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THE INFLUENCE OF VERNALIZATION ON FLOWERING OF *CAMPANULA* 'BIRCH HYBRID' AND *DIANTHUS GRATIANOPOLITANUS* 'BATH'S PINK' AND THE REGULATION OF FLOWERING OF *COREOPSIS GRANDIFLORA* 'SUNRAY' BY VERNALIZATION, PHOTOPERIOD AND LIGHT QUANTITY

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Sonali Ramesh Padhye

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# THE INFLUENCE OF VERNALIZATION ON FLOWERING OF *CAMPANULA* 'BIRCH HYBRID' AND *DIANTHUS GRATIANOPOLITANUS* 'BATH'S PINK' AND THE REGULATION OF FLOWERING OF *COREOPSIS GRANDIFLORA* 'SUNRAY' BY VERNALIZATION, PHOTOPERIOD AND LIGHT QUANTITY

By

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ABSTRACT

THE INFLUENCE OF VERNALIZATION ON FLOWERING OF *CAMPANULA* 'BIRCH HYBRID' AND *DIANTHUS GRATIANOPOLITANUS* 'BATH'S PINK' AND THE REGULATION OF FLOWERING OF *COREOPSIS GRANDIFLORA* 'SUNRAY' BY VERNALIZATION, PHOTOPERIOD AND LIGHT QUANTITY

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Flowering of temperate herbaceous perennials is often regulated by daylength (photoperiod) and low temperature (vernalization) to ensure reproductive success. Greenhouse growers manipulate photoperiod and vernalization to schedule flowering of herbaceous perennials for specific market dates. Our objective was to characterize the influence of vernalization on floral evocation and subsequent flowering characteristics of three herbaceous perennials. We also investigated the effect of photoperiod and photosynthetic daily light integral (DLI) on flowering of *Coreopsis grandiflora* 'Sunray'.

Following vernalization at −2.5 to 20 °C for 0 to 12 weeks, *Campanula* 'Birch Hybrid' exhibited a near-obligate vernalization requirement and all flowering responses studied were affected by the vernalization temperature, duration and their interactions. The minimal and maximal cardinal temperatures for vernalization were <0 °C and between 15 and 17.5 °C, respectively. The optimal vernalization temperatures ($T_{opt}$) depended on the flowering response assessed and ranged between 0 to 12.5 °C for flowering percentage to 5 to 7.5 °C for rate of progress to flowering. Therefore, all relevant flowering responses should be considered when developing and interpreting vernalization models.

*Dianthus gratianopolitanus* 'Bath's Pink' exhibited a facultative vernalization response to 0 to 10 °C and did not vernalize at 15 °C. Complete flowering was achieved following ≥4, ≥3 and 8 weeks at 0, 5 and 10 °C, respectively. Vernalization temperature and duration affected time to anthesis, and number of nodes and flower buds and flowers at anthesis. Based on the minimum durations required to achieve maximum flowering response, the order of efficacy of vernalizing temperature was 5 °C>0°C>>10°C.

*Coreopsis grandiflora* 'Sunray' exhibited a dual induction requirement for floral evocation. The primary induction was fulfilled by either vernalization or short days and secondary induction was stimulated by long days. Following sub-optimal durations of primary induction treatment, vernalization was more effective in promoting floral evocation than short days. DLI influenced flowering percentage, percent reproductive laterals, time to anthesis, number of inflorescences, and plant height at anthesis.

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# Chapter I

# Vernalization: Literature Review

## Vernalization: Literature Review

### Introduction

In the life cycle of a plant, the transition from vegetative to reproductive phases of development can determine the success of flower development, pollination and timing of seed dispersal. Therefore, in most plants, this transition to flowering is tightly regulated by various endogenous and exogenous factors to ensure reproductive success (Amasino, 1996; Amasino, 2005). The endogenous factors affecting flowering consist of developmental age, availability of assimilates and plant hormones (Bernier, 1986). The exogenous factors affecting flowering include environmental cues such as light quality and quantity, stresses such as nutrient deficiency, high temperature and water availability and two primary cues, daylength and vernalization (Fausey, et al., 2001; Mouradov et al., 2002; Simpson and Dean, 2002; Sung and Amasino, 2004a; Yanovsky and Kay, 2003). Together, the factors affecting flowering regulate the flowering time of plants to ascertain the completion of flowering and seed development during favorable environmental conditions (Amasino, 1996).

### History and Definition of Vernalization

It has long been known that winter cereals needed to be planted prior to the end of winter in order to ensure flowering within a year, whereas spring cereals flowered readily after planting in the spring (Chouard, 1960). In 1857, Klippart researched environmental factors responsible for flowering of winter cereals and showed that it was exposure to cold that induced winter cereals to

flower following return to warm temperature (reviewed by Chouard, 1960). In 1898, von Seelhorst and, Gassner, in 1918, extensively studied the winter and spring forms of various species. Gassner discovered the ability of vernalizing imbibed seeds followed by Lysenco's finding that partially imbibed seeds can be vernalized before being mechanically sowed (reviewed by Chouard, 1960). Since spring cereals are called yarovoe in Russian (Chouard, 1960), Lysenco coined the term "yarovizatzya" in Russian to describe conversion of winter cereals into spring cereals by cold (Thomas and Vince-Prue, 1984). Vernum means spring in Latin (Chouard, 1960; Lang 1965). Therefore, vernalization, the Latin translation of "yarovizatzya" essentially means "springization" or conversion of winter annuals into spring annuals (Salisbury and Ross, 1992). The term vernalization has since commonly been used in scientific literature.

Vernalization has been defined as "the process whereby flowering is promoted by a cold treatment given to a fully hydrated seed or to a growing plant" (Amasino, 2002). Additionally, the low temperature treatment intended to induce or promote flowering is also referred to as vernalization treatment (Napp-Zinn, 1987). Vernalization promotes floral evocation, though the actual development of floral primordia generally occurs only following return to higher temperatures (Vince-Prue, 1975). Thus, during vernalization there is a temporal separation between the perception of low temperature and the flowering response. For several woody plants, including lilac, exposure to low temperature promotes appearance of flowers by breaking dormancy. Hence, in these woody plants the role of low temperature is promotion of growth in the pre-formed buds and not the

promotion of floral evocation, indicating that vernalization and dormancy are two distinct phenomena (Chouard, 1960). Interestingly, vernalization and dormancy share some common physiological characteristics such as the effective treatment temperatures and durations, non-transmissibility of the perceived signal, site of low temperature perception and the suggested requisite of mitotic activity (Metzger, 1996). Based on above similarities between vernalization and dormancy, Metzger (1996) postulated that vernalization and dormancy may have common molecular mechanisms, however at present there is no supporting molecular evidence available (Sung and Amasino, 2005).

**Ecological Significance of Vernalization**

The process of sexual reproduction in plants consisting of flowering and seed set is energy-expensive (Sachs, 1987) and therefore, its scheduling in appropriate environmental conditions is essential for the survival of plants. A vernalization requirement imposes that plants remain vegetative in autumn and winter and flower only during the spring or summer following exposure to cold for an adequate duration (Simpson and Dean, 2002). Thus, a vernalization requirement is ecologically advantageous to plants native to the temperate climates with severe winters since it facilitates programming flowering during favorable environmental conditions by "perceiving" seasons (Sung and Amasino, 2004a) and enhances the probability of completion of the plant life cycle and seed set (Sheldon et al., 2000a; Henderson et al., 2003). The life cycle of annuals ends following flowering and seed set therefore, the timing of flowering

in annuals is especially important, making vernalization requirement of particular significance in the life cycle of annuals (Amasino, 2003).

*Arabidopsis thaliana* (L.) Heynh. ecotypes are native to diverse geographical conditions and exhibit natural variation in flowering time and vernalization responses. Therefore, *Arabidopsis* ecotypes are excellent candidates to study the correlation between their geographical origins and responsiveness to vernalization. Following evaluation of 40 *Arabidopsis* ecotypes, Johanson et al. (2000) reported that most late flowering ecotypes that responded to vernalization were from the northern latitudes while most early flowering ecotypes that did not respond to vernalization originated in central and eastern Europe where winters are comparatively mild. However, Johanson et al. (2000) did not find a correlation between flowering time and mean temperatures or altitude in the ecotypes studied. Although favorable seasonal cycles may have had a significant impact on evolution of mechanisms regulating flowering time such as vernalization, variable flowering times may also be advantageous in other environments including locations with severe winters preventing seed germination, those that support multiple generations in a year (Johanson et al. 2000), locations where plant populations may be affected by agricultural activities such as harvest or tillage or succession or environmental stresses such as drought (Mitchel-Olds, 1996). Therefore, evolution of vernalization requirement probably depended not only on the geographical origin, but different microenvironments may also have had a great impact on its evolution, suggesting the complexity of the evolutionary process (Johanson et al., 2000).

