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EFFECTS OF COLD TREATMENT, PHOTOPERIOD, AND FORCING TEMPERATURE ON OENOTHERA FRUTICOSA 'YOUNGII-LAPSLEY' AND STOKESIA LAEVIS 'KLAUS JELITTO'

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ABSTRACT

EFFECTS OF COLD TREATMENT, PHOTOPERIOD, AND FORCING TEMPERATURE ON *OENOTHERA FRUTICOSA* 'YOUNGII-LAPSLEY' AND *STOKESIA LAEVIS* 'KLAUS JELITTO'

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A series of experiments were conducted to determine the effect of cold treatment, photoperiod, and forcing temperature on *Oenothera fruticosa* L. 'Youngii-lapsley' and *Stokesia laevis* (Hill) Greene 'Klaus Jelitto'. 'Youngii-lapsley' had a nearly obligate cold requirement and, following cold treatment, behaved as a facultative long-day plant. Only 3 weeks of cold treatment were required for 100% flowering. Time to flower decreased slightly as cold treatment increased from 3 to 15 weeks. *Stokesia laevis* 'Klaus Jelitto' had a facultative cold requirement and was proposed to be a facultative intermediate-day plant because flowering was most consistent and rapid under photoperiods between 12 and 13 h both before and after cold treatment. Time to flower, flower number, and plant height at flower decreased as temperature increased from 14 °C to 29 °C for both 'Youngii-lapsley' and 'Klaus Jelitto'.

The flowering characteristics of five cultivars of *Oenothera fruticosa* were compared under either incandescent lamps or high-pressure sodium lamps used for daylength extension. Lamp types differed in light intensity as well as spectral quality. Flower number was increased two to three times for 'Youngii-lapsley', ssp. *glauca* and 'Highlight' when high-pressure sodium lamps were used for daylength extension compared to incandescent lamps.

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Literature Review

Introduction

Vernalization is defined as the promotion of flower formation by exposure to low temperatures (Lang, 1965). It is an inductive process, the effects of which generally are not observed until the chilled plant is moved to higher temperatures (Chouard, 1960; Vince-Prue, 1975). Vernalization is not required for flowering of all plant species; most that respond to vernalization originated in temperate latitudes where it is ecologically advantageous for a plant not to flower during the winter months (Roberts and Summerfield, 1987). Some species have a quantitative, or facultative, response to a cold treatment and flower without one, but flower development is hastened after a cold treatment. Plants with either a qualitative or quantitative response to cold treatment are usually long-day plants, although there is no definitive relationship between a vernalization requirement and a specific photoperiod requirement (Vince-Prue, 1975). Most species native to tropical or subtropical regions, where there is no need to avoid a period of lethally cold temperatures, are insensitive to vernalization (Roberts and Summerfield, 1987).

The first scientific work on vernalization was done primarily on winter wheat and rye. Winter races are fall-planted and require the cold temperatures to hasten flowering the following spring. The spring races differ in that they do not have a requirement for cold and will flower soon after sowing. While the phenomenon that we now call vernalization was recognized as early as the mid-

nineteenth century (Klippart, 1857, cited by Whyte, 1948), scientific definition did not occur until 1918 when Gassner (cited by Whyte, 1948) showed that a great variety of plants had a cold requirement for flowering.

Lysenko, working with winter rye, termed the phenomenon in his native Russian "jarovizacija" (a combination of the word for "spring" with a suffix meaning "to make") and translated it himself into English, French, and German as "vernalization" (Whyte, 1948; Chouard, 1960). Lysenko was also the first to propose a model to explain the role of vernalization in plant development. He called it the "theory of phasic development," in which he suggested that plants must pass through two irreversible phases in order to flower: the temperature-sensitive phase (thermophase) and the light-sensitive phase (photophase) (Chouard, 1960; Napp-Zinn, 1984). Today, we know that only a few plants respond exactly according to this theory and it has been soundly rejected (Napp-Zinn, 1984).

Throughout this century, a great deal of information concerning vernalization has been compiled and the characterization of the vernalization response is well described for many species. The genetic and physiological aspects of vernalization, however, have yet to be described in detail. Many genes from several different species are important in vernalization, but their mode of action is unknown.

In this review, I will present a thorough characterization of the vernalization response and a summary of current progress on the underlying genetic and physiological mechanisms.

Characterization of vernalization

Gregory and Purvis defined the fundamental characteristics of vernalization in their many experiments with the rye *(Secale cereale)* cultivar Petkus which consists of two races- winter and spring rye (Whyte, 1948; Chouard, 1960; Vince-Prue, 1975). They observed that both races flower faster when kept in continuous light and that neither will flower if the photoperiod is shorter than 12 hours (in Vince-Prue, 1975). Without a cold treatment (also called "thermoinduction," the actual exposure of a plant to cold temperatures), the winter race flowers in continuous light after 15 weeks when 22 leaves have been formed beneath the floral spike. The spring race flowers in about 7.5 weeks and produces six to seven leaves beneath the floral spike. After a cold treatment, the winter race behaves exactly like the spring race.

The vernalization response is seen as an aftereffect of the low-temperature treatment (Chouard, 1960), and therefore the response is typically measured by the time to anthesis, the leaf number beneath the flower, or both (Purvis, 1961). Currently, there are no genetic or physiological markers that can be used to provide a definitive assay of the progress of vernalization during a low- temperature treatment (Brooking, 1996); hence, the classical methods of measuring the vernalization response (e.g., final leaf number at flower, time to

anthesis) still are used.

Effective temperatures. Effective temperatures for vernalization of many species occur between -5 °C and 15 °C, with a broad optimum between 1 °C and 7 °C (Chouard, 1960; Lang, 1965; Thomas and Vince-Prue, 1984). The response generally increases with temperatures between -4 °C and 1 °C and decreases with temperatures above 9 °C (Purvis, 1961). Although this range is effective for most species, there are exceptions. For example, Easter lily *(Lilium longiflorum* Thunb.*)* 'Ace' is effectively vernalized at temperatures up to 16 °C and partially vernalized at 18 °C. No vernalization occurs at 21 °C (Weiler and Langhans, 1968).

The optimum temperature for vernalization may depend on the length of the cold treatment (Purvis, 1961). For example, experiments with black henbane *(Hyoscyamus niger* L.*)* and 'Petkus' rye showed a higher optimum temperature when the duration was shorter. However, when the cold treatment was extended, any temperature within the vernalization range produced the same effect (Purvis, 1961; Vince-Prue, 1975). In contrast, experiments with the *Cineraria* L. 'Cindy Blue' showed that optimum temperature was little affected by duration of cold treatment (Yeh et al., 1997a).

The optimum temperature also may depend on cultivar or the method by which the vernalization response is assessed. Rawson et al. (1998) studied several cultivars of wheat differing in their vernalization requirements at several temperatures. The moderate winter cultivar Oxley had an optimum vernalization

temperature of 8 to 10 °C when the response was measured as days from sowing to heading, 6 °C when measured as summed thermal time (°Cd) above a base of 0 °C, and 3 °C when measured as minimum final leaf number at heading. Different optima can be obtained when the vernalization response is measured as days from end of cold treatment to heading or days from sowing to heading. The winter cultivar JF87%014 had an optimum temperature of 8 °C when the response was measured as days from the end of cold treatment to heading; 6 °C, from sowing to heading (Rawson et al., 1998).

Robertson et al. (1996) point out that there is a problem in finding an optimum temperature for vernalization because the effectiveness of any temperature in lowering final leaf number is confounded with its effect on vegetative development. They found that the saturataion rate of vernalization in various winter-wheat cultivars (they considered vernalization saturated in treatments in which the number of leaves initiated at the end of the treatment equaled the final number of leaves produced beneath the flower) responded linearly to temperatures between 5 and 14 °C. Vernalization's effectiveness in decreasing the number of leaves below the flower decreased with temperature, and there was no optimum temperature. They suggested that higher temperatures were less effective because the rate of leaf primordium initiation responds more strongly to temperature than does that of vernalization (Robertson et al., 1996).

Duration of cold treatment. Vernalization is a quantitative process whose

intensity increases with the length of the exposure until a saturation response (here defined as no further reduction in time to flower or final leaf number) occurs (Vince-Prue, 1975). The duration of time to saturation is species- and cultivar-specific, but usually will occur between 10 and 100 days of low-temperature treatment (Thomas and Vince-Prue, 1984).

Increasing durations of cold treatment influence other plant characteristics such as height, flower number, and days to flower. Inflorescence number of *Lavandula angustifolia* Mill. grown at 20 °C under a 9-hour day plus a 4-hour night interruption (NI) increased from 2 to 11 as cold duration (at 5 °C) increased from 0 to 15 weeks (Whitman et al., 1996). The increase in flower number with cold treatment was not as dramatic when *L. angustifolia* was grown under 9-hour short days. Flowering did not occur until plants had received a minimum of 10 weeks of cold, and flower number increased only slightly (from approximately 5 to 6) when cold duration was increased from 10 to 15 weeks. Inflorescence number of *Leucanthemum xsuperbum* Bergman ex J. Ingram 'Snowcap' grown at 20 °C under NI increased from 5 to 13 as cold treatment (at 5 °C) increased from 0 to 15 weeks (Runkle et al., 1998). Plant height of 'Snowcap' increased from 14 to 22 cm as cold duration increased from 0 to 12 weeks.

Stability of the vernalization response, or the length of time that a chilled plant maintains the inductive effects of the cold treatment, increases with duration of cold treatment. Picard (1965) found that for *Oenothera biennis* L. var. *Sulfurea,* the vernalized state was lost after exposure to short days (8 hours)

at 18 to 22 °C for 12 to 20 days.

Antivernalization and devernalization. High temperatures (generally above 30 °C) given either before or after the cold treatment can influence the vernalization response. Several researchers observed that when high temperatures were given prior to the cold treatment, there was a nullification of, or a decrease in, the vernalization response (Lang, 1965). This effect was termed "antivernalization." A recent study of the effect of high temperature before cold treatment on subsequent flowering of *Brassica napus* L. var. *annua* (a plant that has a quantitative response to cold) showed that flowering of several genotypes was delayed by the heat treatment (30 °C) (Dahanayake and Galwey, 1998). For example, plants of genotype TB 14 exposed to a 30 °C treatment for 2 days before an 8-week 4 °C treatment flowered 8 days later than unexposed cold-treated control plants. Extending the heat treatment from 2 to 4 days further delayed flowering of this genotype; these plants flowered 13 days later than the control plants (Dahanayake and Galwey, 1998).

Devernalization is the elimination or reduction of the vernalization response when a heat treatment is given immediately after the cold treatment. Temperatures from 20 to 40 °C generally cause devernalization (Napp-Zinn, 1961). Temperatures between 12 and 15 °C cause neither vernalization nor devernalization in many winter cereals (Lang, 1965). Devernalization may occur only when the duration of cold temperatures is relatively short. Purvis and Gregory (1952, cited by Purvis, 1961) found that after 8 or more weeks of cold,

winter rye could not be devernalized. They also noted that even partially vernalized plants could not be devernalized if they were kept at a neutral temperature for a few days before the heat treatment was applied. The vernalization response then was deemed to be "fixed" or "stabilized" (Purvis, 1961; Lang, 1965). Recent work with the cineraria cultivars Cindy Blue and Cindy Dark Red supports the findings of Purvis and Lang (Yeh et al., 1997b). Temperatures of 20 °C or less promoted devernalization when the cold duration (at 6 °C) was 1 or 2 weeks. Devernalization response increased with temperature and length of heat treatment. Cultivars stabilized after 3 to 9 days at 15 °C, even plants that were only partially vernalized. In contrast, *Brassica napus* var. *annua* did not stabilize rapidly even when cooled plants were maintained at 15 °C for one week prior to heat treatment at 30 °C (Dahanayake and Galwey, 1998). In fact, devernalizing heat treatments were more effective after 1 week of stabilization. It was only after 2 weeks at the neutral temperature (15 °C) that devernalization was not as effective.

Not all species can be devernalized by a simple high temperature treatment. A cold-requiring variety of chrysanthemum did not devernalize completely even when exposed to 35 °C for 30 days (Schwabe, 1955). However, this variety was devernalized by a low-intensity light treatment of about 25 foot-candles (given by incandescent bulbs) combined with high temperature (28 °C) for 10 to 20 days although neither treatment alone had a measurable effect (Schwabe, 1957). Napp-Zinn (1960, cited by Lang, 1965) found that, for

Arabidopsis thaliana (L.) Heynh. , devernalization did not occur if plants were kept in light during the high temperature treatment. Lang (1965) cautions that the inhibitory action of high light with respect to devernalization indicated by the results with *Arabidopsis* and chrysanthemum actually may be a general response, since many devernalization experiments were conducted in darkness or weak light.

Plant age. The age at which plants are able to perceive cold temperatures for effective vernalization is very species specific. Some species such as 'Petkus' rye and winter wheat are capable of perceiving low temperatures soon after fertilization, while others such as *Hyoscyamus niger* must be at least 10 days old (Purvis, 1961).

In many cases, younger plants require a longer duration of cold to achieve vernalization saturation than do older plants. When Gott (1950, cited by Purvis, 1961) chilled rye plants of different ages, she found a decline in response when plants were older than 2 weeks. Picard (1965) found that young plants of *O. biennis* (with a minimum of 10 leaves) required 17 weeks of cold treatment (at 11 °C/3 °C D/N) for 100% flowering, while older plants (minimum 50 leaves) required 13 weeks for 100% flowering. Recent work with two cultivars of winter wheat (*Triticum aestivum* L. Pioneer 2548 and Augusta) showed that the estimated minimum days of cold treatment required to reach saturation of the vernalization response decreased linearly with increasing plant age at the beginning of the cold treatment (Wang et al., 1995). These authors suggest an

interchangeability between days of cold treatment and plant age.

Robertson et al. (1996) proposed and tested a model to explain the interaction between plant age and vernalization. The model predicted the final leaf number below the flower based on the effect of temperature on the rate of vernalization saturation and rate of leaf initiation for wheat under continuous and intermittent low temperature. Plants grown under intermittent low temperatures progress toward flowering through a combination of rates, depending on the temperature at any given time. When Robertson et al. (1996) fitted the data of Wang et al. (1995) to their model, they found that the model satisfactorily predicted a range of final leaf numbers between 8 and 21, with an r^2 of 88%. For the authors this model explained to a reasonable extent the interchangeability between plant age and vernalization.

Plant age when the cold treatment is given can influence the stability of vernalization. Younger plants of *O. biennis* had lower vernalization stability than older plants when both were maintained under short days after cold treatment before being moved to inductive long days (Picard, 1965).

Light and vernalization. The interactions between photoperiod, light quality, light quantity, and vernalization are complex and inconclusive. Napp-Zinn (1984) describes the often conflicting results in this area of research as discouraging and points out that there still is too little information. He also indicates that vernalization likely means different things in different species or even in different genotypes of the same species.

Vernalization can be substituted by an appropriate photoperiod in certain cases. Short-day vernalization, or the replacement of a cold treatment by short days, first was studied in the 1930s for 'Petkus' rye (Napp-Zinn, 1984). Wellensiek (1960) was the first to discover short-long day induction in a dicotyledon, *Campanula medium* L., a discovery Chouard (1960) refers to as "Wellensiek's phenomenon." Wellensiek (1960) observed that if a *Campanula* rosette was grown to three months of age and then exposed to at least 4 weeks of warm (20 °C) short days, it would flower when transferred to 16-hour long days. Flowering under short days only was never observed, even after 2 years. Increasing the number of short days did increase the percentage of plants that bolted, but did not regularly decrease the days to bolting. Wellensiek (1960) also noted that "the reaction to warm short days is not as regular as to cold."

An experiment using a winter race of barley (*Hordeum vulgare* L. 'Arabi Abiad') showed unequivocally that short days can substitute for vernalization in this species (Roberts et al., 1988). Photoperiods of 8 or 10 hours given after germination were sufficient to decrease the number of leaves beneath the flower by a factor of 3.3 when compared to that of plants given a 13-hour photoperiod. The minimum number of leaves formed with short-day vernalization is 9 or 10. Photoperiods longer than 13 hours did not increase the number of leaves beneath the flower. However, substitution by short days does not appear to be equal to vernalization by cold treatment since flowers can emerge after formation of 6 or 7 leaves if the plant is cold-treated (Roberts et al., 1988).

Substitution of a cold treatment by long days has been documented for several species. *Pisum sativum* L. genotype *lf e Sn hr* kept in continuous light did not respond to vernalization; in fact, vernalized plants grown under continuous light flower at a node slightly higher than that of unvernalized plants (Murfet and Reid, 1974). When plants are grown under short days (8 hours), vernalization promotes flowering of genotype *lf e Sn hr* 6 nodes lower than that of unvernalized plants. Runkle et al. (1998) found that, for *L.* x*superbum* 'Snowcap', 80% of plants grown at 20 °C under NI flowered without a cold treatment, while 80% of plants grown under a 9 hour short day required a minimum of 3 weeks at 5 °C. When *Campanula longestyla* Fomin. was grown under 16 hour long days, plants flowered without vernalization, but when the photoperiod was reduced to 14 hour, plants required vernalization (Mathon, 1960).

Substitution of a cold treatment with light of particular radiation ratios or of high irradiances also has been demonstrated. Bagnall (1993) showed that for three late-flowering ecotypes of *Arabidopsis* ('Eifel', 'Innsbruck', 'Pitztal'), time to flower and leaf number at flower were halved when nonvernalized plants were grown under fluorescent and incandescent lights (R : FR = 1.0) compared to those grown strictly under fluorescent lighting (R :FR = 5.8). Bagnall terms this phenomenon of flower induction by different ratios of radiation "photoinduction." Work by Chouard during the 1960s revealed that several species, including *Geum urbanum* L. and *Dactylis glomerata* L., flowered without cold when they

were kept under long days or continual light at high irradiances, usually above 14,000 lx. Napp-Zinn (1984) suggests that this effect may be due to photosynthesis, since it often is accompanied by a need for high mineral nutrition.

In certain cases, the duration of light before the cold treatment can have an effect on subsequent flowering. Long days before cold treatment were beneficial for *Oenothera biennis* var. *sulfurea* because they caused a shorter juvenile period (Picard, 1965). Under long days (16 hours) *O. biennis* perceived vernalizing cold temperatures 50 to 60 days after germination, while under short days (8 hours), the species could not perceive cold temperatures for 5 to 6 months at 18 to 22 °C.

Experiments comparing different photoperiods during cold treatment yield inconsistent results. Long days, short days plus a night break, or continual light during cold treatment reduced subsequent flowering of celery, while they enhanced flowering of *Arabidopsis* 'Stockholm' (Napp-Zinn, 1984). For *Campanula medium,* there was no difference between plants exposed to long or short days during the 5 °C cold treatment (Wellensiek, 1960). The photoperiod during the cold treatment also had no influence on subsequent flowering of *O. biennis* (Picard, 1965).

Perception of Vernalization

Early experiments demonstrated that vernalization is perceived in the shoot tip. Evidence for this conclusion came from localized cooling experiments

in which either the roots or shoots were cooled separately while the rest of the plant was kept in noninductive temperatures (Lang, 1965). Gregory and Purvis (1938, cited by Lang, 1965) showed that even fragments of embryos, which consisted of only the shoot tip, could respond to the low temperatures. Grafting studies showed that only when the shoot tip itself had been exposed to low temperatures would flowering occur (Lang, 1965). Metzger (1988) found that maintaining roots of *Thlaspi arvense* L. at 21 °C (while shoots were kept at 4 °C) reduced subsequent stem elongation, and he suggested that cold treatment of tissue other than the apex may be important for integration of reproductive development.

Since the site of perception appears to be in the shoot tip, dividing cells may be necessary for vernalization to occur (Wellensiek, 1964; Lang, 1965). If this supposition is true, then any dividing cell in the plant may be vernalizable. Wellensiek (1964) provided support for this theory when he found that excised leaves of *Lunaria biennis* L. given a cold treatment could regenerate plants that immediately flowered. When he cut off the lowest 0.5 cm of the petiole, where regeneration and thus cell division takes place, the regenerated plants remained vegetative. In contrast to Wellensiek, Metzger (1988) observed that when shoots were regenerated from *Thlaspi arvense* leaf cuttings from which the lower 1 to 2 cm had been removed (which occurred in only 10 to15% of the cuttings), all shoots immediately flowered. He suggested that many cell types, not necessarily only actively dividing cells, may become vernalized. However, since

the outcome of vernalization is the ability to flower and flowering occurs on structures that arise from the shoot apex, it may be coincidental that the apex is considered the site of perception (Metzger, 1988).

Mechanism of Vernalization

To date, very little is known about the mechanism of vernalization. The role of classical plant hormones in the vernalization process is still unclear and evidence for a flower-forming hormone produced by thermoinduced tissue is equivocal. In 1965, Lang stated that studies into the biochemical foundations of vernalization were inconclusive. Almost thirty years later, Hazebroek (1993) wrote, "Essentially nothing is known about the biochemical or molecular processes that occur during vernalization." While attempts to elucidate the mechanism of vernalization have been frustrating, recent genetic studies have provided some new insight and may renew interest in the study of vernalization.

Grafting studies. In several early experiments, the subsequent flowering of nonthermoinduced receptor plants grafted onto thermoinduced donor plants suggested the existence of a graft-transmissible flower-forming material (Lang, 1965). Successful grafts in which the nonthermoinduced receptor flowered were made between many different species and response types. Lang (1965) commented that many of these experiments were performed in inductive long-day conditions for the donor, and that flowering of the receptor therefore may have been due to the transmissible material (also called "florigen") produced in

response to photoperiod and not necessarily just thermoinduction. Direct evidence for a transmissible material formed during thermoinduction came from grafting nonthermoinduced *Hyoscyamus niger* to the short-day plant Maryland 'Mammoth tobacco' (Melchers, 1939, cited by Lang, 1965). The *H. niger* receptor flowered under both long and short days, which indicated that a transmissible material (named "vernalin") was produced by the thermoinduced tissue. Efforts to extract substances from thermoinduced plants that induced flowering in nonthermoinduced plants were unsuccessful (Lang, 1965).

Grafting studies using *Pisum sativum* also have revealed evidence for a transmissible substance. Reid and Murfet (1975) grafted unvernalized or vernalized scions to unvernalized or vernalized stocks and used several different lines (both early- and late-flowering) of *P. sativum*. They found that chilling the stock of late-flowering lines (which contain the *Sn* gene) could promote flowering in unvernalized scions. In fact, vernalization of the stocks for these late-flowering lines more effectively reduced the node number below the flower than vernalization of the scions. They suggested that the *Sn* gene produces a flowering inhibitor and that vernalization suppresses its action. They also suggested that vernalization is active at two sites: the shoot tip and the cotyledons (the only node below the graft).

While flowering of nonthermoinduced receptor plants has been achieved in grafting studies, there were many other studies in which a nonthermoinduced receptor did not flower, regardless of the state of the donor (Lang, 1965).

Chouard (1960) states that, aside from the success of graft transmissibility in *Hyoscyamus,* flowering of a noninduced receptor on a thermoinduced donor is not generally effective and is "only quite rarely successful."

Gibberellins. The application of gibberellins (GAs) to nonthermoinduced plants produces a broad range of responses; for some species, GAs can induce flowering in nonthermoinduced plants, while for others, they result in stem elongation but no flower formation (Lang, 1965). In general, treating seeds with GAs does not effectively induce flowering (Lang, 1965). Stem elongation precedes flower formation in GA-treated plants, while flowering and elongation occur simultaneously after a cold treatment (Lang, 1965).

Applying inhibitors of GA biosynthesis can influence the flowering process. For example, uniconazole, a triazol GA biosynthesis inhibitor applied 3 days before a 20-day cold treatment, delayed both stem elongation and flowering of *Raphanus sativus* L. (Japanese radish), a strongly facultative cold-requiring plant (Nishijima et al., 1997). The effects of uniconazole on stem elongation and flowering reached a maximum at 2 μmol/pot, at which rate flowering was delayed by 23 days and stem elongation was reduced by 48 cm. Increasing the cold treatment from 20 to 40 days moderated the effects of uniconazole only slightly. Days to stem elongation for plants cooled for 40 days were reduced by approximately 13 compared to that of plants cooled for 20 days. Days to flower and stem length were similar for plants given either duration of cold treatment. Timing of the GA inhibitor application was important:

if the application was made during the cold treatment, it reduced stem elongation and delaying flowering, but if it was made at or 5 days after the end of the cold treatment, flowering was not delayed (Nishijima et al., 1997).

Zeevaart (1978) proposed that the response to GA may depend on using the "appropriate GA". For *Myosotis alpestris* F.W. Schmidt, GA_7 induced both stem growth and flowering, while GA_3 induced only stem growth (Michniewicz and Lang, 1962, cited by Zeevaart, 1978). Research of Wittwer and Bukovac (1962) also demonstrated a differential effect on flowering when different GAs were applied to lettuce. Both GA_3 and GA_1 were able to induce 100% flowering, while GA_4 and GA_5 induced 30 to 40% flowering and GA_6 and GA_8 were unable to induce it. Zeevaart (1978) suggested that plants that do not respond to GA might not be receiving the appropriate form.

Gibberellins may influence flowering of those plants with suboptimal cold treatments. In a study on vernalization and exogenous Gas' effect on flower induction of *Brassica oleracea* L. var. *botrytis* (cauliflower), Fernandez et al. (1997) showed that $GA_{(4+7)}$ accelerated flowering of plants grown at 22 °C (weak flower-inducing conditions) but not 10 °C (strong flower- inducing conditions). Plants grown at 10 °C produced a flower after 23 leaves had emerged, regardless of GA application. Plants that were not treated with GA and grown at 22 °C produced a flower after 38 leaves had emerged. Plants that were grown at 22 °C and treated with GA, however, produced a flower after 26 leaves had emerged (Fernandez et al., 1997).

For some species, levels of GAs increase after the cold treatment (Lang, 1965; Zanewich and Rood, 1995). Hazebroek et al. (1993) found that the activity of the enzyme kaurenoic acid hydroxylase, which converts kaurenoic acid to 7-OH kaurenoic acid (two members of the GA biosynthetic pathway), was increased after vernalization. In addition, the up-regulation of the enzyme was localized in the shoot tip and no change was observed in the leaves. For *Brassica napus* 'Crystal', Zanewich and Rood (1995) found that not only were endogenous levels of GAs increased (3.1, 2.3, 7.8, 12.0, and 24.5 times higher for GA_1, GA_3, GA_8, GA_{19}, and GA_{20}, respectively) after vernalization, but also the metabolism of GAs was changed. Vernalized plants showed an increase in the conversion of $[^3H]GA_{20}$ to an $[^3H]GA_1$-like metabolite and reduced conversion of $[^3H]GA_{20}$ or $[^3H]GA_1$ to polar $[^3H]$ metabolites, possibly representing GA glucosyl conjugates.

The association between GAs and the flowering process also has been indicated by GA-deficient mutants. The *rosette* mutant of *Brassica napus* has about one-tenth of the normal level of total GAs and flowers very slowly compared to the wild type (Rood et al., 1989).

Genetics of vernalization

A vernalization requirement is a heritable property. In certain species, crosses between winter and spring races showed that the vernalization character seemed to follow predicted Mendelian ratios (Chouard, 1960). Over the years, genes, linkage groups, and cDNAs that either confer late flowering or are

associated with a vernalization requirement have been identified.

Genes that influence the requirement for vernalization have been identified in several species. For such species as *Triticum*, *Arabidopsis*, and *Pisum*, numbers of genes involved in vernalization have been deduced by the study of segregation ratios. It was postulated that there are four *Triticum* loci, labelled *Vrn-1* to*Vrn-4* (Napp-Zinn, 1987). For *Pisum*, 20 genes initially were identified and were alleles of four individual loci. A dominant allele at either of two loci, *Lf* or *Sn*, confers late flowering. The requirement for vernalization is not always caused by the presence of a dominant allele. Recessive alleles appear to cause a vernalization requirement for *Triticum* and *Arabidopsis* (Napp-Zinn, 1987). In *Arabidopsis thaliana,* the single *FRI* locus is apparently the cause for the difference between the winter ecotypes SF-2 and Le-0 and the spring ecotype Col. After the cold treatment, all ecotypes with the *FRI* locus, either naturally occurring or introduced, flower early (Lee et al., 1993). However, the action of *FRI* is not necessarily based on simply presence or absence of the locus. In the L*er* background, the *FRI* locus causes only a delay in flowering when another locus, *FLC,* is present (Lee et al.,1994). Lee et al. (1994) suggest that *FRI* and *FLC* act to suppress flowering.

The *FRI* locus also confers a strong responsiveness to light quality. Far red light accelerated flowering of plants containing the *FRI* locus when they received no cold treatment (Lee and Amasino, 1995). After a cold treatment, the acceleration of flowering time by far red light was eliminated. Since vernalization

did not influence the effect of far red light on petiole extension, the authors concluded that the effects of vernalization on flowering occur after far red light perception.

In contrast to situations in which one or a few genes are responsible for the vernalization requirement, sometimes there are cases where it appears that many genes may be involved. Teutonico and Osborn (1995) mapped quantitative trait loci (QTL) for flowering time in *Brassica rapa* L. They found two linkage groups associated with variation for flowering time in unvernalized plants. After vernalization, neither of these QTL had a significant effect on flowering time. It is interesting to note that an RFLP locus identified with a clone from the *Arabidopsis* cold-induced gene COR6.6 mapped very close to one of the *B. rapa* QTL (Teutonico and Osborn, 1995). Osborn et al. (1997) compared the QTL for flowering time in *B. rapa* with QTLs in *B. napus* and *A. thaliana*. By aligning map positions they found that the two QTL from *B. rapa* corresponded to two major QTL for flower timing already identified in *B. napus*. The RFLP loci were mapped for both *B. napus* and *A. thaliana* by using *Arabidopsis* probes for flowering time genes and one of the *Brassica* QTL was collinear with the top of chromosome 5 of *Arabidopsis*. This area contains the locus *FLC* and various other flowering loci (Lee et al., 1994). The other *Brassica* QTL showed fractured collinearity with several regions of the *Arabidopsis* genome, one of which was the top of chromosome 4 where *FRI* is located (Lee et al., 1993).

Recently, three mutants with altered vernalization responses were

identified at three independent loci in *Arabidopsis* (Chandler et al., 1996). Each of the mutants was induced by ethyl methane sulphonate (EMS) in the *fca* mutant in the L*er* background, which itself shows late flowering and a strong response to vernalization. The three loci identified were named *VRN1*, *VRN2*, and *VRN3*. Before vernalization, *vrn1-1 fca-1* flowered at the same time as *fca-1* but, after vernalization, flowered about 9 days later (a 42% reduction of the vernalization response); *vrn2-1 fca-1* differed from *vrn1-1 fca-1* because it flowered later than *fca-1* both before and after vernalization. The upper arm of chromosome 3 contained *VRN1* (Chandler et al., 1996).

Chong et al. (1994; 1998) identified two cold-induced cDNAs (*verc*17 and *verc*203) thought to be specific for the vernalization response in wheat. The *verc*17 appeared in vernalized winter wheat tissue only; it was absent in nonthermoinduced and devernalized plants. The *verc*17 cDNA had significant homology with such diverse genes as a L. lactis PIII-type proteinase, maturation protein genes, and a major merozoite surface antigen gene. When wheat plants were transformed with the antisense RNA of the vernalization-specific cDNA *verc*203 under control of the CaMV 35S-promoter, it was found that flowering was greatly delayed (≈160 days to flower for antisense compared to ≈102 days to flower for the natural orientation of the DNA). These results suggest that the VER203 protein has a role in controlling flower development in winter wheat (Chong et al., 1998).

Although progress has been made in identifying genes involved

associated with vernalization, little is known about how their function in creating the phenomenon known as vernalization. Burn et al. (1993) found that flowering in *Thlaspi arvense* and *Arabidopsis thaliana* (late ecotype) could be accelerated significantly if unvernalized plants were treated with 5-azacytidine, a DNA demethylating agent. In contrast, treatment with 5-azaC did not influence flowering of vernalized plants. When the treated plants were allowed to set seed, the resulting plants behaved as if unvernalized. After cold or treatment with 5-azacytidine, both *Nicotiana plumbaginifolia* L. and *Arabidopsis* show reduced levels of 5-methylcytosine in their DNA. The authors suggested that methylation status could be a control mechanism of vernalization. Brock and Davidson (1994) also found that 5-azacytidine as well as gamma rays (thought to be a demethylating agent) could substitute partially for a cold treatment. The 5-aza-C treatment induced significantly earlier flowering in both spring and winter wheats, but the response was pronounced for the winter wheat. The gamma rays effectively reduced flowering time in the winter wheat only.

Recently, the hypothesis that the promotion of flowering by vernalization is mediated by DNA demethylation was tested by transforming *Arabidopsis* plants with a methyltransferase *(MET1)* antisense gene (Finnegan et al., 1998). The authors found that plants homozygous for *MET1* had 15% of normal methylation levels and flowered significantly earlier than untransformed controls. They also found that the promotion of flowering generally was correlated with the extent of demethylation. They tested plants of three methyltransferase antisense families

that differed in DNA demethylation magnitude; plants homozygous for four copies of the antisense gene flowered significantly earlier than those homozygous for three. Demethylation caused by the *MET1* antisense transgene did not prevent a response to vernalization. Cold treatment of antisense plants accelerated flowering compared to that of untreated antisense plants, which indicates that demethylation caused by the *MET1* antisense transgene did not saturate the early flowering response and acted additively with a cold treatment.

