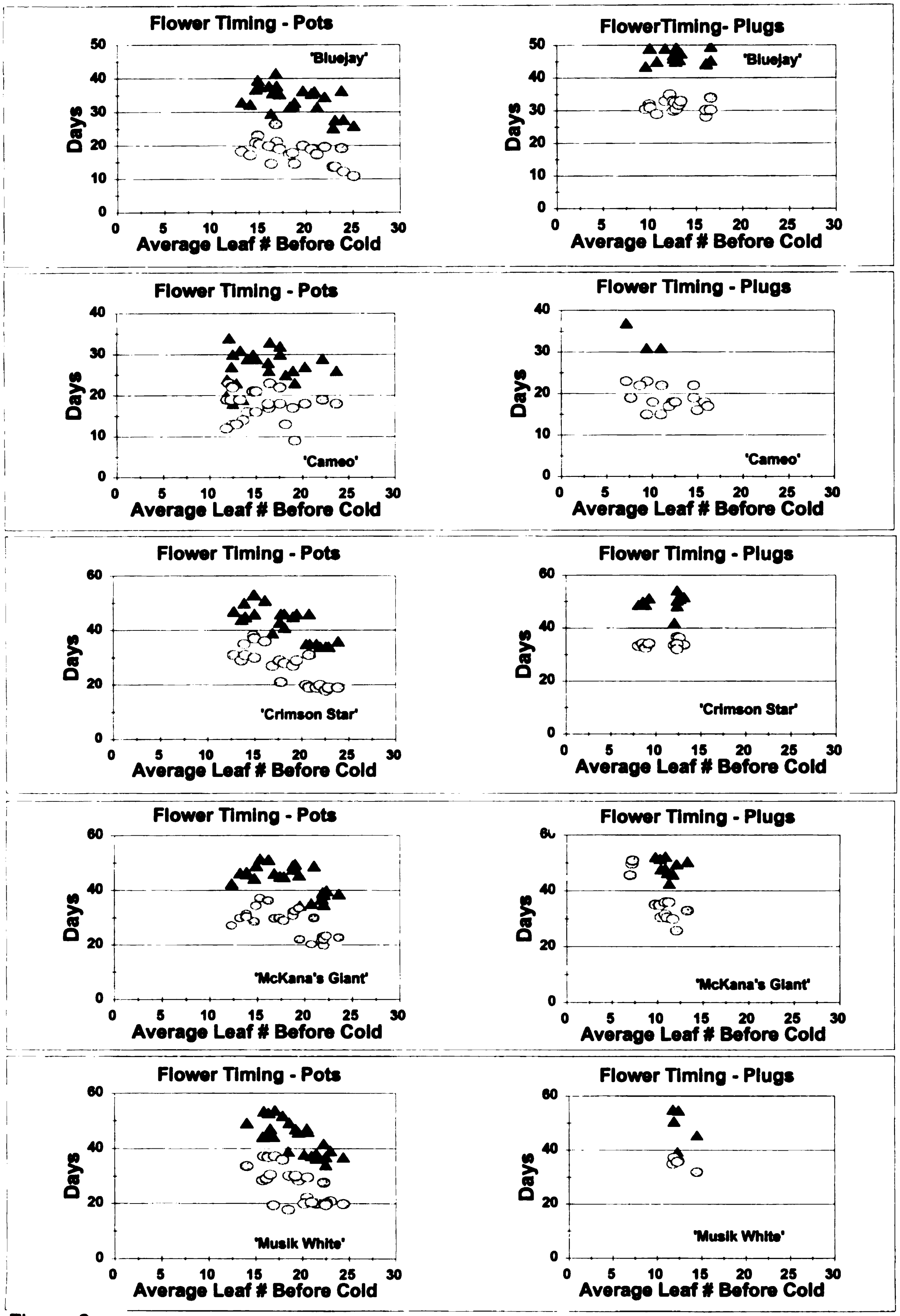

Flower Timing - Pots
'Bluejay'
Days
Average Leaf # Before Cold
FlowerTiming- Plugs
'Bluejay'
Days
Average Leaf # Before Cold
Flower Timing - Pots
'Cameo'
Days
Average Leaf # Before Cold
Flower Timing - Plugs
'Cameo'
Days
Average Leaf # Before Cold
Flower Timing - Pots
'Crimson Star'
Days
Average Leaf # Before Cold
Flower Timing - Plugs
'Crimson Star'
Days
Average Leaf # Before Cold
Flower Timing - Pots
'McKana's Giant'
Days
Average Leaf # Before Cold
Flower Timing - Plugs
'McKana's Giant'
Days
Average Leaf # Before Cold
Flower Timing - Pots
'Musik White'
Days
Average Leaf # Before Cold
Flower Timing - Plugs
'Musik White'
Days
Average Leaf # Before Cold
Figure 6.

Figure 7. The effects of plant age in weeks on the flower number of “Pots” and “Plugs” of ‘Bluejay’, ‘Cameo’, ‘Crimson Star’, ‘McKana’s Giant’, and ‘Musik-White’. Data points represent total average of plants grown under both SD and NI lighting at each age.