A vernalization requirement is absolute in crops such as *Beta vulgaris* L. (sugar beet); without vernalization, plants remain vegetative for years (Salisbury and Ross, 1992). In contrast, when clonally propagated vernalization-requiring herbaceous perennial species such as *Oenothera fruticosa* L. 'Youngii-lapsley' (narrowleaf evening-primrose) are grown without vernalization for a prolonged period of time, a few plants in the population do flower (Clough et al., 2001). This non-uniform vernalization requirement among the plant population may be advantageous since at least a few plants will complete flowering in the absence of a vernalization treatment.

**Relationship Between Vernalization and Acclimation**

Cold acclimation is another plant response observed following exposure to low temperature. Cold acclimation occurs following exposure to cold but non-freezing temperatures and leads to freezing tolerance in plants of temperate origins (Thomashow, 1999). Freezing tolerance is essential for survival of plants where ambient temperatures are below freezing in the winter. Due to common occurrences of sudden decreases in ambient temperatures in autumn and winter in temperate climates, it is imperative that plants acclimate to cold quickly. On the contrary, longer durations of low temperature exposure are needed to meet vernalization requirement presumably since flowering in response to a transient decrease in temperatures would negate the ecological benefit of having a vernalization requirement (reviewed by Sung and Amasino, 2005). *Raphanus sativus* L. 'Chinese Radish Jumbo Scarlet' (radish) is an exception to

vernalization in response to long exposures to thermoinductive temperatures as it vernalizes following 8 d exposure to 6 °C (Erwin et al., 2002). *Arabidopsis* on the other hand vernalizes in response to longer durations at thermoinductive temperatures. The overall differences in the time of initiation and development of cold acclimation and vernalization response following exposure to low temperature in *Arabidopsis* are illustrated in Fig. 1.

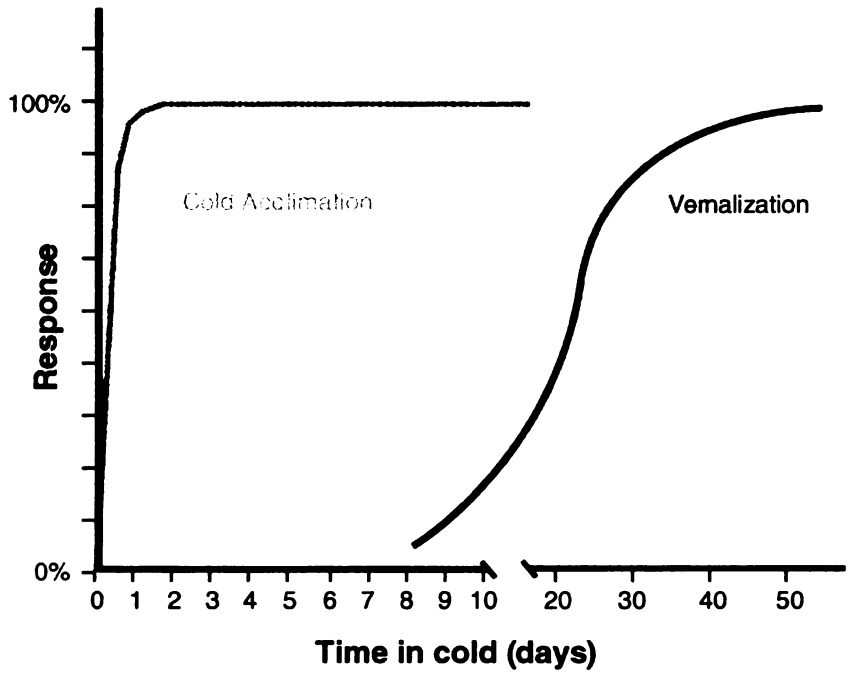


Figure 1. Typical time-course of initiation and development of cold acclimation and vernalization responses in *Arabidopsis thaliana*. Cold acclimation, leading to freezing tolerance occurs within days after exposure to low temperature whereas vernalization requires prolong exposure to low temperature (from Sung and Amasino, 2005).

The molecular mechanism involved in induction of cold acclimation in *Arabidopsis* involves a cascade of cold-induced transcription regulators known as C-repeat binding factors (CBFs), resulting in the induction of proteins that provide

protection from cold (Thomashow, 1999). In addition, phytohormone abscisic acid (ABA) has been known to be associated with cold acclimation responses. The vernalization response of *Arabidopsis* was not affected following overexpression of *CBF*, impairing ABA biosynthesis by mutagenesis or elevation of ABA levels by exogenous application or water stress (Liu et al., 2002). Hence, cold acclimation and vernalization responses are mediated through separate pathways in *Arabidopsis*. However, it is likely that the two pathways share some common components at upstream level, particularly in early perception of cold (reviewed by Sung and Amasino, 2005).

**Vernalization Response Types**

Based on their vernalization responses, plants can be grouped into obligate (qualitative) and facultative (quantitative) response categories (Lang, 1965). Plants with an obligate vernalization response acquire the ability to flower following exposure to vernalization treatment. For example, *Campanula* 'Birch Hybrid' plants did not flower without exposure to cold and therefore, their flowering response was categorized as obligate (Enfield, 2002; Niu et al., 2004). In plants exhibiting a facultative vernalization response, vernalization accelerates flowering and/or improves flowering characteristics including increasing the percentage of plants flowering in the population, synchronization of flowering and increasing flower number. For example, flowering of *Osteospermum ecklonis* (DC.) Nor. (Vanstaden's river daisy) plants was hastened and synchronized following vernalization treatment and vernalized plants had more buds than non-

vernalized plants (Suzuki and Metzger, 2001). It is noteworthy that often plants are categorized as obligate vernalization responsive based on them remaining vegetative for a certain period of growing time following vernalization treatment. Additional growth at warm temperatures may result in flowering of some plants in the non-vernalized population, indicating that botanically, the vernalization response of such species is facultative. However, horticulturally such flowering response may still be considered obligate (Runkle et al., 2001). Clough et al. (2001) categorized the vernalization requirement of *Oenothera fruticosa* 'Youngii-lapsley' as horticulturally obligate, since, in this species, flowering was rare (~0.6%) and delayed without a vernalization treatment.

**Flowering in Response to Vernalization and Photoperiod.** Similar to vernalization, plant responses to photoperiod can also be categorized as obligate and facultative and many plants have a dual regulation of flowering based on their response to vernalization and photoperiod (Vince-Prue, 1975). Typically, plants responding to both vernalization and photoperiod treatment are long day (LD) plants however, *Chrysanthemum xmorifolium* Ramat. (florist's daisy) is an exception and has been known to respond to vernalization and short day (SD) photoperiods (Vince-Prue, 1975). Categorization of plants based on their flowering responses to vernalization and LD photoperiod is presented in Table 1.

Table 1. Flower induction categories based on plant responses to vernalization and long day photoperiod (adapted from Padhye et al., 2005).

| **Long day photoperiod response** | **Vernalization response** | | |
|---|---|---|---|
| | **None** | **Facultative** | **Obligate** |
| **None** | Vernalization treatment or LD photoperiod do not affect flowering. | Vernalization treatment is not required for flowering but improves flowering characteristics such as synchronization of flowering, reducing time to flower and/or improves plant quality characteristics such as number of flowers and number of lateral shoots produced. Plants are day-neutral after vernalization. | Vernalization treatment is required for flowering. Plants are day-neutral after vernalization. |
| **Facultative** | Vernalization treatment is not required for flowering. Flowering characteristics are improved by LD photoperiod. | Both vernalization treatment and LD photoperiod are not required for flowering but improve flowering characteristics. | Vernalization treatment is absolutely required for flowering. LD photoperiod improves flowering characteristics. |
| **Obligate** | Vernalization is not required for flowering. LD photoperiod is required for flowering. | Vernalization treatment is not required for flowering but improves flowering characteristics. LD photoperiod is absolutely required for flowering. | Vernalization treatment and LD photoperiod are absolutely required for flowering |

Following vernalization, the photoperiodic response of some species is altered. For example, non-vernalized *Leucanthemum* x*superbum* (Bergmans ex J. W. Ingram) D. H. Kent 'Snowcap' (Shasta daisy) and *Lobelia* x*speciosa* 'Compliment Scarlet' (hybrid cardinal flower) required LD for flowering however, following vernalization the plants responded facultatively to LD (Runkle et al., 1998; Runkle, 1999a). In addition, vernalization can alter the critical photoperiod for flowering as reported in the case of *Rudbeckia fulgida* Ait. 'Goldstrum' (orange

coneflower). In this *Rudbeckia* cultivar, following vernalization treatment, the critical photoperiod shifted from 14 to 13 h (Runkle et al., 1999b). In *Arabidopsis*, the photoperiod pathway is distinct from the vernalization pathway and both these pathways integrate downstream at genes involved in induction of floral evocation. However, this does not explain the mechanisms involved in altering the photoperiodic response following vernalization reported in many herbaceous perennials.