Studies of the inheritance of early flowering used plants with the *MET1* antisense transgene and showed that demethylation caused by the transgene did not exactly replicate early flowering due to vernalization (Finnegan et al., 1998). For wild type plants, the requirement for vernalization is reset every generation (possibly because of remethylation during gametogenesis or embryogenesis). Like those of wild type plants, progeny of cold-treated or untreated antisense plants flowered at the same time, which shows that the *MET1* antisense transgene does not inhibit the resetting process. However, progeny of plants hemizygous for the transgene (and not given a cold treatment) flowered earlier than progeny of an untransformed plant. Even progeny that did not contain the antisense transgene had reduced levels of methylation and flowered earlier than progeny of untransformed plants. Although this response does suggest that early flowering is caused by lower levels of methylation and not the transgene itself, it also shows that early flowering due to demethylation by *MET1* antisense does not replicate all aspects of vernalization. Finnegan et

al. (1998) suggested that there may be two processes involved if demethylation of DNA is necessary for vernalization-- one involved in the actual demethylation, the other involved in resetting the vernalization requirement each generation. Alternatively, the authors suggest that there may be several methyltransferases that methylate different sites and that the *MET1* antisense transgene affects only one of these.

Conclusion

Although a great deal of information that characterizes the phenomenon of vernalization and the genetics involved has been generated, much is still unknown. The complex interactions between temperature before, during, and after the cold treatment, photoperiod, light intensity, and plant maturity make it a difficult subject to study. Regardless, a greater understanding of the genetic mechanisms for controlling developmental processes should shed light on the mystery of how cold temperatures are perceived and how this stimulus is translated into the ability to flower.

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Growth and Development of *Oenothera fruticosa* Is Influenced by Cold Treatment, Photoperiod, Forcing Temperature, and Plant Growth Regulators

Abstract

The influences of cold treatment, photoperiod, forcing temperature, and plant growth regulators on growth and development of *Oenothera fruticosa* L. 'Youngii-lapsley' were determined. Plants were cooled at 5 °C for 0, 3, 6, 9, 12, or 15 weeks under a 9-h photoperiod and subsequently forced in a 20 °C greenhouse under a 16-h photoperiod. Only one plant in two years flowered without cold treatment. Time to visible bud and flower decreased about 1 week as cold duration increased from 3 to 15 weeks. Response of plants to 10-, 12-, 13-, 14-, 16-, or 24-h photoperiods or a 4-h night interruption (NI) were determined in a 20 °C greenhouse following 0 or 15 weeks of cold treatment at 5 °C. Flowering occurred under all photoperiods only after cold treatment. Time to flower decreased about 2 weeks, flower number decreased, and plant height increased as photoperiod increased from 10 to 24 h. Days to flower under NI were similar to that of plants grown under a 16- h photoperiod. To determine the effect of forcing temperature on flowering of 'Youngii-lapsley', plants were cooled for 15 weeks and then forced under a 16-h photoperiod at 14, 17, 20, 23, 26, or 29 °C. Plants flowered more quickly at higher temperatures but were taller and produced fewer and much smaller flowers. Days to visible bud and flowering were converted to rates, and base temperature (T_b) and thermal time to flowering (degree-days) were calculated as 4.4 °C and 606 °day. The effects of spraying ancymidol, chlormequat, paclobutrazol, daminozide, and uniconazole were tested on plants cooled for 19 weeks and forced at 20 °C under a 16-h

photoperiod. Final plant height was reduced 31% and flower diameter was reduced 36% for plants sprayed with uniconazole. Plants sprayed with all other plant growth regulators were similar to control plants.

Introduction

Many species of *Oenothera* have been cultivated since the early 1800s when they were brought from North and South America to England where they became popular garden plants (Wiesner,1991). *Oenothera fruticosa* L., or sundrops, is native to eastern North America and hardy in USDA zones 4-8. *Oenothera fruticosa* is a day-flowering species that typically flowers in June (Griffiths, 1994; Nau, 1996). In a preliminary screen, the vegetatively propagated cultivar Youngii-lapsley showed great ornamental flowering potted-plant potential with its long natural flowering time (≈4 weeks) and numerous large bright-yellow flowers (Frane, 1999). To our knowledge, no species of *Oenothera* is grown currently as a pot plant; for potted plant production, detailed information on flowering requirements is needed.

Vernalization and photoperiod are two important factors regulating flowering of vegetative propagules. Vernalization can be defined as the promotion of flower formation by a period of low temperatures, generally between -5 and 15 °C, with a broad optimum between 1 and 7 °C (Lang, 1965). A vernalization requirement often is linked to a specific photoperiod requirement, which for many species is usually, but not always, a long-day requirement (Vince-Prue, 1975). Flowering of many herbaceous perennials is influenced by vernalization and photoperiod (Iversen and Weiler, 1994; Whitman et al., 1996,1997; Runkle et al., 1998).

Many early experiments investigating the role and mechanism of vernalization and photoperiod in flowering were performed on various species of *Oenothera* and several different response types were identified. Picard (1965) observed flowering of *O. biennis* var. *sulfurea* De Vries only after a minimum cold treatment (at 11 °C /3 °C day/night [D/N]) of 10 weeks followed by long days ≥12 h. Other species of *Oenothera*, including *O. suaveolens, O. longiflora,* and *O. stricta,* had facultative requirements for vernalization followed by an obligate requirement for long days (Chouard, 1960). *Oenothera rosea* L'Herit. ex Ait. is a facultative long-day plant with no response to vernalization (Chouard, 1960). In preliminary experiments, 'Youngii-lapsley' appeared to be a facultative long-day plant with an obligate vernalization requirement (Frane, 1999).

Knowledge of the effect of temperature on plant development is important to accurately time crop production. Without photoperiod or vernalization effects, the rate of development increases linearly with temperature within a limited range of growing temperatures (Roberts and Summerfield, 1987). Such responses are also evident in photoperiod- and vernalization-sensitive species when the separate effects of photoperiod and vernalization are removed from those of postvernalization temperatures. The relationship between temperature and rate of development toward flowering (1/DTF where DTF is days to flower) can be described as follows:

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

where b_0 and b_1 are constants and T is temperature (°C) (Roberts and Summerfield, 1987).

The base temperature (T_b), or the temperature at or below which the rate of progress toward flowering is zero, and thermal time, or the number of units (°days) of thermal time above a base temperature, required for flowering can be calculated from the constants in Equation 1 as follows:

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

$$°days = 1/b_1 \quad [3]$$

Growing temperature can affect plant appearance as well as flower timing. For *Antirrhinum majus* L. 'Jackpot', there was an increase in stem length, spike length, and number of florets as temperature decreased from 21 to 10 °C (Maginnes and Langhans, 1961). Flower size decreases with increasing temperature in *Galinsoga parviflora* Cavan. and *Agrostemma githago* L. (Knapp, 1957); *Campanula carpatica* Jacq. 'Blue Clips' (Whitman et al., 1997); *Gaillardia xgrandiflora* Van Houtte 'Goblin', *Leucanthemum xsuperbum* Bergman ex J. Ingram 'Snowcap', *Coreopsis grandiflora* Hogg ex Sweet 'Sunray' and *Rudbeckia fulgida* Ait. 'Goldsturm' (Yuan et al., 1998).

A potential problem in growing *O. fruticosa* 'Youngii-lapsley' as a potted plant is its relatively tall natural height (33-54 cm). Control of height by plant growth regulator (PGR) treatment may be required to produce high-quality potted plants. Although we have been unable to find literature on the effect of PGRs on *O. fruticosa,* chlormequat (up to 3.0 μg) applications to the root zone after

chilling had no effect on stem elongation or flowering of *O. biennis* (Picard, 1967). According to Picard (1967), chlormequat in doses between 50 and 650 μg applied to the apical meristem actually promoted stem elongation, while a dose of 750 μg decreased it. Daminozide in doses up to 550 μg applied to the root zone after chilling decreased stem elongation and delayed flowering, the effect increasing with larger doses. A dose of 650 μg of daminozide completely inhibited flowering (Picard, 1967).

The objectives of our study were (1) to determine and quantify the effects of cold treatment, photoperiod, and forcing temperature on flowering of *O. fruticosa* 'Youngii-lapsley', and (2) to determine the effect of five commercially available PGRs on flowering and final plant height of 'Youngii-lapsley'.

Materials and Methods

General. Plants of *Oenothera fruticosa* 'Youngii-lapsley' were received as rooted stem cuttings in 72-cell plug trays on 29 Oct. 1997 (Year 1) and 23 Sept. 1998 (Year 2) from the same commercial producer. Upon arrival at Michigan State University, all plants were held in plug trays and exposed to natural day lengths (lat. 43 °N;≈11-12 h) for 2 or 5 days (Year 1 or 2, respectively) until the experiments began. Cold treatments were delivered to plants held in plug trays in coolers set at 5 °C where plants were illuminated for 9 h·day^{-1} with cool-white fluorescent lamps (F96T12/CW/VHO, Philips, Somerset, N.J.) at approximately 10 μmol·m^{2}·s^{-1} and watered as necessary (approximately 2 times per week) using well water acidified with sulfuric acid (H_2SO_4) to a pH of approximately 6.0.

Before placement in the greenhouses (set at 20 °C day/night [D/N] unless otherwise stated), plugs were transplanted into 13-cm square plastic pots (1.1-L volume) with a soilless medium containing sphagnum peat moss, composted pine bark, vermiculite, and perlite (Strong-lite High Porosity Mix, Strong-Lite, Pine Bluff, Ark.). Pots were spaced at approximately 22 plants/m^2. Plants were fertilized at every irrigation with a nutrient solution of well water (EC of 0.70 $mS \cdot cm^{-1}$ and 105, 35, and 23 $mg \cdot L^{-1}$ Ca, Mg, and S, respectively) acidified with H_2SO_4 to a titratable alkalinity of 130 $mg \cdot L^{-1}$ $CaCO_3$ and water soluble fertilizer providing 125-12-125-13 N-P-K-Ca $mg \cdot L^{-1}$ (30% ammoniacal N) plus 1.0-0.5-0.5-0.5-0.1-0.1 $mg \cdot L^{-1}$ (Fe, Mn, Zn, Cu, B, Mo) (MSU Special, Greencare, Chicago, Ill.). The target range was 0.5-1.2 $mS \cdot cm^{-1}$ for electrical conductivity (EC) and 5.8-6.2 for pH. In the second year, it was necessary to drench all plants transplanted after 1 Jan. 1999 twice with a 750 mg $N \cdot L^{-1}$ liquid fertilizer and KCO_3 to raise the pH and EC into the desired ranges.

Cold duration (Expt. 1). Six durations of cold treatment were tested (0, 3, 6, 9, 12, or 15 weeks). Experiments were initiated on 31 Oct. 1997 (Year 1) and 28 Sept. 1998 (Year 2). Initial node counts on aerial shoots were not taken in Year 1 for any experiment. In Year 2, the initial number of nodes was counted at transplant and averaged 18.1. A 16-h photoperiod was provided by four-hundred-watt high-pressure sodium lamps (HPS) switched on at 0800 HR and off at 2400 HR. HPS lamps provided both photoperiod and supplemental lighting with a photosynthetic photon flux *(PPF)* of 50 $\mu mol \cdot m^2 \cdot s^{-1}$ at plant height starting

at 0800 and continuing until the outside *PPF* exceeded 400 μmol·m^{-2}·s^{-1}. If the outside *PPF* then dropped below 200 μmol·m^{-2}·s^{-1} lamps were again turned on until 2400 HR. Ten plants were removed from the cooler after each treatment, transplanted, and placed in the greenhouse.

Photoperiod (Expt. 2). Seven photoperiods (10, 12, 13, 14, 16, and 24 h and a 4-h NI) and two cold treatments (0 or 15 weeks) were tested. Seventy plants were transplanted on 2 Nov. 1997 (Year 1) and 28 Sept. 1998 (Year 2) and placed in a greenhouse. Ten plants were used per treatment and treatments were assigned randomly each year to greenhouse benches. The remaining plants were left in the plug tray and placed in the cooler. After 15 weeks of cold treatment, 70 plants were transplanted on 18 Feb. 1998 (Year 1) and 12 Jan. 1999 (Year 2) and placed in the same photoperiod treatments as the first set of 70 plants. In Year 2, plants averaged 18.0 nodes.

Opaque black cloth was pulled at 1700 HR and opened at 0800 HR every day on all benches so all plants received a similar daily light integral. Continual photoperiods were delivered by day-extension lighting with incandescent lamps that were turned on at 1700 HR and turned off after each photoperiod was completed. The 4-h NI was provided by incandescent lamps that were turned on at 2200 HR and turned off at 0200 HR. Day-extension and NI lighting with incandescent lamps provided 1 to 3 μmol·m^{-2}·s^{-1} at canopy level. Supplemental lighting was provided by HPS lamps (which delivered approximately 50

μmol·m^{2}·s^{-1} at canopy level) between 0800 HR and 1700 HR as described for Expt.1.

Forcing temperature (Expt. 3). On 28 Sept. 1998, plants left in the plug tray were placed directly into the cooler for 15 weeks until 12 Jan. 1999, when they were transplanted. At transplant, plants averaged 19.4 leaves. Ten plants were placed into each of six greenhouses set to 14, 17, 20, 23, 26, or 29 °C. Plants received a continual 16-h photoperiod provided by HPS lamps that were on from 0800 HR to 2400 HR and delivered approximately 90 μmol·m^{2}·s^{-1} at plant height.

Plant growth regulators (Expt. 4). Five commercially available plant growth regulators (PGRs) were tested for their ability to control final plant height of 'Youngii-lapsley'. The PGRs and rates tested included daminozide (butanedioic acid mono-[2,2-dimethyl hydrazide]) at 5000 mg·L^{-1}, paclobutrazol ([2*R*,3*R*+2*S*,3*S*]-1-[4-chlorophenyl-4,4-dimethyl-2-[1,2,4-triazol-1-yl]]) at 30 mg·L^{-1}, chormequat ([2-chloroethyl] trimethylammonium chloride) at 1500 mg·L^{-1}, uniconazole ([*E*]-[*S*]-1-[4-chlorophenyl]-4,4-dimethyl-2-[1,2,4-triazol-1-yl]-pent-1-ene-3-ol) at 15 mg·L^{-1} and ancymidol (α-cyclopropyl-α-[4-methoxyphenyl]-5-pyrimidinemethanol) at 100 mg·L^{-1}. Plants were given a 19-week cold treatment at 5 °C and on 6 Feb. 1999 were transplanted and placed in a greenhouse set at 20 °C. A 16-h photoperiod was provided by HPS lamps, as described for Expt. 1. The daily average temperature between transplant and average date of flowering was 20.6 °C and the average daily light integral was 14.9 mol·m^{2}·day^{-1}. Plants averaged 18.2 nodes at the beginning of the experiment and were

allowed to establish for 10 days before the first PGR spray. Eight plants were sprayed with each of the chemicals until the solution dripped off the leaves (approximately 3 ml·plant^{-1}). Following the first spray, plants were sprayed every 10 days until the first flower opened. In total, there were three applications per PGR. Eight plants were not sprayed with any chemical and served as the control.

Preliminary postharvest evaluation. The 10 plants in the 15-week cold treatment of Expt. 1 (Year 1) were moved to an environmentally controlled chamber on 3 Apr. 1998, approximately 1 week after the first flower opened. The average chamber temperature (measured with a thermometer in a water bath) was 24.6 °C. Plants were given a 24-h photoperiod delivered with both cool-white fluorescent and incandescent lamps at approximately 90 μmol·m^2·s^{-1} (7.8 mol·m^2·day^{-1}), watered as needed (usually every day), and were monitored for 12 days for the number of open, flowers, abscised, and spent flowers. Number of new open flowers was calculated by subtracting the number of open flowers from the previous day from the number of flowers open on the current day and adding the current day's number of spent and abscised flowers.

Data collection and analysis. The data collected for all other experiments (Expts. 1-4) included date of first visible flower bud (without dissection) and date of first open flower. Days to visible bud, days to flower, and days from visible bud to flower were calculated for all experiments. At first open flower, total plant height (excluding the container), number of visible flowers and buds, and number of

final nodes on the main stem were recorded. In Year 1, the number of final nodes was counted to the last visible leaf. In Year 2, the number of final nodes was counted to the last visible leaf and included the initial leaf count, the number of new nodes produced during forcing was calculated by subtracting the initial number of leaves from the final number of leaves, and the number of new nodes beneath the first flowering lateral was calculated by subtracting the number of initial nodes from the number of nodes beneath the first flowering lateral. The number of new nodes beneath the first flowering lateral was calculated only for Expts. 2 and 3. Number of lateral shoots and number of flowering lateral shoots were recorded for Year 2 only. Flower diameter was recorded for Expts. 3 and 4. For all experiments, plants that did not have visible buds after 15 weeks of forcing were considered non-flowering and were discarded. Plants that died during the experiment were discarded and not included in the results or calculations.

All experiments were completely randomized designs. Data were analyzed using the general linear models procedure (PROC GLM)(SAS Institute, Cary, N.C.) for analysis of variance and linear regression procedure (PROC REG) for regression models. Duncan's mean separation test was used to analyze the PGR experiment (Expt. 4) because of the discontinuous nature of the treatments. All other experiments were analyzed with linear and quadratic trend contrasts. The cold duration and photoperiod experiments (Expts. 1 and 2, respectively) were replicated in time, and each experiment was conducted once

per year. The forcing temperature and PGR experiments (Expts. 3 and 4, respectively) were each conducted only once in Year 2.

Greenhouse temperature control and daily light integral collection. Air temperature was monitored in each greenhouse with 36-gauge (0.127-mm-diameter) thermocouples connected to a CR 10 datalogger (Campbell Scientific, Logan, Utah). The datalogger collected temperature data every 10 s and recorded the hourly average. Average daily air temperatures from transplant to average date of flowering were calculated for every treatment in each experiment and those for Expts. 1 and 2 are shown in Tables 1 and 2. Average daily temperatures for Expt. 3 are shown in Table 3. Data for Expt. 4 are recorded above. Temperatures in each greenhouse were controlled by a Priva environmental computer (model CD750; Priva, De Lier, The Netherlands). Actual average daily air temperatures were calculated and used in all analyses.

Average daily light integrals were collected at the same interval as temperatures at canopy level by using a quantum sensor (LI-COR) connected to the CR 10 datalogger. Daily light integrals were only collected for Expt. 1 in Year 2.

Results and Discussion

Cold duration (Expt.1). Without a cold treatment, only one out of 10 plants flowered in Year 1 and no plants flowered in Year 2 (Figure 1A). This plant is the only one that has flowered without cold in any of our experiments (1 out of 180 plants tested) and its flowering behavior was quite different from all other plants

examined. This plant flowered 42 to 54 days slower (Figure 1B), produced 47 to 55 more leaves (data not shown), was 23 to 34 cm shorter (Figure 3D) and produced about 25% the number of flowers that other plants did for Year 1 (Figure 3A). Because flowering without cold treatment was extremely rare, it was considered an anomaly and was not included in the analysis. We conclude that 'Youngii-lapsley' can be classified as having an obligate requirement for vernalization with the acknowledgment that the system is "leaky" and on occasion a few plants will flower without cold treatment.

In the first year, days to visible bud decreased quadratically ($P \leq 0.001$) and days to flower decreased linearly ($P \leq 0.001$) as cold duration increased from 3 to 15 weeks (Figures1B and 1C). In the second year, days to visible bud decreased linearly ($P \leq 0.001$) and days to flower decreased linearly ($P \leq 0.001$) as cold duration increased from 3 to 15 weeks (Figures1B and C). While statistically significant, the decrease in time to flower was slight, 7 to 10 days. While there were significant year ($P \leq 0.01$), cold duration, and year X cold duration interaction ($P \leq 0.001$) effects on days from visible bud to flower, the differences were small (Figure 1D); the largest difference in either year was four.

Flowering of 'Youngii-lapsley' was very uniform. While there was a significant year effect ($P \leq 0.001$) on days to visible bud, days to flower, and days from visible bud to flower ($P \leq 0.05$), these differences were relatively small. For example, excluding the single uncooled plant that flowered in Year 1, the

greatest difference between the average of any of these variables in either year was 7 days; the smallest, zero. Within each cold-duration treatment for either year, all plants flowered within 3 to 9 days.

In either year, there were no significant trends for the number of final nodes measured to the last visible leaf (data not shown). In Year 2, the number of new nodes produced during forcing decreased linearly ($P \leq 0.001$) from 36 to 28 nodes (Figure 2). For time to flower, the slight change in time to flower that occurred with increasing durations of cold treatment was only slightly correlated ($r^2 = 0.37$) with the decrease in the number of new nodes produced during forcing (Year 2).

In Year 1, the number of flowers and buds counted at initial flower opening increased quadratically ($P \leq 0.001$) with increased cold duration (Figure 3A). In Year 2 the trend was also quadratic ($P \leq 0.05$) but flower number was similar for plants cooled for 3 weeks or 15 weeks and highest flower counts were observed on plants cooled for 6 or 12 weeks (Figure 3A). Thrips infested plants cooled for 6 or 9 weeks in Year 2, which could have accounted for some of the decreased number of flowers of plants cooled for nine weeks. Plants cold-treated for 15 weeks in Year 1 were forced during February and March, while plants cooled for 15 weeks in Year 2 were forced during January and February, and so the higher flower number seen in Year 1 could be due to naturally higher light levels during forcing. Increasing light intensity has been shown to increase number of flowers. For *Dendranthema xgrandiflorum* Ramat. grown under a 12-

h day, flower number increased from 7.2 to 19.0 as daily light integral increased from 10.4 to 29.9 $mol \cdot m^{-2} \cdot day^{-1}$ (Warrington and Norton, 1991).

A count of the number of flowering lateral shoots and total (vegetative and reproductive) lateral shoots in Year 2 showed that the former increased linearly ($P \leq 0.01$) with increased durations of cold treatment (Figure 3B) while the latter was similar for all durations of cold treatment (data not shown). The percentage of flowering laterals increased from 69 to 78 as cold increased from 3 to 15 weeks in Year 2 (Figure 3C).

Plants in Year 2 averaged 11 cm shorter over all cold duration treatments than those grown in Year 1 (Figure 3D). In both years there was a significant ($P \leq 0.001$) quadratic trend for the influence of cold duration on final plant height.

Photoperiod (Expt. 2). Without a cold treatment, no plants flowered under any photoperiod in either year. After 15 weeks at 5 °C, all plants flowered under all photoperiods. There was no significant year effect on days to visible bud. However, there was a significant ($P \leq 0.001$) year effect on days to flower as well as a significant ($P \leq 0.001$) year X photoperiod interaction. The differences between days to flower over the two years were small; the greatest difference between the average was six days under the 10-h photoperiod. Days to flower and days to visible bud decreased quadratically ($P \leq 0.001$) by 14 (Year 1) or 19 (Year 2) as photoperiod increased from 10 to 24 h (Figure 4A and C). Under 4-h NI, days to flower were 40.4 in both years. Days to flower under NI were similar

to that of plants grown under photoperiods of 16 h in both years and 24 h in Year 1.

There was a significant ($P \leq 0.001$) year effect as well as a significant ($P \leq 0.001$) year X photoperiod interaction for days from visible bud to flower. In Year 1, days from visible bud to flower decreased linearly ($P \leq 0.001$) with increasing photoperiod (Figure 4B). However, in Year 2, the trend was quadratic ($P \leq 0.001$). Actual differences were relatively small (< 7 days).

From these results, we conclude that 'Youngii-lapsley' is a facultative long-day plant since it flowered under all tested photoperiods, but flowering was accelerated by two weeks under the longer photoperiods. The fact that no plants flowered without cold in either year supports our conclusion that 'Youngii-lapsley' has a nearly obligate vernalization requirement.

The final number of nodes produced under each photoperiod did in general reflect the change in flower timing. In Year 1, the final node number decreased linearly ($P \leq 0.001$) from 38 to 30 under photoperiods $\leq$14 to 24 h, respectively (Figure 5A). However, in Year 2, the change in final node numbers was quadratic ($P \leq 0.05$; Figure 5A). Final number of nodes in Year 2 decreased from 59 to 47 as photoperiod increased from 10 to 24 h. The number of new nodes produced during forcing in Year 2 (Figure 5B) decreased quadratically ($P \leq 0.001$) from 42 to 29 as photoperiod increased from 10 to 24 h. There were no significant trends for the number of new nodes produced beneath

the first flowering lateral (data not shown). Under NI, number of nodes to the final leaf was similar to that of plants grown under 16 or 24 h for either year.

The number of flowers and buds at first flower decreased markedly (quadratically; $P \leq 0.001$) with increasing photoperiod (Figure 6A). For example, in Year 2, the average number of flowers decreased from 129 to 36 as photoperiod increased from 10 to 24 h. There was a significant ($P \leq 0.001$) year effect but the year X photoperiod interaction was not significant. In general, plants grown in Year 2 produced slightly more flowers. The number of flowers appears to be influenced strongly by light levels. For example, plants cooled for 15 weeks and grown under a 16-h photoperiod in Expt. 1 provided by day extension with high-pressure sodium lamps produced 97 flowers while plants in Expt. 2 also cooled for 15 weeks and provided a 16-h photoperiod with day extension from incandescent lamps produced 47 flowers (Year 2). It was estimated that day extension with incandescent lamps reduced the daily light integral by 22 % compared to day extension with high-pressure sodium lamps. This estimation was found by subtracting the amount of light available during daylight hours (0800 to 1700 HR) (as measured by a LI-COR quantum sensor placed on the bench at canopy height) from the amount of light available during the full 16-h photoperiod of day extension with high-pressure sodium lamps, and assuming that incandescent lamps (with an output of 1-3 $\mu mol \cdot m^2 \cdot sec^{-1}$) contribute essentially no light beyond the compensation point.

The number of flowering lateral shoots and total lateral shoots decreased quadratically ($P \leq 0.01$ for the number of flowering lateral shoots; $P \leq 0.05$ for the number of total vegetative shoots) with increasing photoperiod, while the percentage flowering lateral shoots changed little (Figure 6B and C). The change in the number of lateral shoots under the different photoperiods had a marked effect on plant appearance. Plants grown under photoperiods ≤14 h were very leafy and floriferous while those grown under photoperiods ≥14 h appeared spindly with a sparse flower display. Plants grown under NI had as many total and flowering lateral shoots as plants grown under 16 h.

There was a small but significant ($P \leq 0.05$) year and photoperiod ($P \leq 0.001$) effect on final plant height but the year X photoperiod interaction was not significant (Figure 6E). In Year 1, there were no significant trends for the effect of photoperiod on plant height. In Year 2, plant height increased quadratically ($P \leq 0.01$); differences in height were small, increasing from 42 to 48 cm as photoperiod increased from 10 to 24 h. Under NI, height was similar to that of plants grown under 24- and 16- h photoperiods for both years and a 14- h photoperiod for Year 1.

Forcing temperature (Expt. 3). All plants flowered except for one plant in the 14 °C treatment. Actual temperatures for each greenhouse section during different developmental stages are shown in Table 3. Days to visible bud, days from visible bud to flower, and days to flower decreased as temperature increased from ≈15 to ≈30 °C (Figure 7A-C). Increasing temperature from 15.2 to 20.6 °C

accelerated flowering more than increasing it from 20.6 to 29.8 °C. For example, days to flower decreased 24 d from 60 to 36 as temperature increased 5.4 °C from 15.2 to 20.6 °C, but only 11 d from 36 to 25 as temperatures increased 9.2 °C from 20.6 to 29.8 °C. Plants grown at 20.6 °C flowered approximately 5 days faster than plants cooled for 15 weeks in Expt. 1 (Year 2) which were subsequently grown at 20.8 °C (both under a 16-h photoperiod). A possible explanation for this difference is that the plants in Expt. 3 were provided approximately 40 μmol·m^{-2}·s^{-1} more supplemental light than plants in Expt.1. Such an increase in supplemental lighting using high-pressure sodium lamps probably would increase plant temperature, potentially as much as 0.5 °C (Faust and Heins, 1997). If true, then these plants would be expected to develop more quickly, since temperature strongly influences plant development.

The number of final nodes were not significant when counted to the last visible leaf. However, when the new nodes produced during forcing were counted, there was a significant ($P \leq 0.001$) linear trend (data not shown). The number of new nodes decreased from 36 to 24 as temperature increased from 15.2 to 29.8 °C.

Flowering of 'Youngii-lapsley' was very uniform in this experiment, although variability increased as temperature decreased. All plants grown at 29.8 °C flowered within 3 days of each other; at 15.2 °C, within 8 days.

There were significant linear relationships between temperature and rate of progress toward visible bud, from visible bud to flowering, and flowering

(Figure 7D-F). The parameters for the equations from the linear regression analysis are given in Table 4. The reciprocal of the linear regression function described the relationship between temperature and developmental rate well (Figure 7A-C). Base temperatures for each developmental stage ranged from 1.5 to 7.3 °C (Table 4). The degree-days to flower were 606 when days-to-flower data were used alone and 619 when data for days from transplant to visible bud and days from visible bud to flower were added. The concepts of degree-days and base temperature (T_b) are useful in commercial greenhouse production because they allow prediction of flowering time under fluctuating temperature conditions when average temperature is known (Roberts and Summerfield, 1987). By using the average daily temperature (T_a), the days required to complete a developmental stage can be calculated as °days/(T_a - T_b). Differences between predicted and observed days for any developmental stage and temperature were no greater than 5 (data not shown).

The number of flowers decreased ≈84% as temperature increased from 15.2 to 29.8 °C (Figure 8A). The relationship between number of flowers and temperature was linear ($P < 0.001$). Flower diameter decreased from 7.0 to 4.0 cm as temperature increased, which corresponds to a threefold difference in flower area (assuming that flower shape is circular) (Figure 8B). There are similar effects of temperature on flower size of other herbaceous perennials such as *Coreopsis grandiflora (*Hogg ex Sweet.) 'Sunray', *Leucanthemum* x*superbum* (Bergman ex J. Ingram) 'Snowcap', *Rudbeckia fulgida* (Ait.) 'Goldsturm' (Yuan

et al., 1998), *Campanula carpatica* 'Blue Clips' (Whitman et al., 1997), *Coreopsis verticillata* L. 'Moonbeam', *Campanula* 'Birch Hybrid' and others (Frane, 1999).

Height decreased 35% in a quadratic fashion ($P < 0.001$) as temperature increased from 15.2 to 23.8 °C (Figure 8C). As forcing temperature increased from 23.8 to 29.8 °C, there was almost no change in plant height.

Plant growth regulators (Expt.4). There were no statistical differences among the growth regulators or the control for days to visible bud, number of new leaves, or number of flowers (data not shown). Days to flower ranged from 39 to 41 (Figure 9A). Plants sprayed with ancymidol, paclobutrazol, or chlormequat flowered approximately one day earlier than the control. Days to flower were similar for control plants and plants sprayed with daminozide or uniconazole. Days from visible bud to flower were approximately 20 for non-sprayed plants and plants sprayed with daminozide, uniconazole, or chlormequat and 19 for plants sprayed with ancymidol or paclobutrazol (data not shown).

Final plant height was reduced 31% for plants sprayed with uniconazole compared to that of the control plants (Figure 9B). No other PGR had a significant effect on final plant height although higher rates of daminozide and paclobutrazol may have reduced height significantly. Uniconazole reduced flower diameter 36% compared to that of control plants (Fig 9C). The flowers on plants sprayed with uniconazole were noticeably smaller: the 36% decrease in diameter resulted in a 58% decrease in flower area. Flower diameters were similar for all plants treated with one of the other PGRs and for the control.

While daminozide and chlormequat did not reduce final plant height, plants sprayed with these PGRs as well as uniconazole had shorter lateral stems compared to that of control plants (personal observation). Shorter lateral stems changed the plants from their natural conical shape to cylindrical.

If a standard height/diameter ratio of 1.5 to 2 (height = pot plus plant; diameter = diameter at top of plant or at greatest width) suggested for a high-quality potted plant (Sachs et al., 1976) was used, spraying uniconazole at 15 $mg \cdot L^{-1}$ was only slightly successful for reducing height. The 30-cm height achieved for plants sprayed with uniconazole resulted in a height/diameter ratio of approximately 2.1 (pot height = 11 cm and diameter was approximated at 1.5 X width of pot [13 cm]). Control plants had a height/diameter ratio of 2.7. A disadvantage of using this PGR and rate is the large reduction in flower size. Further studies using different rates of uniconazole and perhaps other PGRs individually or in combination are needed to identify PGRs and rates that effectively control height without such a drastic reduction in flower size and change in plant shape.