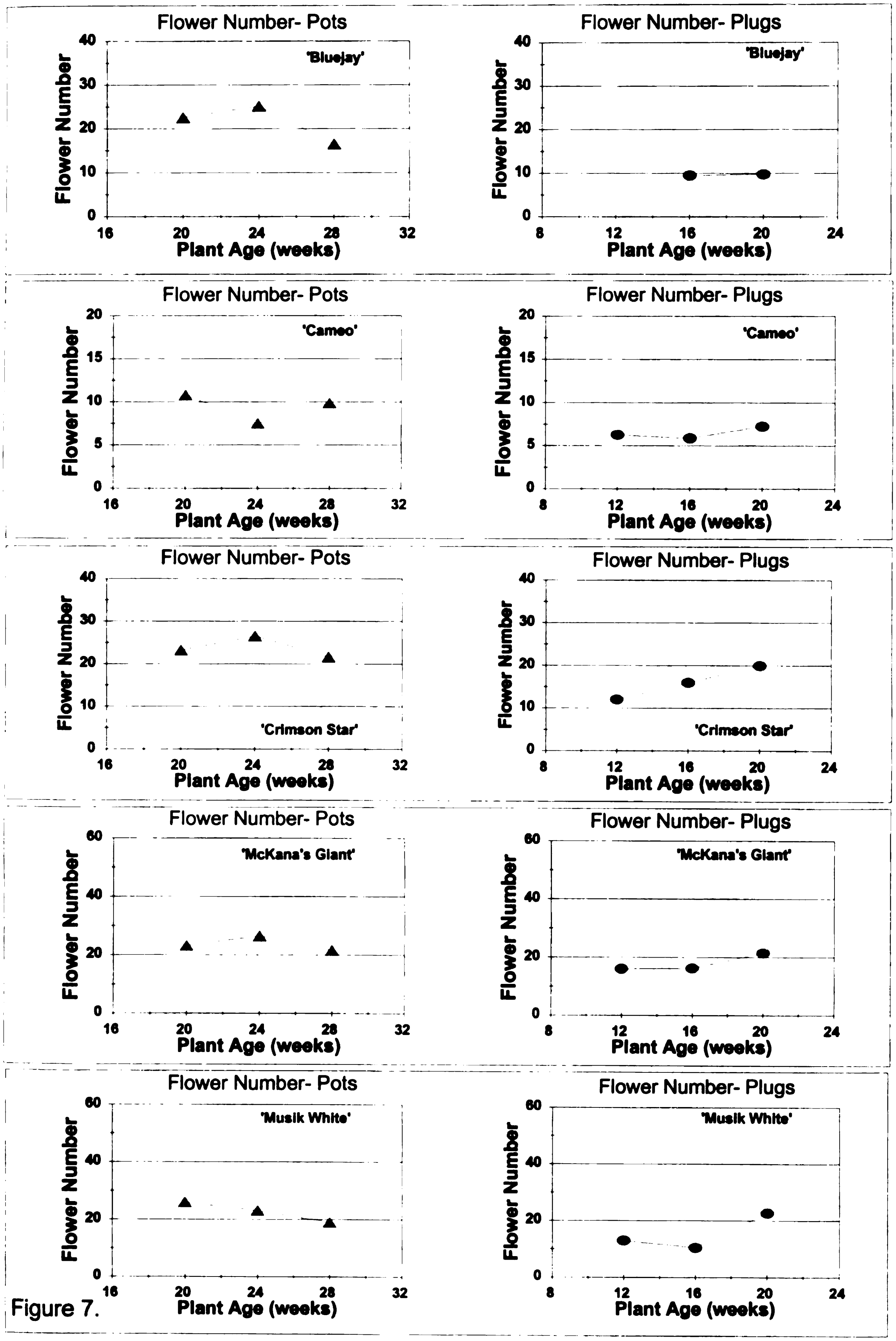
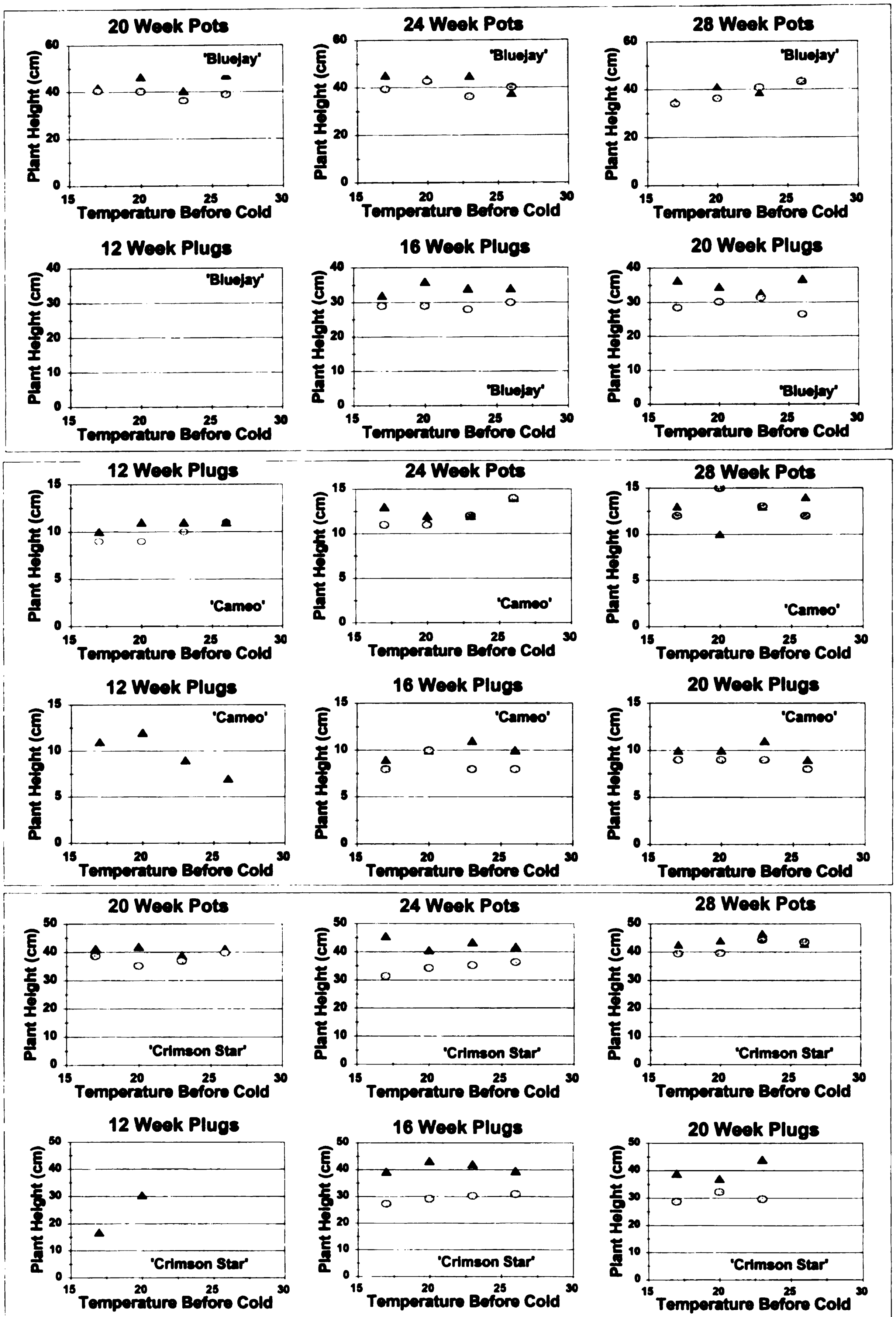

Flower Number- Pots
'Bluejay'
Flower Number
Plant Age (weeks)
Flower Number- Plugs
'Bluejay'
Flower Number
Plant Age (weeks)
Flower Number- Pots
'Cameo'
Flower Number
Plant Age (weeks)
Flower Number- Plugs
'Cameo'
Flower Number
Plant Age (weeks)
Flower Number- Pots
'Crimson Star'
Flower Number
Plant Age (weeks)
Flower Number- Plugs
'Crimson Star'
Flower Number
Plant Age (weeks)
Flower Number- Pots
'McKana's Giant'
Flower Number
Plant Age (weeks)
Flower Number- Plugs
'McKana's Giant'
Flower Number
Plant Age (weeks)
Flower Number- Pots
'Musik White'
Flower Number
Plant Age (weeks)
Flower Number- Plugs
'Musik White'
Flower Number
Plant Age (weeks)

Figure 7.

Figure 8. The effects of temperature before cold treatment, and photoperiod before cold for "Pots", and photoperiod after cold for "Plugs" on plant height of 'Bluejay', 'Cameo', 'Crimson Star', 'McKana's Giant', and 'Musik-White'. (○) represents plants grown under SD, (△) represents plants grown under NI.



20 Week Pots
24 Week Pots
28 Week Pots
'Bluejay'
12 Week Plugs
16 Week Plugs
20 Week Plugs
'Cameo'
'Crimson Star'
Plant Height (cm)
Temperature Before Cold

Figure 8.

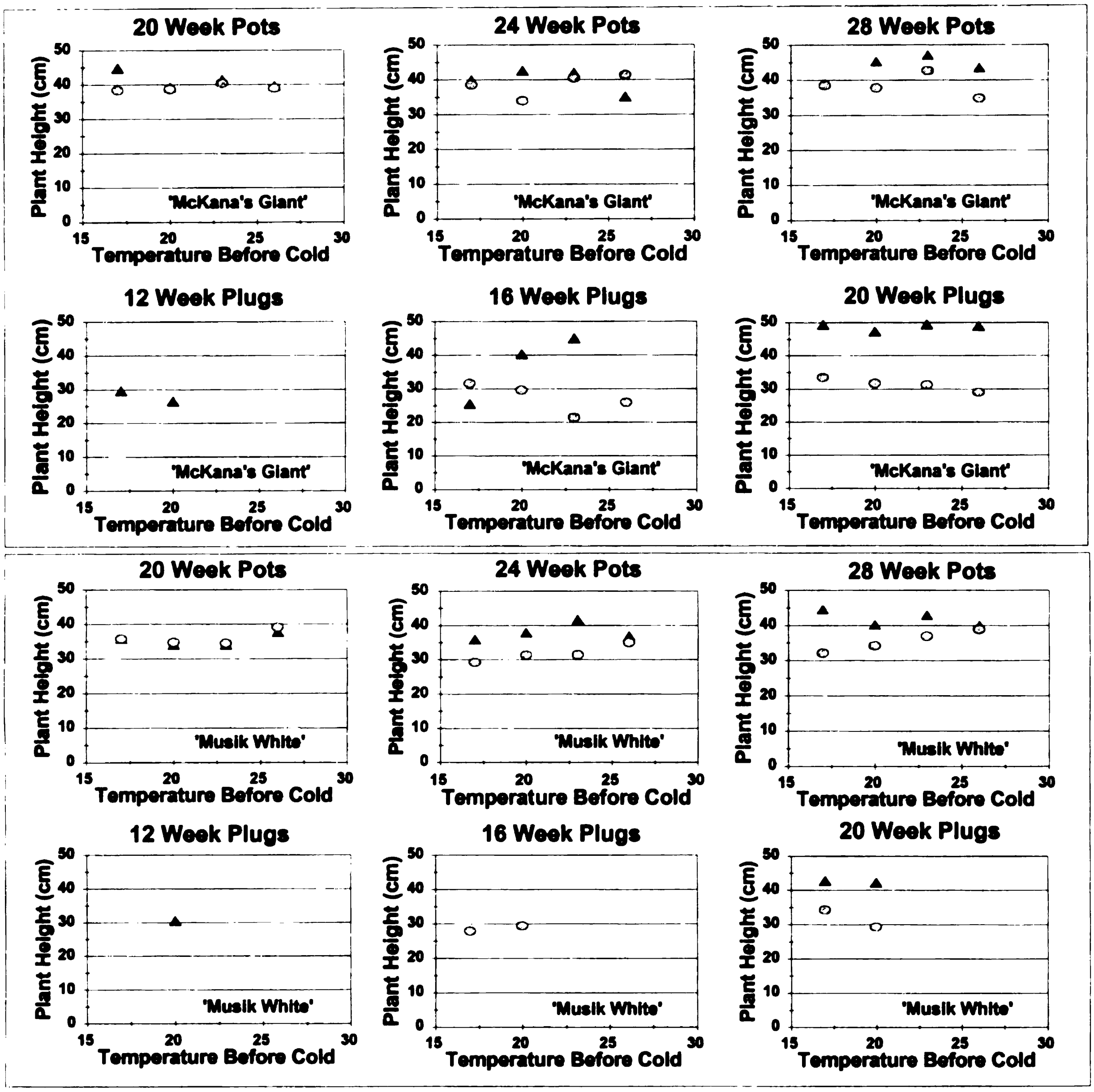


Figure 8, (con't).