## Perception of Vernalization

**Prerequisites for Vernalization.** Wellensiek (1962, 1964) illustrated that vernalized growing leaves and roots of *Lunaria annua* L. (moneyplant) regenerated into flowering shoots whereas, leaves fully grown prior to vernalization treatment regenerated into vegetative shoots. Therefore, it was inferred that mitotic activity is a prerequisite for vernalization (Wellensiek, 1964). Contrasting results were reported by Metzger (1988) when similar regeneration experiments were performed with *Thlaspi arvense* L. (field pennycress). Mature leaves excised from vernalized *Thlaspi* plants regenerated in flowering plants, thereby questioning the prerequisite of mitotic division for the perception of vernalization. Metzger (1988) postulated that mitotic activity may not be required for perceiving vernalization but it may be necessary for the perpetuation of the vernalized state. Although the possibility of localized mitosis existed in fully expanded *Thlaspi* leaves, it was later postulated that DNA replication may still occur in those leaves (Michaels and Amasino, 2000). Since DNA replication

often plays a role in reprogramming gene expression, Michaels and Amasino (2000) concluded that DNA replication, rather than cell division, is required for vernalization to occur.

Juvenility is the early developmental phase reported in various species during which plants are unable to respond to favorable exogenous factors promoting flowering such as photoperiod and vernalization (Thomas and Vince-Prue, 1984). Hence, overcoming juvenility is a prerequisite for perceiving vernalization treatment in such species. In species such as *Hedera helix* L. (English ivy) the juvenile and adult phase is easily distinguishable due to differences in leaf morphology and arrangement (Amasino, 2002). However, in many herbaceous perennial species such as *Coreopsis grandiflora* Hogg ex Sweet 'Sunray' (largeflower tickseed), the number of leaves or nodes unfolded is typically used to determine the end of juvenile period due to the lack of visual indicators. Juvenility of *Coreopsis grandiflora* 'Sunray' seedlings apparently ends with the formation of 8 nodes (16 leaves) and, subsequently, plants perceive vernalization treatment (Yuan et al., 1998).

**Localization of Vernalization.** Lang (1965) reviewed following localized cooling and grafting experiments performed to determine the primary site of perception of vernalization. When various plant parts were cooled locally, vernalization occurred when buds, embryos and excised meristems were specifically cooled. Additionally, grafting various parts of vernalized plants onto non-vernalized plants revealed that flowering occurred only when vernalized apical tissue was grafted on an non-vernalized plant. Additionally, excision of

vernalized apical tissue and replacing it with apical tissue from a non-vernalized plant did not promote flowering. Taken together, these results suggest that shoot apical tissue, presumably the meristem, was the primary site of perception of thermoinductive temperatures during vernalization. However, in these studies, it was impossible to differentiate if the vernalization response occurred at the shoot apical meristem (SAM), young leaves, or both (Searle et al., 2006). Determining the site of vernalization was further complicated by reports that young leaves of *Lunaria annua* (Wellensiek 1964) and mature leaves of *Thlaspi arvense* could be vernalized (Metzger, 1988). Consequently, depending on the species, the localization of vernalization is in the SAM and/or leaves. Based on this conclusion, the regulation of vernalization may involve the ability of SAM to respond to floral stimulus and/or the ability of leaves to generate a floral stimulus (McDanitel, 1985 cited by Searle et al., 2006).

**Thermosensory Pathway.** Exposure to low temperatures results in many biochemical changes including changes in plasma membrane fluidity, modification of membrane lipid composition, rearrangement of microfilaments, $Ca^{2+}$ fluxes and cascades of protein phosphorylation (reviewed by Sung and Amasino, 2005). Evidence indicates that $Ca^{2+}$ fluxes and cascades of protein phosphorylation play a role in activation of cold acclimation response in plants (Thomashow, 2001). As discussed earlier, cold acclimation and vernalization pathways are distinct in *Arabidopsis*. The only possible molecular link between these two pathways is *HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES*1 (*HOS1*), which seems to be the upstream repressor of genes involved

in cold acclimation and vernalization pathway (Lee et al., 2001). However, the components and mechanism of thermosensory pathway of *Arabidopsis* are yet to be determined.

**Time-keeping Mechanism.** In addition to temperature sensing, vernalization also involves a time-keeping mechanism that measures the duration of exposure to thermoinductive temperatures during vernalization. Based on their functions, the thermosensory pathway and time-keeping mechanism probably share some common features. Although the molecular components involved in this time-keeping mechanism are still unknown, recent investigation of quantitative regulation of *Arabidopsis* vernalization genes have provided some insight about this mechanism that will be discussed later.

## Effective Thermoinductive Temperatures

The efficacy of vernalization treatment depends on temperatures and insufficient vernalization can result in incomplete flowering. The thermoinductive temperatures can be described as cardinal temperatures for vernalization with minimum ($T_{min}$), maximum ($T_{max}$) and optimum ($T_{opt}$) values (Atherton et al., 1990; Yan and Hunt, 1999). $T_{min}$ and $T_{max}$ describe the upper and lower thresholds at which the rate of progress to vernalization is zero (Atherton et al., 1990). $T_{min}$ and $T_{max}$ are species-specific and range between >−4 °C and <17 °C, respectively (Lang, 1965). $T_{opt}$ describes the range of optimum thermoinductive temperatures at which maximum vernalization response is achieved (Atherton et al., 1990). Salisbury and Ross (1992) reported that the $T_{opt}$ of Secale cereale L.

'Petkus' (winter rye) is about 0 to 10 °C following 6-week vernalization treatment, however it appears that based on highest relative flowering, $T_{opt}$ of *Secale cereale* 'Petkus' is between 0 to 8 °C (Fig. 2).

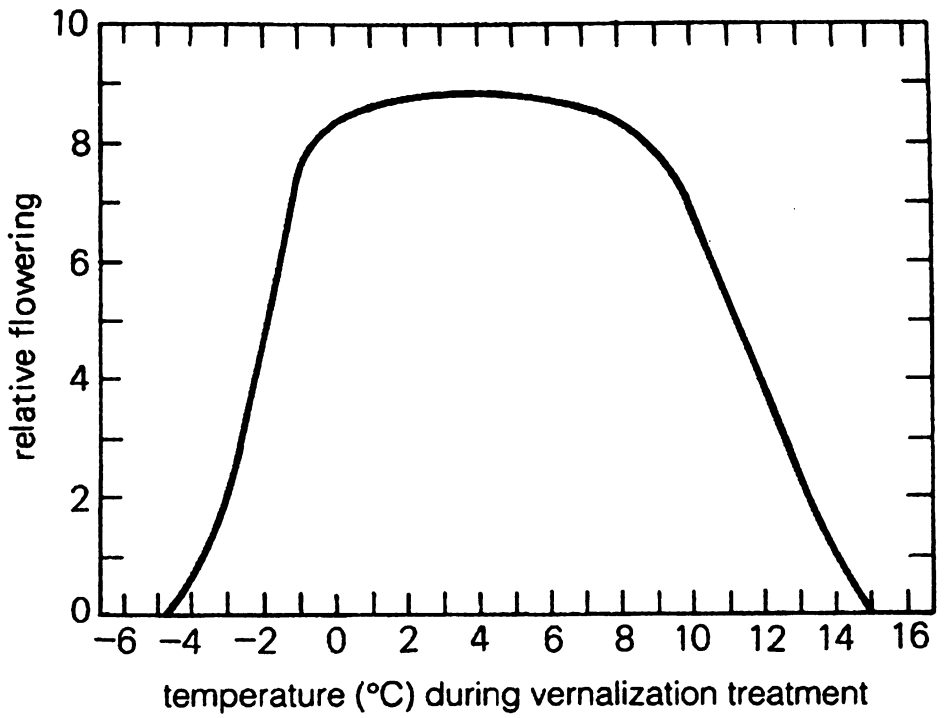


Figure 2. $T_{opt}$ of *Secale cereale* 'Petkus' as a function of vernalization temperature provided to imbibed seeds for 6 weeks (from Salisbury and Ross, 1992).

Similar to $T_{min}$ and $T_{max}$, $T_{opt}$ is also species-specific. For example, the $T_{opt}$ resulting in the greatest flowering response of *Veronica spicata* L. 'Red Fox' (spiked speedwell) and *Isotoma axillaris* Lindl. (blue star) were −2.5 °C and 5 to 10 °C, respectively (Fausey, 2005). $T_{opt}$ is relative and even within a species, it depends on the duration of the vernalization treatment. For example, *Veronica spicata* 'Red Fox' flowered completely following 4 weeks at −2.5 to 0 °C however,

after 6 weeks, the effective thermoinductive temperature range broadened to −2.5 to 2.5 °C, and further to −2.5 to 7.5 °C after 8 weeks (Fausey, 2005). Additionally, by definition, $T_{opt}$ is also based on the vernalization response being assessed (Rawson et al., 1998). For example, when vernalization response was assessed as minimum days from sowing to heading, the $T_{opt}$ for *Triticum aestivum* L. 'Dollarbird' (common wheat) was 19 °C. However, when vernalization response was assessed as the minimum thermal time from sowing to heading above the base temperature of 0 °C, $T_{opt}$ of the same wheat cultivar was 6 °C (Rawson et al., 1998).