Preliminary postharvest evaluation. During 12 days of postharvest evaluation, 'Youngii-lapsley' maintained a good floral display. The number of open flowers reached a maximum of 53 on day 5 (Figure 10). After day 5, the number of open flowers decreased to an average of 17 on day 12. At the end of the study, plants were in adequate condition (personal observation). In another postharvest evaluation using plants of 'Youngii-lapsley' cooled for 6 weeks,

plants had a maximum of 26 open flowers and maintained at least 20 open flowers for the first ten days of the study (data not shown), only a few more flowers than plants had at the end of the 12-day study. New flowers continued to open under low light, although at a decreasing rate (Figure 10). The number of spent flowers ranged from 4 to 21 but was relatively constant during the 12 days of the study, averaging approximately 10 per day. Over 30 flowers per plant abscised on day two; from day three onward, there were never fewer than 10 abscised flowers per plant each day. The relatively high number of flowers abscised could decrease the potted plant potential of 'Youngii-lapsley' since they are unattractive and need to be removed during marketing and home display.

Summary

On the basis of these results, *O. fruticosa* 'Youngii-lapsley' can be classified as a facultative long-day plant with an obligate vernalization requirement. This species of *Oenothera* is the first that we know of with this combination of vernalization and photoperiod requirements.

Flowering occurred consistently after a minimum cold treatment of 3 weeks followed by any photoperiod $\geq$10 h. Increasing the duration of cold treatment up to 15 weeks resulted in slightly faster flowering and more flowering lateral shoots. Although time to flower is approximately 2 weeks faster under a 16-h or longer photoperiod than a 10-h photoperiod, flower number decreased and height increased as photoperiod increased from 10 to 24 h.

Plants were most attractive when grown under photoperiods $\leq$14 h

because of the high numbers of vegetative and flowering lateral shoots and thus were better suited as potted plants than those grown under longer photoperiods. Since this is a plant that responds strongly to light levels, as seen when the flower number doubled when the daily light integral doubled (Expt. 1 and 2, Year 2), supplemental lighting is necessary to produce high-quality potted plants during winter.

Forcing temperatures between 17 and 20 °C are recommended for forcing plants to flower within 5-6 weeks from the start of transplant. However, since there is an increase in the number of flowers with a decrease in temperature, temperatures $\leq$20 °C would be best for potted plant production. Uniconazole at 15 mg·L^{-1} can be used to reduce final plant height $\approx$30% but also will decrease flower diameter by 36%. In preliminary postharvest trials, 'Youngii-lapsley' performed well indoors. A good floral display was maintained for the 12 days of the trial.

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Table 1. Average daily air temperatures and daily light integrals from date of transplant to average date of flowering for *Oenothera fruticosa* 'Youngii-lapsley' after each duration of cold treatment

Year	Cold duration (weeks at 5 °C)					
	0	3	6	9	12	15
	Average air temperatures during forcing (°C)					
1997-1998	19.9	20.0	19.7	19.7	19.9	20.2
1998-1999	-[a]	21.1	20.9	20.1	20.3	20.8
	Average daily light integral ($mol \cdot m^{-2} \cdot day^{-1}$)					
1997-1998	-	--[b]	--	--	--	--
1998-1999	-	12.6	11.2	10.6	10.6	11.6

[a] No plants flowered (one dash).
[b] Measurement not taken (two dashes).

Table 2. Average daily air temperatures from date of transplant to average date of flowering for *Oenothera fruticosa* 'Youngii-lapsley' under each photoperiod

Year	Weeks at 5 °C	Photoperiod (h)						
		10	12	13	14	16	24	NI[z]
		Average air temperature during forcing (°C)						
1997-98	0	-[y]	-	-	-	-	-	-
	15	21.1	20.8	20.6	21.9	21.8	21.1	21.1
1998-99	0	-	-	-	-	-	-	-
	15	21.0	20.8	20.7	20.9	20.6	21.1	21.2

[z] 9-h photoperiod plus 4-h night interruption (NI).
[y] No plants flowered.

Table 3. Actual temperatures of houses for the time of forcing for three developmental stages: transplant to visible bud, visible bud to flower, and transplant to flower.

Temperature setting (°C)	Developmental stage (days)		
	Transplant to visible bud	Visible bud to flower	Transplant to flower
14	15.4	15.0	15.2
17	18.2	18.3	18.2
20	20.5	20.7	20.6
23	23.7	23.8	23.8
26	26.7	27.1	26.8
29	29.6	29.9	29.8

Table 4. Parameters of linear regression analysis relating forcing temperature to rate of progress from transplant to visible bud (VB), visible bud to flowering (VB to FLW) and transplant to flowering (FLW) for *Oenothera fruticosa* 'Youngii-lapsley'; 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_1) (1/d)/C	T_b(°C)	°days	r^2
Transplant to VB	-3.56E-3 ± 7.99E-3[z]	2.45E-3 ± 3.49E-4	1.5	408	0.91**
VB to FLW	-3.42E-2 ± 1.56E-3	4.72E-3 ± 6.77E-5	7.3	211	0.99***
Transplant to FLW	-7.21E-3 ± 2.75E-3	1.65E-3 ± 1.20E-4	4.4	606	0.97***

[z] Standard error.

,*Significant at $P < 0.01$, or 0.001, respectively.

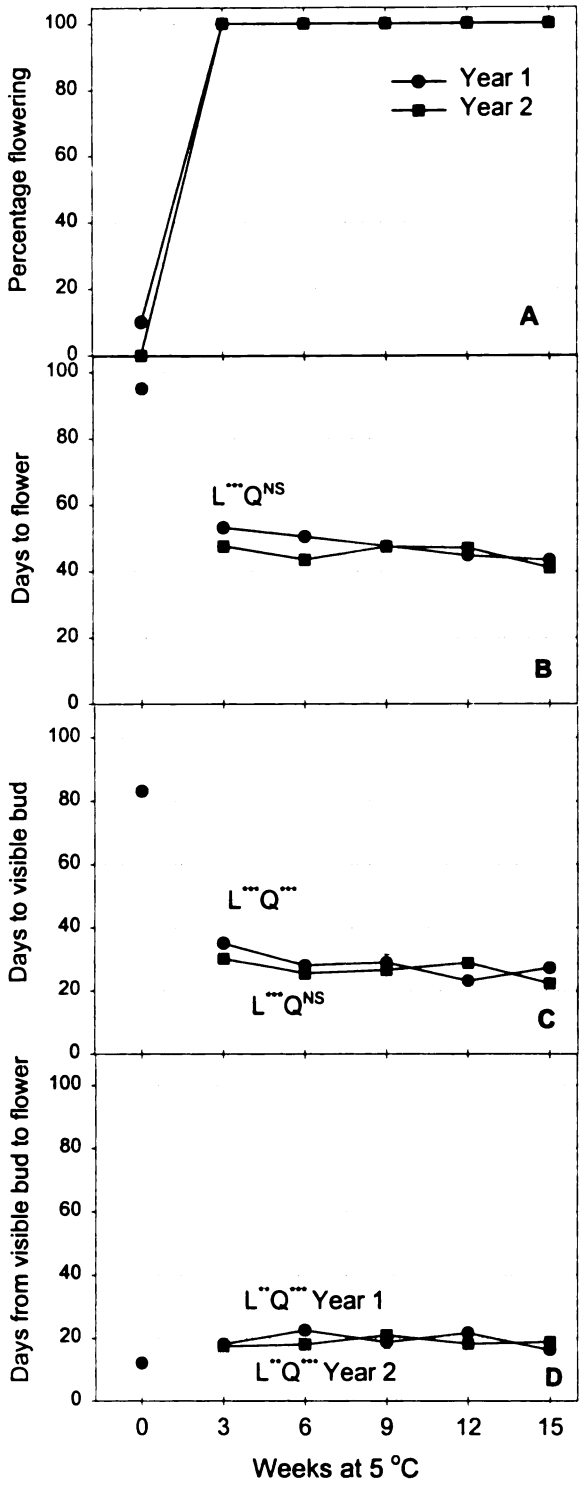

Figure 1. Responses of *Oenothera fruticosa* 'Youngii-lapsley' to various durations of cold treatment at 5 °C for percentage flowering (A), days to flower (B), days to visible bud (C), and days from visible bud to flower (D) in Year 1 (●) or Year 2 (■). Error bars represent 95% confidence intervals. All symbols represent the mean of 9 or 10 plants except for Year 1, 0 week cold treatment, when only one plant flowered. L = linear; Q = quadratic trends. ^{NS,**,***}Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

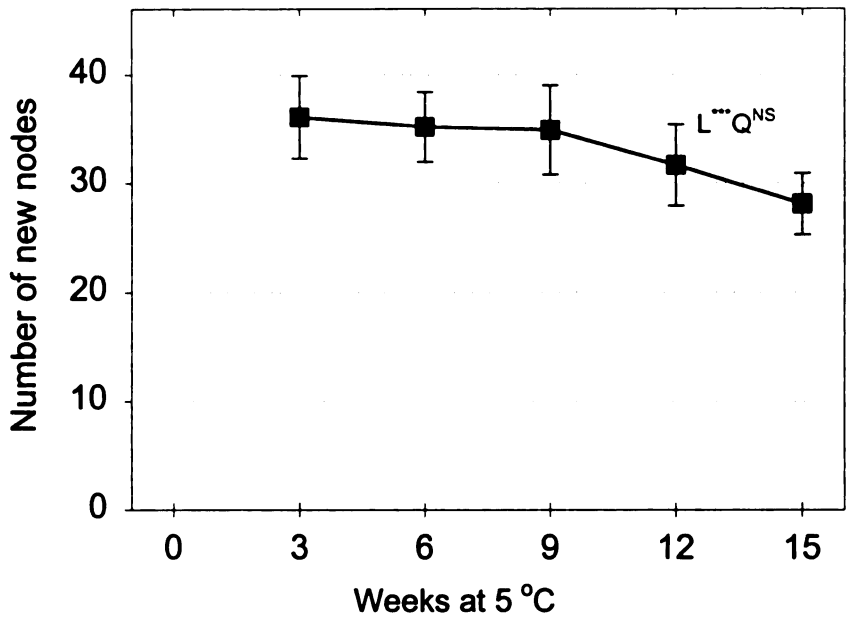

Figure 2. Effect of cold duration at 5 °C on number of new nodes produced during forcing in Year 2. Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

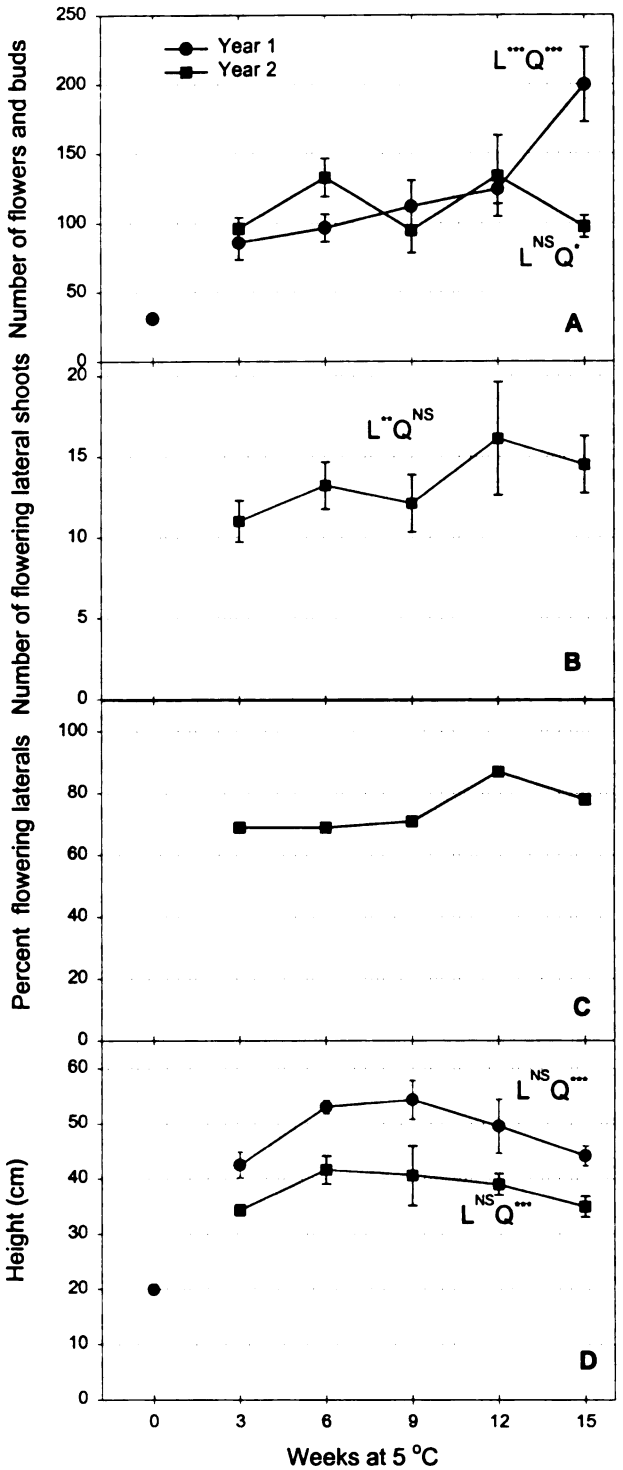

Figure 3. Effects of cold duration at 5 °C on number of flowers and buds (A), number of flowering lateral shoots (B), percent flowering lateral shoots (C), and plant height (D) for *Oenothera fruticosa* 'Youngii-lapsley' in Year 1 (●) or Year 2 (■). All symbols represent the mean of 9 or 10 plants except for Year 1, 0 week cold treatment, when only one plant flowered. Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

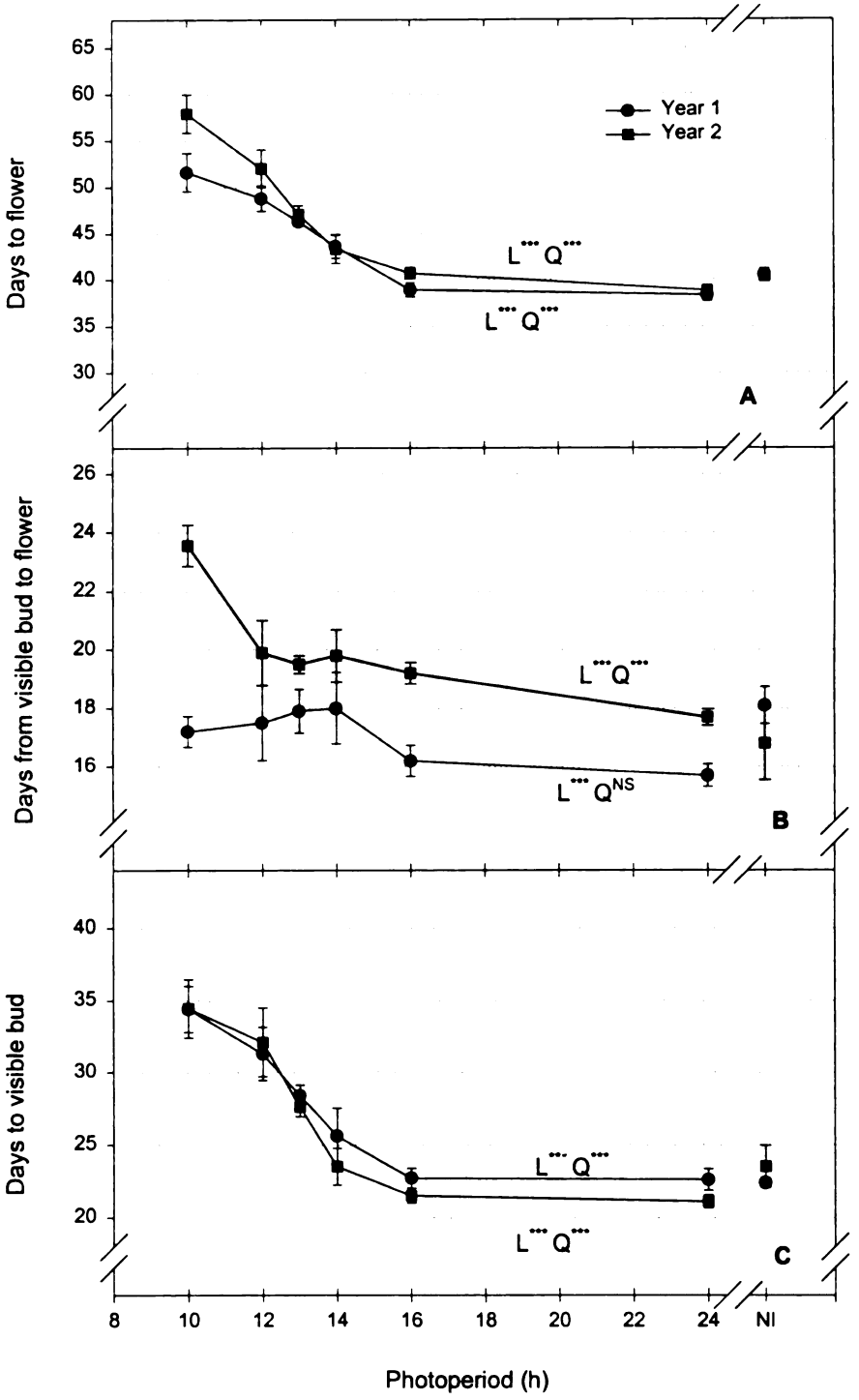

Figure 4. Responses of *Oenothera fruticosa* 'Youngii-lapsley' to various photoperiods after 15 weeks of cold treatment at 5 °C. Continual photoperiod treatments consisted of 9-h natural days extended with light from incandescent lamps. NI = 4-h night interruption. Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

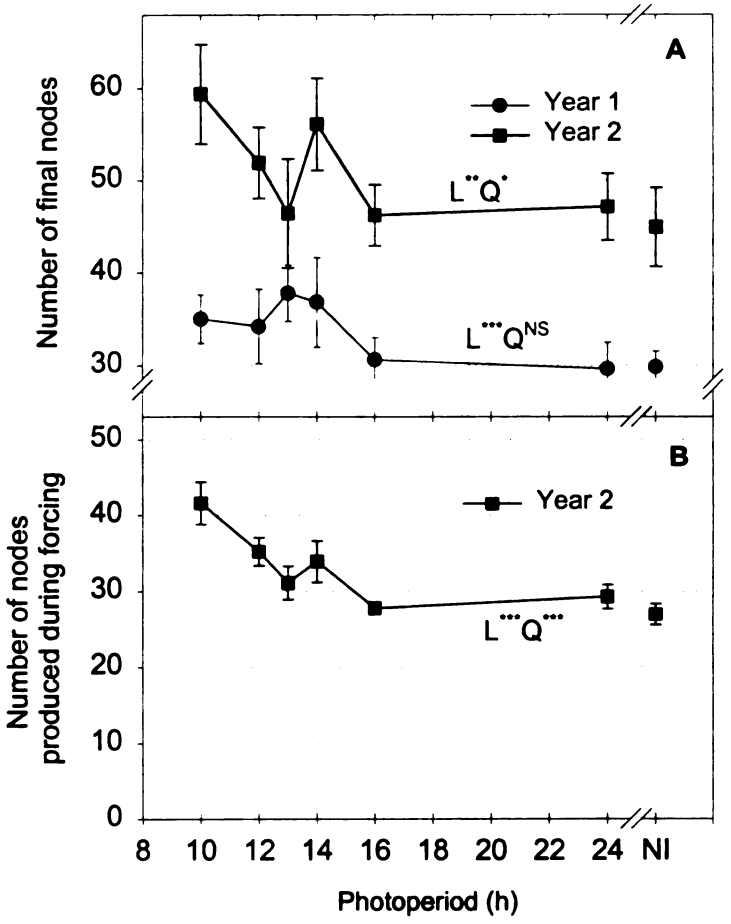

Figure 5. Effects of various photoperiods after 15 weeks at 5 °C on number of final nodes (A) and number of nodes produced during forcing (B) for *Oenothera fruticosa* 'Youngii-lapsley' in Year 1 (●) or Year 2 (■). Continual photoperiod treatments consisted of 9-h natural days extended with light from incandescent lamps. NI = 4-h night interruption. Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

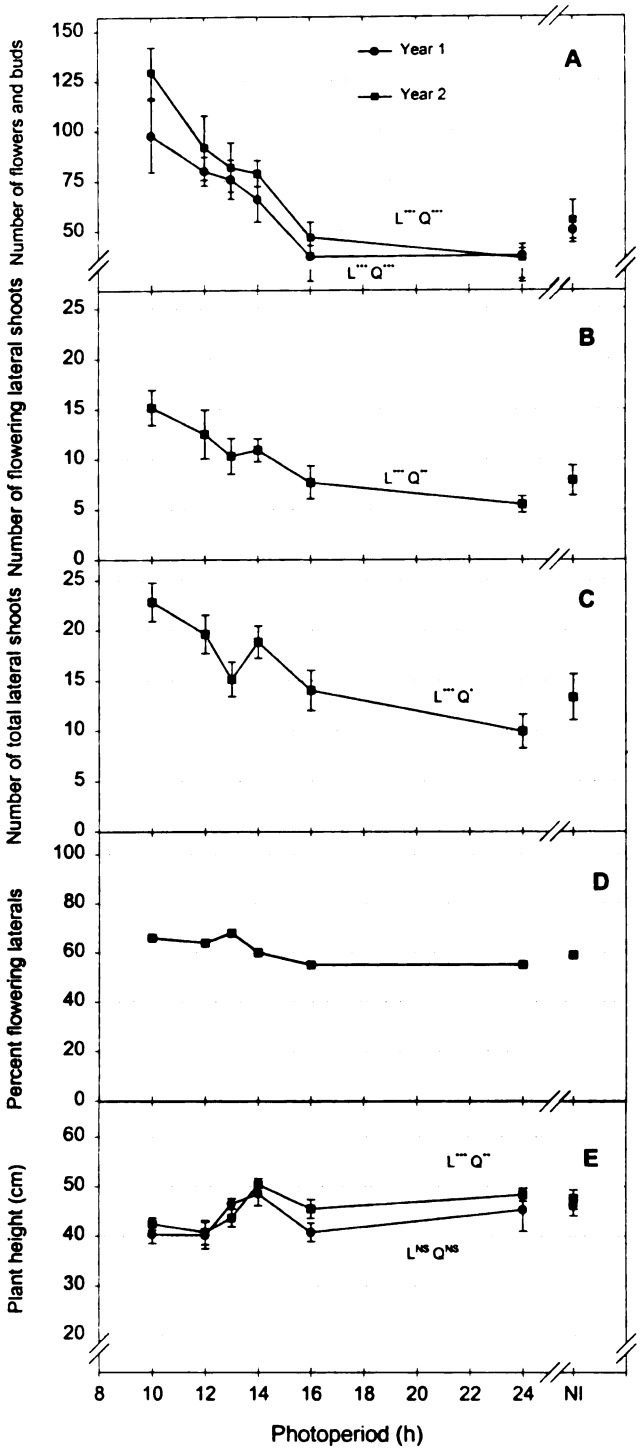

Figure 6. Effects of various photoperiods after 15 weeks at 5 °C on number of flowers and buds (A), number of flowering lateral shoots (B), number of total lateral shoots (C), percent flowering laterals (D), and plant height (E) for *Oenothera fruticosa* 'Youngii-lapsley' in Year 1 (●) or Year 2 (■). Continual photoperiod treatments consisted of 9-h natural days extended with light from incandescent lamps. NI = 4-h night interruption. Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***} Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

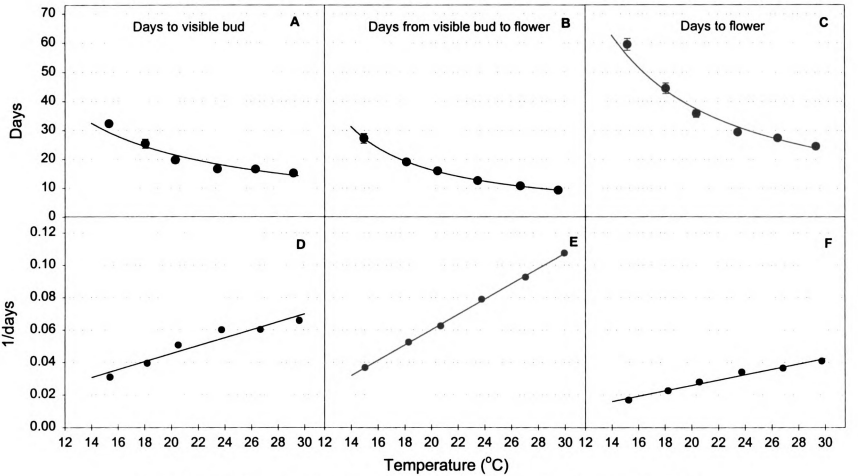

Figure 7. Effects of temperature on time to (A, B, C) and rate of progress toward flowering (D, E, F) in *Oenothera fruticosa* 'Youngii-lapsley'. The parameters of linear regression lines are presented in Table 4. The quadratic regression lines in graphs A, B, and C are the reciprocals of correlated linear regression lines in graphs D, E, and F.

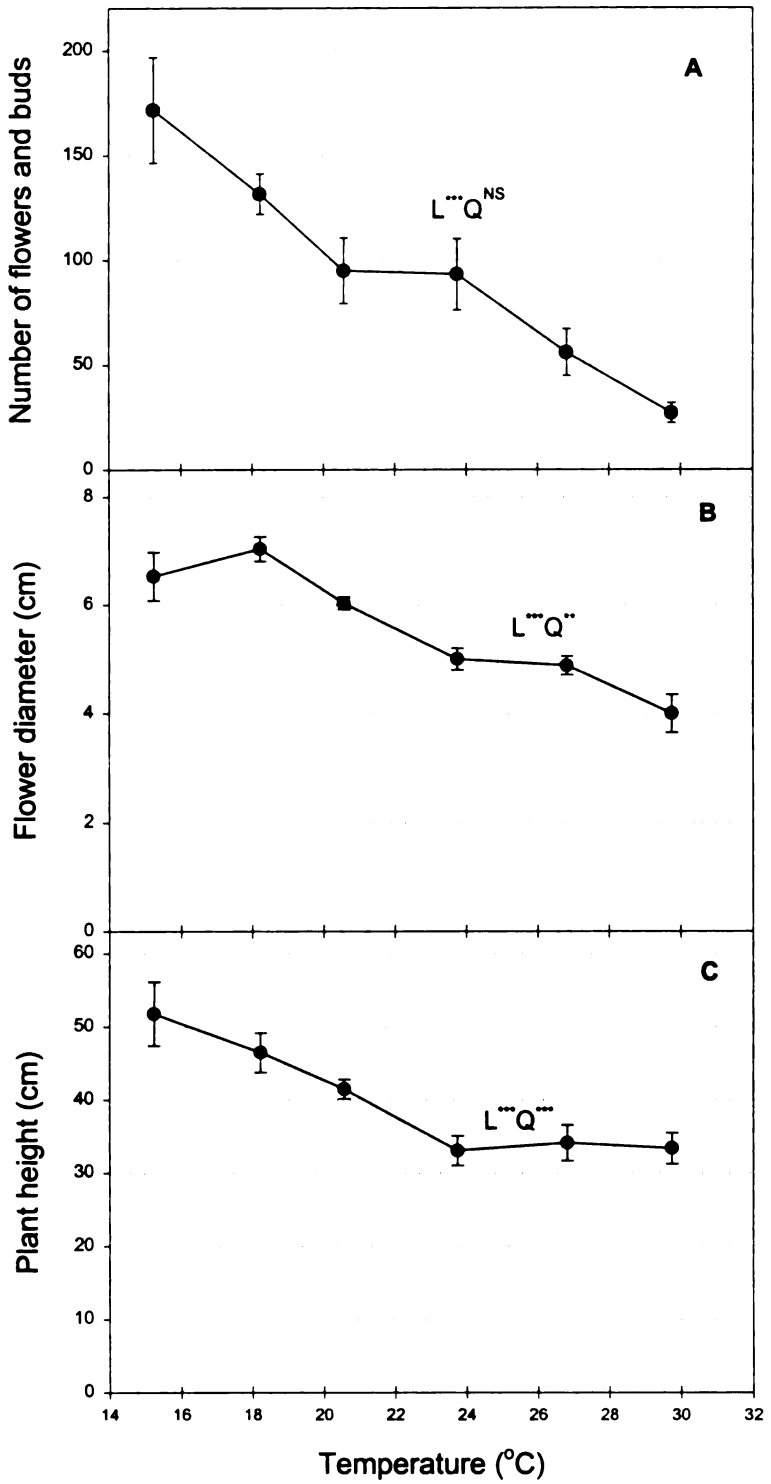

Figure 8. Effects of temperature on numbers of flowers and buds (A), flower diameter (B), and plant height (C) for *Oenothera fruticosa* 'Youngii-lapsley'. Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,**,***}Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

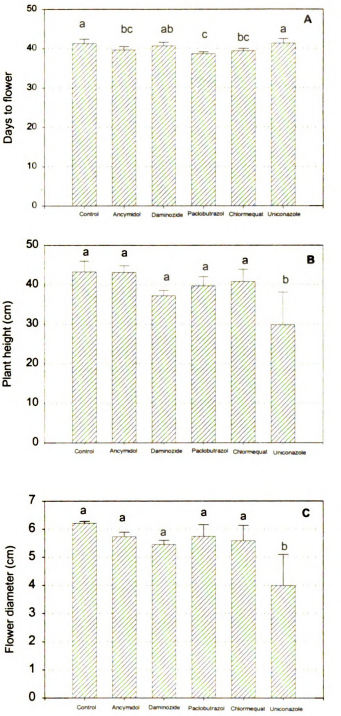

Figure 9. Effects of various plant growth regulators on days to flower (A), plant height (B), and flower diameter (C) for *Oenothera fruticosa* 'Youngii-lapsley'. Rates of PGRs were: Ancymidol (100 mg·L^{-1}), Daminozide (5000 mg·L^{-1}), Paclobutrazol (30 mg·L^{-1}), Chlormequat (1500 mg·L^{-1}) and Uniconazole (15 mg·L^{-1}). Error bars represent 95% confidence intervals.

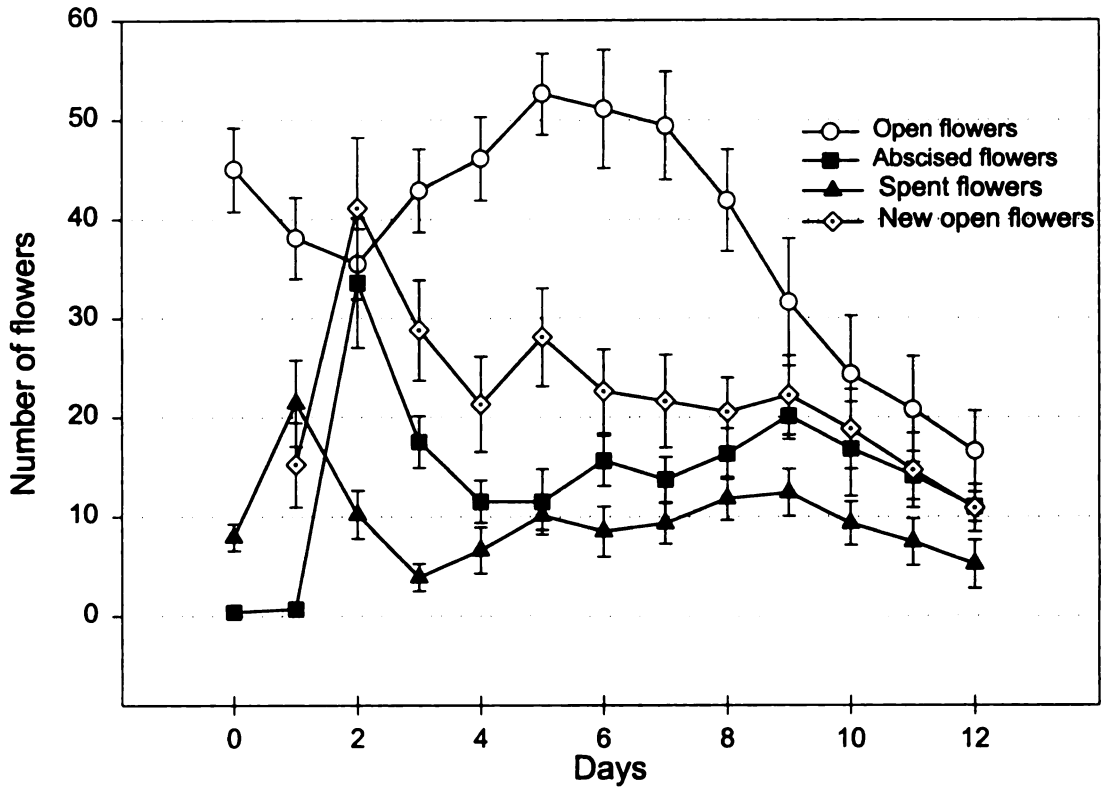

Figure 10. Postharvest responses of *Oenothera fruticosa* 'Youngii-lapsley' for 12 days at 24.6 °C under a 24-h photoperiod. Error bars represent 95% confidence intervals.