CHAPTER V.
THE EFFECTS OF PHOTOPERIOD, COLD TREATMENT, AND FORCING TEMPERATURE ON THE GROWTH AND FLOWERING OF *GAURA LINDHEIMERI* 'WHIRLING BUTTERFLIES'.

Introduction

The genus *Gaura*, a member of the *Onagraceae* (Evening Primrose) family, includes about eighteen native American species of annual, biennial and perennial plants (Bailey, 1976). All eighteen species are native to Texas, Louisiana, and Mexico. *G. lindheimeri* Engelm & A. Gray 'Whirling Butterflies' is found growing in climates ranging from USDA zones 5 to 10, and is versatile, and resilient. *Gaura* usually performs best in hot, dry, full sun locations, and interest in the species has increased along with the popularity of low-maintenance gardening and water conservation.

Most *Gaura* species are tall and bushy and range in height from 3 to 4 feet. They are free-flowering plants that bloom for most of the growing season. The foliage may turn a deep purple color as temperatures decrease in the fall and winter. Although individual plants do not spread rapidly, *Gaura* self-sows seed freely. There is also a pink-flowered cultivar of *Gaura lindheimeri* called 'Siskiyou Pink'. The foliage is dark green with pink veins and has a mottled appearance. This cultivar originated at the Siskiyou Rare Plant Nursery in Oregon as a chance seedling.

Plant response to environmental stimuli, such as temperature and photoperiod, depends upon the species and genotype of the plant. Many perennial plants require a period of cold, or vernalization, for optimal flower development (Jones, 1992). Vernalization has been defined as a cold treatment that promotes subsequent flowering when it is given to imbibed seeds, bulbs, or whole plants (Vince-Prue, 1975). For plants with an obligate requirement for vernalization, flower buds will not form until the requirement for cold is fulfilled and the plants are returned to higher temperatures.

Cold temperatures promote flowering in many plants by breaking dormancy of existing buds when reproductive structures are present prior to the onset of low temperatures. Many woody perennials and flowering bulbs such as azaleas, hydrangeas, and tulips for example, form their flower buds in the summer or fall and remain dormant throughout the winter (Thomas and Vince-Prue, 1984). The floral primordia of *Paeonia* are initiated soon after anthesis of the current year's flowers and require a minimum of four weeks at 5.6 °C to break dormancy (Byrne and Hadley, 1986). Full reproductive development, or anthesis, will not occur without a subsequent cold treatment. This would be considered a breaking of dormancy, not vernalization (Thomas and Vince-Prue, 1984).

In some plants, such as *Brassica oleracea* (Brussels sprouts) and *Dicentra spectabilis*, flower differentiation occurs during the cold, or cool treatment (Thomas, Vince-Prue, 1984). In *Allium cepa* (onion), and *Iris* cv. Wedgewood, low temperatures are also required during floral initiation. In *Matthiola incana*, low temperatures must continue until the buds are visible in order the development to continue upon return to higher temperatures (Kohl, 1957).

For some plants, a cold treatment will speed the process of flowering but is not necessary to initiate flowering. This is termed a facultative, or quantitative response to cold. The hastening of flowering can be measured directly by calculating time to flower and also has been correlated with a reduction of the number of leaves formed before flowering in grains (Chouard, 1960). No information was found in the literature that documented the response of *Gaura lindheimeri* 'Whirling Butterflies' to cold treatment.

The requirement for vernalization is commonly, but not only, found in long day plants (Thomas and Vince-Prue, 1997). Many plants that have a cold requirement, such as *Coreopsis* 'Sunray', also require subsequent treatment with long days. In some cold-requiring plants, such as *Campanula medium,* short days may substitute for, enhance, or be required instead of cool temperatures in order to achieve anthesis (Roberts et al.,1988). The low temperature requirement for flowering in Japanese winter wheat varieties Norinn 27 and 8 is completely replaced by short-day treatments (Krekule, 1987). There was no available information in the literature on the response of *Gaura lindheimeri* 'Whirling Butterflies' to photoperiod.

The rates of many plant developmental processes, and the timing of phenological stages, are strongly temperature dependent (Jones, 1992). Thus, production time for any crop is related to temperatures supplied during forcing (Whitman et al., 1996). Under long-day conditions, average daily temperature is the primary factor influencing of flower development. Although increasing the temperature to speed up the crop may seem desirable, negative consequences may result from growing the crop too warm. Delayed flowering and reduced plant vigor and flower size can result at high temperatures. At temperatures below a species-specific minimum (T_b), the time to flower (f) is infinite (Roberts and Summerfield, 1987). Temperatures above a ceiling value (T_{opt}) delay flowering.

When developmental time is converted to a rate by taking the reciprocal, the relationship between mean temperature and rate of development is often linear in the range between T_b and T_{opt} (Roberts and Summerfield, 1987). Thus, the relationship between the rate of development toward flowering (1/DTF, where DTF is the days to flower) and temperature can be described as follows

$$1/DTF = b_0 + b_1 * T \qquad [1]$$

(Yuan et al., 1998). Using the constants b_0 and b_1, the base temperature, T_b, and degree-days (°days) can be calculated as follows:

$$T_b = -b_0/b_1 \qquad [2]$$

$$°days = 1/b_1 \qquad [3]$$

(Yuan et al., 1998). Base temperature is the temperature at, or below, which, the rate of progress toward flowering is zero, and degree-days represent the thermal time required for flowering (Yuan et al., 1998). The base and optimum temperatures vary within and between species and are related to climatic origin (Roberts and Summerfield, 1987). The effects of temperature have been documented for a limited number of herbaceous perennials, such as *Campanula carpatica* 'Blue Clips', and *Rudbeckia fulgida* (Whitman et al., 1997, Yuan et al., 1998), but no such available information was found for *Gaura lindheimeri* 'Whirling Butterflies'.

The objective of our study was to 1) measure the influence of cold treatment, photoperiod and forcing temperature on flower number, flower diameter, leaf number and plant height of *Gaura lindheimeri* 'Whirling Butterflies' 2) establish the optimum cold treatment duration, photoperiod, and forcing temperature that induced the most complete and rapid flowering. Percentage of plants flowering, time to flower, flower quantity and size were evaluated.

Materials and Methods

General. Vegetative cuttings with an average of 7 to 10 nodes in 72-cell trays (50-ml cell volume) were received from a commercial producer during the first week of November 1996 and 1997. Plants were grown in a commercial soilless medium composed of composted pine bark, horticultural vermiculite, Canadian sphagnum peat moss, processed bark ash, and washed sand (MetroMix 510, Scotts-Sierra Horticultural Products Company, Marysville, Ohio, year 1). Plants were top-watered with well water acidified (two parts H_3PO_4 plus one part H_2SO_4, which provided $\approx$2.5 mol $P \cdot m^{-3}$) to a titratable alkalinity of approximately 130 mg calcium bicarbonate per liter and fertilized with 14N-0P-6K_2O ($mol \cdot m^{-3}$) from potassium nitrate (14-0P-55K_2O) (Vicksburg Chemical Co., Vicksburg, MS) and ammonium nitrate (34N-0P-0K_2O) (Cargill, Lexington, KY). Fertilization and acidification rates were adjusted in response to weekly soil test results, so regimens varied during experiments. Our target range for soil pH was 5.8 to 6.2 and, and 0.5 to 1.2 for soil EC.

Supplemental lighting from high-pressure sodium lamps provided a photosynthetic photon flux (PPF) of approximately 50 $\mu mol \cdot m^{-2}\ s^{-1}$ at plant level when the ambient greenhouse PPF dropped to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ between 800 hr to 1700 hr daily. Supplemental lighting was terminated when PPF exceeded 400 $\mu mol \cdot m^{-2}\ s^{-1}$.