During vernalization, the exposure to thermoinductive temperatures and observed flowering response are temporally separated and hence, there is no other indicator of completion of the vernalization process besides the subsequent flowering. Since insufficient vernalization due to the use of non-inductive temperatures or short durations can elicit incomplete flowering, empirical studies investigating the effective vernalization temperatures and durations are essential to ensure successful flowering. $T_{opt}$ for various flowering responses has been empirically determined in field crops including *Triticum aestivum*, *Daucus carota* L. (carrot) and *Allium cepa* L. (onion; Brooking I.R., 1996; Robertson et al., 1996; Yan and Hunt, 1999; Streck, 2003) and select potted flowering plants including *Cineraria sp.* and *Lilium longiflorum* Thunb. (Easter lily; Lange and Heins, 1990; Yeh et al., 1997a; Streck and Schuh, 2005).

**Quantitative Nature of Vernalization Response**

As discussed earlier, the duration of exposure to thermoinductive temperatures affects the intensity of the flowering response and increasing the duration of vernalization treatment increases the flowering response until saturation (Lang, 1965). This quantitative nature of vernalization has been documented in plants exhibiting obligate and facultative vernalization responses and has been demonstrated by investigating various flowering parameters including percentage of flowering plants in a population, node or leaf development until flowering, time to flower (Fig. 3), number of flowers and reproductive laterals (Fausey, 2005; Lang,1965; Lange and Heins, 1990).

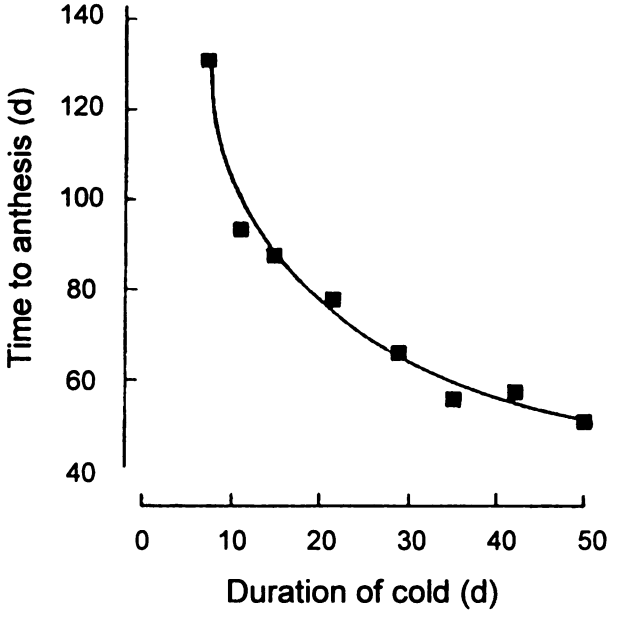


Figure 3. The quantitative vernalization response of Petkus winter rye assessed by measuring time to flower computed after the end of thermoinductive treatments (adapted from Vince-Prue, 1975).

Typically, at a constant thermoinductive temperature, the optimum duration of vernalization treatment changes based on the response being assessed. For example, 4-week treatment at −2.5 °C was sufficient for achieving 100% flowering of *Veronica spicata* 'Red Fox' however, longer durations at −2.5 °C increased the number of reproductive laterals, improving the ornamental value at flowering. Therefore, the choice of appropriate duration of vernalization treatment should be selected to maximize all flowering responses of significance.

**Memory of Vernalization**

Once achieved, the vernalized state is maintained for a prolonged period of time until conditions favorable for flowering arise (Lang, 1965). Thus, a cellular "memory" of the winter or exposure to prolonged cold is maintained through several mitotic divisions until environmental conditions are favorable. Meiosis resets the vernalization requirement in annuals and biennials, requiring progeny of vernalized plants to go through vernalization again to attain the capacity to flower (Michaels and Amasino, 2000). Thus, the vernalized state is epigenetically regulated. Perennials require vernalization to flower in the next season without going through meiosis, hence probably have a distinct vernalization resetting mechanism. Vince-Prue (1975) proposed that the vernalization requirement may be reset in perennials by devernalization (discussed below) of some buds during summer, by the inability of some buds to vernalize during vernalization treatment, by vernalization not being an autocatalytic process, or by a combination of all these processes.

**Devernalization**

The complete or partial reversion of vernalization is termed as devernalization and exposure to high temperatures following vernalization has a devernalizing effect that increases with the temperature and duration of the exposure (Lang, 1965). Devernalization has been documented in many crops including *Lactuca serriola* (wild lettuce; Marks and Prince, 1979), *Daucus carota* (Hiller and Kelly, 1979), *Brassica oleracea* var. *botrytis* (cauliflower; Fujime and Hirose, 1980), *Brassica oleracea* var. *gongylodes* (kohlrabi; Wiebe et al., 1992), *Apium graveolens* var. *dulce* (celery; Booi and Meurs, 1993), *Allium fistulosum* (Japanese bunching onion; Yamasaki et al., 2000) and *Cineraria sp.*, a potted flowering crop (Yeh et al., 1997b). The ranges of vernalizing, neutral (having neither vernalizing nor devernalizing effect) and devernalizing temperatures can be fairly close to one another and are species-specific (Lang, 1965). Attaining saturation of vernalization typically renders the high temperature devernalization ineffective (Lang, 1965). Additionally, exposure to neutral temperatures after non-saturating vernalization treatment and prior to high temperature exposure prevents devernalization. This effect is known as stabilization of vernalization and has been demonstrated in *Hyoscyamus niger* L. (henbane), *Secale cereale* 'Petkus' and *Arabidopsis* (reviewed by Lang, 1965). In some species certain conditions are either essential for devernalization or to maximize the devernalizing effect including particular stage of development, preventing seed germination by water supply restriction and low light intensity or absence of light during high temperature exposure (Lang, 1965). For example, *Lactuca serriola*

plants can not be devernalized but seeds can be devernalized by exposure to 25 °C only prior to germination and in the absence of light (Marks and Prince, 1979).

**Substitution of Vernalization Requirement**

In some species, the vernalization requirement can be completely or partially substituted by exposure to SD or LD photoperiods. SD can substitute for vernalization requirement in some LD plants, hence such species can be considered as short-long-day plants or vernalization requiring LD plants (Vince-Prue, 1975). Based on experiments investigating the effect of inductive treatments of SD or vernalization prior to exposure to LD in *Campanula medium* and *Silene armeria* L. (sweet-William catchfly), it was postulated that these inductive treatments operated through different pathways and resulted in the same outcome (reviewed by Vince-Prue, 1975). However, Dubcovsky et al. (2006) reported that in winter wheat, which flowers in response to SD or vernalization followed by LD, exposure to SD downregulated vernalization responsive flowering inhibitor, *VRN2* and flowering promoter *VRN1* was upregulated only after exposure to LD. Therefore, the mechanism of substitution of vernalization by SD involves regulation of vernalization responsive genes.

Ketellapper and Barbaro (1966) reported that *Coreopsis grandiflora* 'Single Mayfield Giant' could be induced to flower either by vernalization or SD treatment followed by LD. In this study, vernalization was more effective than SD in inducing more plants in the population to flower and flowering was faster after vernalization than SD treatment. Damann and Lyons (1993) reported that

*Coreopsis grandiflora* 'Sunray' did not flower in response to 10-week SD treatment (9-h photoperiod) followed by LD however, Runkle et al. (unpublished data) found that 100% *Coreopsis grandiflora* 'Sunray' flowered following the same treatment. Exposure to LD can partially substitute for vernalization requirement in Easter lily. In the field, Easter lily bulbs receive some vernalization prior to harvest and exposure to LD can substitute for partial vernalization (Rees, 1987). High daily light integral also partially substituted for vernalization in *Salvia* x*superba* Stapf 'Blaukonigin' (perennial sage; Waaseth et al., 2006).

**Vernalization in *Arabidopsis***

*Arabidopsis thaliana* has been used as a model system to study flowering and in recent years, significant progress has been made in understanding the genetic and molecular basis of flowering in *Arabidopsis*. In *Arabidopsis*, four pathways involved in transition of the vegetative SAM to reproductive meristem have been identified. These pathways include vernalization pathway, autonomous or constitutive pathway, photoperiod pathway and GA-dependant pathway (Simpson et al, 1999) (Fig. 4). Flowering of *Arabidopsis* is promoted by exposure to vernalization and LD in a facultative manner (Napp-Zinn, 1969). The vernalization pathway and photoperiod pathway integrate the external stimuli to coordinate flowering with favorable environmental conditions. In contrast, the autonomous pathway and GA-dependant pathway act independent of the environmental conditions. There is some redundancy and cross-talk between all

the flowering pathways ensuring success in flowering under diverse environmental and developmental conditions (Amasino, 1996).