Flowering Characteristics of Several Cultivars of *Oenothera fruticosa* under Two Different Lamp Types for Daylength Extension

Abstract

Flowering characteristics of *Oenothera fruticosa* L. 'Fireworks', 'Highlight', 'Summer Solstice', 'Youngii-lapsley,' and ssp. *glauca* were determined. Two nursery sources were used for ssp. *glauca* and are indicated by a label ("A" or "B"). All plants were cooled for 15 weeks at 5 °C and subsequently grown under a 16-h photoperiod provided by either high-pressure sodium (HPS) or incandescent (INC) lamps to deliver daylength extension in 20 °C greenhouses. Light quantity was increased approximately 27% when day extension was delivered by HPS lamps rather than INC lamps. Data collected included days to flower, number of flowers and buds at first flower, number of nodes on the main stem and final plant height. Lamp type had little to no influence on days to flower. Day extension with HPS rather than INC lamps increased flower number approximately two times for 'Youngii-lapsley' and ssp. *glauca* A, three times for ssp. *glauca* B, and 3.5 times for 'Highlight'. Day extension with INC lamps increased plant height at flower in ssp. *glauca* B and 'Youngii-lapsley'. *Oenothera fruticosa* ssp. *glauca* shows particular promise as a potted flowering plant.

Introduction

Oenothera fruticosa L. 'Youngii-lapsley' is a facultative long-day plant with an obligate vernalization requirement and potential as a flowering potted plant (Frane, 1999; chapter 2). Several other choice selections from this species include *O. fruticosa* 'Fireworks', *O. fruticosa* 'Highlight', *O. fruticosa* 'Summer Solstice' and *O. fruticosa* ssp. *glauca*.

Because 'Youngii-lapsley' manifests flowering potted plant potential we were interested in investigating that of other *O. fruticosa* cultivars and *O. fruticosa* ssp. *glauca*.

Different lamp types used for day extension profoundly affect flowering characteristics of *O. fruticosa* 'Youngii-lapsley'. However, a 16-h photoperiod provided by day extension lighting with incandescent (INC) lamps reduced the daily light integral approximately 22% compared to day extension lighting with high-pressure sodium (HPS) lamps. 'Youngii-lapsley' plants grown under the HPS lamps produced two times the number of flowers produced on plants grown under the INC lamps. Warrington and Norton (1991) found that increasing the daily light integral increased the rate of floral development and number of flower buds in *Dendranthema xgrandiflorum* (Ramat.) Kitamura, *Raphanus sativus* L., *Zea mays* L., and *Cucumis sativus* L.

The two light types differ in photosynthetic photon flux *(PPF)* and in spectral quality. Incandescent lamps emit a mixture of red and far-red light that is richer in far-red than red (ratio of red 600-700 nm to far-red 700-800 nm <1

[Lane et al., 1965]). High- pressure sodium lamps emit a mixture also but with much more red than far-red light (Thimijan and Heins, 1983). For *Campanula carpatica* Jacq. 'Blue Clips', *Coreopsis grandiflora* Hogg ex Sweet 'Early Sunrise', *and Coreopsis verticillata* L. 'Moonbeam', time to flower did not differ for plants grown under a 16-h day above the irradiance saturation point, with day-extension delivered by HPS, INC, metal halide, or cool-white fluorescent lamps (Whitman et al.,1998). However, the different lamp types did influence final plant height. *Campanula carpatica* 'Blue Clips' and *C. grandiflora* 'Early Sunrise' had significantly longer stems when grown under INC lamps compared to HPS, cool-white fluorescent, or metal halide.

The objectives of this study were to determine (1) flowering characteristics (e.g., time to flower, flower number, final plant height) and (2) determine the effect of using different lamp types for day-extension lighting for *O. fruticosa* 'Fireworks', 'Highlight' 'Summer Solstice', 'Youngii-lapsley', and ssp. *glauca*.

Materials and Methods

Plant material. Plants were obtained from various commercial producers on different dates between 28 Aug. 1998 and 28 Oct. 1998. The dates of arrival at Michigan State University, cold treatment initiation and forcing, average daily temperature, average daily light integral from transplant to average day of flowering, and form of starting plant material for each cultivar are shown in Table 5. We used two nursery sources for *O. fruticosa* ssp. *glauca*; one source labeled the plants as *O. fruticosa* ssp. *glauca*, and the other labeled them as *O.*

tetragona. According to Griffiths (1994), *O. tetragona* is the same as *O. fruticosa*. In the horticultural industry, many *Oenothera* cultivars including 'Fireworks', 'Highlight', and 'Summer Solstice' are labeled as *O. tetragona* but are actually cultivars of *O. fruticosa* (Griffiths, 1994). The flowering behavior and growth habit of the two sets of *O. fruticosa* ssp. *glauca* plants were quite different and for clarity, we have labeled them *O. fruticosa* ssp. *glauca* A and *O. fruticosa* ssp. *glauca* B, the latter referring to the plants received as *O. tetragona*.

Upon arrival at MSU, bare-root plants of 'Fireworks' and 'Summer Solstice' were placed into bulb trays in which the root masses were covered with soilless medium and put immediately in the cooler. *O. fruticosa* 'Highlight' were received as very small field-grown divisions and allowed to grow in 13-cm (1.1-L) pots under natural daylengths (lat. 43 °N) for 15 days at 20 °C before transfer to the cooler. To control an existing *Botrytis sp.* infestation, *O. fruticosa* ssp. *glauca* A were held for 15 days in the 2.5-inch pots in which they arrived before being transferred to the cooler. 'Youngii-lapsley' and ssp. *glauca* B were held in plug trays under natural daylengths for five or six days, respectively.

Cold treatments. Cold treatments were delivered to plants held in plug trays, bulb trays in which the root masses were covered in soilless medium (Stronglite High Porosity Mix, Stronglite, Pine Bluff, Ark.), or 13-cm pots ('Highlight' only) in coolers set at 5 °C. Within the coolers, plants were illuminated for 9 $h \cdot day^{-1}$ with cool-white fluorescent lamps (F96T12/CW/VHO, Philips, Somerset, N.J.) at approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and watered as necessary using well water acidified

with sulfuric acid (H_2SO_4) to a pH of approximately 6.0. All plants were given a 15-week cold treatment at 5 °C and subsequently grown in greenhouses set at 20 °C (day/night [D/N]).

Plant culture. Before placement in the greenhouses, plugs or bare-root plants were transplanted into 13-cm square plastic pots (1.1-L volume) with a soilless medium containing sphagnum peat moss, composted pine bark, vermiculite, and perlite (Strong-lite High Porosity Mix, Strong-Lite, Pine Bluff, Ark.). Since 'Highlight' already was transplanted into the pots, they were transferred from the cooler to the greenhouse. Pots were spaced at approximately 22 plants/m^2. Plants were fertilized at every irrigation with a nutrient solution of well water (EC of 0.70 $mS \cdot cm^{-1}$ and 105, 35, and 23 $mg \cdot L^{-1}$ Ca, Mg, and S, respectively) acidified with H_2SO_4 to a titratable alkalinity of 130 $mg \cdot L^{-1}$ $CaCO_3$ and water soluble fertilizer providing 125-12-125-13 N-P-K-Ca $mg \cdot L^{-1}$ (30% ammoniacal N) plus 1.0-0.5-0.5-0.5-0.1-0.1 $mg \cdot L^{-1}$ (Fe, Mn, Zn, Cu, B, Mo) (MSU Special, Greencare, Chicago, Ill.). The target range was 0.5-1.2 $mS \cdot cm^{-1}$ for electrical conductivity (EC) and 5.8-6.2 for pH. In the second year, it was necessary to drench all plants transplanted after 1 Jan. 1999 twice with a 750 mg $N \cdot L^{-1}$ liquid fertilizer and KCO_3 to raise the pH and EC into the desired ranges.

Photoperiod and lamp types. All plants were provided a 16-h photoperiod delivered by continual day-extension lighting with either HPS or INC lamps. Day-extension lighting with INC lamps (which provided approximately 1-3 $\mu mol \cdot m^2 \cdot s^{-1}$ at canopy level) were delivered under opaque blackcloth pulled at 1700 HR and

opened at 0800 HR every day. The INC lamps were turned on at 1700 HR and turned off at 2400 HR. Day-extension lighting with HPS lamps (which provided approximately 50 μmol·m^{-2}·s^{-1}) turned off at 2400 HR and then turned on the following day at 0800 HR, fluctuating on and off during daylight hours using the following regimen: at 0800, HPS lamps turned on and continued until the outside *PPF* exceeded 400 μmol·m^{-2}·s^{-1}. If the outside *PPF* then dropped below 200 μmol·m^{-2}·s^{-1} lamps were again turned on until 2400 HR. Plants in the treatment in which daylength was extended with INC lamps received supplemental lighting from HPS lamps in the same fashion during the hours between 0800 and 1700 HR.

Data collected included date of visible bud (without dissection) and first open flower. Days to visible bud and flower and visible bud to flower were calculated for all experiments. At first open flower, total plant height (excluding the container), number of visible flowers and buds, and number of final nodes on the main stem were recorded.

Greenhouse temperature control and daily light integral collection. In each greenhouse air temperature was monitored with 36-gauge (0.127-mm-diameter) thermocouples connected to a CR10 datalogger (Campbell Scientific, Logan, Utah). The datalogger collected temperature data every 10 s and recorded the hourly average. Temperatures in each greenhouse were controlled by a Priva environmental computer (model CD750; Priva, De Lier, The Netherlands). The average daily light integrals were recorded at canopy level using quantum

sensors (LI-COR) connected to the CR10 datalogger. Average daily air temperatures from transplant to average date of flowering were calculated for every treatment in each experiment. Average daily light integral data from transplant to average date of flowering were collected only for plants under day-extension with HPS lamps. It was estimated that day extension with INC lamps reduced the daily light integral 22 % compared to high-pressure sodium lamps. This estimation was found by subtracting the amount of light available during daylight hours (0800 to 1700 HR) (as measured by a LI-COR quantum sensor placed on the bench at canopy height) from the amount of light available during the full 16-h photoperiod provided by day-extension with HPS lamps, and assuming that INC lamps (with an output of 1-3 $\mu mol \cdot m^2 \cdot sec^{-1}$) contribute essentially no light beyond the compensation point.

Data analysis. The experiment was a completely randomized design. Data were analyzed using the general linear models procedure (PROC GLM)(SAS Institute, Cary, N.C.) for analysis of variance.

Results and Discussion

All plants flowered under each lamp type used for day extension. All cultivars flowered within a relatively small range of days (41 to 51) (Table 6). However, final plant height ranged between 24 and 45 cm and number of flowers produced between 12 and 502. Plant appearance differed markedly between cultivars grown under different lamp types (Figure 11). There were no significant effects of lamp type on final node number for any cultivar.

The influence of lamp type on flowering time was not great for any cultivar. Time to visible bud was one and two days faster under INC lamps for 'Youngii-lapsley' and 'Highlight', respectively (data not shown). Time from visible bud to flower was two or seven days faster under INC lamps for 'Fireworks' or ssp. *glauca* A, respectively, and two days faster under HPS lamps for 'Summer Solstice'. Lamp-type influence on days to visible bud and days from visible bud to flower was not significant for all other cultivars. For 'Summer Solstice', 'Fireworks', and ssp. *glauca* A, time to flower was three, four, and six days slower, respectively, for plants grown under HPS lamps rather than INC lamps (Table 2). Time to flower was one or two days faster for plants grown under HPS day-extension lighting compared to that under INC lamps for 'Highlight' or ssp. *glauca* B, respectively. Lamp type had no significant effect on days to flower for 'Youngii-lapsley'. It is surprising that the extra light provided by day extension with HPS lamps did not result in consistently faster flowering times for all cultivars. Increasing *PPF* has been shown to decrease time to flower for many species, including *Raphanus sativus* L., and *Zea mays* L. (Warrington and Norton, 1991) and is likely due to an increase in plant temperature observed with an increase in *PPF* (Faust and Heins, 1997). When *Campanula carpatica* 'Blue Clips', *Coreopsis grandiflora* 'Early Sunrise', and *Coreopsis verticillata* 'Moonbeam' were grown under HPS, INC, metal halide, and cool-white fluorescent lamps at similar photosynthetic photon fluxes, lamp type did not influence time to flower (Whitman et al., 1998).

Lamp type did not influence plant height on any cultivar except that of ssp. *glauca* B and 'Youngii-lapsley' under incandescent lamps, it increased 3 or 10 cm, respectively, compared to that when HPS lamps were used. Whitman et al. (1998) observed that plant height was increased significantly for *Campanula carpatica* 'Blue Clips' and *Coreopsis grandiflora* 'Early Sunrise' when INC lamps rather than when HPS, metal halide, or cool-white fluorescent lamps were used for day-extension lighting.

High-pressure sodium lamps used as day-extension lighting increased flower number 2, 2, 3, and 3.5 times compared to that under INC lamps for , 'Youngii-lapsley', *O. fruticosa* ssp. *glauca* A, *O. fruticosa* ssp. *glauca* B, and 'Highlight' respectively. Both light quantity and quality have been shown to influence flower number of various species. For example, Warrington and Norton (1991) found that for *Dendranthema* x*grandiflorum* (Ramat.) Kitamura, grown under a 12-h day, flower number increased from 7.2 to 19.0 as daily light integral increased from 10.4 to 29.9 $mol \cdot m^2 \cdot day^{-1}$. *Rosa* x*hybrida* 'Samantha' plants produced 27-71% more flowers when grown with supplemental light from HPS lamps (at a *PPF* of 70 to 75 $\mu mol \cdot m^2 \cdot s^{-1}$ one meter above of the pots at night) compared to those grown under HPS lamps fitted with a blue gel filter which reduced the R : FR ratio from 1:0.95 to 1:2 (Roberts et al., 1993). It is interesting to note that two cultivars ('Fireworks' and 'Summer Solstice') showed no effect of lamp type on flower number.

Although they were received labeled as the same subspecies, *O. fruticosa* ssp. *glauca* A and B behaved very differently when grown similarly and it was not obvious that they were the same species upon inspection. Plants labeled "B" flowered two to 10 days faster, were 8 or 9 cm shorter, and produced ≈1.5 to 2 times more flowers than those labeled "B". Plants labeled "B" were much more uniform than "A", which was expected since ssp. *glauca* B was vegetatively propagated while ssp. *glauca* A was propagated by seed. Based on standard deviation, variation for days to flower for A was 5.9 when plants were grown under HPS lamps and 3.6 when they were grown under INC; for B, 0.9 and 1.3, respectively. A preexisting infestation of *Botrytis sp.* on *O. fruticosa* ssp. *glauca* A could have contributed to the lower flower number and slower flowering time and greater variability. Otherwise, we cannot explain their different flowering behaviors.

Because of its flowering characteristics, *O. fruticosa* ssp. *glauca* has potential as potted plant crop and may be better suited as one than 'Youngii-lapsley'. Subspecies *glauca* B was naturally short and floriferous, particularly when grown under a 16-h photoperiod provided by day-extension lighting with HPS lamps. Without plant growth regulators (PGR), this subspecies has a height/diameter ratio of 2.0, which is in the desired range of 1.5 to 2.0 for a potted flowering plant, as suggested by Sachs (1976). This ratio can be compared to the natural height/diameter ratio of 2.7 for 'Youngii-lapsley'.

Most of the other cultivars showed some potential but had major drawbacks. 'Fireworks' was naturally short but produced few flowers. However, in a preliminary study, rooted stem cuttings of 'Fireworks' cooled for eight weeks and subsequently grown under a 16-h photoperiod provided by HPS for day extension attained a height of 26 cm, produced 159 flowers, and showed excellent potted plant potential. 'Highlight' could have potted plant potential if the height were controlled by PGR. 'Summer Solstice' lacked potential because of its natural tallness (40 cm) and relatively low flower count (46 to 59).

Summary

Different lamp types used for daylength extension had only small effects on time to flower but, for some cultivars, greatly influenced flower number. High-pressure sodium lamps used for day-extension lighting drastically increased flower number of 'Highlight', 'Youngii-lapsley', and *O. fruticosa* ssp. *glauca* A and B. Because of the large variability between *O. fruticosa* ssp. *glauca* produced by different sources, it is advisable to screen stock-plant material before wide-scale production of this species is attempted. For production of 'Youngii-lapsley', 'Highlight', and ssp. *glauca*, using HPS lamps for day extension is recommended for producing more attractive plants. Subspecies *glauca* merits attention for its pot plant potential.

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Table 5. Starting plant material; dates of arrival; cold treatment initiation; forcing; average daily temperature, and average daily light integral for *Oenothera fruticosa* 'Fireworks', 'Highlight', 'Youngii-lapsley', 'Summer Solstice', and *O. fruticosa* ssp. *glauca*. The labels "A" and "B" after *O. fruticosa* ssp. *glauca* refer to different sources for plant material. Lamp type = type of lamp used to deliver daylength extension for 16-h photoperiods. HPS = high-pressure sodium lamps; INC = incandescent lamps

Cultivar	Starting plant material	Date of arrival	Date cold treatment began	Date forcing began	Lamp type	Average daily temperature (°C)	Average daily light integral ($mol \cdot m^2 \cdot day^{-1}$)
Fireworks	bare-root	2 Sept. 1998	2 Sept. 1998	15 Dec. 1998	INC HPS	20.5 19.8	7.9[z] 10.1
Summer Solstice	bare-root	2 Sept. 1998	2 Sept. 1998	15 Dec. 1998	INC HPS	20.5 20.2	8.1 10.4
Highlight	bare-root	15 Sept. 1998	30 Sept. 1998	16 Jan. 1999	INC HPS	20.7 21.1	8.9 11.5
Youngii-lapsley	72-cell	23 Sept. 1998	28 Sept. 1998	12 Jan. 1999	INC HPS	20.6 20.8	9.0 11.6
ssp. *glauca* A	2.5-inch	28 Aug. 1998	14 Sept. 1998	31 Dec. 1998	INC HPS	20.5 20.5	8.4 10.8
ssp. *glauca* B	94-cell	28 Oct. 1998	4 Nov. 1998	21 Feb. 1999	INC HPS	20.7 20.4	13.2 16.9

[z] Daily light integral was estimated for plants grown under daylength extension with incandescent (INC) lamps. Light data were only recorded for plants grown under daylength extension with high-pressure sodium (HPS) lamps.

Table 6. Flowering characteristics of several cultivars of *Oenothera fruticosa* and *O. fruticosa* ssp. *glauca* grown at 20 °C under 16-h photoperiods, delivered by day extension with either high-pressure sodium (HPS) lamps or incandescent (INC) lamps. All cultivars were treated with 15 weeks at 5 °C. Labels of significance refer to intracultivar comparisons for lamp type

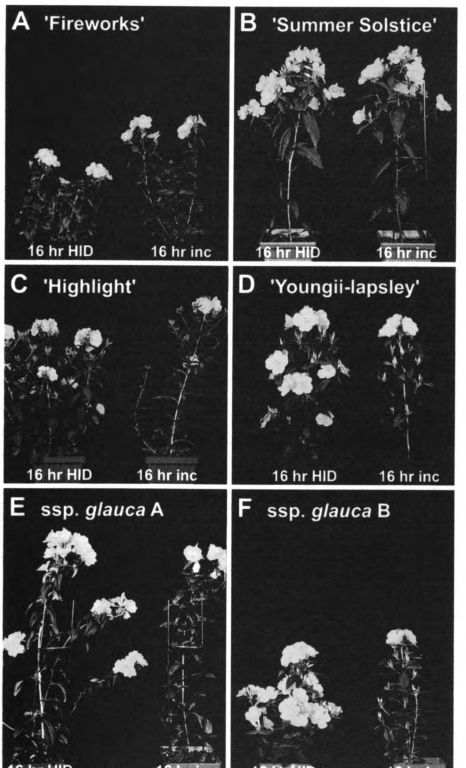
Cultivar	Lamp yype	No. of flowering plants	Days to flower	Plant height (cm)	No. of flowers and buds
Fireworks	INC	4	47	25	12
	HPS	5	51	24	23
				Significance	
			***	NS	NS
Summer Solstice	INC	10	45	40	46
	HPS	10	48	40	59
				Significance	
			***	NS	NS
Highlight	INC	7	41	36	145
	HPS	8	40	36	502
				Significance	
			*	NS	***
Youngii-lapsley	INC	10	41	45	47
	HPS	10	41	35	97
				Significance	

Table 6 cont'd.

			NS	***	***
ssp. *glauca* A	INC	6	44	42	35
	HPS	8	50	40	71
			Significance		
			*	NS	*
ssp. *glauca* B	INC	10	42	34	51
	HPS	10	40	31	150
			Significance		
			**	*	***

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

Figure 11. Cultivars of *Oenothera fruticosa* and *O. fruticosa* ssp. *glauca* grown at 20°C under a 16-h photoperiod provided by a 9-h natural day plus 7-h day extension with either high-pressure sodium lamps (labeled in the photographs as HID for high-intensity discharge) or incandescent lamps (inc). The labels "A" and "B" after ssp. *glauca* refer to different nursery sources.


A 'Fireworks'
16 hr HID
16 hr inc
B 'Summer Solstice'
16 hr HID
16 hr inc
C 'Highlight'
16 hr HID
16 hr inc
D 'Youngii-lapsley'
16 hr HID
16 hr inc
E ssp. glauca A
16 hr HID
16 hr inc
F ssp. glauca B
16 hr HID
16 hr inc

Effects of Cold Treatment, Photoperiod, and Forcing Temperature on Growth and Development of *Stokesia laevis* 'Klaus Jelitto'

Abstract

The influences of cold treatment, photoperiod, and forcing temperature on growth and development of *Stokesia laevis* 'Klaus Jelitto' were determined. Plants were cooled at 5 °C for 0, 3, 6, 9, 12, or 15 weeks under a 9-h photoperiod and subsequently forced in a 20 °C greenhouse under a 16-h photoperiod. Percentage flowering increased and time to visible bud and to flower decreased with increasing cold durations. Photoperiods of 10, 12, 13, 14, 16, 24 h, or a 4-h night interruption (NI) were tested in a 20 °C greenhouse following 0 or 15 weeks of cold treatment at 5 °C. Before cold treatment, plants flowered under photoperiods between 10 h and 14 h and one plant flowered under 24 h in Year 2. Percentage flowering, time to visible bud and to flower decreased with increasing photoperiod. After cold treatment, plants flowered under all photoperiods (except 24 h in Year 1). Percentage flowering, time to visible bud and to flower, and flower number decreased with increasing photoperiod. Plant height increased with increasing photoperiod. *Stokesia laevis* 'Klaus Jelitto' may best be described as an intermediate-day plant since highest flowering percentage and fastest time to flower were observed under photoperiods between 12 and 13 h both before and after cold treatment. To determine the effect of forcing temperature on flowering of 'Klaus Jelitto', plants were cooled for 15 weeks and then forced under a 16-h photoperiod at 14, 17, 20, 23, 26, or 29 °C. Time to visible bud and to flower increased and percentage

flowering decreased at higher forcing temperatures. Plant height and flower number decreased greatly as temperature increased.

Introduction

Stokesia laevis (Hill) Greene is an herbaceous perennial native to the southeastern coastal plain of South Carolina, Louisiana, and the panhandle of Florida (Gunn and White, 1974). Plants produce large (approximately 10 cm diameter) lavender blue flowers usually between July and October, but in warmer climates, such as in the southern United States, flowering can occur year-round (Fischer,1998). 'Klaus Jelitto' is a vegetatively propagated cultivar of *Stokesia laevis* with large, light lavender-blue flowers (Fischer,1998). *Stokesia laevis* is used for home perennial gardens, and rarely, for commercial cut flower production (Nau, 1996). Since 1970, *Stokesia laevis* has been investigated for its potential as an oilseed crop (Gunn and White, 1974). The achenes of *Stokesia laevis* contain 40% oil, and of that, 70% is epoxyoleic acid (also known as vernolic acid) which is easily converted to epoxy oil, a material used in the manufacture of plastics (Gunn and White, 1974; Perdue et al., 1986).

Flower induction in many plant species is controlled by vernalization and photoperiod. The promotion of flowering by cold treatment is defined as vernalization while photoperiodism is the control of flowering by daylength (Lang, 1965). Both vernalization and photoperiod requirements can be facultative or obligate. Vernalization and photoperiod are inductive processes and once floral induction has occurred, plants can be moved to noninductive conditions, and flowering will still occur (Lang, 1965). Modifying the length of the cold treatment or photoperiods after induction has occurred can dramatically influence plant

appearance. For example, plants of *Leucanthemum* x*superbum* Bergmans ex J. Ingram 'Snowcap' cooled for 15 weeks at 5 °C produced an average of 3 inflorescences under a 10-h photoperiod while they produced an average of 11 inflorescences under 16 h (Runkle et al., 1998). As cold treatment duration increased from 0 to 15 weeks, inflorescence number increased by approximately three times for plants of 'Snowcap' subsequently grown under a 4-h night interruption (NI), (Runkle et al., 1998). Such manipulations of cold treatments and photoperiod are important for producing high-quality crops. In a preliminary experiment, 'Klaus Jelitto' was tested either under a 9-h short day or a 4-h NI before and after a 15-week cold treatment at 5 °C. 'Klaus Jelitto' was tentatively proposed to be a facultative short-day plant with an obligate requirement for cold treatment (Frane, 1999).

Knowledge of the effect of temperature on plant development is important to accurately time crop production. In the absence of the effects such as photoperiod or vernalization, in general, the rate of development increases linearly with temperature within a limited range of growing temperatures (Roberts and Summerfield, 1987). Such responses are also evident in photoperiod- and vernalization-sensitive species when the separate effects of photoperiod and vernalization are removed from those of post-vernalization temperatures. The relationship between temperature and rate of development toward flowering (1/DTF where DTF is days to flower) can be described as follows:

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

where b_0 and b_1 are constants and T is temperature (°C) (Roberts and Summerfield, 1987).

The base temperature (T_b) , or the temperature at or below which the rate of progress toward flowering is zero, and thermal time, or the number of units (°days) of thermal time above a base temperature required for flowering can be calculated from the constants in Equation 1 as follows:

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

$$°days = 1/b_1 \quad [3]$$

Besides influencing rate of flowering, temperature influences other aspects of growth and development such as number of flowers and plant height at flower. For *Antirrhinum majus* L. 'Jackpot', there was an increase in stem length, spike length and number of florets as temperature decreased from 21 to 10 °C (Maginnes and Langhans, 1961). Flower bud number, plant height, and flower diameter of *Coreopsis grandiflora* Hogg ex Sweet 'Sunray', *Leucanthemum xsuperbum* 'Snowcap', and *Rudbeckia fulgida* Ait. 'Goldsturm' decreased as forcing temperature increased from 15 to 27 °C (Yuan et al., 1998) We could find no literature on forcing temperatures for 'Klaus Jelitto' or any cultivar of *Stokesia laevis*.

The objectives of these experiments were to describe and quantify the effects of cold treatment, photoperiod, and forcing temperature on growth and development of *Stokesia laevis* 'Klaus Jelitto'.

Materials and Methods

General. Plants of *Stokesia laevis* 'Klaus Jelitto' were received as crown divisions or rooted basal cuttings in either 2.5-inch pots or in 36-cell plug trays on 15 Oct. 1997 (Year 1) and 28 Aug. 1998 or 16 Oct. 1998 (Year 2) from two separate commercial producers. Upon arrival at Michigan State University, all plants were held in the 2.5- inch pots or plug trays and exposed to natural daylengths (lat. 43 °N;≈11-12 h) until the experiments began. Cold treatments were delivered to plants held in 2.5-inch pots or plug trays in coolers set at 5 °C. Within the coolers, plants were illuminated for 9 $h \cdot day^{-1}$ with cool-white fluorescent lamps (F96T12/CW/VHO, Philips, Somerset, N.J.) at approximately 10 $\mu mol \cdot m^2 s^{-1}$ and watered as necessary (approximately 2 times per week) using well water acidified with sulfuric acid (H_2SO_4) to a pH of approximately 6.0.

Before placement in the greenhouses (set at 20 °C day/night [D/N] unless otherwise stated), plugs were transplanted into 13-cm square plastic pots (1.1-L volume) with a soilless medium containing sphagnum peat moss, composted pine bark, vermiculite, and perlite (Strong-lite High Porosity Mix, Strong-Lite, Pine Bluff, Ark.). Pots were spaced at approximately 22 plants/m^2. Plants were fertilized at every irrigation with a nutrient solution of well water (EC of 0.70 $mS \cdot cm^{-1}$ and 105, 35, and 23 $mg \cdot L^{-1}$ Ca, Mg, and S, respectively) acidified with H_2SO_4 to a titratable alkalinity of 130 $mg \cdot L^{-1}$ $CaCO_3$ and water soluble fertilizer providing 125-12-125-13 N-P-K-Ca $mg \cdot L^{-1}$ (30% ammoniacal N) plus 1.0-0.5-0.5-0.5-0.1-0.1 $mg \cdot L^{-1}$ (Fe, Mn, Zn, Cu, B, Mo) (MSU Special, Greencare, Chicago,

Ill.). The target range was 0.5-1.2 $mS \cdot cm^{-1}$ for electrical conductivity (EC) and 5.8-6.2 for pH. In the second year, it was necessary to drench all plants transplanted after 1 Jan. 1999 twice with a 750 mg $N \cdot L^{-1}$ liquid fertilizer and KCO_3 to raise the pH and EC into the desired ranges.

Cold duration (Expt. 1). Six durations of cold treatment were tested (0, 3, 6, 9, 12, or 15 weeks). Experiments were initiated on 18 Oct. 1997 (Year 1) and 7 Oct. 1998 (Year 2). No initial node count was taken in Year 1. In Year 2, the initial number of nodes was counted at transplant and averaged 9.8 nodes. Ten plants were removed from the cooler after each treatment duration, transplanted and placed in the greenhouse.

A 16-h photoperiod was provided by four-hundred-watt high-pressure sodium lamps (HPS) switched on at 0800 HR and off at 2400 HR. HPS lamps provided both the photoperiod and supplemental lighting with a photosynthetic photon flux *(PPF)* of 50 $\mu mol \cdot m^{2} \cdot s^{-1}$ at plant height starting at 0800 and continuing until the outside *PPF* exceeded 400 $\mu mol \cdot m^{2} \cdot s^{-1}$. If the outside *PPF* then dropped below 200 $\mu mol \cdot m^{2} \cdot s^{-1}$ lamps were again turned on until 2400 HR.

Photoperiod (Expt.2). Seven photoperiods (10, 12, 13, 14, 16, 24 h and a 4-h night interruption [NI]) and two cold treatment durations (0 or 15 weeks at 5 °C) were tested. Seventy plants were transplanted on 18 Oct. 1997 (Year 1) and 28 Oct. 1998 (Year 2) and placed in a greenhouse. Ten plants were used per treatment and treatments were assigned randomly each year to greenhouse benches. The remaining plants were left in the 2.5-inch pots and placed in the

cooler. After 15 weeks of cold treatment, seventy plants were transplanted on 31 Jan. 1998 (Year 1) and 14 Feb. 1999 (Year 2) and placed in the same photoperiod treatments as the first set of seventy plants. Plants averaged 10.8 nodes in Year 1 and 10.3 nodes in Year 2.

Opaque black cloth was pulled at 1700 HR and opened at 0800 HR every day on all benches so all plants received a similar daily light integral. Continual photoperiods were delivered by day-extension lighting using incandescent lamps which were turned on at 1700 HR and turned off after each photoperiod was completed. The 4-h night interruption was provided by incandescent lamps which were turned on at 2200 HR and turned off at 0200 HR. Day-extension and NI lighting using incandescent lamps provided approximately 1-3 μmol·m^{2}s^{-1} at canopy level. Supplemental lighting during daylight hours was given as described for Expt. 1, except that HPS lamps were terminated at 1700 HR.

Forcing Temperature (Expt. 3). On 7 Oct. 1998, plants left in plug trays were placed directly into the cooler for 15 weeks until 20 Jan. 1999, when they were transplanted. At transplant, plants averaged 10.1 leaves. Ten plants were placed into each of 6 greenhouses set to 14, 17, 20, 23, 26, and 29 °C. Plants received a continual 16-h photoperiod provided by HPS lamps that remained on continuously from 0800 HR to 2400 HR and delivered approximately 90 μmol·m^2·s^{-1}.

Data collection and analysis. The data collected for all experiments included date of first visible bud (without dissection) and date of first open flower. Days to

visible bud, days to flower, and days from visible bud to flower were calculated for all experiments. At first open flower, total plant height (excluding the container), number of visible flower buds, and number of final nodes on the main stem were recorded. The number of final nodes was counted to the last visible leaf and when the initial node count was taken, included the number of initial nodes. The number of new nodes produced during forcing was calculated by subtracting the initial number of leaves from the final number of leaves, and the number of new nodes beneath the first flower (in Year 2) was calculated by subtracting the number of initial nodes from the number of nodes beneath the first flower. For all experiments, plants that did not have visible bud after 15 weeks of forcing were considered non-flowering and were discarded. Plants that died during the experiment were discarded and not included in the results.

All experiments were completely randomized designs. Data were analyzed using the general linear models procedure (PROC GLM) (SAS Institute, Cary, N.C.) for analysis of variance and linear regression procedure (PROC REG) for regression models. All experiments were analyzed with Linear and Quadratic trend contrasts. The cold duration and photoperiod experiments (Expts.1 and 2, respectively) were replicated in time, and conducted once per year. The forcing temperature experiment (Expt. 3) was conducted only once, in Year 2.