Plants were exposed to cold treatments in a 5 $\pm$ 0.5 °C cooler illuminated for 9 h •day $^{-1}$ with cool-white fluorescent lamps (VHOF96T12; Philips, Bloomfield, N.J.), as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE). While in the cooler, plants were watered two to three times a week as needed with well water acidified (H_2SO_4) to an approximate pH of 6.0.

Critical photoperiod (Expt. 1). Upon arrival, 70 seedlings were removed from the plug-trays and transplanted into 1.1-L (5" U.S.) square pots. Ten plants were placed under 10, 12, 13, 14, 16, and 24 hours of continuous light and 9 hours of natural light plus a 4-hour night interruption (NI) from 2200 to 0200 HR. Photoperiods were completed with incandescent lamps at 1 to 3 μmol·m^{-2} s^{-1}. For the continual photoperiodic treatments, lamps provided day-extensions; they were turned on at 1700 HR and turned off after each photoperiod was completed. Photoperiod treatments were assigned randomly to benches in the greenhouse. The remaining 70 seedlings were placed in the cooler for 15 weeks. After the cold treatment, plants were transplanted into 1.1-L (5" U.S.) square pots. Ten plants were placed under each of seven previously mentioned photoperiod treatments. Dates of the first visible bud, and first open flower were recorded for each plant, and days from transplant to visible bud and flowering were calculated. At first flower, total plant height, visible flower bud number, and node number on the main stem were recorded.

Greenhouse temperature was set at 20 °C. Air temperatures on each bench were monitored with 36-gauge (0.013-mm-diameter) type E thermocouples connected to a CR10 datalogger (Campbell Scientific, Logan, UT). The datalogger collected temperature data every 10 seconds and recorded the hourly average. To provide uniform temperature conditions, the datalogger controlled at 1500-W electric heater under each bench that provided supplemental heat as needed throughout the night. Actual average daily air temperatures from the beginning of forcing to the average date of flowering were calculated for each species.

Cold treatments (Expt 2). Ten plants were removed from the plug-tray and transplanted into 1.1-L (5" U.S.) pots, and were then placed in the greenhouse under 16 hours of extended day light from high-pressure sodium lamps. The remaining fifty plants were placed in the cooler for 3, 6, 9, 12, and 15 weeks. After each cold duration treatment, ten plants were removed and transplanted into 1.1-L (5" U.S.) square pots, and placed in the greenhouse under 16 hours of extended day light from high-pressure sodium lamps. The greenhouse was lit from 0700 to 0800 HR and from 1700 to 2300 HR at approximately 50 $\mu mol \cdot m^{-2} s^{-1}$ as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE) to provide the proper photoperiod. Data were recorded as described for Expt. 1.

Forcing temperature treatments (Expt. 3). Fifty plants were placed in a 5 °C cooler for 12 weeks, and then transplanted into 1.1-L (5" U.S.) square pots. Ten plants were placed in each of five greenhouses set to 17, 20, 23, 26, and 29 °C. Plants received natural day-lengths plus day-extension lighting from 0700 to

0800 HR and from 1700 to 2300 HR at approximately 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (HPS lamps) as measured by a LICOR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, NE) to provide a 16-hour photoperiod.

Air temperatures on each bench were monitored with 36-gauge (0.013-mm-diameter) type E thermocouples connected to a CR10 datalogger (Campbell Scientific, Logan, UT). The datalogger collected temperature data every 10 seconds and recorded the hourly average. The datalogger collected temperature data every 10 seconds and recorded the hourly average. Actual average daily air temperatures from the beginning of forcing to the average date of flowering were calculated for each species.

Results and Discussion

Critical photoperiod (Expt. 1).

Gaura lindheimeri responded as a facultative long-day plant both years it was studied. However, long days were nearly obligate both years *Gaura* was studied. For example, under the 10-hour photoperiod, without a cold treatment, only 10% of plants flowered during year 1 and no plants flowered during year 2 (Table 1, Figure 1). After 15 weeks of cold, 40-50% of plants flowered under the 10-hour photoperiod both years (Tables 1, Figure 1). Although some plants eventually flowered under the 10-hour photoperiod, and all plants flowered under the 12-hour photoperiod after cold treatment. However, the plants remained prostrate under these short photoperiods (Table 1, Figure 1). Under the 13-hour photoperiod, plant morphology was upright, as opposed to the plants grown under the 10- and 12-hour photoperiods. All plants flowered under all other photoperiods with and without cold. Without a cold treatment, longer photoperiods significantly accelerated flowering, especially during year 1. For example, visible bud was accelerated by 16 days as the photoperiod increased from 10 to 24 hours of continuous light (Table 1, Figure 1). The difference in days to visible bud in relation to photoperiod was smaller during year 2, however, significant interactions were observed between photoperiod, weeks of cold, and the two separate years of data (Table 1).

Height at first flowers increased as photoperiod lengthened. Plants under the 24-hour photoperiod were about 70-cm tall, and required support, possibly due to a far-red light effect (Table 1, Figure 2). The number of flower buds per plant was not significantly different between photoperiod treatments at and above the 12-hour photoperiod (Table 1). Cold treatment, however, doubled the number of flower buds under all photoperiods (Table 1, Figure 2).

Cold treatments (Expt. 2).

Vernalization was not required for flowering of *G. lindheimeri.* Without a cold treatment, all plants flowered under 16 hours of light, as in experiment 1 (Tables 1 and 2, Figures 2 and 4). Days to visible bud was variable between the two years of data (Table 2). For example, during year 1, days to visible bud increased slightly from 47 to 53 days, but decreased from 48 to 28 days during year 2 as the duration of cold treatment increased from 0 to 15 weeks (Table 2, Figure 4).

Flower number was significantly affected by cold treatment, but during year 1, data varied with treatment and no trends were observed. Year 2, flower number increased by 50% as the duration of cold treatment increased from 0 to 15 weeks (Table 2, Figure 4). Plant node development and plant height were also variable during year 1, but decreasing trends with increased cold were observed for both node number and height during year 2 (Table 2). Cold decreased plant node development prior to flowering by about three leaves and plant height decreased by 12 cm (Table 2, Figure 4).

Forcing Temperature (Expt.3).

Plants flowered faster at temperatures up to 26 °C, but heat-delay was observed at 29 °C. The time to flower after beginning LD for *Gaura lindheimeri* 'Whirling Butterflies' was dependent upon forcing temperature: about 7 weeks at 64°F (17°C), 6 weeks at 68°F (20°), or 5 weeks at 74°F (23°C). Days to visible bud decreased from 32 to 22 days as temperature increased to 26 °C, but was delay by six days at 29 °C (Table 3, Figure 5). Days from visible bud to flower decreased linearly from 19 to 13 days as temperature increased to 29 °C with no delay observed (Table 3). All plants flowered under all temperature treatments.

There were significant linear and quadratic relationships between temperature and rate of progress toward visible bud, days from visible bud to flowering, and days to flower for *G. lindheimeri*. The rate to visible bud and flowering followed a statistically significant quadratic pattern, whereas days from visible bud to flower followed a linear pattern (Table 4). With a 10 °C rise in temperature, there was only an approximate 30% decrease in time to flower. The base temperature and degree-days for days to visible bud, days from visible bud to flower, and days to flower were determined using equations [2] and [3]. Base temperature and degree-days can be used to predict the flowering date in commercial greenhouse conditions in which temperatures fluctuate (Yuan et al., 1998). The base temperatures calculated for 'Whirling Butterflies' were below zero for all events, ranging from -3.4 for days to visible bud to -11.8 for days to flower, suggesting that flower buds may have formed while plants were still in the cooler (Table 4).

There was a decrease in flower diameter, measured across the open face of the flower, as the forcing temperature increased. Flowers at the highest temperature were smaller, although no gross flower deformities or doubling were observed at 29 °C. Flower diameter decreased linearly, by about 35%, with increasing temperature (Table 3, Figure 6). Reducing forcing temperature from 29 to 17 °C promoted a six-fold increase in bud number at first flower. Bud number at first flower followed a statistically significant quadratic pattern in relation to temperature.

Plants grown at 17 °C had an average of 77 flower buds at the time of first flower as opposed to plants grown at 29 °C, which had an average of only 12 flower buds (Table 3, Figure 6).

Plant node development and plant height decreased linearly as temperature increased. Final leaf number decreased by five nodes, and plant height decreased by 16 cm as temperature increased from 17 to 29 °C (Table 3, Figure 6).