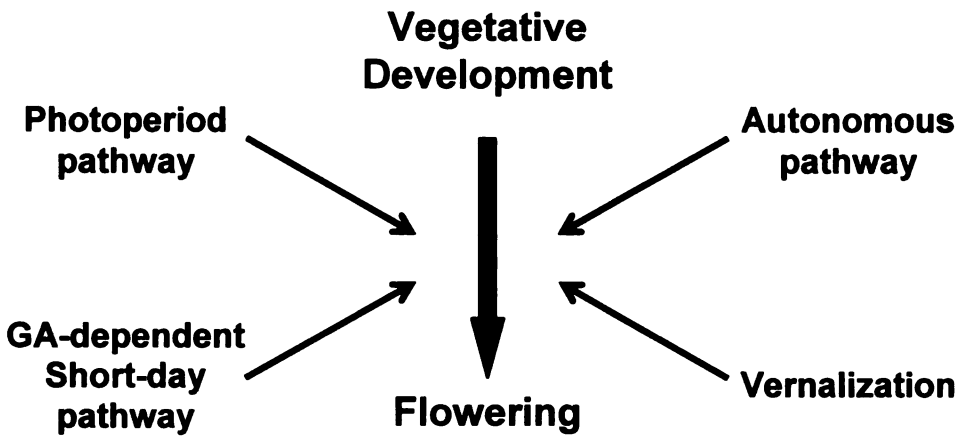


Figure 4. Pathways regulating transition from vegetative development to flowering in *Arabidopsis thaliana* (from Michaels and Amasino, 2000).

As mentioned earlier, *Arabidopsis* has a wide geographic distribution resulting in natural variation in flowering time. Based on the natural variation in vernalization requirements, *Arabidopsis* ecotypes can be grouped into two categories: early flowering ecotypes that behave as summer annuals and late flowering ecotypes that behave as winter annuals. Vernalization has no effect on flowering of the early flowering ecotypes whereas, vernalization accelerates flowering of the late flowering ecotypes (Fig. 5). This natural diversity in the vernalization responsiveness of *Arabidopsis* ecotypes has been exploited in many studies to understand the genetic and molecular basis of the vernalization response. Studying *Arabidopsis* mutants with altered vernalization response has been another common approach used to identify key regulatory genes involved in vernalization. Extensive studies with early and late flowering ecotypes and vernalization mutants have resulted in identification and characterization of

*FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*), two key genes involved in *Arabidopsis* vernalization pathway.

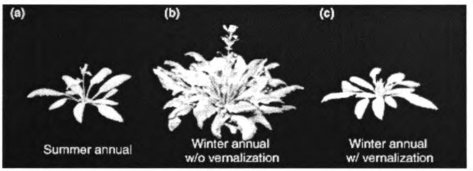


Figure 5. Natural variability in flowering time of *Arabidopsis thaliana* ecotypes. (a) Summer annual types are naturally early flowering and do not respond to vernalization treatment, (b) flowering of winter annual types is delayed without vernalization treatment, (c) winter annual types flower rapidly similar to summer annuals following vernalization treatment (from Sung and Amasino, 2004a).

***FRIGIDA* (*FRI*).** Napp-Zinn (1955) first described the *FRIGIDA* (*FRI*) locus responsible for delayed flowering in late-flowering *Arabidopsis* ecotypes by analyzing the progeny of early-flowering and late-flowering ecotypes (cited by Johanson, 2000) and further studies showed that *FRI* is a dominant gene (Napp-Zinn, 1987). Cloning of *FRI* revealed that a single copy of *FRI* is present in *Arabidopsis* and the predicted FRI protein is not homologous to any known protein (Johanson, 2000). Johanson et al. (2000) identified three lesions in *FRI* by studying 40 *Arabidopsis* ecotypes with varying flowering responses and the presence of at least one *FRI* lesion was the primary basis for the spring annual flowering response in the evaluated ecotypes. The study by Johanson et al. (2000) also showed that the lesions in *FRI* occurred as at least two independent

evolutionary events, indicating a strong selection against vernalization in some environments.

***FLOWERING LOCUS C* (*FLC*).** Segregation analysis revealed the requirement of a dominant allele on chromosome 5 for late flowering phenotype of dominant *FRI* and this locus was named *FLOWERING LOCUS C* (*FLC*) (Koornneef et al., 1994). *FLC* expression has been detected in leaves, apical tissue and roots of *Arabidopsis* (Michaels and Amasino, 1999). *FLC* is a dominant MADS box transcription factor responsible for repression of flowering in the absence of vernalization in late flowering *Arabidopsis* ecotypes (Michaels and Amasino, 1999; Sheldon et al., 1999). *FLC* is upregulated in vernalization-responsive mutants and *FLC* levels mostly remain unchanged in vernalization-nonresponsive mutants (Sheldon et al., 1999). *FRI* increases *FLC* mRNA levels in late flowering *Arabidopsis* lines and following vernalization, *FLC* mRNA levels are undetectable. This downregulation of *FLC* correlates with elimination of the late flowering phenotype (Michaels and Amasino, 1999). *FLC* acts downstream of *FRI* (Michaels and Amasino, 2001) and vernalization eliminates the *FRI*-mediated elevation of *FLC* expression, resulting in early flowering phenotype. *FRI* and *FLC* regulate the vernalization response in *Arabidopsis* by acting synergistically, delaying flowering in late flowering ecotypes in the absence of vernalization and a loss of function mutation in *FRI* or *FLC* results in early flowering (Michaels and Amasino, 1999).

**Dose-dependant response of *FLC*.** In non-vernalized ecotypes or mutants of *Arabidopsis*, vernalization response depends on the levels of *FLC*

transcripts and the duration of cold required to saturate vernalization response is proportional to the extent of downregulation of *FLC* transcripts (Sheldon et al., 2000b). Michaels and Amasino (2000) reported that by adding additional copies of *FLC*, late flowering *Arabidopsis* containing *FRI* can be converted into a true biennial that does not flower without a vernalization treatment. Thus, *FLC* acts in a dose-dependent (rheostat-like) manner to delay flowering in the absence of vernalization (Fig. 6) (Michaels and Amasino, 2000). Therefore, differences in molecular mechanism may not be required for the regulation of obligate versus facultative vernalization response, only different levels of flowering repressor can be adequate (Sung and Amasino, 2005).

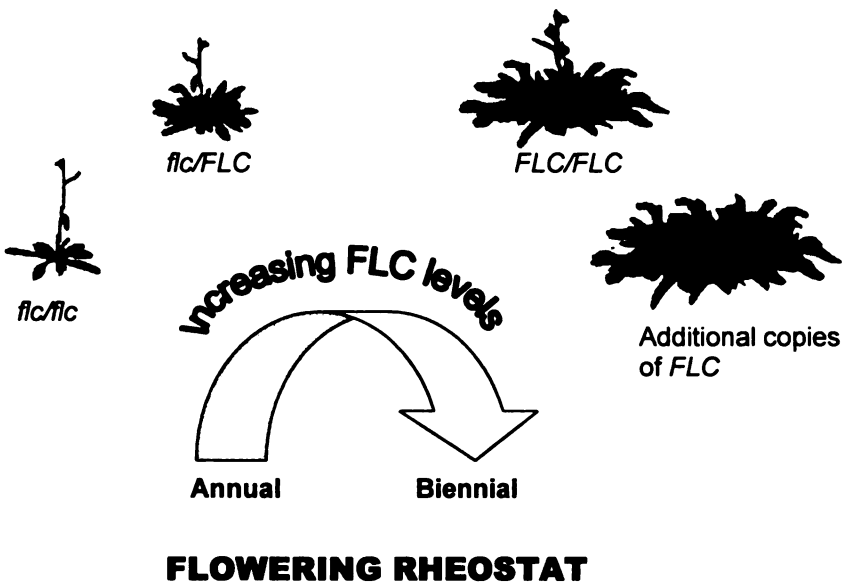


Figure 6. Dose-dependent (rheostat-like) response of *FLC* in delaying flowering in *Arabidopsis thaliana*. In *FRI* background, flowering is delayed based on the number of *FLC* copies and transgenic plants with additional *FLC* copies behave as biennials in absence of vernalization treatment (from Michaels and Amasino, 2000).

**Role of *FLC* in Quantitative Nature of Vernalization Response.** As discussed earlier, vernalization is a quantitative process and with increasing durations at thermoinductive temperatures, vernalization response increases until saturation. In *Arabidopsis*, there exists a quantitative relationship between the duration of vernalization treatment, flowering time and *FLC* transcript levels, indicating that *FLC* plays a key role in quantitative vernalization response (Sheldon et al., 2000b). Until recently, it was unclear whether the degree of *FLC* downregulation resulting in quantitative vernalization response was due to the extent of initial *FLC* repression or the degree of maintenance of *FLC* repression during the subsequent plant development. Recently, Sheldon et al. (2006) reported that following vernalization treatment, the extent of initial repression of *FLC* depends on the duration of exposure to thermoinductive temperatures.