Greenhouse temperature control and daily light integral collection. Air temperature was monitored in each greenhouse with 36-gauge (0.127-mm-

diameter) thermocouples connected to a CR 10 datalogger (Campbell Scientific, Logan, Utah). The datalogger collected temperature data every 10 s and recorded the hourly average. Average daily air temperatures from transplant to average date of flowering for Expts. 1 and 2 are shown in Tables 7 and 8. Average daily temperatures for three developmental stages of plants in Expt. 3 are shown in Table 9. Temperatures in each greenhouse were controlled by a Priva environmental computer (model CD750; Priva, De Lier, The Netherlands). Actual average daily air temperatures were calculated and used in all analyses.

Average daily light integral data were collected only for Expt. 1 in Year 2. The average daily light integrals were recorded at canopy level (at the same interval as temperature data) using quantum sensors (LI-COR) connected to the CR 10 datalogger. It was estimated that plants in Expt. 2 received approximately 78% of the daily light integral that plants received in Expt. 1. This estimation was found by subtracting the amount of light available during daylight hours (0800 to 1700 HR) from the amount of light available during the full 16-h photoperiod of day extension with high-pressure sodium lamps. Supplemental lighting was similar between the houses from 0800 to 1700 HR and it was assumed that day-extension lighting with incandescent lamps (with an output of 1-3 μmol·m^{2}·sec^{-1}) contribute essentially no light beyond the compensation point.

Results and Discussion

Cold duration (Expt. 1). Fifty to 60 % of 'Klaus Jelitto' plants flowered without a cold treatment (Figure 12A). In Year 1, percentage flowering reached 100 after

6 weeks of cold treatment (Figure 12A) while in Year 2, flowering percentage did not reach 100 until plants had received 15 weeks cold treatment.

Days to visible bud and days to flower decreased quadratically ($P \leq 0.01$ for days to flower; $P \leq 0.001$ for days to visible bud) in Year 1, although there were no significant trends for either variable in Year 2 (Figures 12B, C). In Year 1, time to flower decreased greatly as cold duration increased from zero to 6 weeks (112 to 79 days). The decrease in days to flower as cold duration increased from 6 to 15 weeks was slight (79 to 74 days), thus it appears that vernalization was saturated after 6 weeks of 5 °C treatment. Increasing cold duration generally decreased variability for flower timing. For example, variability based on standard deviation decreased from 23.8 to 11.3 days as cold duration increased from zero to 15 weeks in Year 2. Based on these results, we conclude that 'Klaus Jelitto' has a facultative requirement for vernalization.

There was a large difference between the two years for plants that did not receive any cold treatment but only small differences between the years for plants cooled ≥3 weeks. Plants without a cold treatment flowered slowly (112 days) in Year 1 while in Year 2, they flowered relatively quickly (76 days). Starting plant material was different between the years: 2.5-inch pots in Year 1, and 36-cell plugs in Year 2 each received from a different nursery. Although no initial node count was taken in Year 1, the initial node count of plants in 36-cell plug trays averaged 9.8, which was very similar to the node counts found for plants in 2.5-inch pots in both Year 1 and Year 2 (10.8 and 10.2, respectively),

which would have been similar to the node count for plants in Expt. 1, in Year 1. We can not readily explain the different flowering behavior between the noncooled plants in the two years. The experiments were initiated within 11 days of each other between the years and, although we do not have the light data for Year 1, we assume that plants in each year received relatively similar amounts of light. Temperature was carefully controlled; the greatest difference between the years was 1.2 °C, thus temperature probably did not cause the differences in flowering that we observed between the years or between the different cold duration treatments.

There were small but significant differences for the effect of cold treatment on days from visible bud to flower. In Year 1, days from visible bud to flower decreased quadratically ($P \leq 0.01$), while in Year 2, there was an increasing linear ($P \leq 0.01$) trend (Figure 12D). Although significant, the differences in time from visible bud to flower between different cold durations within a year or between the years were relatively small: the greatest difference between treatments within a year and treatments between years was 11 days.

While the change in flower timing with increased cold treatment was only clearly observed in Year 1, the number of nodes counted to the last visible leaf did reflect earlier floral initiation with increasing cold treatment in both years (Figure 13A). There were no significant differences for the final or new node number beneath the first flower in Year 2, which reflects the lack of significant linear or quadratric trends observed in Year 2. The greatest decrease in final

and new number of nodes (31 to 26 for final nodes in Year 2) occurred as cold duration increased from zero to six weeks, after which the change in number of nodes was very small (26 to 25 for final nodes in Year 2) as cold duration was increased to 15 weeks. In addition, variability of node number (both final and new nodes) decreased as cold duration increased. These observations are consistent with the hypothesis that vernalization of 'Klaus Jelitto' is saturated after 6 weeks of cold treatment. The number of new nodes in Year 2 was almost identical to the number of final nodes in Year 1. This was most likely due to the fact that initial leaf counts were not taken in Year 1, and the leaves present on the plant at the beginning of the experiment had probably senesced by the time the plant flowered. The number of final nodes in Year 1 most likely represented the number of new nodes produced during forcing. It is interesting to note that the faster flowering observed for plants without a cold treatment in Year 2 was not reflected by either the final or new number of nodes.

There were large differences in number of flower buds between the years (Figure 13B). There were no significant trends for the effect of cold duration on the number of flower buds in Year 1, but there was a significant ($P \leq 0.01$) linear increase in Year 2. The number of flower buds per plant was variable but, increased from five for noncooled plants to 13 for plants cooled for 15 weeks.

There was little effect of cold duration on plant height. In Year 1, height decreased linearly ($P \leq 0.001$) from 59 to 49 cm as cold duration increased from

zero to 15 weeks (Figure 13C). There were no significant differences for height in Year 2.

Photoperiod (Expt. 2) Without a cold treatment, in Year 1, 'Klaus Jelitto' plants flowered only under photoperiods between 12 and 14 h; flowering percentage declined from 75 to 16 in this photoperiod range (Figure 14A). Percentage flowering was much higher for noncooled plants in the second year where all plants flowered under photoperiods between 10 and 13 h and one plant each flowered under 14 and 24 h. No plants flowered under night interruption (NI) without cold treatment in either year. While plants did not flower under 16 h without a cold treatment in this experiment, 50 and 60% flowered without a cold treatment in Expt.1 (in Year 1 and Year 2, respectively). Plants in Expt. 2 only received about 78% of the light received by the plants in Expt. 1 which could explain some of the difference. It has been observed for several cold-requiring species that high light levels can induce flowering without cold treatment. Blondon and Chouard (1965) found that a cold-requiring clone of *Dactylis glomerata* L. flowered under a 16-h photoperiod without a cold treatment when provided with a 16-h day at a photosynthetic photon flux of 280 μmol·m^{2}·sec^{-1} (24.2 mol·m^{2}·day^{-1}).

In Year 2, the plants that flowered under 10 h and the single plant that flowered under 14 h were very slow to flower (135 or 139 days, respectively) compared to plants under 12 or 13 h photoperiods, which flowered in 81 or 86 days, respectively (Figures 14B, C, D). The plant that flowered under the 24-h

photoperiod flowered in 74 days. The two plants that flowered under 14 h in Year 1 flowered 63 days faster than the one plant that flowered under 14 h in Year 2. Average daily temperature was only 1 °C higher in Year 2 compared to Year 1, and can not explain this difference. The experiment in Year 2 started only 10 days later than in Year 1, thus it is not likely that differing light levels caused the difference. We have no explanation for the large difference in percentage flowering or time to flower between the two years for noncooled plants. We speculate that if we had left the plants on the bench for longer than 100 days, more plants would have eventually flowered.

After the cold treatment, percentage flowering was 90 or 100 under 10 h and decreased to 0 or 10 under 24 h in Year 1or 2, respectively (Figure 15A). Eighty and 90 percent of the plants flowered under NI in Year 1 and 2, respectively.

Following cold treatment, days to visible bud and days to flower changed little between photoperiod treatments except for the one group of plants under 10 h in Year 1 (Figures 15B, C). In Year 1, plants grown under 10 h flowered in 122 days, while plants grown under 10 h in Year 2 flowered in 79 days. In Year 2, plants under 10 h were only approximately 10 days slower than plants under other treatments. We have no explanation for the large differences for plants under 10 h between the years. Days from visible bud to flower changed little between photoperiods. In Year 1, there was a small tendency for increasing

days from visible bud to flower while in Year 2, there was a small tendency for decreasing days from visible bud to flower with increasing photoperiod (Figure 15D). However, differences in days from visible bud to flower between each photoperiod were small, as were the differences between years. The greatest difference between the years for days to visible bud to flower before or after cold treatment was 11 days. Days to visible bud, day from visible bud to flower, and days to flower for plants grown under NI were similar to plants grown under photoperiods ≥14h in both years.

In general, cold-treated plants flowered only slightly faster than flowering non-cold-treated plants, although the effect of cold treatment on flower timing differed for plants grown under different photoperiods. For example, for plants grown under 12 h in Year 1, days to flower decreased ≈2 weeks after cold treatment, while days to flower decreased 8 weeks after cold treatment for plants grown under 10 h in Year 2 (Figures 14B,15B). Variability generally decreased following cold treatment. For example, the standard deviation for days to flower of plants grown under 13h before and after cold treatment in Year 2 decreased from 13.2 to 3.5.

The number of final and new nodes for plants without a cold treatment decreased linearly ($P \leq 0.001$) in Year 2, and quadratically ($P \leq 0.001$) after a cold treatment in Year 1 (Figures 16A,B). There were no significant trends for either the number of final or new nodes without a cold treatment in Year 1, or

following a cold treatment in Year 2. The decrease observed in both final and new nodes as photoperiod increased from 10 to 24 h reflects the decrease in flower timing seen in Year 1. In Year 2, there were no significant trends for the number of final or new nodes beneath the flower following cold treatment.

The lower flowering percentage observed under photoperiods ≥14 h together with the slower time to flower under photoperiods ≤10 h both before and after a cold treatment suggest that 'Klaus Jelitto' does not behave similar to either a short-day plant response or a long-day plant response. On the basis of these results, we conclude that 'Klaus Jelitto' is best described as a facultative intermediate-day plant. Allard (1938) described intermediate-day plants as those which flower when the day is neither too long nor too short. For example, *Mikania scandens* L. flowered only under photoperiods of 12 to 16 h (Allard, 1938). Only a few species, including *Mikania scandens* L., *Phaseolus polystachios* (L.) B.S.P., *Eupatorium hyssopifolium* var. *hyssopifolium* L., a variety of *Saccharum spontaneum* L. (28N. G. 292) (Allard, 1938), *Salsola komarovii* Ijin (Takeno et al., 1995), and *Echinacea purpurea* (L.) Moench 'Magnus' (E. Runkle, unpublished data) have been documented as intermediate-day plants. Thomas and Vince-Prue (1997) suggested that intermediate-day plants may be a variation of the short-day type response since short day plants also need some light in each day to flower. However, the daylength limitations for short-day plants are fairly wide, while for intermediate-day plants, the range of

effective photoperiods is typically quite narrow. Alternatively, Sachs (1956) suggested that intermediate-day plants may actually have dual daylength requirements for flowering but the long-day and short-day ranges overlap, so that intermediate photoperiods satisfy both photoperiodic requirements. Studies are needed to test these hypotheses for the intermediate-day response observed for *Stokesia laevis* 'Klaus Jelitto'

There was no consistent effect of photoperiod on flower bud number of non-cold treated plants (Figure 16C). After cold treatment, the number of flower buds decreased linearly ($P \leq 0.001$ in Year 1; $P \leq 0.01$ in Year 2) with increasing photoperiod in both years. Flower bud number increased after a cold treatment only for plants grown under 12-h photoperiods. Cooled plants that flowered under NI produced as many flowers as those grown under ≥14 h in both years.

Plant height at first flower for plants with or without a cold treatment increased markedly as photoperiod increased from 10 and 14 h (Figure 16D); for cold-treated plants in Year 1, plant height increased from 27 to 47 cm. Plant height for cooled plants grown under NI was similar to cooled plants grown under 13 h in Year 1 and similar to cooled plants grown under photoperiods ≥14h in Year 2.

The effect of photoperiod on plant height and number of flowers drastically changed the appearance of the plants grown under different photoperiods. Those grown under photoperiods ≤12 h were short, produced many flowers, and

could make a very acceptable potted flowering plant. However, when plants were grown under photoperiods ≥13 h, the plants were too tall and produced too few flowers to be considered as a potted flowering plant. Knowledge of effective plant growth regulators would be beneficial for production of 'Klaus Jelitto' if plants are to be produced under long photoperiods. However, if plants of 'Klaus Jelitto' are produced for cut-flower production, long photoperiods may be more appropriate for the longer stems that result under longer photoperiods.

Flowering of 'Klaus Jelitto' was extremely variable. There were large differences in percent flowering between years for both Expt. 1 and 2 (Figures 12A, 14A). Percentage flowering in Expt. 1 was generally higher in Year 1 compared to that in Year 2, but for Expt. 2, percentage flowering was much higher in Year 2 compared to that in Year 1. For example, in Expt. 2, 100% of plants flowered under 10, 12, and 13 h in Year 2 while no plants flowered under 10 h and only at 75 or 30% plants flowered under 12 or 13 h, respectively, in Year 1. This result was surprising since the plant material used for this experiment came from the same nursery and had similar starting plant sizes in both years (10.8 nodes in Year 1 and 10.3 nodes in Year 2). In addition to variability for percent flowering, there were wide ranges in days to flower even within a single treatment group. For example, all plants cooled for 15 weeks in Expt. 1 (Year 2) flowered over a range of 35 days, an unacceptably large period of time for flowering of a crop in the floriculture industry.

Forcing Temperature (Expt. 3) Percentage flowering decreased from 100 at temperatures ≤20 °C, to 90 at temperatures between 23 and 26 °C, and to 50 at 29 °C (data not shown). Days to visible bud, days from visible bud to flower, and days to flower of 'Klaus Jelitto' decreased with increasing temperatures (Figures 17A, B, C). Plants forced at 15.9 °C flowered in 90 days while plants forced at 29.9 °C flowered in 42 days.

Rates of progress to visible bud, from visible bud to flower, and to flower were linear within the studied temperature range (Figures 17D, E, F). The parameters for the linear regression analysis are shown in Table 10. The reciprocal of the linear regression lines (shown in Figures 17A, B, C) described the relationship between temperature and the time required to reach each developmental stage well. Base temperatures ranged from 3.2 to 4.2. The degrees days required to reach first open flower were 1104 when calculated from the constants obtained from the linear regression analysis with day to flower data and 1035 when the degree days was calculated by adding the data from transplant to visible bud and visible bud to flower. Degree-days and base temperature are important concepts in production of flowering plants since they allow for prediction of flowering time in environments where the temperatures are fluctuating, and the mean temperature is known (Roberts and Summerfield, 1987). Actual average days to flower did not differ from predicted days to flower more than 3 days for any temperature (Figure 17C).

The number of final and new nodes to the last visible leaf decreased quadratically ($P \leq 0.05$) as temperature increased (Figure 18A), although the actual decrease was small (29 to 23 for final nodes). The quadratic trend of the decrease in final and new nodes relates to the quadratic pattern observed for the decrease in days to visible bud, days from visible bud to flower, and days to flower as temperatures increase (Figures 17A, B, C). There were no significant differences for the number of final or new nodes beneath the flower.

Flower number decreased ≈82% as temperature increased from 15.9 to 29.9 °C (Figure 18B). Others have observed that flower number decreases with increasing forcing temperature; examples include *Coreopsis grandiflora* 'Sunray', *Rudbeckia fulgida*.'Goldsturm', *Leucanthemum* x*superbum* 'Snowcap' (Yuan et al., 1998), and *Campanula carpatica* Jacq. 'Blue Clips' (Whitman et al., 1997).

Plant height decreased dramatically with increasing temperature (Figure 18C). Tallest plants (average height of 59 cm) were those forced 18.4 °C and the shortest plants (average of 21 cm) were forced at 29.9 °C. Plant height of many species increases as the difference between day temperature and night temperature increases (DIF) (Erwin and Heins, 1995). The DIF to which plants of 'Klaus Jelitto' were subjected during the developmental stages of transplant to visible bud, visible bud to flower, and transplant to flower are presented in Table 11. The largest values for DIF were observed in the house set at 17 °C (actual temperature averaged 18.4 °C during the time between transplant and average

day to flower), which corresponds to the temperature setting where tallest plant heights observed, thus DIF may have contributed to some of the differences in plant height.

Summary

Stokesia laevis 'Klaus Jelitto' has a facultative requirement for vernalization. Time to flower decreased as cold treatment increased. Before cold treatment, flowering generally occurred only under photoperiods between 10 and 14 h. However, when plants were grown without a cold treatment under 16 h in high light conditions, such as provided by high-pressure sodium lamps in Expt.1, flowering occurred but at relatively low percentages (50 to 60%). After cold treatment, plants flowered under all photoperiods but flowering percentage decreased as photoperiod increased from 14- to 24-h. In Year 1, no plants flowered under 24 h. Time to flower was slowest under 10 h and decreased as photoperiod increased. Based on this study, plants of 'Klaus Jelitto' flower most rapidly, completely, and uniformly after a cold treatment under photoperiods between 12 and 13 h and can best be described as a facultative intermediate-day plant. Time to flower and percentage flowering decreased with increasing forcing temperature. A forcing temperature of 18 °C and photoperiods between 12-13 h are recommended for maximizing percentage flowering and flower number.

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Table 7. Average daily air temperatures and daily light integrals from date of transplant to average date of flowering of *Stokesia laevis* 'Klaus Jelitto' after each duration of cold treatment

Year	Cold Duration (weeks at 5 °C)					
	0	3	6	9	12	15
	Average air temperatures during forcing (°C)					
1997-1998	19.8	19.9	19.7	19.8	19.7	20.4
1998-1999	21.0	20.6	20.6	20.5	20.4	20.9
	Average daily light integral ($mol \cdot m^{-2} \cdot day^{-1}$)					
1997-1998	--[z]	--	--	--	--	--
1998-1999	12.6	11.0	11.0	11.2	12.6	14.8

[z] Measurement not taken.

Table 8. Average air temperatures from date of transplant to average date of flowering of *Stokesia laevis* 'Klaus Jelitto' under each photoperiod

Year	Weeks at 5 °C	Photoperiod (h)						
		10	12	13	14	16	24	NI[z]
		Average air temperature during forcing (°C)						
1997-98	0	--[y]	20.3	20.5	19.8	--	--	--
	15	21.3	20.7	20.6	20.5	21.6	--	20.9
1998-99	0	21.1	20.7	20.8	20.8	--	21.5	--
	15	20.4	21.1	20.7	20.9	20.4	20.4	21.4

[z] 9-h photoperiod plus 4-h night interruption.
[y] No plants flowered.

Table 9. Actual temperatures of houses for the time of forcing for three developmental stages: Transplant to Visible Bud, Visible Bud to Flower, and Transplant to Flower

Temperature Set Point (°C)	Developmental Stage (days)		
	Transplant to Visible Bud	Visible Bud to Flower	Transplant to Flower
14	15.1	16.7	15.9
17	18.2	18.6	18.4
20	20.6	21.3	21.0
23	23.8	23.3	23.6
26	26.8	27.2	27.0
29	29.7	30.0	29.9

Table 10. Parameters of linear regression analysis relating forcing temperature to rate of progress from transplant to visible bud (VB), visible bud to flowering (VB to FLW) and transplant to flowering (FLW) for *Stokesia laevis* 'Klaus Jelitto'. 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_1) (1/d)/C	T_b(°C)	°days	r^2
Transplant to VB	-7.60E-3 ± 7.44E-3 [z]	2.10E-3 ± 3.25E-4	3.6	476	0.89**
VB to FLW	-7.59E-3 ± 2.52E-3	1.79E-3 ± 1.08E-4	4.2	559	0.98***
Transplant to FLW	-2.93E-3 ± 1.57E-3	9.06E-4 ± 6.78E-5	3.2	1104	0.97***

[z] Standard error.

,* Significant at $P \leq 0.01$, or 0.001, respectively.

Table 11. The difference between day and night temperature (DIF) of plants of *Stokesia laevis* 'Klaus Jelitto' grown under different forcing temperatures and three developmental stages: from transplant to visible bud (VB), from VB to flowering (FLW), and from transplant to FLW

Temperature setting (°C)	Transplant to VB	VB to FLW	Transplant to FLW
14	0.11	2.03	1.08
17	1.38	1.95	1.69
20	0.69	1.37	1.03
23	0.71	1.04	0.88
26	0.46	0.97	0.76
29	0.32	0.67	0.50

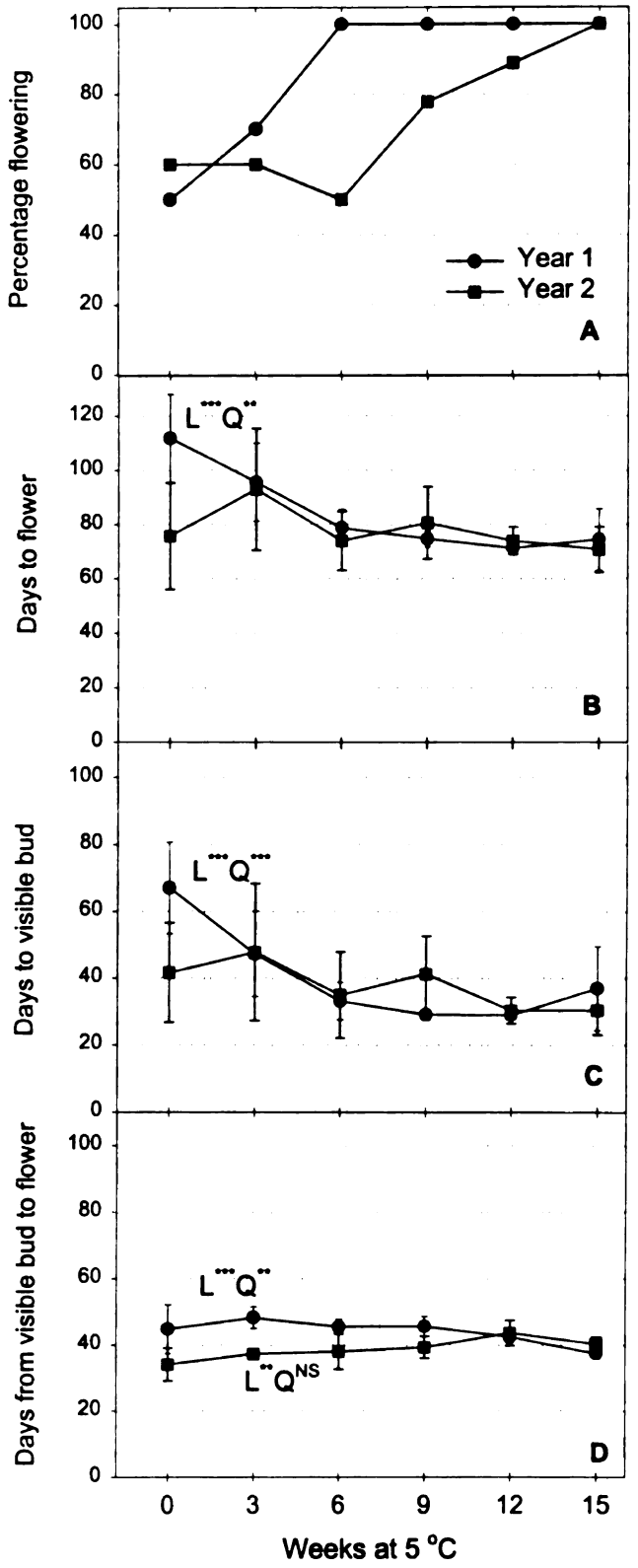

Figure 12. Effects of various durations of cold treatment at 5 °C on percentage flowering (A), days to flower (B), days to visible bud (C), and days from visible bud to flower (D) in *Stokesia laevis* 'Klaus Jelitto' in Year 1 (●) and Year 2 (■). Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,**,***}Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

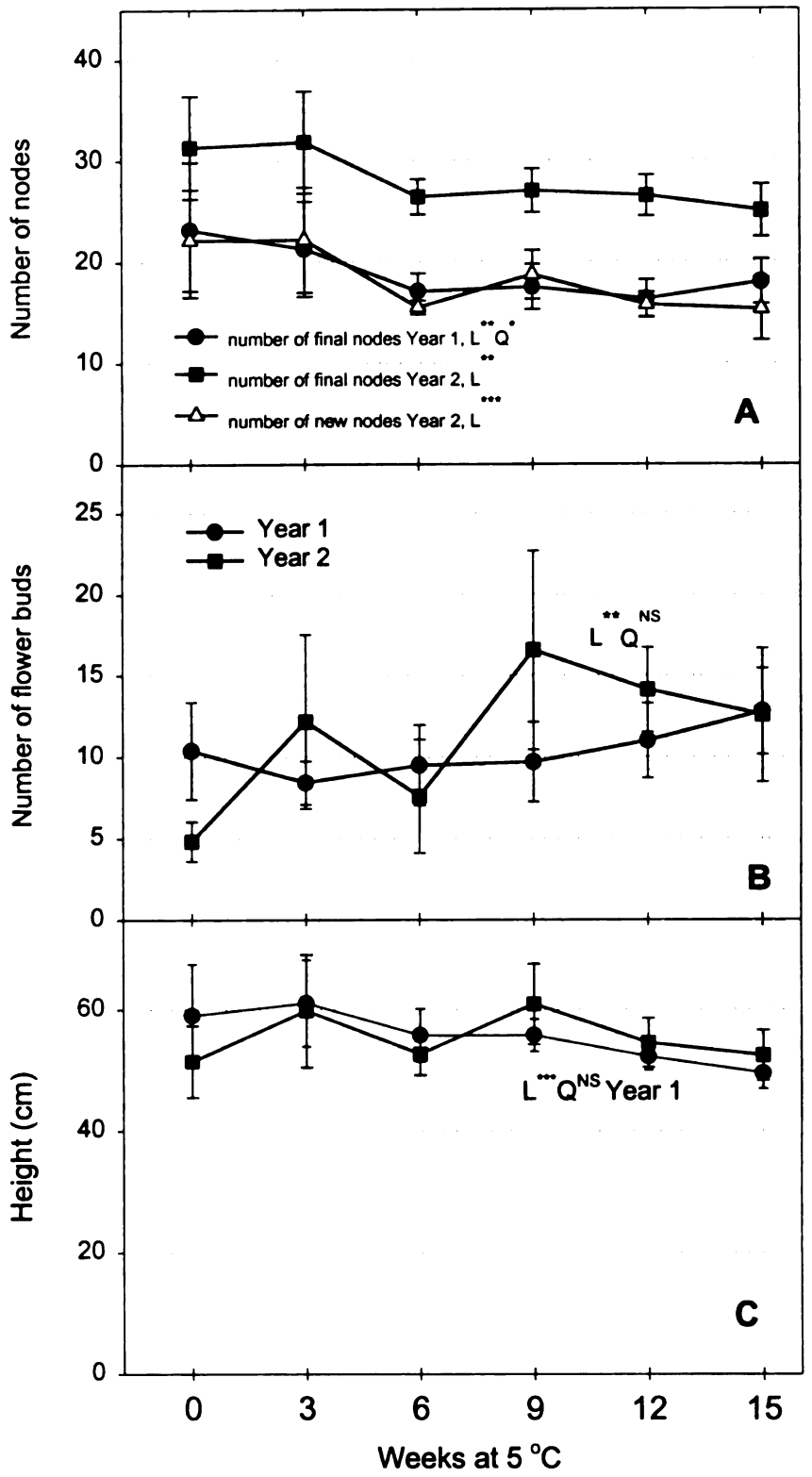

Figure 13. Effects of cold duration at 5 °C on number of nodes (A), number of flower buds (B), and plant height (C) in *Stokesia laevis* 'Klaus Jelitto' in Year 1 (●) and Year 2 (■). Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

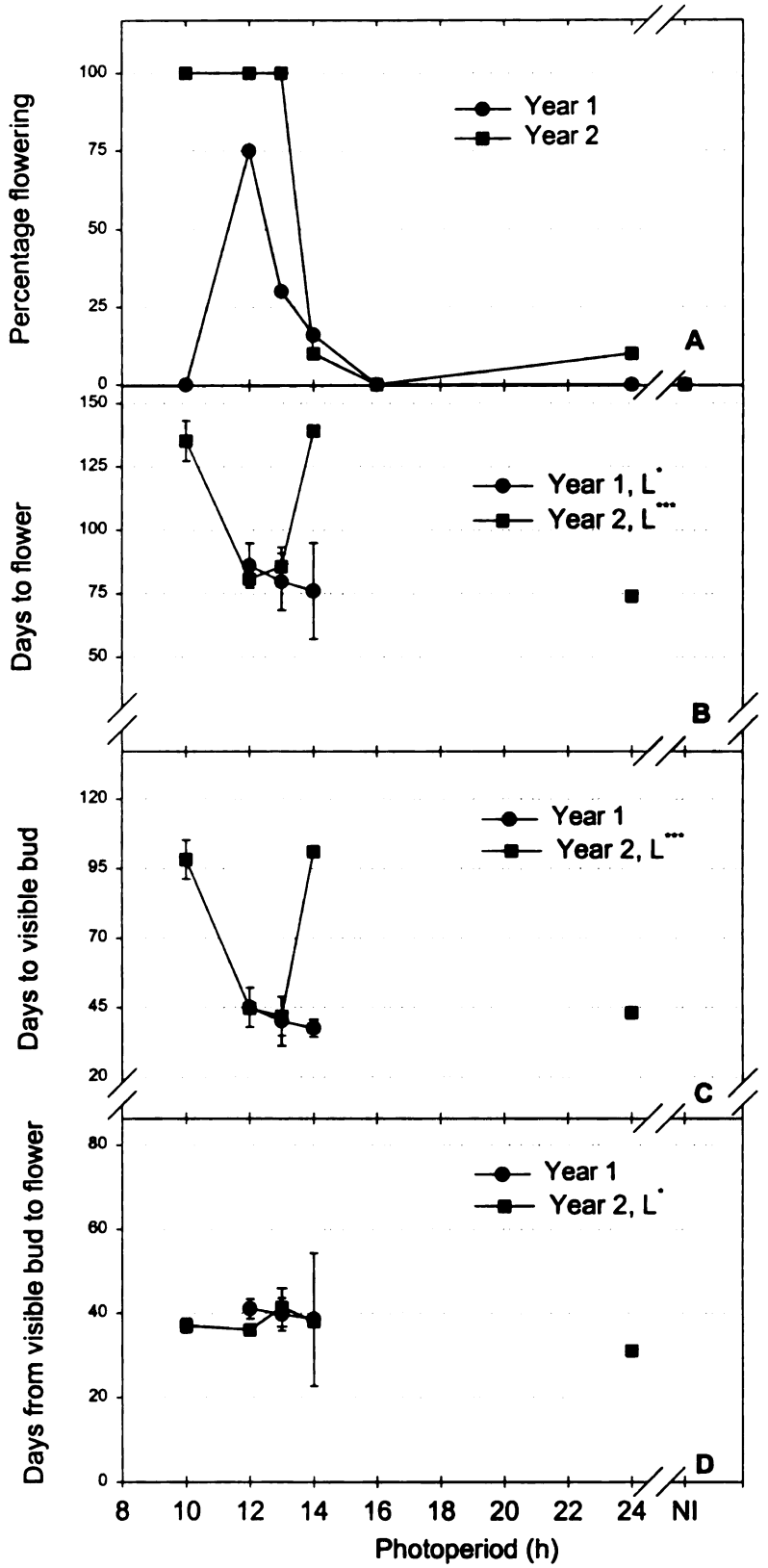

Figure 14. Effects of various photoperiods without a cold treatment on percentage flowering (A), days to flower (B), days to visible bud (C), and days from visible bud to flower (D) in *Stokesia laevis* 'Klaus Jelitto' in Year 1 (●) and Year 2 (■). Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