Conclusion

Vince-Prue defines the critical photoperiod of a species as the daylength at which 50% of the plants flower (Vince-Prue, 1975). However, Roberts and Summerfield (1987) define critical photoperiod for short-day plants as the photoperiod at or below which the time to flower is minimal and is not affected by variations in day-length. Conversely, they define critical photoperiod for long-day plants as the photoperiod above which time to flowering is minimal and not affected by further increased in photoperiod, and below which flowering is delayed (Roberts and Summerfield, 1987). In commercial horticulture, however, it is necessary to have the ability to keep all plants vegetative, or to promote flowering of all plants of a certain species as desired. Therefore, we have proposed an alternative definition of critical photoperiod; critical photoperiod may be the photoperiod at which 100% of plants flower, and at which the time to flower is minimal and is not affected by variations in day-length (Whitman et al., 1996a, 1996b, 1998). Under photoperiods of 12 hours or less, 100% of *Campanula carpatica* 'Blue Clips' remained vegetative (Whitman et al., 1996b). Under 14 hours, 60% of plants flowered, while all plants flowered under 16 hours of continuous light or a 4-hour night-interruption from 10 p.m. to 2 a.m. (Whitman et al., 1996b). It was concluded that the critical photoperiod for *C. carpatica* 'Blue Clips' was 16 hours.

Percentage flowering for 'Whirling Butterflies' reached 100% for plants that were grown under photoperiods $\geq$ 12 hours, both with and without a cold treatment. Thus, 12 hours would be considered the critical photoperiod for *G. lindheimeri.* Extended exposure to 5 °C increased the percentage flowering under the short-day photoperiods, but did not entirely eliminate the requirement for longer photoperiods. In addition, the plants grown under 10-hr photoperiods were diminutive and prostrate in their growth habit and would not be acceptable for sale. When lighting *Gaura* with incandescent bulbs, photoperiods longer than 16 hours are not recommended due to excessive stem elongation. Plants given a cold treatment, then grown under 13 hours of light were consistently of higher quality compared to those grown under longer photoperiods when incandescent lighting was used. Plants were shorter and more compact that those grown under longer photoperiods, and had a higher flower number than plants grown under both shorter and longer photoperiods. We recommend a 13-hour continuous photoperiod when using incandescent bulbs.

Plants in experiment 2 were grown using 16 hours of light from high-pressure sodium lamps as opposed to incandescent bulbs in experiment 1. Thus, plants received a higher daily light integral in experiment 2, which resulted in plants with more lateral branching, more flower buds, and less undesirable stem elongation. Plants in experiment 2 had, in most cases, 50% more flower buds than those grown with incandescent lighting in experiment 1 (Tables 1 and 2, Figures 1 and 2).

The requirement for cold treatment differs among species and can often be categorized by the relationship between exposure to cold and time of floral initiation. Our experiments show that exposure to a period of low temperatures is not required for promoting flowering in *Gaura lindheimeri* 'Whirling Butterflies'. One hundred percent flowering was achieved without a cold treatment when grown with 16 hours of high-intensity lighting. However, a cold treatment did promote increased lateral branching, and flower number, as well as the overall plant appearance.

Although all plants of *G. lindheimeri* flowered under all temperature treatments, high temperatures had a negative effect on flower number and size. Similar responses to temperature have been reported for other species, such as tomato. In two varieties of tomato, heat-sensitive Pusa Ruby and heat-tolerant CL-1311, many floral anomalies such as stigma exertion without anthesis, empty flowers, and persistent flowers without fruit-set were observed in when grown under a 35 / 30 °C day/night temperature regimen (Lohar and Peat, 1998). Abnormalities such as flower doubling occurred for plants of *Campanula* 'Birch Hybrid' when grown at constant day night temperatures of 29 °C (Frane, unpublished data).

No flower abnormalities were observed in this study for plants of 'Whirling Butterflies' grown at 29 °C, but flower size and vigor were both dramatically reduced.

Overall, plants grown a lower temperatures had more and larger flowers and were taller, but took longer to reach flowering. Plants grown at the highest temperature were too weak and diminutive in size for 1.1L (5" U.S.) pots, whereas the plants grown at the coolest temperature may have been too large for them. Settings of 17 to 20°C are recommended to force *Gaura lindheimeri* 'Whirling Butterflies'. Plants flowered slightly faster in the temperature range of 20 to 23 °C than did those grown at lower temperatures, but those grown at 17 °C were highly attractive. These results for *Gaura lindheimeri* are similar to the responses of *Coreopsis grandiflora*, *Gaillardia xgrandiflora*, *Leucanthemum xsuperbum*, and, *Rudbeckia fulgida* (Yuan et al., 1998), *Lavendula angustifolia* 'Munstead' (Whitman et al., 1996a), and several species of *Aquilegia* (chapter 2).

Based on time-to-flower and flower number, and overall plant appearance, we recommend 6 to 9 weeks of cold at 5 °C for production of *Gaura lindheimeri* 'Whirling Butterflies'. After the cold treatment, provide long-days, which can be delivered by natural or extended photoperiods $\geq$ 13 hours with a minimum light intensity of 1 to 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (10 footcandles). Light from high-pressure sodium lamps is preferred over incandescent. Provide plants with supplemental lighting from HPS lamps during dark winter climates. Supplemental lighting at approximately 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (500 footcandles) will increased flower number, thus plant quality. Force plants at temperatures between 17 and 20 °C (64 and 74 °F).

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Table 1. The effects of photoperiod and cold treatment on flowering cooled plugs of *Gaura lindheimeri* 'Whirling Butterflies'. Plants were cooled for 0 or 15 weeks in a controlled environment chamber set at 5°C. After cold treatment, plants were grown in a greenhouse set at 20 °C.

Photo-period	Weeks cold 5C	Year	Percentage flowering	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final leaf number	Final plant height (cm)
10	0	1	10	46	29	75	4	13	36
12	0	1	100	37	25	62	35	14	64
13	0	1	100	35	21	55	47	14	55
14	0	1	100	32	22	54	54	15	59
16	0	1	100	30	22	52	50	16	72
24	0	1	100	31	27	58	43	18	74
NI	0	1	100	30	27	58	45	25	63
10	15	1	40	30	19	49	34	11	41
12	15	1	90	30	17	47	42	13	49
13	15	1	100	28	18	46	48	13	51
14	15	1	100	29	17	46	52	15	60
16	15	1	100	29	19	48	44	15	60
24	15	1	100	30	23	53	37	16	68
NI	15	1	100	26	20	46	41	16	46
10	0	2	0	-	-	-	-	-	-
12	0	2	100	25	17	42	21	12	34
13	0	2	100	25	19	43	26	16	33
14	0	2	100	26	19	42	34	21	51
16	0	2	100	27	17	44	33	25	61
24	0	2	100	28	18	46	30	23	61
NI	0	2	100	25	16	41	21	21	43
10	15	2	50	28	17	45	10	23	16
12	15	2	100	31	11	42	41	25	38
13	15	2	100	26	13	39	69	26	32
14	15	2	100	25	14	39	65	27	48
16	15	2	100	24	14	38	67	21	50
24	15	2	100	23	14	37	58	14	54
NI	15	2	100	22	13	35	57	15	60
Significance									
Year				NS	NS	NS	NS	***	***
Weeks of cold 5C				NS	NS	NS	***	NS	***
Year x Weeks of cold 5C				NS	NS	NS	***	***	*
Photoperiod				***	NS	NS	***	***	***
Year x Photoperiod				***	NS	NS	*	***	**
Week x Photoperiod				***	NS	NS	NS	*	NS
Year x Weeks cold x Photoperiod				***	NS	NS	NS	**	**
Contrasts for Photoperiods and Cold									
NI vs 16 hr 0 weeks cold				NS	NS	NS	NS	**	***
NI vs 24 hr 15 weeks cold				NS	NS	NS	NS	NS	*
NI vs 16 hr 0 weeks cold				NS	*	*	NS	**	*
NI vs 24 hr 15 weeks cold				NS	NS	NS	*	NS	*
P Linear for 0 weeks of cold				NS	NS	NS	**	**	NS
P Quadratic for 0 weeks of cold				NS	NS	NS	*	NS	NS
P Linear for 15 weeks of cold				NS	NS	NS	*	ns	***
P Quadratic for 15 weeks of cold				NS	*	NS	NS	NS	NS

NS, *, **, *** Non-significant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 2. The effects of cold treatment on flowering cooled plugs of *Gaura lindheimeri* 'Whirling Butterflies'. Plugs were cooled for 0, 3, 6, 9, 12, and 15 weeks in a controlled environment chamber set at 5 °C. After cold treatment, plants were forced in a greenhouse set at 20°C under 16 hours of light from HPS lamps.