**Genes Regulating *FLC* Repression.** Mutagenesis identified early flowering and vernalization insensitive mutants *vernalization1* (*vrn1*) and *vernalization2* (*vrn2*) (Chandler, 1996). *VRN1* encodes a DNA-binding protein with B3 domains (Levy et al., 2002) and *VRN2* encodes a zinc finger protein homologous to *Drosophila* polycomb group (PcG) protein, Su(Z)12 (Gendall et al., 2001). Since in PcG mutants of *Drosophila*, gene repression occurs but fails to be maintained, it was proposed that VRN2 played a similar role in *Arabidopsis* and was not responsible for repressing *FLC* during vernalization treatment but was involved in maintaining the repressed state of *FLC* after the plants were returned to warmer temperatures (Gendall et al., 2001). Levy et al. (2002) suggested that similar to VRN2, the role of VRN1 was to maintain the repressed

state of *FLC*. However, recently Sheldon et al. (2006) showed that both VRN1 and VRN2 activities are needed for maximal repression of *FLC* during vernalization and inactive VRN1 or VRN2 reduce vernalization response, with VRN2 having a greater effect than VRN1. Sheldon et al. (2006) also showed that VRN1 and VRN2 are not required to maintain the repressed state of *FLC* although the authors suggested the possibility that VRN1 and VRN2 may maintain *FLC* repression initiated by other factors in wild type plants.

Mutagenesis also identified *vernalization independent 3* (*vin3*) in which vernalization response is completely blocked. *VIN3* gene has been cloned and encodes a PHD (plant homeodomain) finger containing protein, which is known to be involved in chromatin remodeling (Sung and Amasino, 2004b). *VIN3* can distinguish between transient exposure to cold and prolonged cold exposure and is expressed only after prolonged exposure to cold (Sung and Amasino, 2004b) Therefore, it is postulated that *VIN3* plays a key role in affecting flowering time after the end of the winter (Sung and Amasino, 2004a). Since *VIN3* is expressed only after exposure to cold for a duration needed to satisfy vernalization requirement, it can serve as a marker of vernalization duration essential for successful flowering.

**Epigenetic Regulation of *FLC*.** As discussed earlier, vernalization is epigenetically regulated and its "cellular memory" persists through mitotic divisions and is reset upon meiosis. In *Arabidopsis*, after vernalization *FLC* is downregulated and its levels remain unchanged through mitosis. The *FLC* levels are reinstated after meiosis, reforming the vernalization requirement. Therefore,

*FLC* acts as an epigenetic switch regulating vernalization response (Michaels and Amasino, 1999). Vernalization causes sequence-specific changes in the histone modification of *FLC* and these changes result in repression of *FLC* (Bastow et al., 2004). The changes in *FLC* histone modification include deacetylation of lysine 9 (K9) and lysine 14 (K14) on histone H3 (Sung and Amasino, 2004b), followed by dimethylation of lysine 27 (K27) and K9 on histone H3 (Bastow, 2004; Sung and Amasino, 2004b). The discovery of *VRN1*, *VRN2* and *VIN3* have provided insight into the mechanism of histone modifications of *FLC* by these three genes, leading to proposed models of epigenetic regulation of *FLC*.

Based on the initially proposed role of *VRN1* and *VRN2* in maintenance of *FLC* repression, Sung and Amasino (2004a; 2005) suggested a model of epigenetic regulation of vernalization in *Arabidopsis*. According to this model, during prolonged exposure to low temperatures, *VIN3* initiates *FLC* histone deacetylation, and hence the initial *FLC* repression. This histone deacetylation generates an environment favorable for methylation of K27 and K9 on histone H3 involving *VRN1-/VRN2*-containing complex. It has been proposed that methylation of Histone H3 at K9 promotes stable heterochromatin formation by *HETEROCHROMATIN PROTEIN1* (*HP1*) in animals. Since the repression of *FLC* following vernalization is unstable in *Arabidopsis hp1* mutants, *HP1* may be involved in maintaining *FLC* repression following vernalization. Thus, the epigenetic regulation of vernalization involves a cascade of histone modifications of *FLC* following vernalization, resulting in mitotically stable repression of *FLC*

heterochromatin serving as the "cellular memory" of the winter. Recently, Sheldon et al. (2006) showed that methylation of K27 and K9 on H3 may not be required for maintaining *FLC* repression after the end of vernalization treatment and postulated that other proteins may be involved in maintaining *FLC* repression. Further studies are necessary to determine the components involved in the mechanism of epigenetic regulation of *FLC*.

**Downstream Target Genes of *FLC*.** In vernalization-responsive ecotypes of *Arabidopsis*, flowering is delayed by *FLC*-mediated repression of floral integrator genes *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*)/ *AGAMOUS LIKE20* (*AGL20*) (Lee et al., 2000) and *FLOWERING LOCUS T* (*FT*) (Michaels et al., 2005). In their proposed flowering regulation model, Michaels et al. (2005) noted that *FLC*-mediated repression of *SOC1* is stronger than that of *FT*. *SOC1* and *FT* promote flowering by activating floral meristem identity genes such as *LEAFY* (*LFY*) and *APETALA* (*AP1*) (reviewed by Sung and Amasino, 2005). Additionally, *FT* also regulates floral meristem identity genes by activating *SOC1* (Michaels et al., 2005). A schematic diagram showing the primary genes involved in *FLC*-mediated vernalization pathway in *Arabidopsis* is presented in Fig. 7.

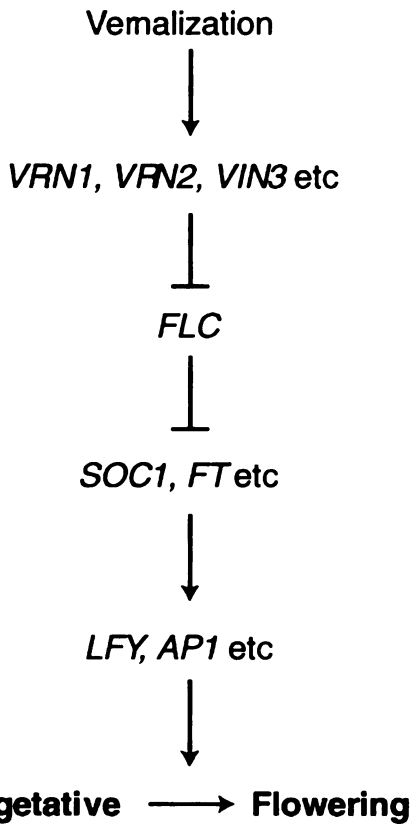


Figure 7. Vernalization pathway regulated by FLC in *Arabidopsis thaliana*. Vernalization induces *VRN1*, *VRN2* and *VIN3* genes which are repressors of *FLC*. *FLC* in turn is a repressor of *SOC1* and *FT* which are promoters of meristem identity genes such as *LFY* and *AP1* (from Sung and Amasino, 2005).

***FLC* Regulation by Autonomous Pathway Genes.** The autonomous pathway consists of *FCA*, *FY*, *FPA*, *FVE*, *LUMINIDEPENDENS* (*LD*) and *FLOWERING LOCUS D* (*FLD*) genes. Mutants of autonomous pathway genes are delayed in flowering irrespective of the photoperiod (Koornneef et al., 1991) and flowering of these mutants is hastened by vernalization treatment (Lee and Amasino, 1995). The delayed flowering of autonomous pathway mutants suggested that the autonomous pathway genes may play a role of in promotion of flowering. Furthermore, the hastening of flowering of autonomous pathway mutants following vernalization treatment indicated that these flowering promoter

genes acted in a pathway parallel to the vernalization pathway (reviewed by Henderson et al., 2003). Higher levels of *FLC* RNA in autonomous pathway mutants suggested that these genes repress *FLC* and this role was substantiated by complete suppression of the late flowering phenotype in double mutants of *flc* and autonomous pathway genes (reviewed by Henderson et al., 2003). Recently it was shown that autonomous pathway consists of a group of genes involved in a non-linear pathway regulating the expression of *FLC* (reviewed by Sung and Amasino, 2005).

**Other Genes Regulating *Arabidopsis* Vernalization Response.** Significant progress has been made in understanding the molecular regulation of *FLC*-mediated vernalization pathway in *Arabidopsis*. However, the role of additional regulators of *Arabidopsis* vernalization pathway remains to be elucidated. Some of these additional regulatory genes in *Arabidopsis* vernalization pathway are briefly described below.

Although *FRI* is the key gene involved in upregulating *FLC* in late flowering *Arabidopsis* ecotypes, additional regulators of *FLC* may exist. For example, in *Sy-0* accession of *Arabidopsis*, *AERIAL ROSETTE 1* (*ART1*) upregulated *FLC* by a mechanism distinct from that of *FRI* and *ART1* and *FLC* synergistically repressed flowering (Poduska, 2003). Although both *FRI* and *ART1* upregulate *FLC*, the similarity of their mechanism is unknown (reviewed by Henderson et al., 2003).