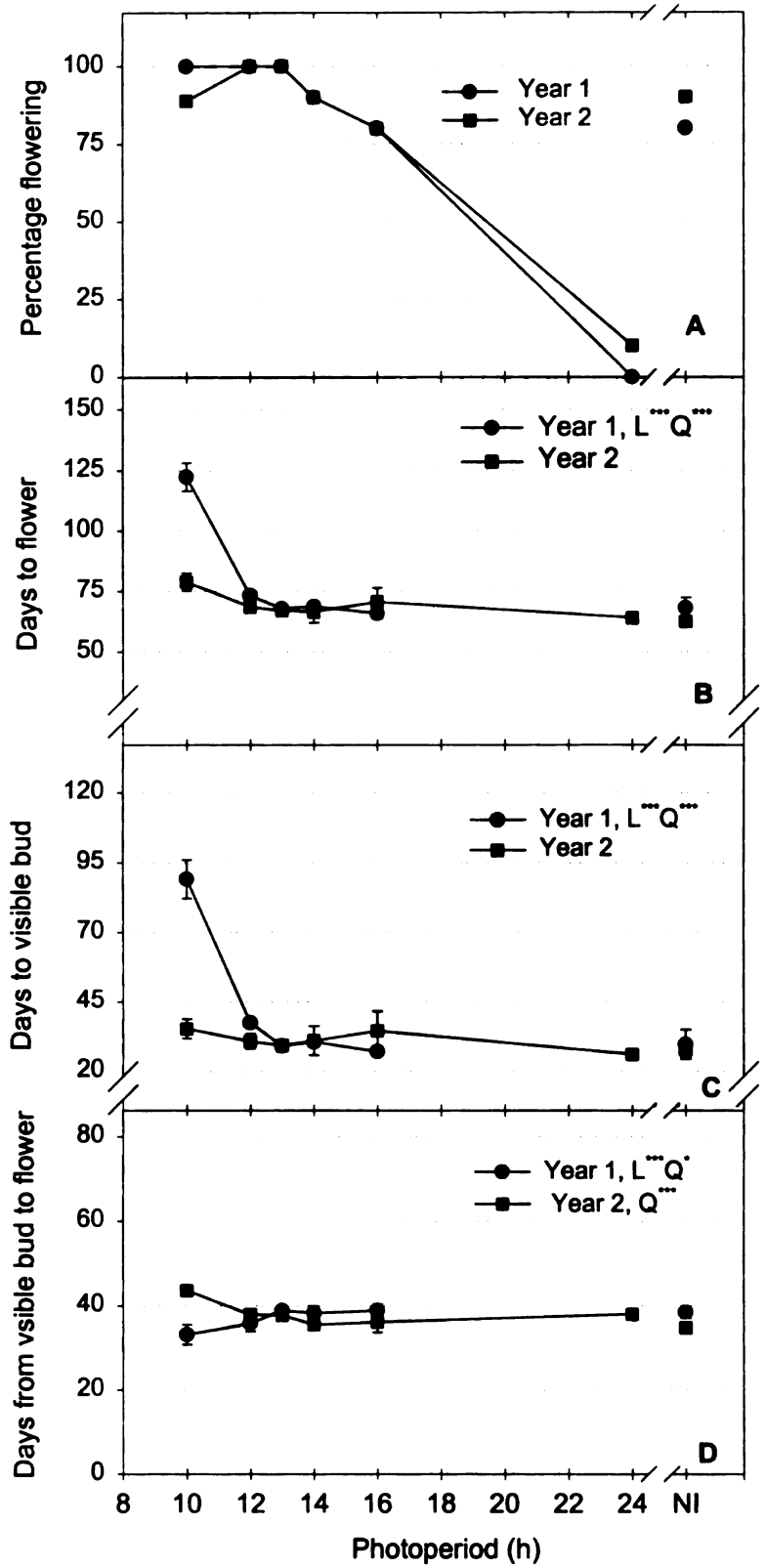

Figure 15. Effects of various photoperiods after a 15 week cold treatment at 5 °C on percentage flowering (A), days to flower (B), days to visible bud (C), and days from visible bud to flower (D) in *Stokesia laevis* 'Klaus Jelitto' in Year 1 (●) and Year 2 (■). Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

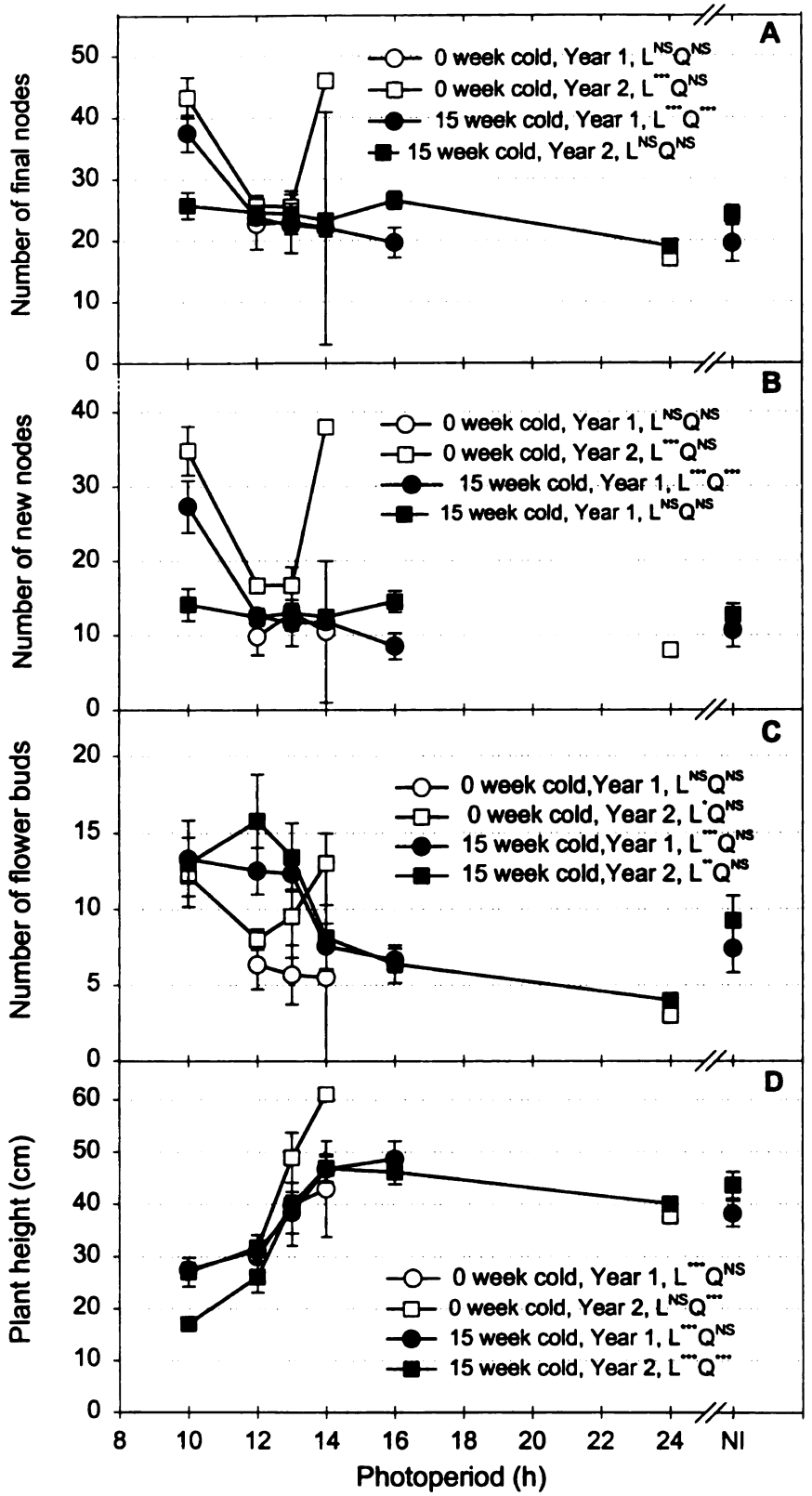

Figure 16. Effects of various photoperiods with and without a 15-week cold treatment at 5 °C on number of final nodes (A), number of new nodes (B), number of flower buds (C), and plant height (D) in *Stokesia laevis* 'Klaus Jelitto' in Year 1 (●) and Year 2 (■). Error bars represent 95% confidence intervals. L = linear; Q = quadratic trends. ^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

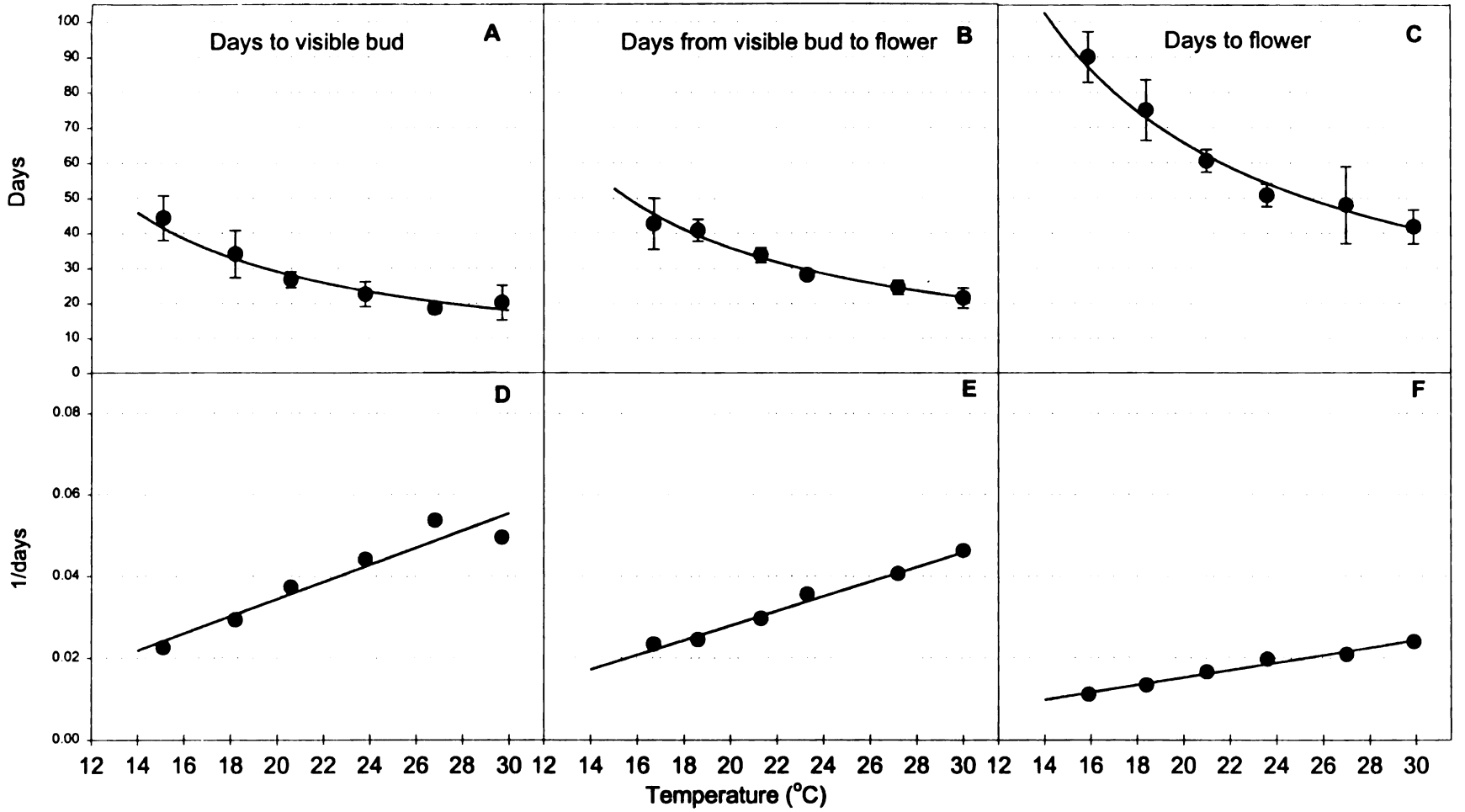

Figure 17. Effects of temperature on time to (A, B, C) and rate of progress toward flowering (D, E, F) in *Stokesia laevis* 'Klaus Jelitto'. The parameters of linear regression lines are presented in Table 10. The quadratic regression lines in graphs A, B, and C are the reciprocals of correlated linear regression lines in graphs D, E, and F.

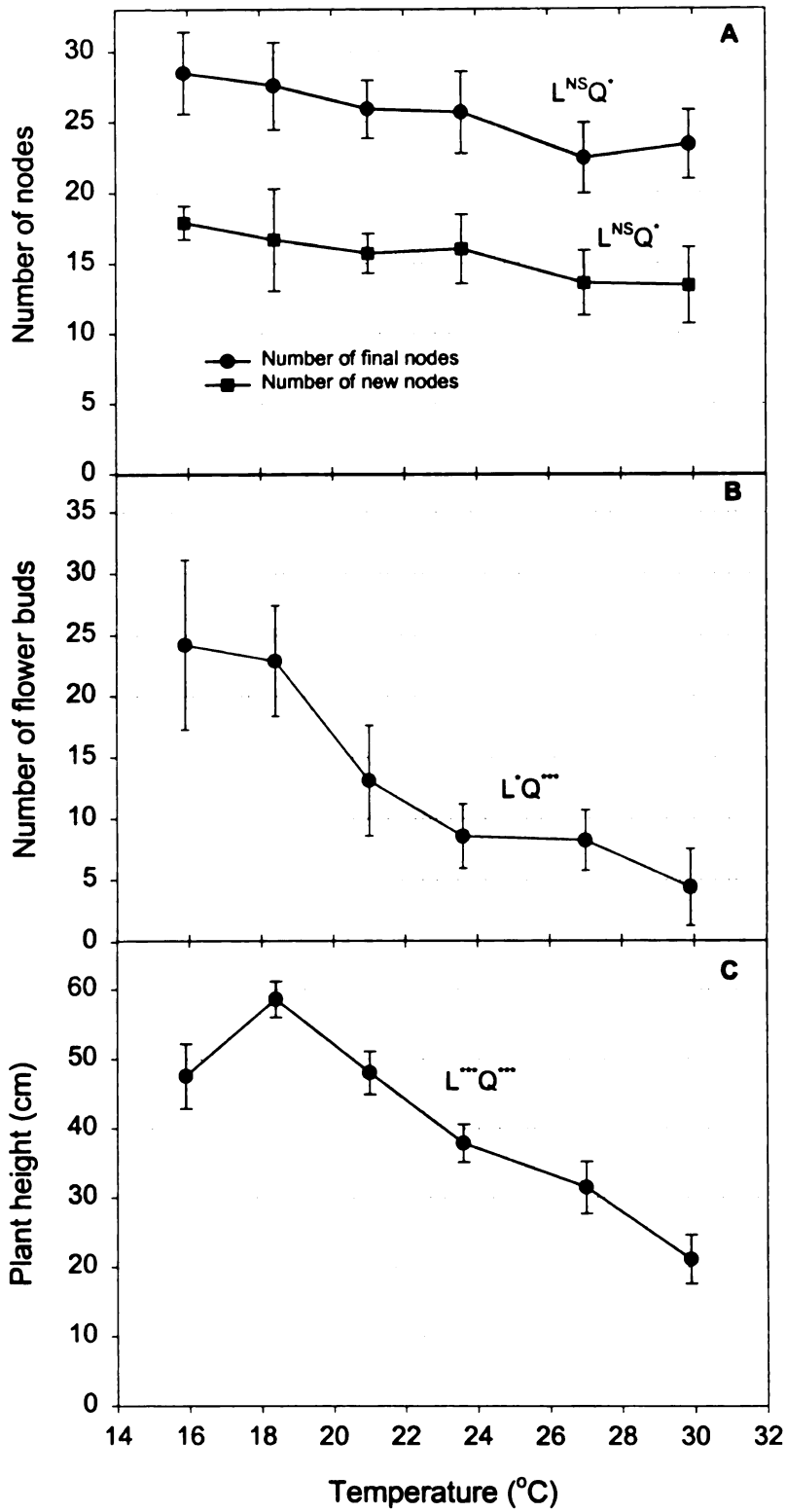

Figure 18. Effects of temperature on number of nodes (A), number of flower buds (B), and plant height (C) in *Stokesia laevis* 'Klaus Jelitto'. L=linear; Q=quadratic trends. ^{NS,*,**,***} Nonsignificant or significant at $P \leq 0.05, 0.01$, or 0.001, respectively.

Appendix A:

Comparison of *Stokesia laevis* Cultivars Grown under Two Different Lamp Types for Daylength Extension

Introduction

Stokesia laevis (Hill) Greene is an herbaceous perennial native to the southeastern United States from South Carolina to Louisiana, including the panhandle of Florida (Gunn and White, 1974). With its large (≈10 cm diameter), lavender-blue flowers, *Stokesia laevis* 'Klaus Jelitto' demonstrated good potential as a potted flowering plant in preliminary studies and forcing experiments (Frane, 1999; chapter 4). 'Klaus Jelitto' has a facultative requirement for vernalization and is best described as a facultative intermediate-day plant (chapter 4). Several cultivars of *S. laevis* exist including yellow-flowered 'Mary Gregory', white-flowered 'Alba' and 'Silver Moon', and 'Purple Parasols' which has violet flowers. 'Klaus Jelitto', 'Blue Danube', 'Wyoming', 'Blue Star' and 'Omega Skyrocket' all have lavender-blue flowers, as does the straight species (Armitage, 1989; Fischer, 1998). Based on the potential observed for 'Klaus Jelitto', we were interested in investigating the flowering characteristics of other cultivars of *Stokesia laevis* in container production.

Different lamp types used for day-extension were found to influence flowering characteristics of *S. laevis* 'Klaus Jelitto'. 'Klaus Jelitto' plants cooled for 15 weeks and subsequently grown under a 16-h photoperiod with day extension from HPS lamps produced approximately two times the number of flowers produced on plants grown under the INC lamps. In addition, plants of 'Klaus Jelitto' without a cold treatment were found to flower at 50-60% under a 16-h photoperiod when HPS lamps were used for day-extension when no plants

flowered without cold treatment under a 16-h photoperiod when INC lamps were used for day-extension. It was estimated that a 16-h photoperiod provided by day-extension lighting with incandescent (INC) lamps provided a daily light integral that was 78% of the daily light integral provided by day-extension with high-pressure sodium lamps (see chapters 1 - 3 for explanation of estimation). Warrington and Norton (1991) found that increasing the daily light integral increased the rate of floral development and number of flower buds in *Dendranthema xgrandiflorum* (Ramat.) Kitamura, *Raphanus sativus* L., *Zea mays* L., and *Cucumis sativus* L.

High-pressure sodium and INC lamps also differ in spectral quality as well as intensity. Incandescent lamps emit a mixture of red and far-red light which is richer in far-red than red (ratio of red 600-700 nm to far-red 700-800 nm < one [Lane et al., 1965]). High pressure sodium lamps emit a mixture of red and far-red light with far less far-red than red light compared to incandescent lamps (Thimijan and Heins, 1983).

The objectives of this study were to (1) determine flowering characteristics (e.g. time to flower, flower number, final plant height) and (2) determine the effect of using different lamp types for day-extension lighting for *S. laevis* 'Alba', 'Blue Danube', 'Klaus Jelitto', 'Mary Gregory', and 'Purple Parasols'.

Protocol

Experimental Design: completely randomized design

Cultivars: 'Alba', 'Blue Danube', 'Klaus Jelitto', 'Mary Gregory', 'Purple Parasols', and 'Wyoming'

See Table 12 for starting plant material, dates of arrival, cold treatment, and forcing, as well as average daily temperature and average daily light integral during forcing. Daily light integral data were only recorded for plants under day extension with HPS lamps.

Cold Treatment:

15 weeks at 5°C

Photoperiod and lamp types: All cultivars were treated with a 16-h photoperiod composed of 9 h of natural light plus a 7-h daylength extension. Natural sunlight was supplemented with light from high-pressure sodium lamps. The high-pressure sodium lamps were turned on every morning at 0800 when natural light (measured on outside of greenhouse roof) fell below 400 μmol·m^{2}·sec^{-1}. High-pressure sodium lamps were terminated when ambient light level was greater than 400 μmol·m^{2}·sec^{-1} and were not re-initiated until ambient light level fell below 200 μmol·m^{2}·sec^{-1}.

Daylength extension was given with either:

1) incandescent lamps (INC) (1-3 μmol·m^{2}·sec^{-1})

2) high pressure sodium lamps (HPS) (40 μmol·m^{2}·sec^{-1})

RESEARCH PROTOCOL:

Upon arrival at MSU, plants were held under natural daylengths at 20 °C until the experiment began. Bare-root plants were immediately transplanted into bulbtrays and the root masses were covered with soilless media. Plants were cooled at 5 °C either in bulbtrays, plug trays, or 2.5-inch pots. Following cold treatment, plants were potted into 13-cm pots and placed in 20 °C greenhouses under a 16-h photoperiod delivered by daylength extension from either INC or HPS lamps.

Data collected included:

1) Initial node count
2) Date of visible bud
3) Date of flowering
4) Number of flower buds at first flower
5) Final node number beneath the first flower
6) Plant height at first flower
7) Number of new nodes were calculated by subtracting the number of initial nodes from the final nodes.
8) Days from visible bud to flower were calculated by subtracting the days to visible bud from days to flower

Summary of Results

In general, the type of lamp used for daylength extension had little effect on flower timing for cultivars of *Stokesia laevis*. There were few statistical differences for flower timing, and where statistical significance did occur, the differences were small (Table 13). For example, 'Purple Parasols' grown under INC lamps flowered approximately 5 days faster than plants grown under HPS lamps. Lamp type influenced plant height of only two cultivars: 'Klaus Jelitto' was approximately 6 cm taller under HPS lamps compared to INC lamps while 'Purple Parasols' was 11 cm taller under INC lamps compared to HPS lamps. Lamp type had a stronger influence on number of flower buds. Bud number increased 1.5 to 2 times for 'Blue Danube', 'Klaus Jelitto' and 'Purple Parasols' grown under HPS lamps compared to INC lamps.

Flowering percentage varied greatly (20 to 100%) between cultivars. 'Wyoming' and 'Alba' had particularly low flowering percentages 20 to 60% and 30 to 60%, respectively, for 'Wyoming' and 'Alba',. 'Purple Parasols' had a moderate flowering percentage of 70 to 80%. The lower flower percentages observed for these cultivars may have been related to small initial plant size. Plants of 'Alba' and 'Purple Parasols' arrived in a 72-cell tray which were considerably smaller than the starting material of most of the other cultivars (2.5-inch pots). Starting material for 'Wyoming' was single-eye bare-root divisions with no more than 6 leaves.

Although most cultivars flowered in between 61 to 85 days, had 6 to 13 flower buds and were between 39 and 52 cm tall, there were several exceptions. 'Alba' was the slowest cultivar to flower (> 100 days). 'Mary Gregory' and 'Wyoming' attained heights at first flower of approximately 27 and 29 cm (when data for each cultivar were pooled for both lamp types due to lack of significance) and were much shorter than other cultivars. Starting plant material size did not appear to influence flower number since 'Alba' and 'Purple Parasols', which had some of the smallest initial plant sizes had considerably more flowers than other cultivars. Armitage (1989) observed that 'Alba' was less floriferous than other cultivars in garden performance, but we observed the opposite when container produced.

Potential as a potted flowering plant varied widely among cultivars. 'Klaus Jelitto' and 'Purple Parasols' showed the greatest potential with numerous large flowers of either light lavender-blue for 'Klaus Jelitto' or deep violet for 'Purple Parasols'. In addition, 'Klaus Jelitto' had an attractive branching habit. 'Mary Gregory' showed some potential with its naturally short height and small dark green leaves but produced only a few small yellow flowers. 'Alba', 'Wyoming', and 'Blue Danube' lacked potential for various reasons: 'Alba' and 'Wyoming' had very low flowering percentages and for 'Alba' the time needed to produce a flowering plant was excessively long, while 'Blue Danube' was spindly, had few flowers, and tended to fall over.

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Frane, A.J. 1999. Temperature effects on timing and bud development of *Coreopsis verticillata* 'Moonbeam' and flower induction of long-day perennials under different night temperatures. MS Thesis, Michigan State Univ., East Lansing.

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Thimijan, R. and R.D. Heins. 1983. Photometric, radiometric, and quantum light units of measure: A review of procedures for in interconversion. HortScience.18: 818-822.

Warrington, I.J. and R.A. Norton. 1991. An evaluation of plant growth and development under various daily quantum integrals. J. Amer. Soc. Hort. Sci.116(3): 544-551.

Table 12. Starting plant material; number of plants per treatment; dates of arrival, cold treatment initiation, forcing, as well as average daily temperature and average daily light integral for *Stokesia laevis* 'Alba', 'Blue Danube', 'Klaus Jelitto', 'Mary Gregory', 'Purple Parasols' and 'Wyoming'. Lamp type = type of lamp used to deliver daylength extension for 16 h photoperiods. HPS = high pressure sodium lamps; INC = incandescent lamps

Cultivar	Starting plant material	No. of plants per treatment	Date of arrival	Date cold treatment began	Date forcing began	Lamp type	Average daily temperature (°C)	Average daily light integral ($mol \cdot m^2 \cdot day^{-1}$)
Alba	72-cell	10	14 Sep. 1998	28 Sep. 1998	16 Jan. 1999	INC	20.5	12.2[z]
						HPS	20.3	15.7
Blue Danube	2.5"	10	--	7 Oct. 1998	23 Jan. 1999	INC	20.5	11.2
						HPS	20.7	14.3
Klaus Jelitto	2.5"	10	16 Oct. 28 Aug. 1998	28 Oct. 7 Oct. 1998	14 Feb. 20 Jan. 1999	INC	20.4	11.5
						HPS	20.9	14.8
Mary Gregory	2.5"	10	--	7 Oct. 1998	21 Jan. 1999	INC	20.6	11.2
						HPS	20.8	14.3
Purple Parasols	72-cell	10	17 Sep. 1998	27 Sep. 1998	16 Jan. 1999	INC	20.6	10.5
						HPS	20.8	13.4
Wyoming	bare-root	5	2 Sep. 1998	2 Sep. 1998	15 Dec. 1998	INC	20.5	8.7
						HPS	20.4	11.1

[z] Average daily light integrals for plants grown under daylength extension with incandescent (INC) lamps were estimated. Light data were recorded only for plants grown under daylength extension with high-pressure sodium (HPS) lamps.

Table13. Flowering characteristics of several cultivars of *Stokesia laevis* grown at 20 °C under 16 h photoperiods, delivered by day-extension with either high pressure sodium (HPS) lamps or incandescent (INC) lamps. All cultivars were treated with 15 weeks at 5 °C. Labels of significance refer to intracultivar comparisons for lamp type. Number of new leaves refers to the number of new leaves beneath the first flower

Cultivar	Lamp Type	Percent Flowering	Days to visible bud	Days from visible bud to flower	Days to flower	Number of new leaves	Plant height (cm)	No. of flower buds
Alba	INC	30	83	38	121	14	41	11
	HPS	60	67	37	104	5	39	22
	Significance		NS	NS	NS	•	NS	NS
Blue Danube	INC	100	30	34	66	11	45	6
	HPS	100	26	35	61	11	42	9
	Significance		••	••	NS	NS	NS	••
Klaus Jelitto	INC	80	35	36	71	8	46	6
	HPS	100	30	40	71	7	52	13
	Significance		NS	•	NS	NS	•	•
Mary Gregory	INC	90	36	35	71	7	28	7
	HPS	100	28	35	64	6	27	8
	Significance		NS	NS	NS	NS	NS	NS
Purple Parasols	INC	70	36	38	61	4	52	11
	HPS	80	25	37	66	2	41	24

Table 13 cont'd.

	Significance		*	NS	*	**	***	***
Wyoming	INC	60	53	32	85	7	27	4
	HPS	20	37	35	72	5	36	8
	Significance		NS	NS	NS	NS	NS	NS

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

Appendix B:

Effects of Cold Treatment, Phototperiod, and Forcing Temperature in *Anemone hupehensis*, *Penstemon digitalis* 'Husker Red', and *Saxifraga arendsii* 'Triumph'

Introduction

Flowering of many temperate herbaceous perennials is influenced by four main factors: juvenility, vernalization, photoperiod, and forcing temperature. All of these factors influence both the quantity and quality of flowering. Juvenility is a developmental plant state during which plants are unable or only poorly able to perceive inductive conditions such as vernalization and photoperiod. Vernalization is the promotion of flowering by cold treatment, while photoperiodism is the control of flowering by daylength. Plants can have obligate or facultative responses to vernalization and photoperiod. Forcing temperature influences flower timing as well as plant quality features such as plant height, flower number, and flower diameter. Vernalization and photoperiod also influence plant quality features such as plant height and flower number.

As herbaceous perennials have gained popularity during the past 15 years, the interest in producing them has also increased. Since consumers prefer to buy plants that are in flower, and most herbaceous perennials are not in flower in May and early June when consumers are doing most of their shopping, producers are interested in the flowering requriements of these species so that they can produce flowering plants in May and June.

The objective of these experiments was to determine the influence of vernalization, photoperiod, and forcing temperature on three herbaceous perennial species: *Anemone hupehensis*, *Penstemon digitalis* 'Husker Red', and

Saxifraga arendsii 'Triumph'. Information from these experiments can be used to create production schedules.

Protocol

(1) Cold Duration Experiment

Experimental Design: completely randomized design

Cold treatment: 0, 3, 6, 9, 12, and 15 weeks at 5 °C. Research protocol: 10 plants were immediately potted into 13-cm pots with soilless medium and placed in a 20 ° C greenhouse where they were provided a 16-h photoperiod delivered by a natural day supplemented and day extension from high-pressure sodium lamps. Plants were cooled in the plug tray in coolers set at 5 ° C. Upon completion of the desired cold treatment duration, 10 plants were transferred to the greenhouse, potted into the 13-cm pots and placed in the same greenhouse as those plants which did not receive a cold treatment.

Data collected: 1) initial node count (Year 2 only)

2) date of visible bud

3) date of first open flower

4) number of flower buds at flower

5) plant height at flower

6) number of nodes to either last visible leaf or beneath the first flower

(2) Photoperiod Experiment

Experimental design: completely randomized design

Cold treatments: 0 or 15 weeks at 5 °C.

Photoperiods: 10, 12, 13, 14, 16, 24, and a 4-h night interruption from 10:00 p.m. to 2:00 a.m.

Research Protocol: Half of the plants were immediately placed in coolers set at 5 °C for 15 weeks. The remaining half were potted into 13-cm pots and placed in a 20 °C greenhouse and ten plants were placed under each photoperiod. Plants were forced for 15 weeks after which plants not in visible bud were discarded. Photoperiods were provided by a 9-h natural day and day extension from incandescent lamps to the desired photoperiod. Between 8:00 a.m. and 5:00 p.m. supplemental light light from high-pressure sodium lamps was given when the ambient photosynthetic photon flux (*PPF*) first fell below 400 μmol·m^{-2}·sec^{-1}. High-pressure sodium lamps were turned off once *PPF* rose above 400 μmol·m^{-2}·sec^{-1} and did not turn on again until ambient *PPF* fell to 200 μmol·m^{-2}·sec^{-1}. After 15 weeks of cold treatment, the other half of the plants were moved to the greenhouse, potted into the 13-cm and placed in the photoperiod treatments exactly as those without a cold treatment.

Data collected: same as in Cold Duration Experiment.

(3) Forcing Temperature Experiment.

Experimental design: completely randomized design

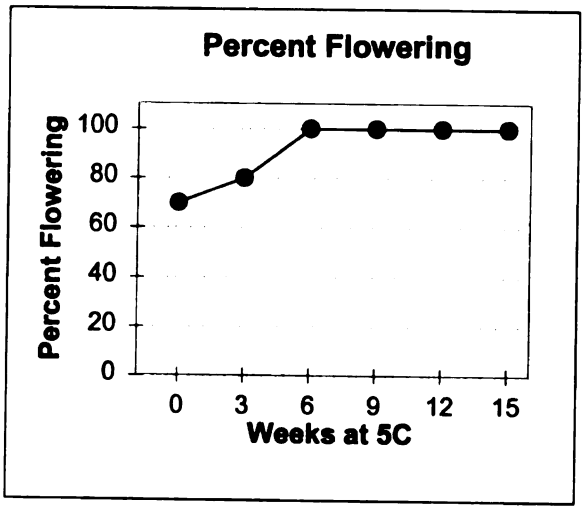
Cold Treatment: 15 weeks at 5 ° C.

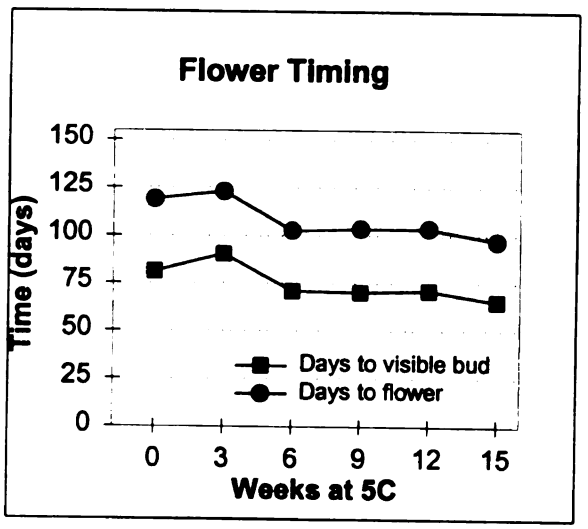
Temperatures: 14, 17, 20, 23, 26, and 29 ° C (constant day and night temperature).

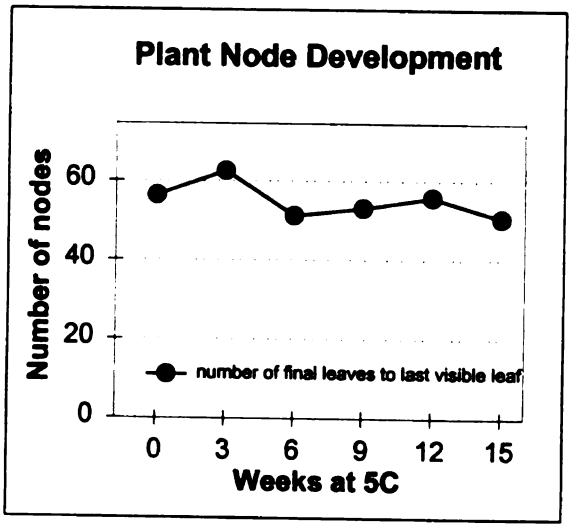
Research protocol: All plants were given a 15 week cold treatment at 5 ° C. Following cold treatment, ten plants were potted into 13-cm pots with soilless medium and placed in each greenhouse set at one of the six temperatures. Plants were provided with a continuous 16-h photoperiod using high-pressure sodium lamps.