Weeks of 5C	Year	Percentage flowering	Initial leaf number	New leaf number	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final leaf number	Final plant height (cm)
0	1	100	8	11	26	21	47	85	19	48
3	1	100	10	13	32	19	50	86	23	51
6	1	100	8	11	28	18	46	67	19	37
9	1	100	7	14	31	21	53	86	21	45
12	1	100	8	11	35	17	52	69	19	39
15	1	100	7	10	35	18	53	81	18	51
0	2	100	8	13	26	17	44	41	21	50
3	2	100	8	13	20	17	37	49	21	51
6	2	100	8	10	19	18	38	58	18	39
9	2	100	8	9	19	16	35	86	17	40
12	2	100	8	9	19	15	34	85	17	40
15	2	100	8	8	12	17	28	85	17	38
Significance										
Year				**	***	**	***	***	**	NS
Weeks of cold 5C				***	**	NS	NS	***	NS	***
Year x Weeks of cold 5C				**	***	NS	***	***	NS	NS
Contrasts										
0, 3, 6, 9, 12, and 15 weeks 5C										
P_{Linear}				***	NS	NS	NS	***	*	**
$P_{Quadratic}$				*	NS	NS	NS	***	NS	NS

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

Table 3. The effects of forcing temperature on flowering of cooled plugs of *Gaura lindheimeri* 'Whirling Butterflies'. Plugs were cooled for 12 weeks in a controlled environment chamber set at 5 °C.

Set temp. (C)	Actual temp. Force to VB	Actual temp Force to FLW	Days to visible bud	Days from visible bud to flower	Days to flower	Flower number	Final leaf number	Flower diameter (cm)	Final plant height (cm)
17	17.2	17.6	32***	19***	47 ***	77***	13***	4.4***	28***
20	20.3	20.1	26	16	41	63	12	4.1	24
23	23.7	23.9	23	15	39	66	10	4.0	20
26	26.4	26.2	22	15	35	44	10	3.6	17
29	29.2	29.2	22	13	41	13	8	2.9	12
Contrasts									
14, 17, 20, 23, and 26 °C									
	P_{Linear}		NS	***	NS	***	*	***	***
	$P_{quadratic}$		**	**	**	***	*	NS	**

NS, *, **, *** Non-significant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 4. Parameters of linear regression analysis relating forcing temperature to rate of progress to visible bud (VB), to first open flower (FLW), and from VB to FLW in *Gaura lindheimeri* 'Whirling Butterflies'. Intercept and slope were used to calculate base temperature (T_b) and degree-days (°days).

Developmental stage (d)	Intercept (b_0) 1/d	Slope (b_0) (1/d)/C	T_b (°C)	°days	r^2
Forcing to VB	0.0054473 ± 0.001480[z]	0.001566 ± 0.000215	-3.4	638	0.96
VB to FLW	0.0295019 ± 0.003049	0.001559 ± 0.000322	-18.9	641	0.96
Forcing to FLW	0.0088113 ± 0.000758	0.000747 ± 0.000114	-11.8	1337	0.88

[z] Standard error.

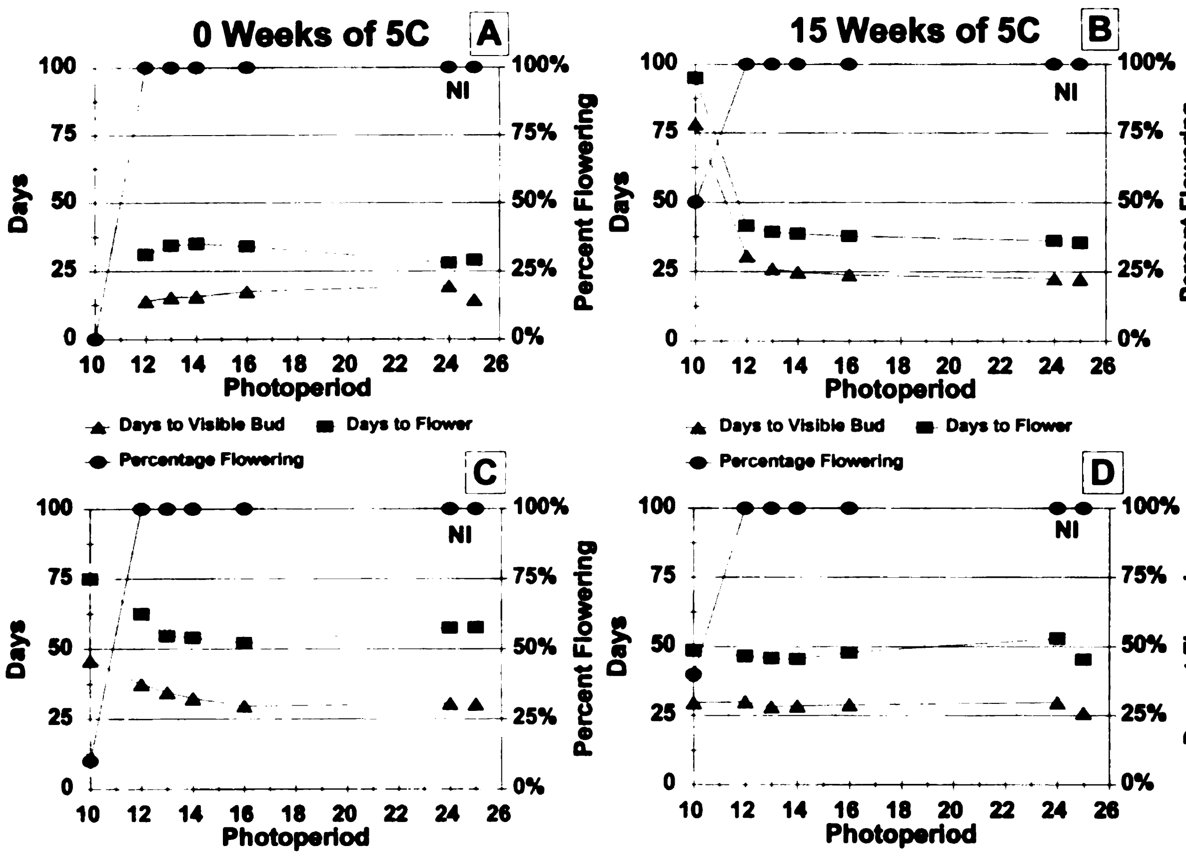

Figure 1. The effects of cold treatment and photoperiod on the flowering of *Gaura lindheimeri* 'Whirling Butterflies'. Percent flowering, days to visible bud, and days to flower for (A) year 1, 0 weeks of cold, (B) year 1, 15 weeks of cold, (C) year 2, 0 weeks of cold, and (D) year 2, 15 weeks of cold.

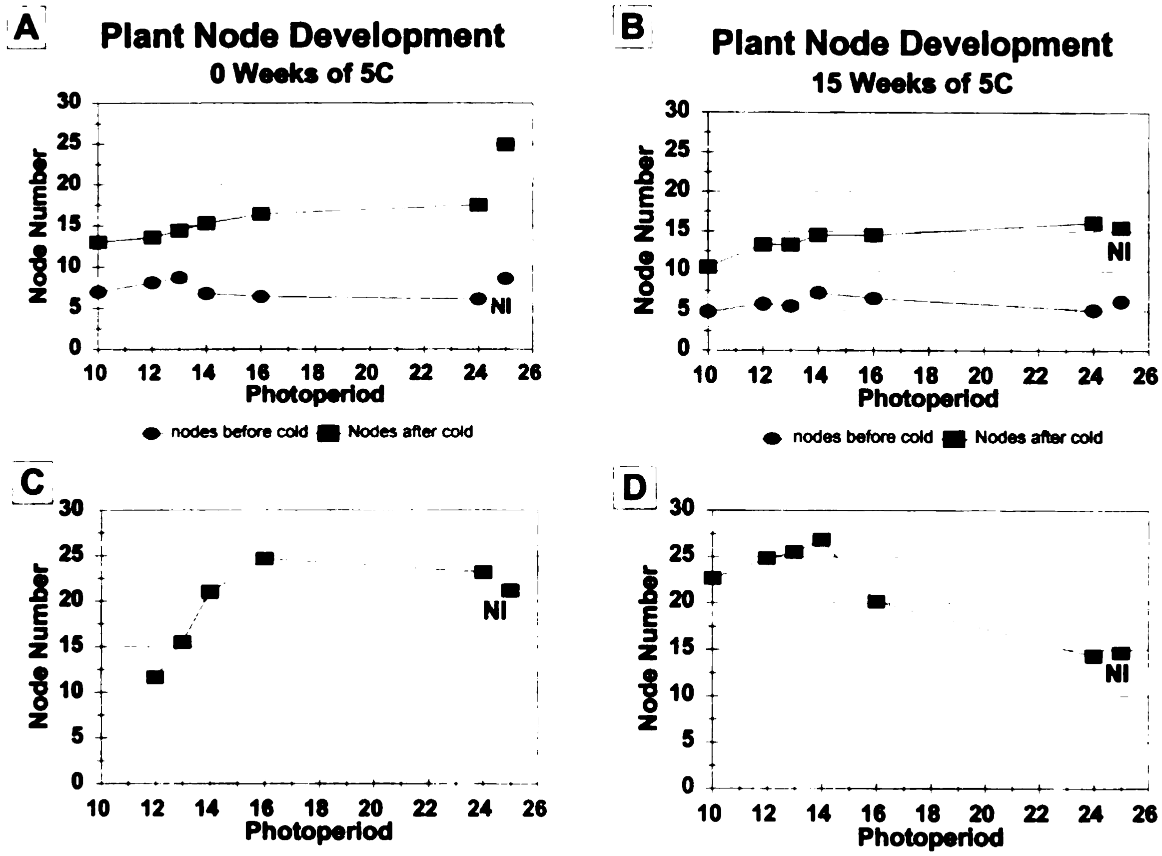

Figure 2. The effects of cold treatment and photoperiod on node development of *Gaura lindheimeri* 'Whirling Butterflies'
Number of nodes at first flower for (A) year 1, 0 weeks of cold, (B) year 1, 15 weeks of cold, (C) year 2, 0 weeks of cold, and (D) year 2, 15 weeks of cold.