While the role of *FLC* as a key regulatory gene of vernalization pathway in *Arabidopsis* remains unchallenged, evidence of an *FLC*-independent

vernalization pathway also exists. Michaels and Amasino (2001) reported that when *flc* null mutants were grown under SD, their flowering was promoted by vernalization, indicating the existence of an *FLC*-independent pathway. This promotion of flowering under SD was completely blocked in *vin3 flc* null double mutant, suggesting that *VIN3* is involved in *FLC*-dependent and *FLC*-independent vernalization pathways (Sung and Amasino, 2004b). The molecular mechanisms involved in this *FLC*-independent vernalization pathway remains to be elucidated.

It was previously discussed that *VIN3* plays a key role in *FLC* repression. However, overexpression of *VIN3* does not abolish the vernalization requirement for promotion of flowering and hence, the process of *FLC* repression may involve additional regulatory factors (Sung and Amasino, 2004b). Also, the regulatory factors involved in the induction of *VIN3* in response to prolonged exposure to thermoinductive temperatures still remain unknown.

**Vernalization in Wheat**

The vernalization pathway in wheat has been shown to be distinct from the *FLC*-mediated vernalization pathway in *Arabidopsis*. In both *Triticum monococcum* L. (diploid wheat) and *Triticum aestivum* (tetraploid wheat), *VRN1* and *VRN2* are the two key regulatory genes governing the vernalization response. Note that *VRN1* and *VRN2* in wheat are distinct from *VRN1* and *VRN2* in *Arabidopsis*. Dominant alleles of *VRN1* are required for spring annual growth habit whereas, dominant alleles of *VRN2* are required for winter annual

growth habit of wheat (Yan et al., 2003; Yan et al., 2004). In the absence of vernalization treatment, *VRN2* delays flowering by repressing *VRN1* and vernalization treatment promotes flowering by downregulating *VRN2*, thereby relieving the block on *VRN1* (Yan et al., 2003; Yan et al., 2004) (Fig. 8). Thus, *VRN2* in wheat seems to play a role similar to that of *FLC* in *Arabidopsis*. Presence of dominant alleles of *VRN1* and *VRN2* results in spring annual growth habit in wheat as dominant alleles of *VRN1* can not be repressed by *VRN2* (Yan et al., 2003). Orthologues of wheat vernalization genes have been reported in *Hordeum vulgare* L. (barley; Dubcovsky et al., 1998) and therefore, it has been postulated that the vernalization pathway mediated by *VRN1* and *VRN2* genes is conserved in monocots.

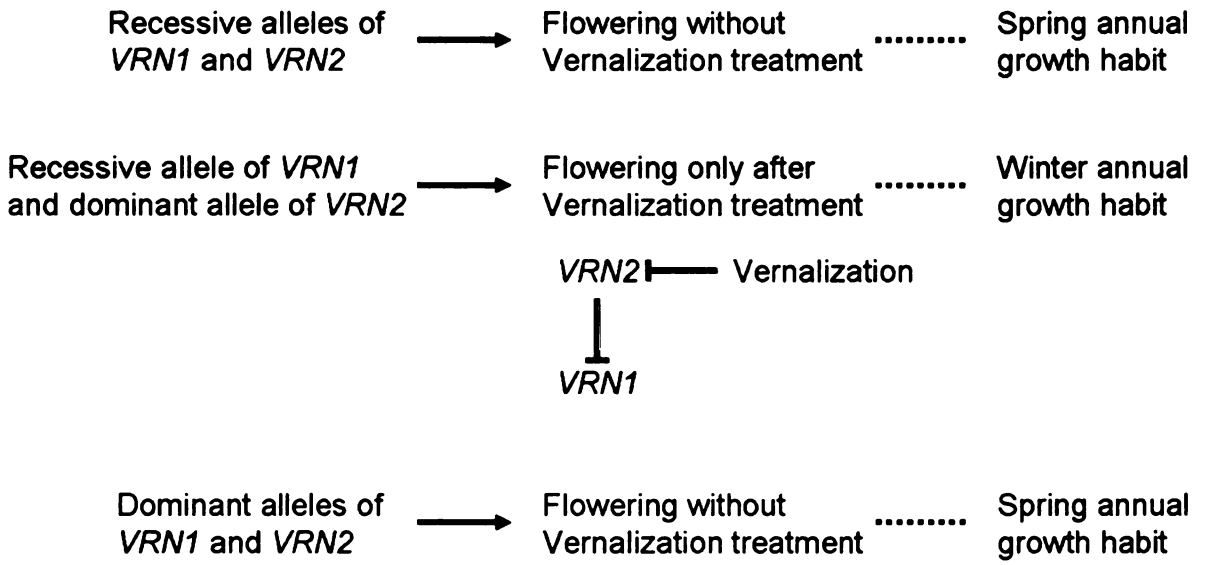


Figure 8. Vernalization pathway mediated by *VRN1* and *VRN2* is responsible for spring annual and winter annual growth habit in wheat.

**Future Perspectives**

The molecular mechanism of vernalization in *Arabidopsis* involving *FLC* regulation has been shown to extend to some other species belonging to the Brassicaceae family including *Brassica juncea* (L.) Czern. (Indian mustard; Martynov and Khavkin, 2004), *Brassica napus* L. (rape; Tadege et al., 2001), *Brassica oleracea* L. var *capitata* (cabbage; Lin et al., 2005), *Brassica rapa* (turnip; Schranz et al., 2002) and Thellungiella *halophila* O. E. Schulz (salt cress; Fang et al., 2006). In these species, homologues or orthologues of *FLC* have been found and are believed to mediate the vernalization response. Thus, so far the *FLC*-mediated vernalization pathway is limited to the members of Brassica family and has not been extended to the plethora of vernalization-responsive species from other families. As previously mentioned, the vernalization pathway in monocots including wheat and barley is distinct from the vernalization pathway in dicots, essentially represented by select Brassicaceae species. Studies investigating the molecular mechanism of vernalization in a winder range of species could account for the molecular basis of vernalization response in plants.

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# CHAPTER II:

# THE FLOWERING RESPONSE BEING ASSESSED REGULATES THE OPTIMUM TEMPERATURE FOR VERNALIZING *CAMPANULA* 'BIRCH HYBRID'

**The Flowering Response Being Assessed Regulates the Optimum Temperature for Vernalizing *Campanula* 'Birch Hybrid'**

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*Additional index words.* cardinal temperatures for vernalization, herbaceous perennial, thermal time to flower

**Abstract**

The objective of this study was to characterize the effect of vernalization temperature and duration on various flowering responses of *Campanula* 'Birch Hybrid'. Clonally propagated plants of *Campanula* 'Birch Hybrid' were exposed to −2.5, 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 or 20 °C for 0, 3, 5, 7, 9 or 12 weeks and subsequently grown at 20 °C in a greenhouse. *Campanula* 'Birch Hybrid' exhibited a near-obligate vernalization requirement and all flowering responses studied were affected by the vernalization temperature, duration and their interaction. The minimal and maximal cardinal temperatures for vernalization were <0 °C and between 15 and 17.5 °C, respectively. At each treatment duration, the range of optimal temperatures ($T_{opt}$) varied based on the flowering response assessed and ranged between 0 to 12.5 °C for flowering percentage to 5 to 7.5 °C for rate of progress to flowering. Additionally, $T_{opt}$ for flowering time

also varied when analyzed as rate of progress to flowering, time to flower from the end of temperature treatments, total time to flower measured from the start of temperature treatments and thermal time to flower measured from the initiation of temperature treatments as average daily temperature above the base temperature. For example, following 12-week treatment, $T_{opt}$ for thermal time to flower was 7.5 °C and for time to flower and total time to flower the range of $T_{opt}$ broadened to 2.5 to 12.5 °C. Since the flowering response assessed altered the $T_{opt}$, this study reiterates the significance of considering all relevant flowering responses while developing and interpreting vernalization models.

**Introduction**

Vernalization is defined as the promotion of flowering following cold treatment (Chouard, 1960; Vince-Prue, 1975; Thomas and Vince-Prue, 1984). The intensity of vernalization response is a function of the interaction between thermoinductive temperature and duration of exposure. The thermoinductive temperatures vary by species (Lang, 1965) and have been described as cardinal temperatures for vernalization with minimum ($T_{min}$), optimum ($T_{opt}$) and maximum ($T_{max}$) values (Atherton et al., 1990; Yan and Hunt, 1999). $T_{min}$ and $T_{max}$ are minimum and maximum temperatures at which the rate of vernalization is zero. The range of vernalizing temperature defined by $T_{min}$ and $T_{max}$ is species-dependant and quite broad (−4 to 17 °C) (Table 1). $T_{opt}$ is the range of vernalizing temperatures at which highest vernalization response is achieved and

is also species-specific (Table 1). By definition, $T_{opt}$ is relative and is based on the vernalization response being assessed.