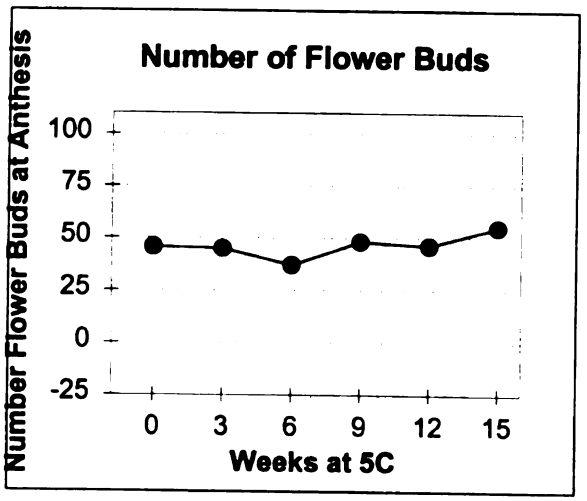
Data collected: same as for Cold Duration Experiment and Photoperiod Experiment.

Anemone hupehensis









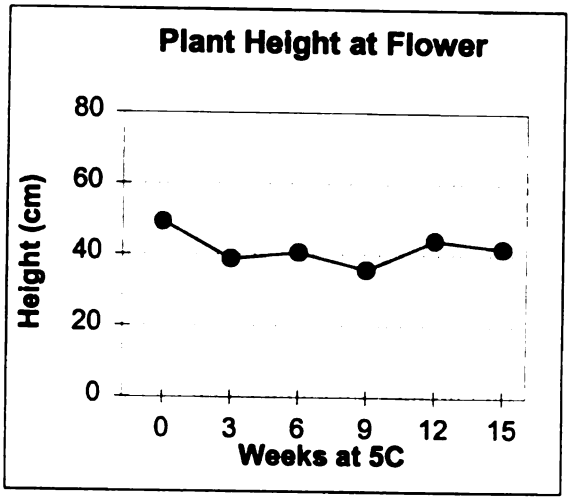


Figure 19. Effects of cold treatment in *Anemone hupehensis* in Year 1.

Anemone hupehensis

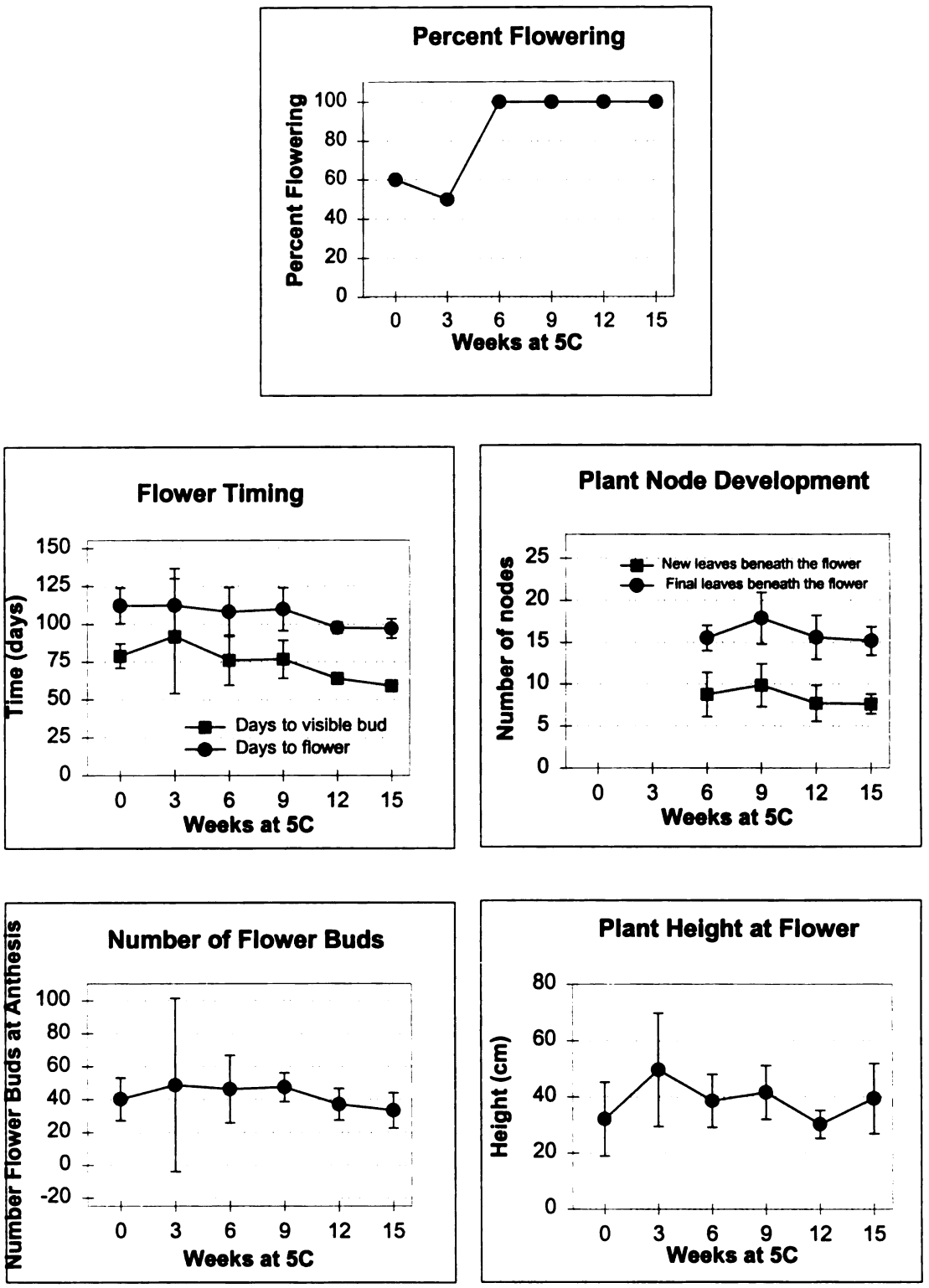


Figure 20. Effects of cold treatment in *Anemone hupehensis* in Year 2. Error bars show 95% confidence intervals.

Anemone hupehensis

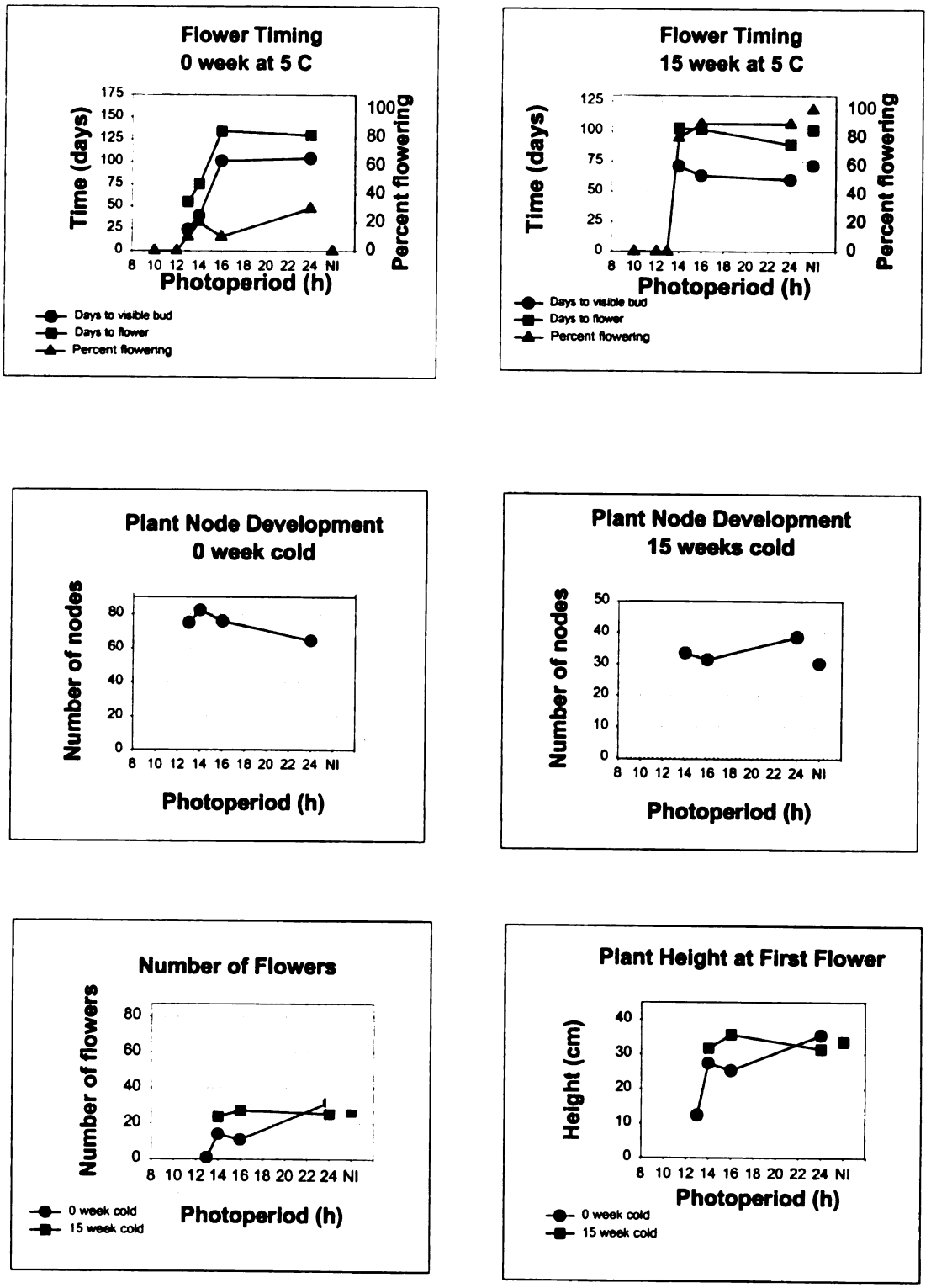


Figure 21. Effects of various photoperiods with and without a 15 week cold treatment at 5 °C in *Anemone hupehensis* in Year 1.

Anemone hupehensis

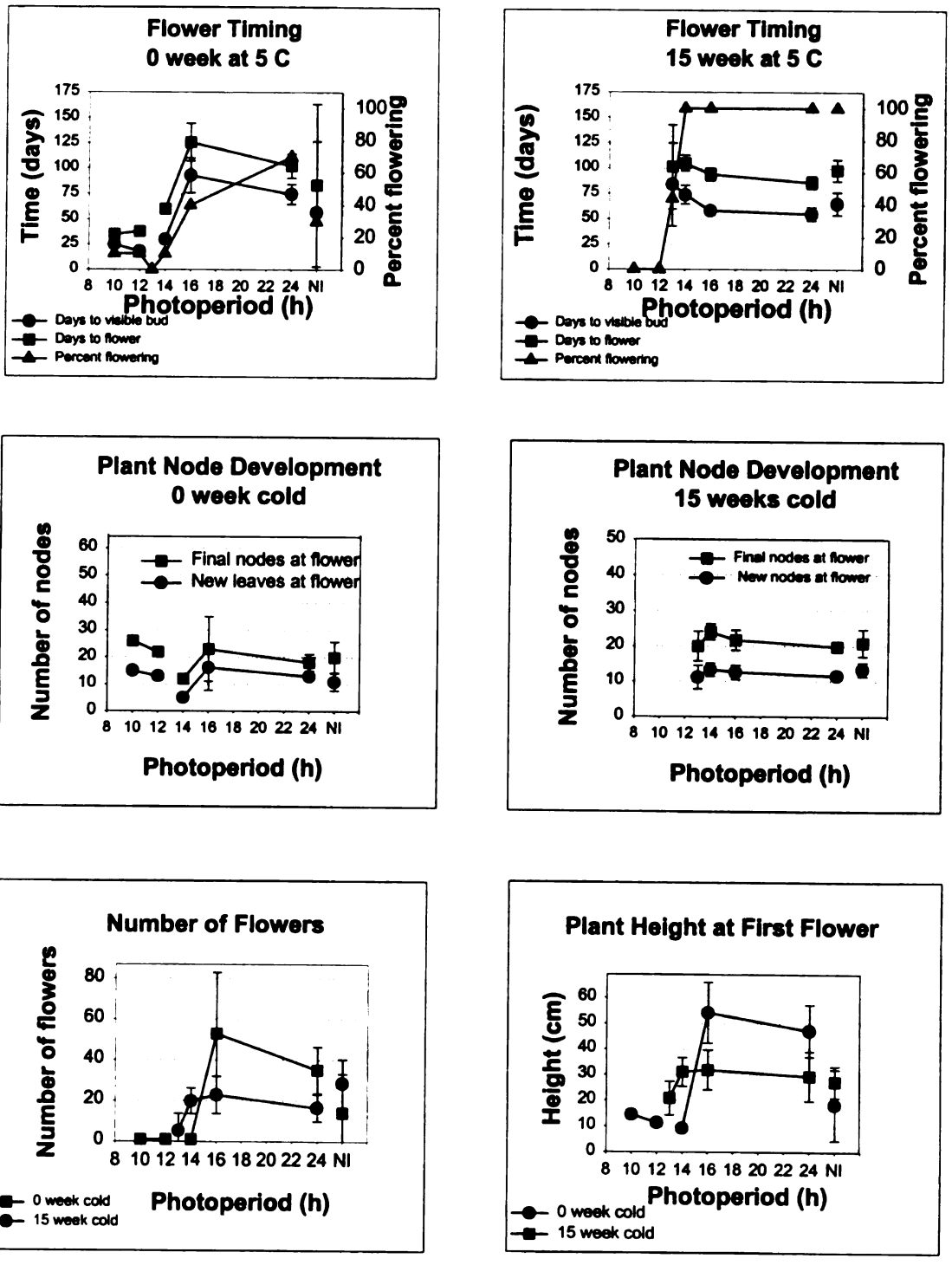


Figure 22. Effects of various photoperiods with and without a 15 week cold treatment at 5 °C in *Anemone hupehensis* in Year 2. Error bars show 95% confidence intervals.

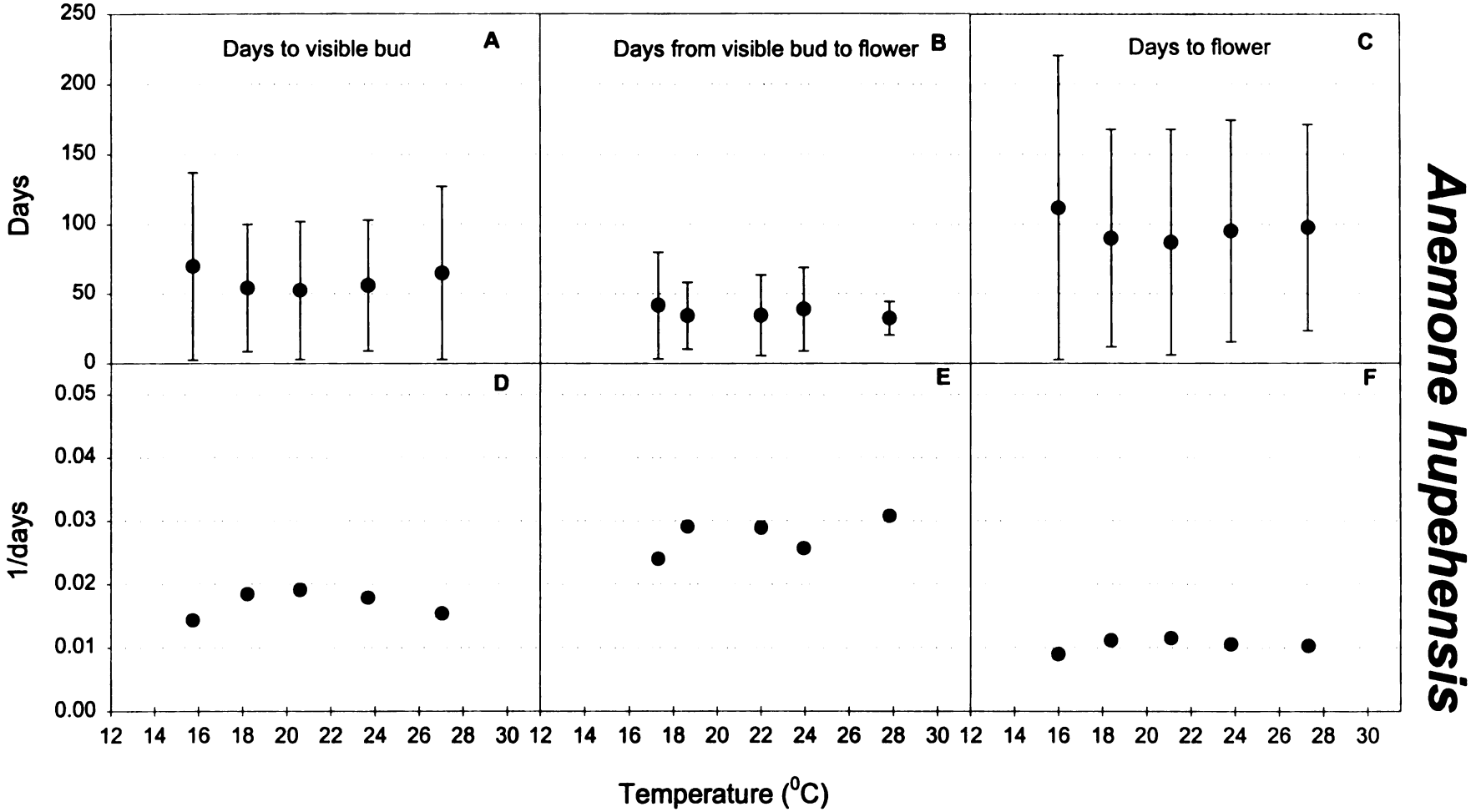


Figure 23. Influence of forcing temperature on flowering time (A,B,C) and rate of progress toward flowering (D,E,F) in *Anemone hupehensis*. Error bars represent 95% confidence intervals.

Anemone hupehensis

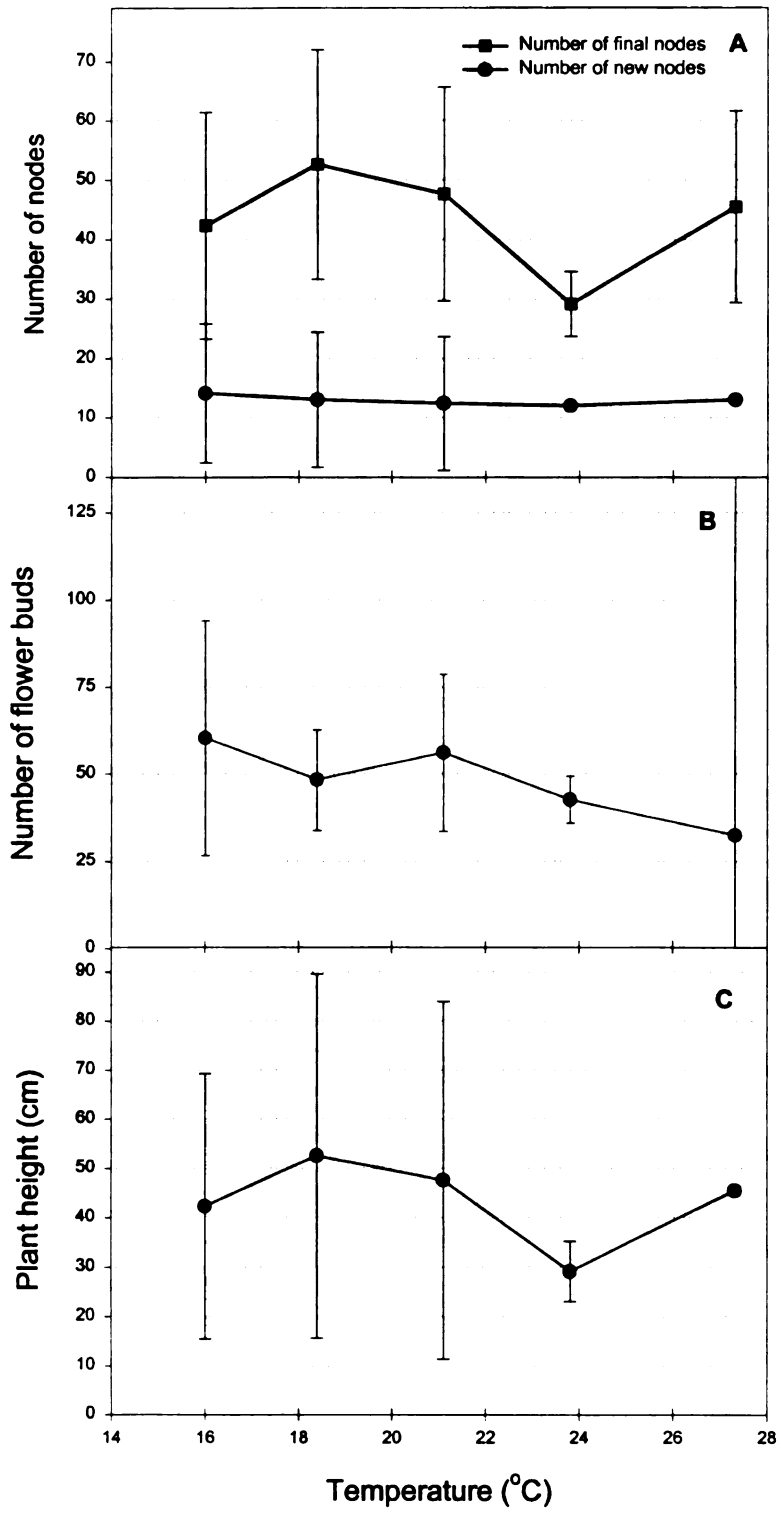


Figure 24. Influence of forcing temperature on number of nodes (A), number of flower buds (B), and plant height at first flower (C) in *Anemone hupehensis*. Error bars represent 95% confidence intervals.

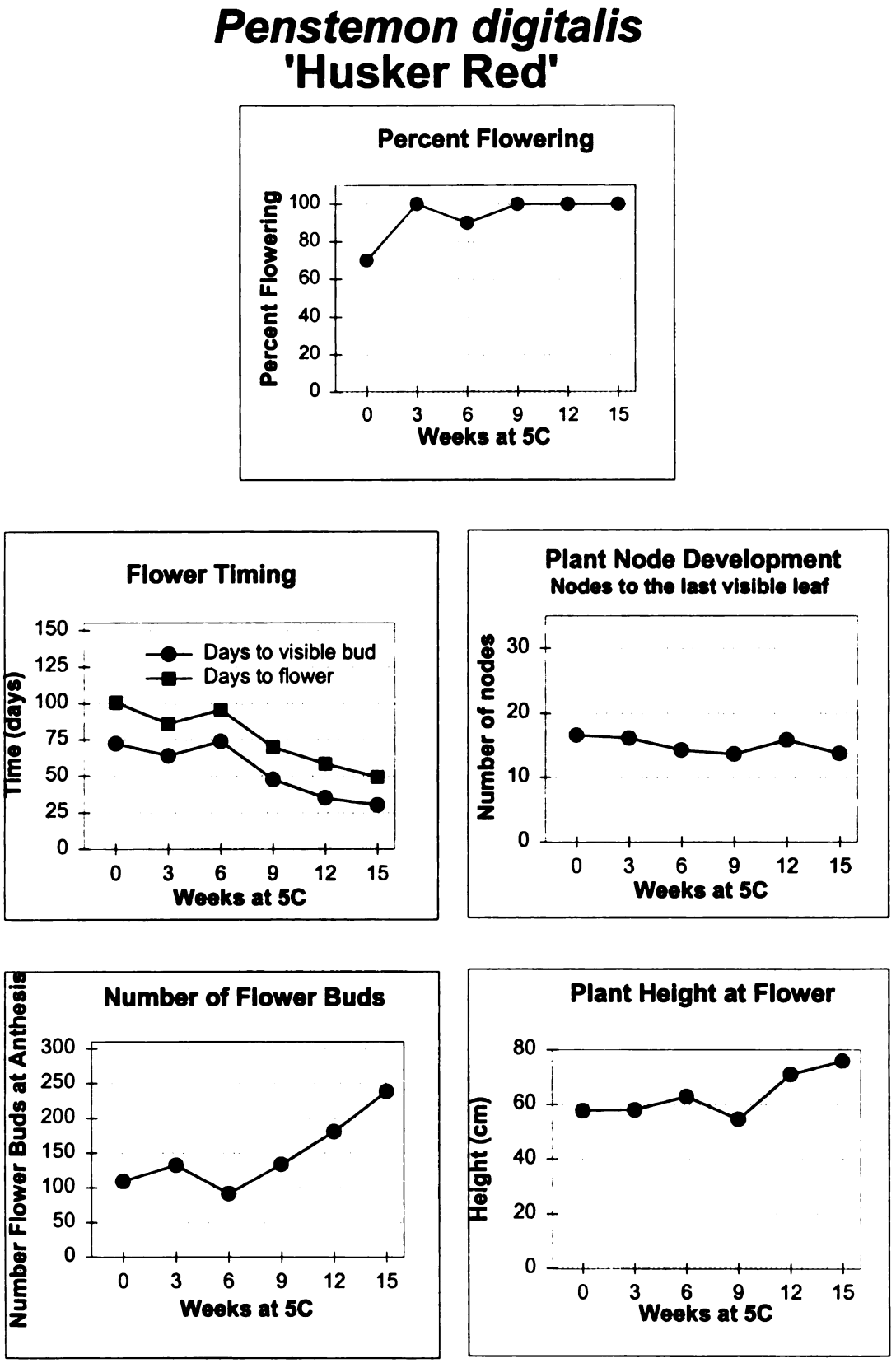
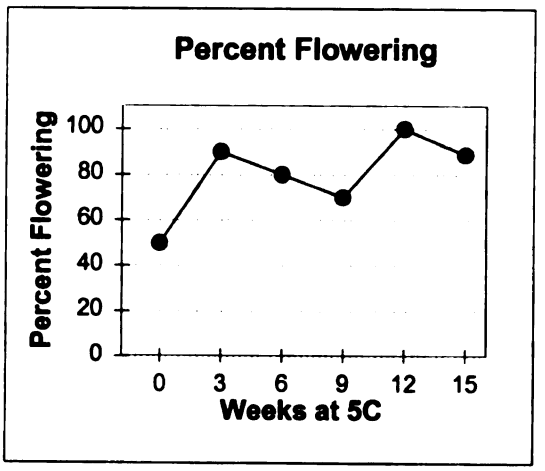
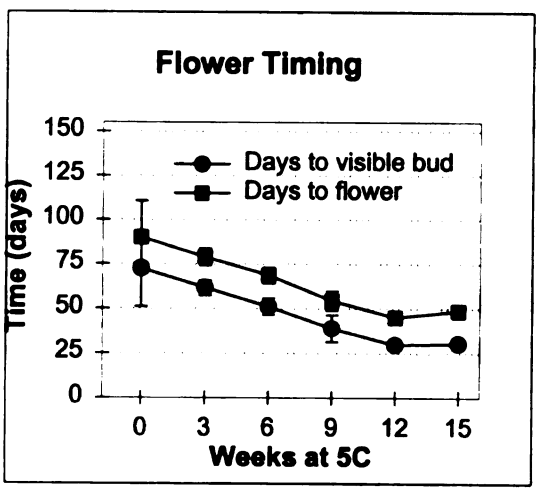
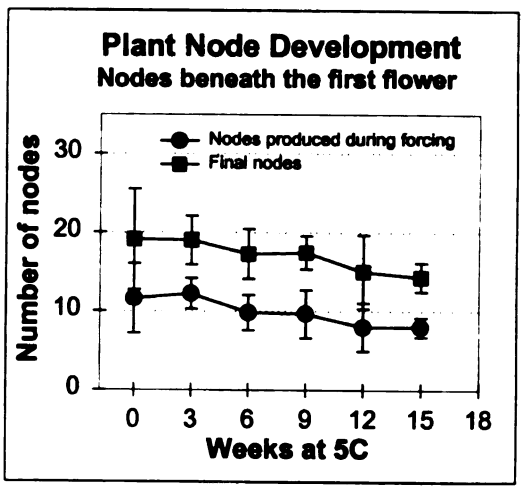


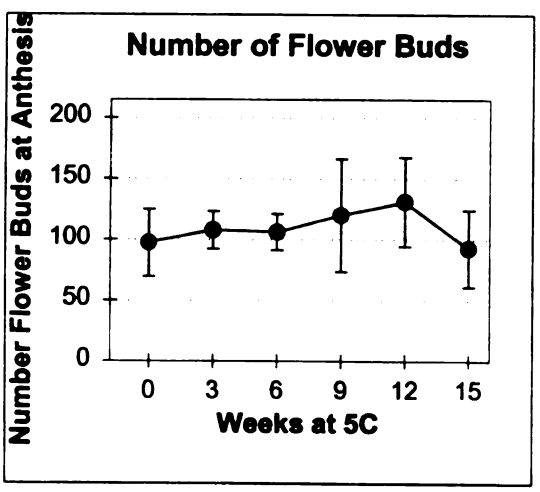
Figure 25. Effects of cold treatment in *Penstemon digitalis* 'Husker Red' in Year 1.

Penstemon digitalis 'Husker Red'









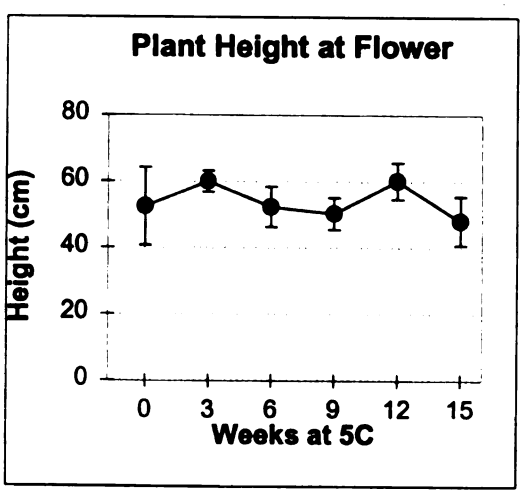


Figure 26. Effects of cold treatment in *Penstemon digitalis* 'Husker Red' in Year 2. Error bars show 95% confidence intervals.

Penstemon digitalis 'Husker Red'

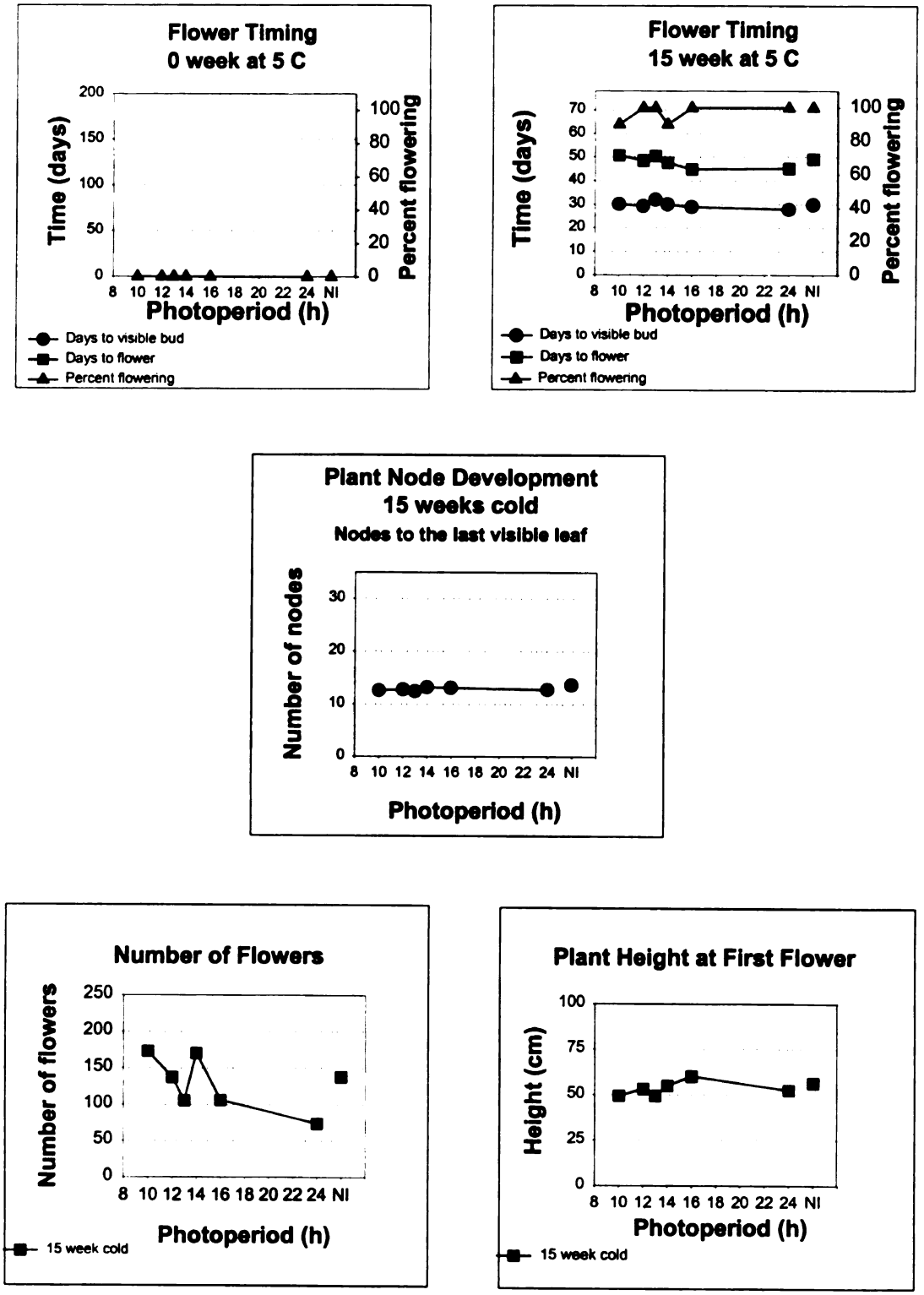


Figure 27. Effects of various photoperiods with and without a 15-week cold treatment at 5 °C in *Penstemon digitalis* 'Husker Red' in Year 1.