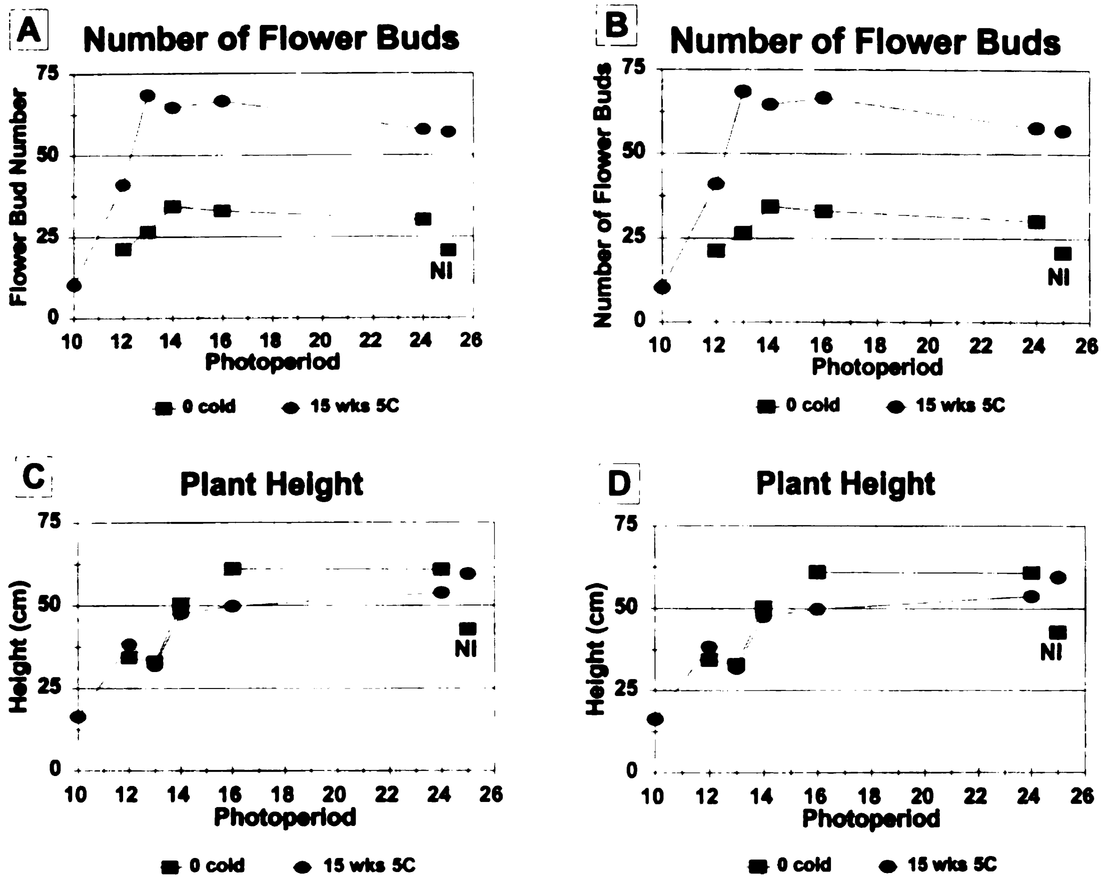

Figure 3. The effects of cold treatment and photoperiod on the flower bud number and plant height of *Gaura lindheimeri* 'Whirling Butterflies'. Flower bud number for (A) year 1 and (B) year 2. Total plant height for (C) year 1 and (D) year 2.

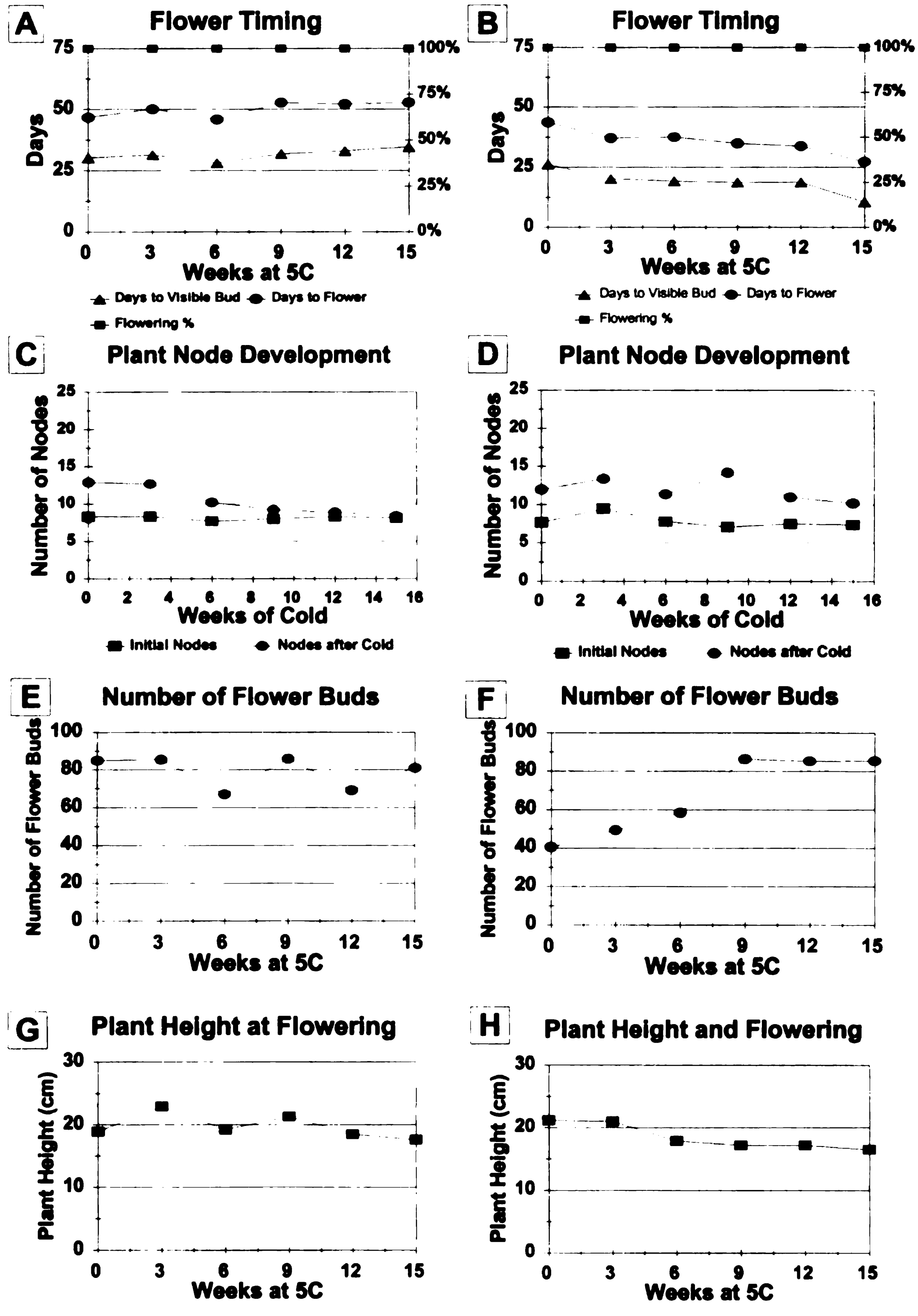

Figure 4. The effects of different durations of cold treatment on the flowering, plant node development, flower number, and plant height of Gaura lindheimeri Whirling Butterflies'. Flowering percentage, days to visible bud, and days to flower for (A) year 1 and (B) year 2. Number of nodes at first flower for (C) year 1 and (D) year 2). Number of flower buds at first flower for (E) year 1 and (F) year 2. Plant height at first flower for (G) year 1 and (H) year 2.

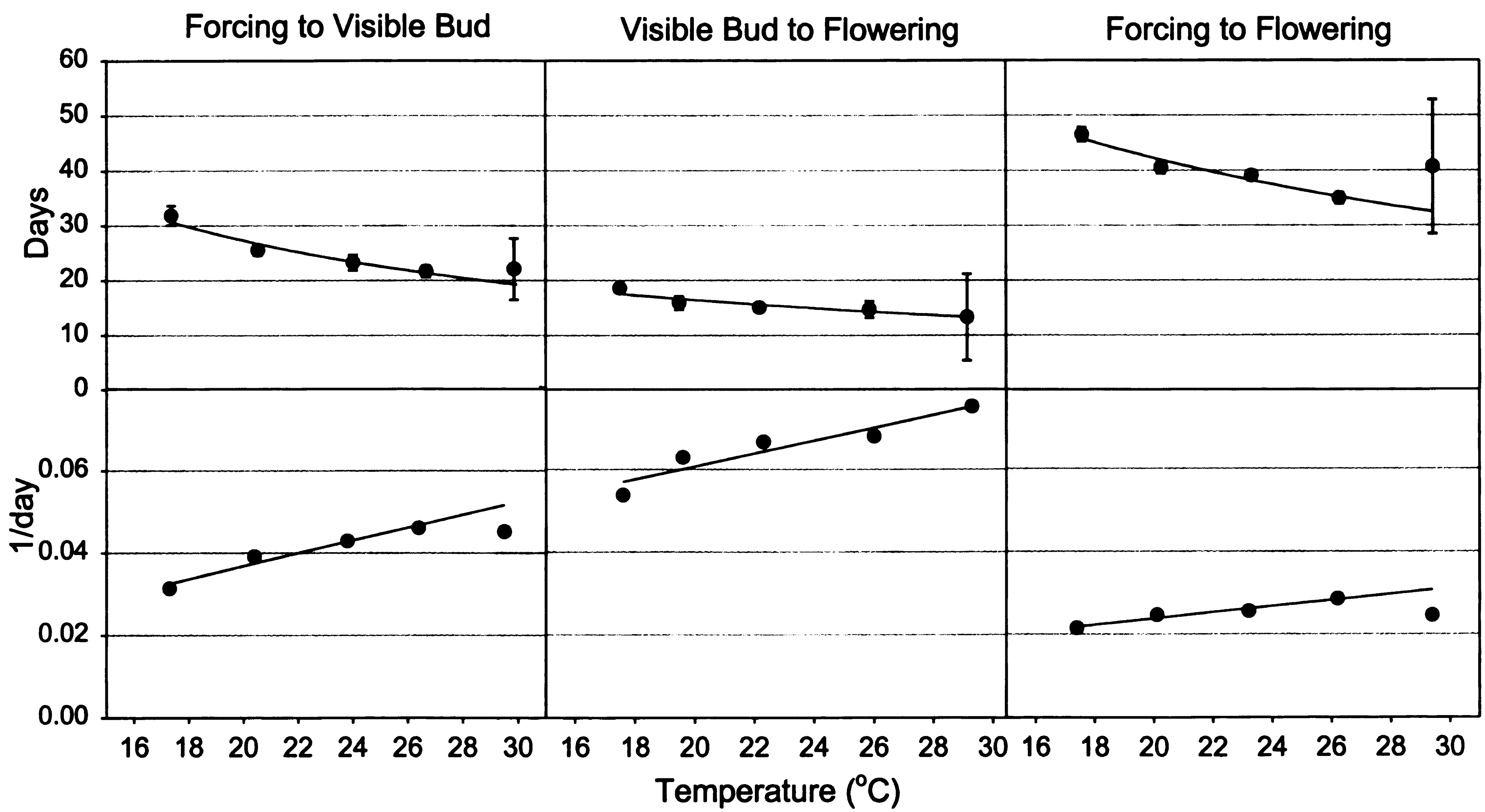

Figure 5. Influence of forcing temperature on time and rate toward flowering. Lines represent predicted values from the regression equations. Base temperature (Tb) and cumulative thermal time (CTT) necessary to complete the indicated developmental stage were calculated from linear regression analysis.

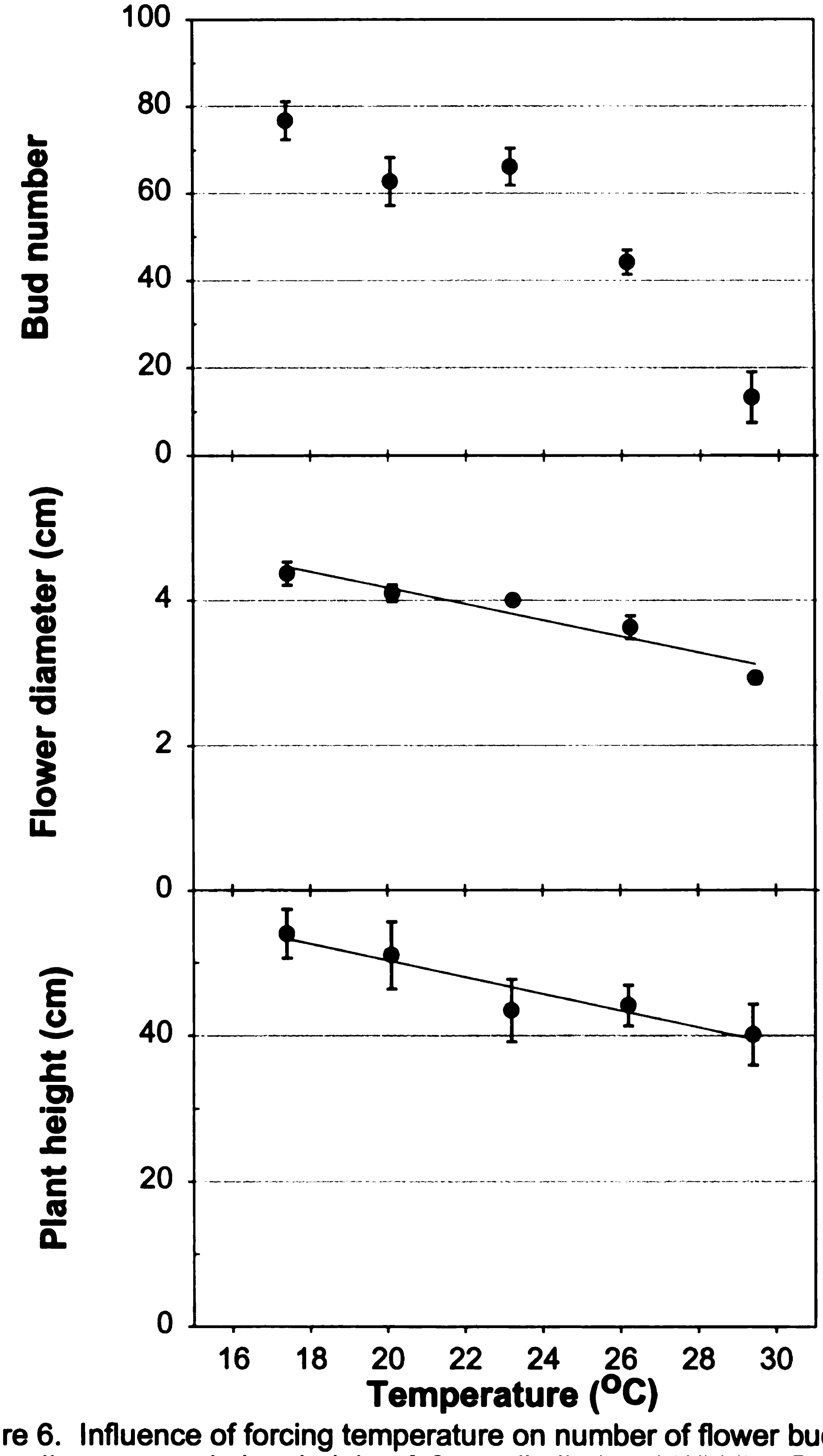

Figure 6. Influence of forcing temperature on number of flower buds, flower diameter, and plant height of *Gaura lindheimeri* 'Whirling Butterflies'. Eachmeasurment was takenat first flower. The points indicate the actual data points and the straight lines were calculated from linear regression analysis. Error bars show standard deviation.