Penstemon digitalis 'Husker Red'

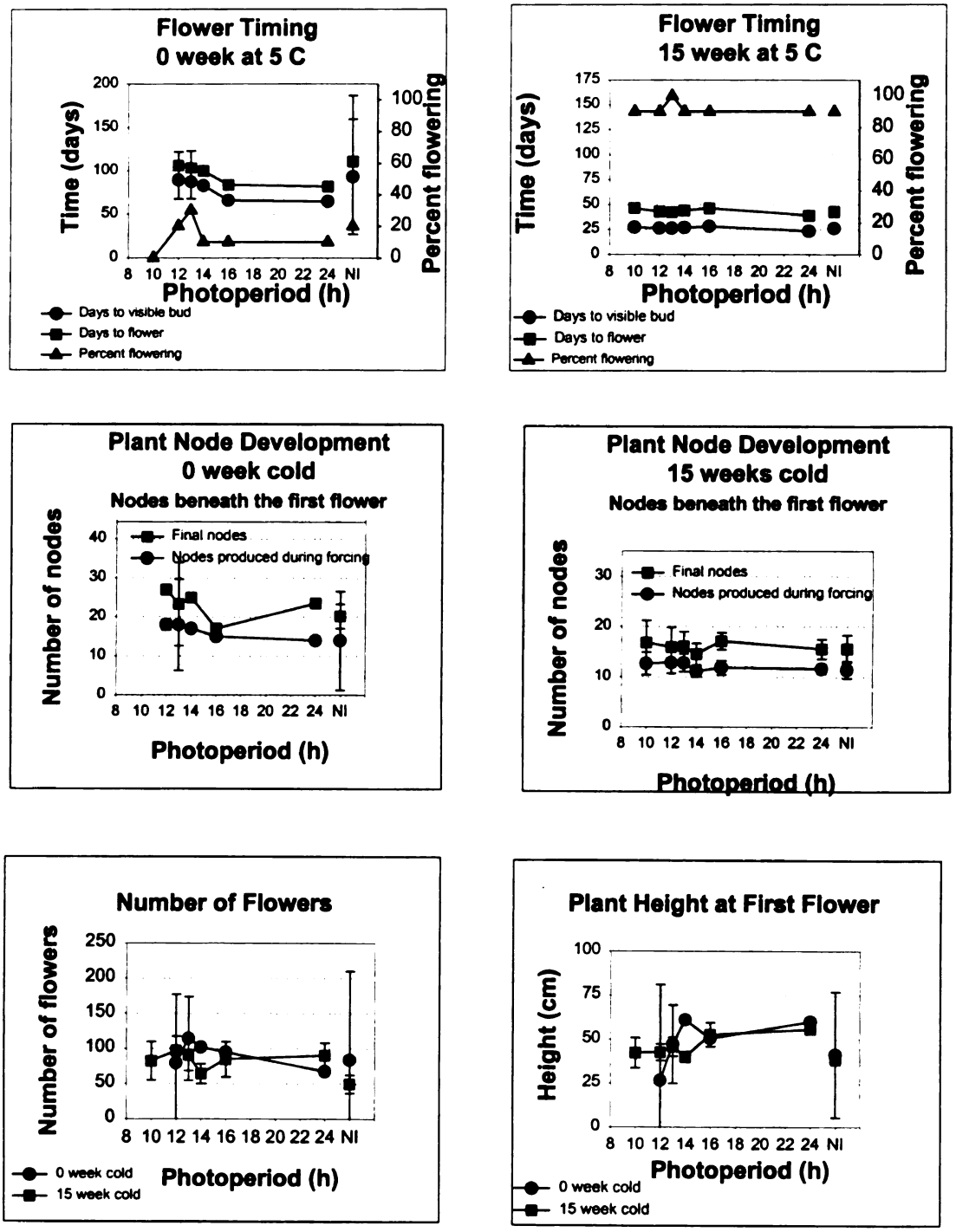


Figure 28. Effects of various photoperiods with and without a 15-week cold treatment at 5 °C in *Penstemon digitalis* 'Husker Red' in Year 2. Error bars show 95% confidence intervals.

Penstemon digitalis
'Husker Red'

Figure 29. Influence of forcing temperature on flowering time (A, B, C) and rate of progress toward flowering (D, E, F) in *Penstemon digitalis* 'Husker Red'. Error bars represent 95% confidence intervals.

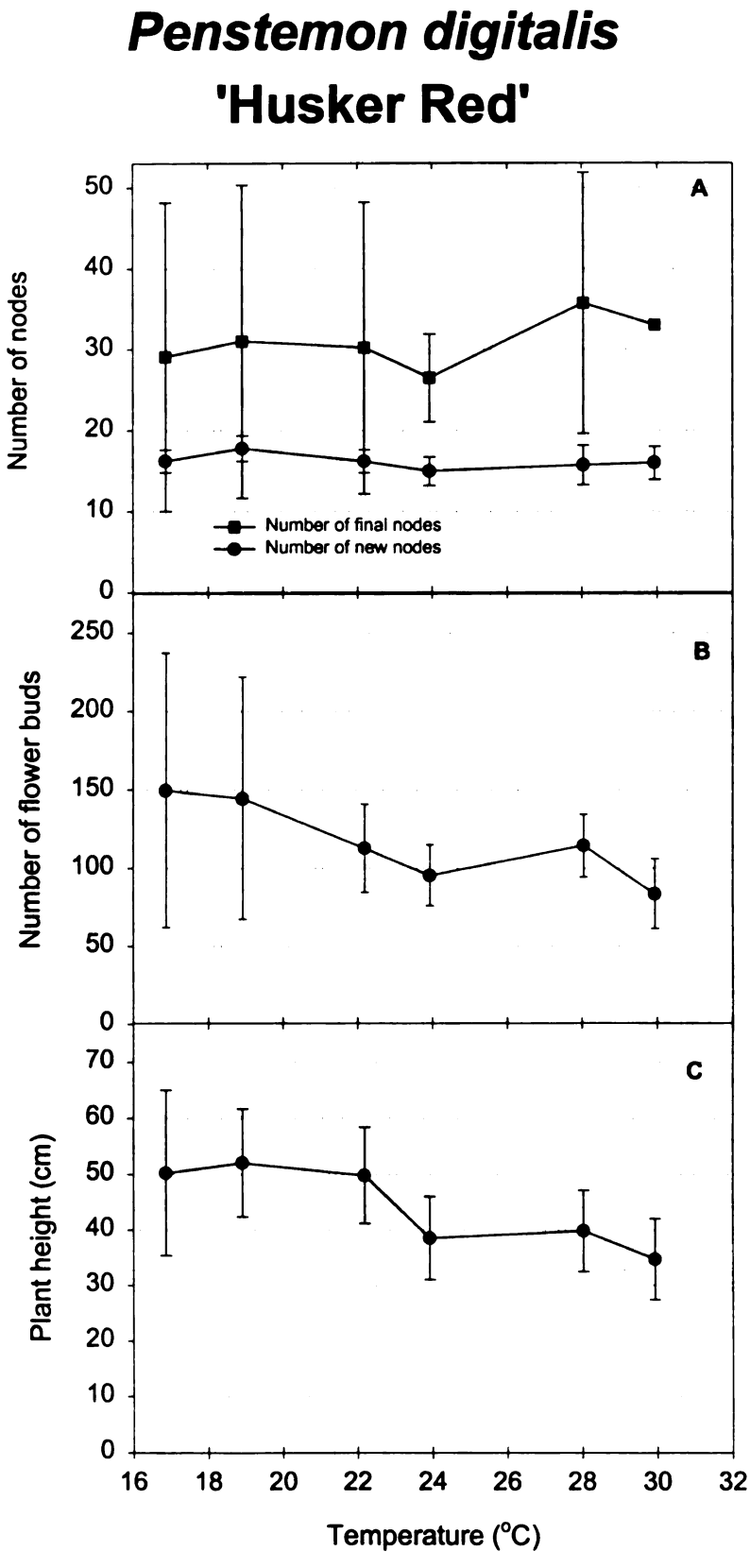


Figure 30. Influence of forcing temperature on number of nodes (A), number of flower buds (B), and plant height at first flower (C) in *Penstemon digitalis* 'Husker Red'. Error bars represent 95% confidence intervals.

Saxifraga arendsii 'Triumph'

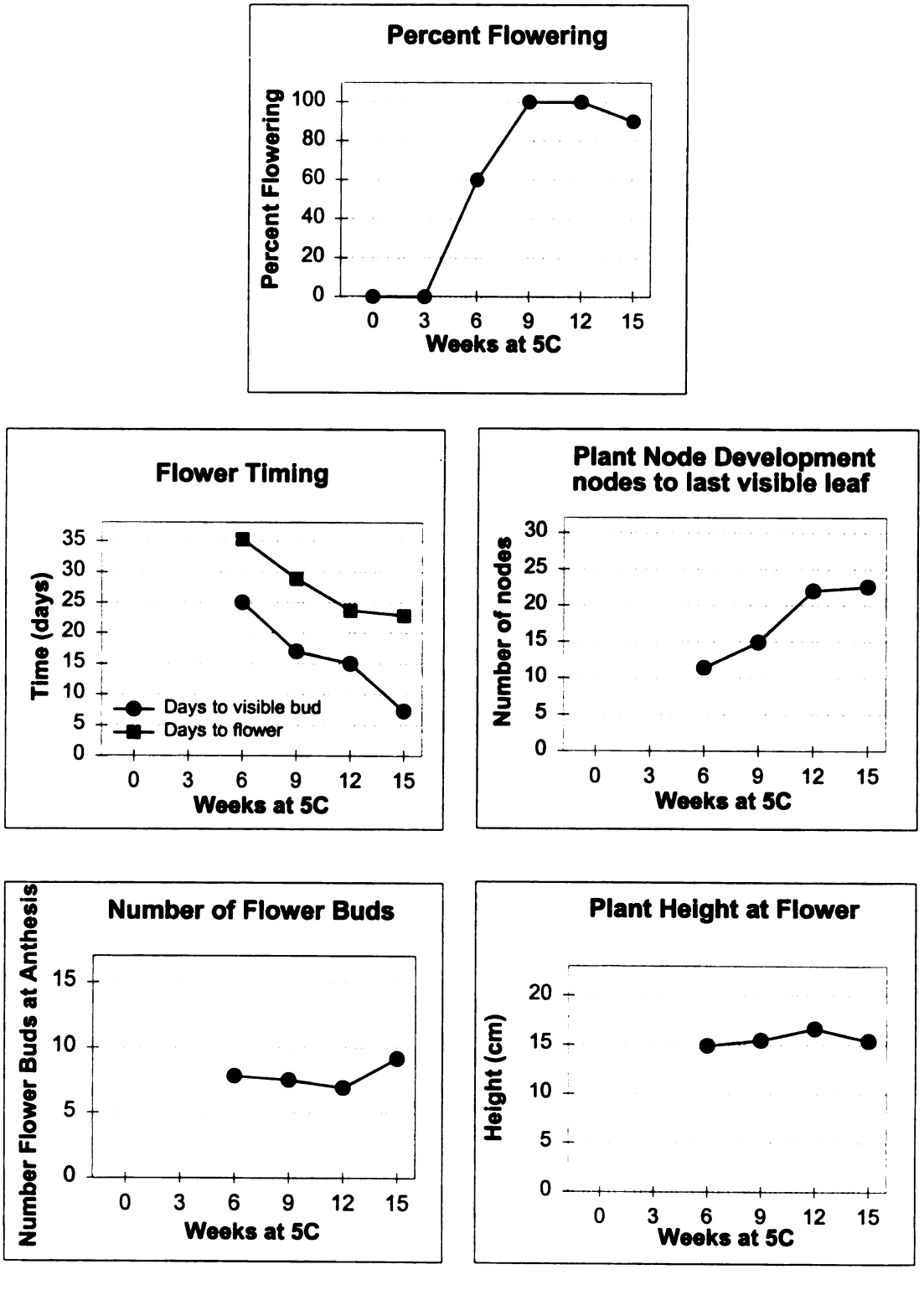


Figure 31. Effects of cold treatment in Saxifraga arendsii 'Triumph' in Year 1.

Saxifraga arendsii 'Triumph'

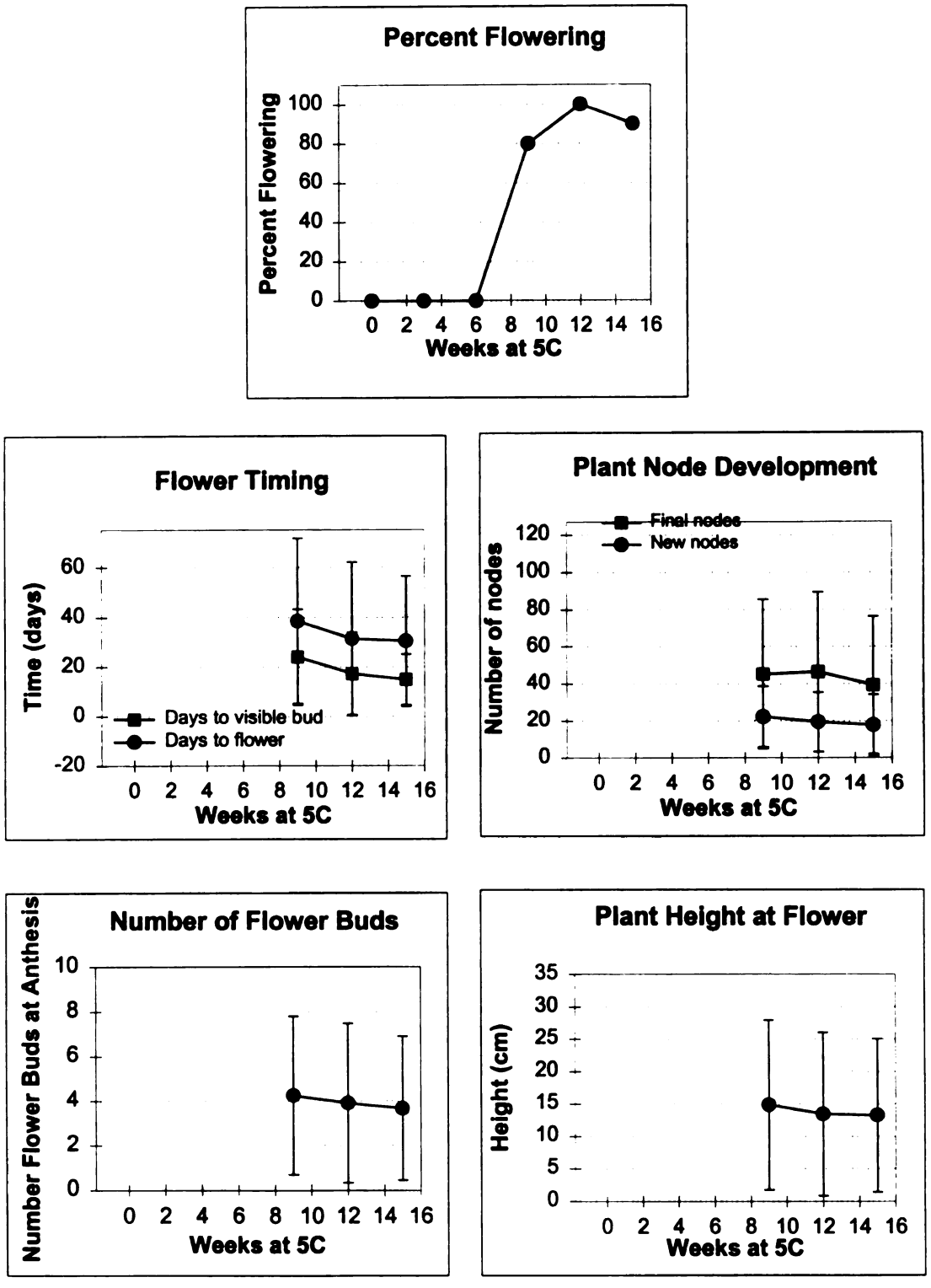
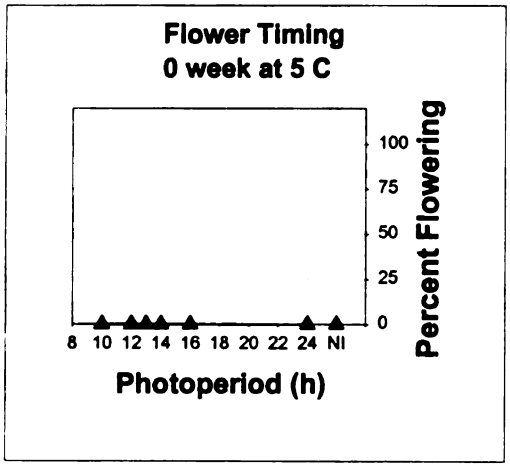
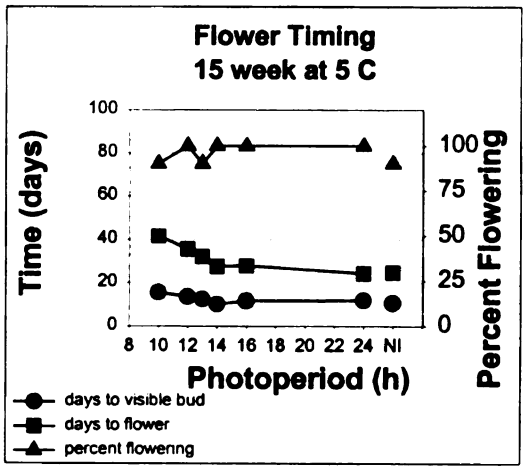
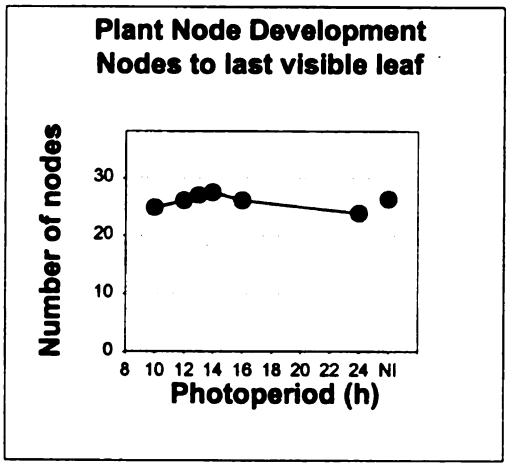


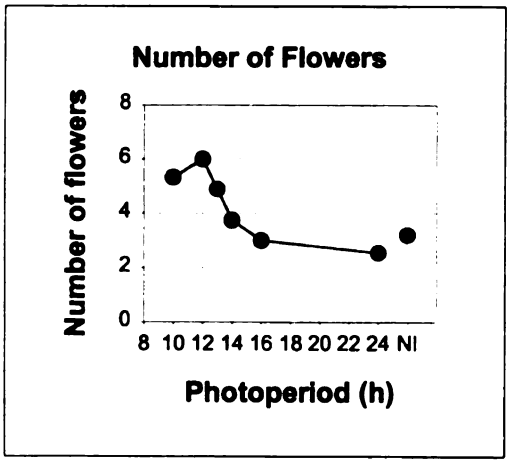
Figure 32. Effects of cold treatment in *Saxifraga arendsii* 'Triumph' in Year 2. Error bars show 95% confidence intervals.

Saxifraga arendsii 'Triumph'









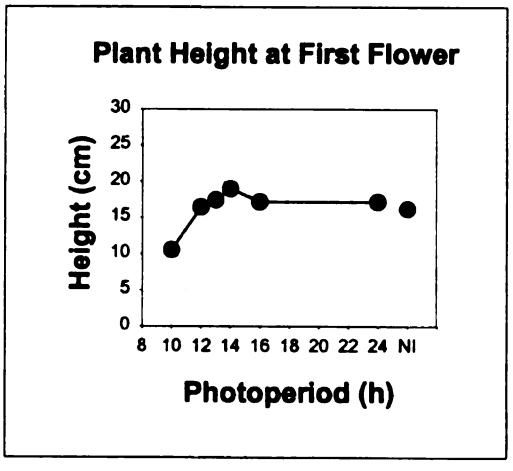
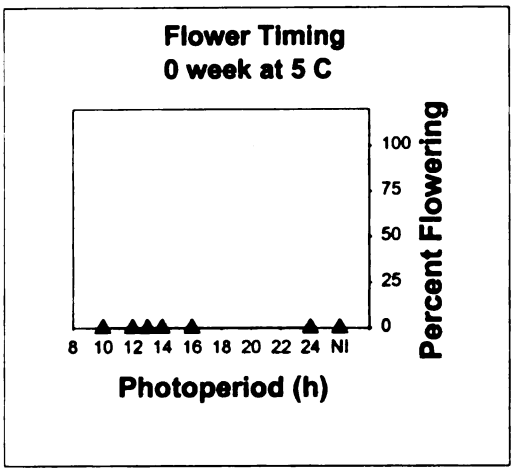
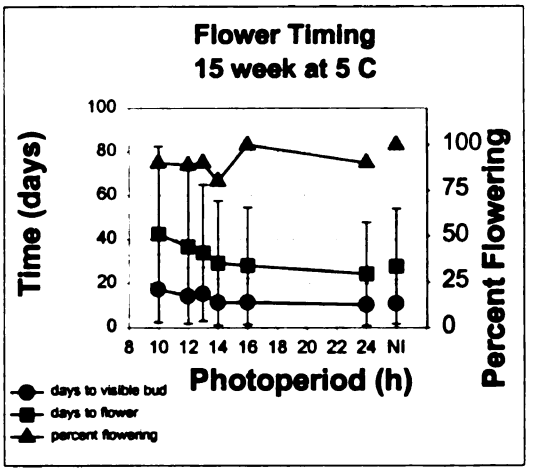
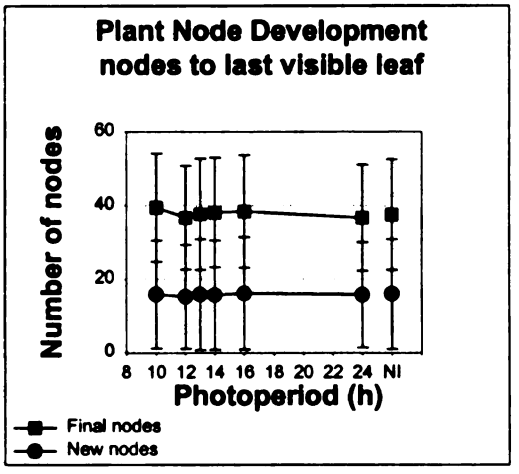


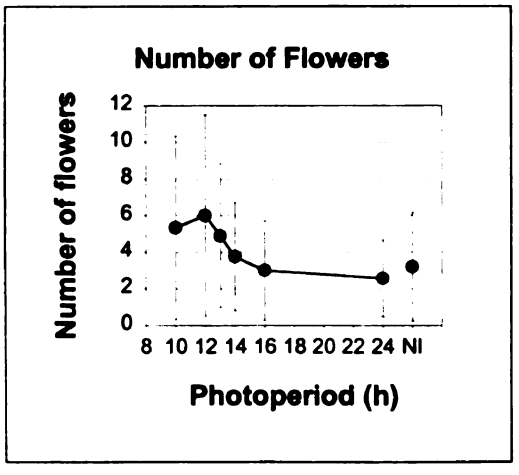
Figure 33. Effects of various photoperiods with and without a cold treatment in in *Saxifraga arendsii* 'Triumph' in Year 1.

Saxifraga arendsii 'Triumph'









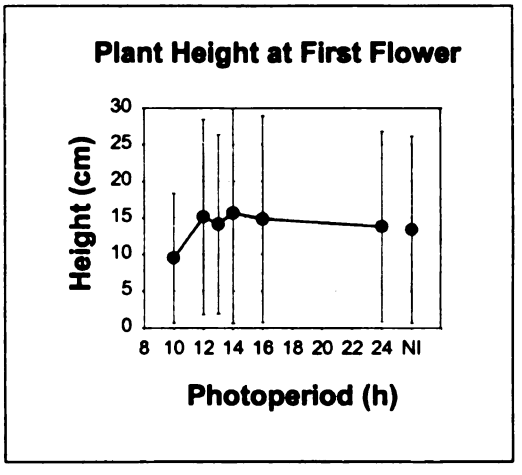


Figure 34. Effects of various photoperiods with and without a cold treatment in in *Saxifraga arendsii* 'Triumph' in Year 2. Error bars show 95% confidence intervals.

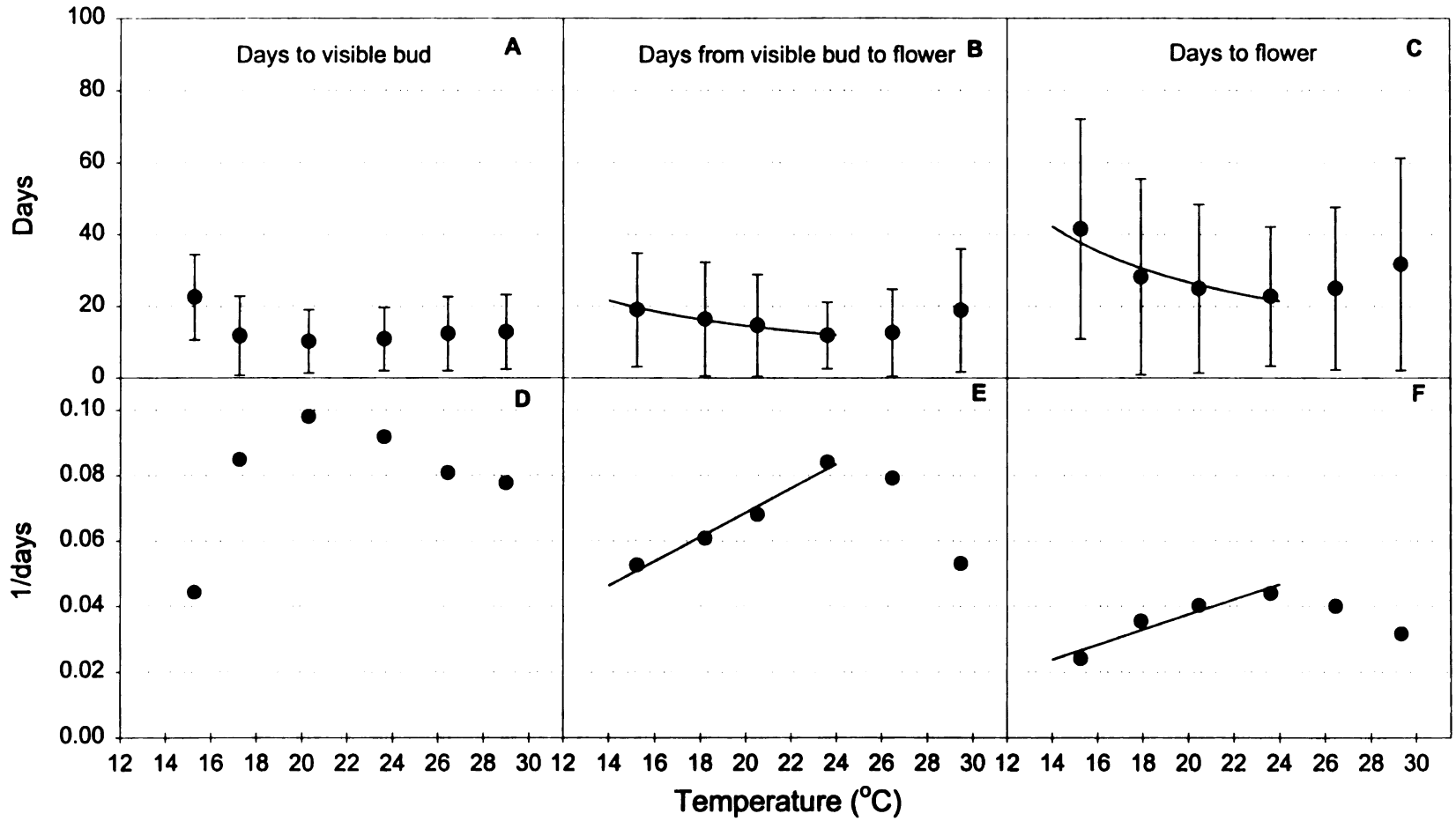


Figure 35. Influence of forcing temperature on flowering time (A, B, C) and rate of progress toward flowering (D, E, F) in *Saxifraga arendsii* 'Triumph'. Error bars represent 95% confidence intervals.

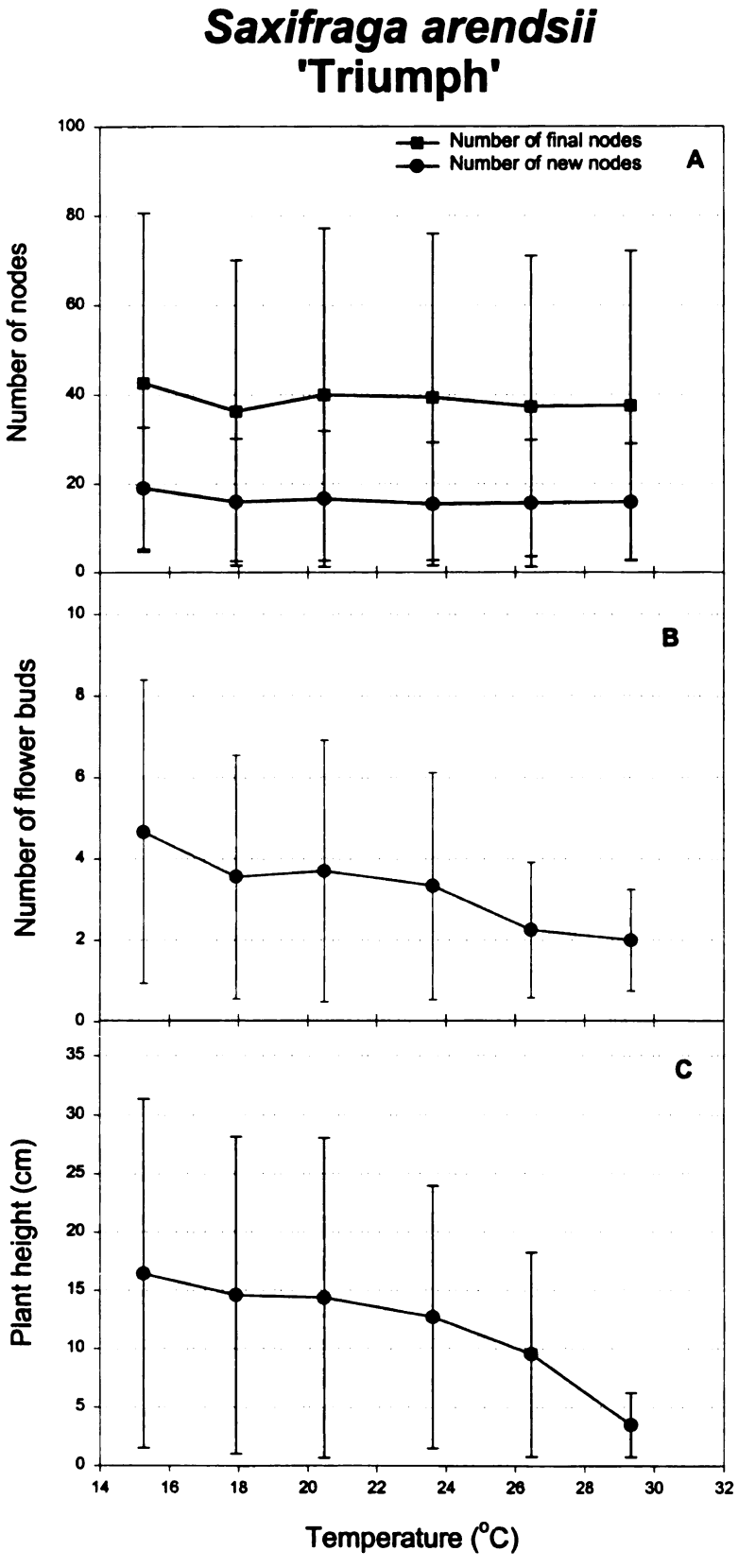


Figure 36. Influence of forcing temperature on number of nodes (A), number of flower buds (B), and plant height at first flower (C) in *Saxifraga arendsii* 'Triumph'. Error bars represent 95% confidence intervals.