The cardinal temperatures for vernalization are also relative due to their dependence on treatment duration (Lang, 1965). Duration of thermoinductive treatment has a quantitative effect on vernalization response until saturation and therefore, increasing durations of thermoinductive treatment progressively increase flowering response until the response saturates (Lang, 1965). Optimum temperature and duration for vernalization have been characterized for several field crops including *Triticum aestivum* L., *Daucus carota* L. and *Allium cepa* L. (Brooking, 1996; Robertson et al., 1996; Streck, 2003; Yan and Hunt, 1999) and select potted flowering plants including *Cineraria* sp. and *Lilium longiflorum* Thunb. (Lange and Heins, 1990; Streck and Schuh, 2005; Yeh et al., 1997). Flowering responses studied include the number of plants flowering in a population (Clough et al., 2001; Lange and Heins, 1990), node or leaf number at flowering (Brooking I.R., 1996; Clough et al., 2001; Lange and Heins, 1990; Rawson et al., 1998; Robertson et al., 1996; Suzuki and Metzger, 2001), rate of flowering (Streck, 2003; Yan and Hunt, 1999), time to flower (Clough et al., 2001; Rawson et al., 1998; Streck et al., 2003; Suzuki and Metzger, 2001; Yan and Hunt, 1999), thermal time to flower (Rawson et al., 1998), number of buds and flowers at flowering (Clough et al., 2001; Suzuki and Metzger, 2001) and percent reproductive lateral nodes (Fausey, 2005). Although many studies have determined $T_{opt}$ based on thermoinductive temperature, treatment duration and

one or more flowering responses listed above, to our knowledge, no study has yet characterized $T_{opt}$ for all the listed flowering responses in one species.

*Campanula* 'Birch Hybrid', a hybrid of *Campanula portenschlagiana* Schult. and *Campanula poscharskyana* Degen., is an herbaceous perennial that has been reported to exhibit a qualitative vernalization response (Enfield, 2001; Niu et al., 2004). *Campanula* 'Birch Hybrid' is a good candidate for investigating the vernalization responses since it can be clonally propagated and produces large numbers of propagules from a few stockplants. While stockplants are held in a controlled environment, propagation and thermoinductive treatments can be scheduled at varying times so that all treated plants can be transferred and grown in a greenhouse simultaneously. Thus, all treated plants can be grown under the same temperature and light conditions regardless of treatment duration as demonstrated by Fausey (2005). *Campanula* 'Birch Hybrid' has a day-neutral photoperiodic response and can be vernalized as small rooted cuttings. Niu et al. (2004) characterized the vernalization response of *Campanula* 'Birch Hybrid' at 0 to 10 °C for 0 to 8 weeks based on flowering percentage, time to visible bud and flower, number of flowering shoots and number of flower buds per shoot and found that temperature of 0 to 10 °C was equally effective in promoting flowering following 6- or 8-week treatments. However, investigation of the responses of *Campanula* 'Birch Hybrid' to a broader range of temperatures and durations is lacking and the cardinal temperatures for a range of vernalization responses are not established. The objective of this study was to characterize the effect of various thermoinductive temperatures and durations on different flowering

responses of *Campanula* 'Birch Hybrid'. We also determined the $T_{min}$ and $T_{max}$ for vernalization of *Campanula* 'Birch Hybrid' and characterized the $T_{opt}$ for a range of flowering responses.

**Materials and Methods**

*Stockplant Management and Propagule Culture.* Clonally propagated *Campanula* 'Birch Hybrid' stockplants were grown for ~5 weeks in 13-cm square plastic containers filled with commercial soil-less medium (Sure-Mix; Michigan Grower Products, Galesburg, Mich.) and were rejuvenated by dividing and repotting as necessary through the experiment. The stockplants were grown in a controlled environment chamber set at 22 °C under a 13-h photoperiod provided from 0600 to 1900 HR by incandescent (60A-130V; Philips, Somerset, N.J.) and fluorescent lamps (VHOF96T12; Philips, Bloomfield, N.J.) (~150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux; *PPF*). Stockplants were watered when necessary with acidified well water ($H_2SO_4$ to a titratable alkalinity of ~140 $mg \cdot L^{-1}$ $CaCO_3$) containing nutrients (40 N, 4 P, 40 K, 5 Ca, 0.3 Fe, 0.2 Mn, 0.2 Cu, 0.03 B, 0.03 Mo, 0.2 Zn $mg \cdot L^{-1}$; MSU Special, Greencare Fertilizers, Chicago).

Shoot-tip cuttings with 4 to 6 nodes were taken starting 13 May 2003 and 17 September 2003 for replication 1 and 2 of the study, respectively. Additional cuttings were taken 3, 5, 7, 9, and 12 weeks later during each replication. Cuttings were dipped in a commercial rooting hormone (Dip 'N Grow; Clackamas, Ore.) containing 1 $g \cdot L^{-1}$ indole-3-butyric acid and 0.5 $g \cdot L^{-1}$ naphthalene acetic acid and rooted in 72-cell trays (50-mL cell volume; Landmark Plastic

Corporation, Akron, Ohio) containing 50% commercial peat-perlite media (Sure-Mix; Michigan Growers Products, Galesburg, Mich.) and 50% coarse perlite (Therm-O-Rock; East Inc., New Eagle, Pa.). Cuttings were rooted for 20 d under mist and weaned for 4 d by manual watering in a glass propagation house set at 23 °C air temperature and 26 °C media temperature, maintained by providing bottom heat, and 0.3 kPa vapor pressure deficit generated by injecting water vapor in the air. A blackout system open from 0800 to 1700 HR maintained a 9-h photoperiod in the propagation environment and the light was provided by natural sunlight. Following propagation, plants were grown for an additional 33 d in a controlled environment chamber maintained at 20 °C under the same lighting and watering regimen as the stockplants.

*Temperature Treatments*. For each replication, 57 d from the start of rooting, plants were transferred to controlled environment chambers set at −2.5, 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 or 20 °C for 0, 3, 5, 7, 9 or 12 weeks. Incandescent and fluorescent lamps provided 20 (−2.5 to 2.5 °C) and 100 (5 to 20 °C) $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* for 11 h. During temperature treatments, plants were watered with acidified well water with nutrients described above. Plants were transferred to the temperature treatments on different dates for each treatment duration, such that all of the treatments ended on the same day (1 October 2003 and 5 February 2004 for replications 1 and 2, respectively).

*Plant Culture and Climate Control.* Following temperature treatments, nine plants per treatment combination and an additional nine plants as non-vernalized controls were potted in 13-cm square plastic containers filled with commercial soil-less medium (Sure-Mix) and grown in a glass greenhouse set at 20 °C under a 16-h photoperiod. High-pressure sodium lamps provided supplemental lighting (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*) when ambient light was below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and ceased after ambient light exceeded 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When necessary, plants were watered with reverse osmosis water containing nutrients (125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe, 0.5 Mn, 1.0 Cu, 0.3 B, 0.1 Mo $mg \cdot L^{-1}$; MSU Special, Greencare Fertilizers, Chicago).

Greenhouse environmental control was regulated by a climate-control computer (Priva, Model CD750; De Lier, The Netherlands). Air temperature was measured at plant height on every greenhouse bench by type E thermocouples (TT-E-40; Omega engineering, Stamford, Conn.) placed in aspirated tubes and *PPF* at plant height was measured at two locations by line quantum sensors containing 10 photodiodes (Apogee Instruments, Logan, Utah). Temperature and light sensors were connected to a CR10 datalogger (Campbell Scientific, Logan, Utah) and data were collected every 10 s and hourly averages were calculated and recorded in a computer. The average daily temperature (ADT) and daily light integral were calculated from the day of potting for 15 weeks and were 20.8 and 21.0 °C and 9.0 and 13.2 $mol \cdot m^{-2} \cdot d^{-1}$ during replication 1 and 2, respectively.

*Data Collection and Analysis.* Number of nodes on each plant were counted prior to and at the end of temperature treatments. Time to develop one node during vernalization was computed and its reciprocal was used as the rate of node development for regression analysis using PROC REG in SAS (SAS Institute, Cary, N.C.). Data from the two replications were not significantly different and therefore were pooled in the analysis. The base temperature for node development and the thermal time (°C d) required for developing one node were computed as described by Roberts and Summerfield (1987).

The date of first open flower was recorded and number of days from the end of temperature treatments to first open flower were reported as time to first open flower. Plants without an open flower after 105 d in the greenhouse were considered vegetative. Rate of progress to flowering was computed by taking the reciprocal of time to first open flower and the rate of progress to flowering of vegetative plants was reported as zero. Since vegetative plants were eliminated from flowering time analysis, even though the same dataset was used to calculate rate of progress to flowering and time to flower, the analyses of these two responses differed for the treatments that did not achieve 100% flowering. Total time to flower was computed as days from the beginning of temperature treatments to first open flower. The thermal time (°C d) to flower of individual flowering plants was calculated as accumulation of ADT above the estimated base temperature for leaf development from the beginning of temperature treatments to the day of first open flower.

The number of vegetative and reproductive laterals was counted one week after first open flower and percent reproductive laterals was computed. One week after first open flower, the number of open flowers were counted and additionally during replication 1, for every 4$^{th}$, 5$^{th}$ and 6$^{th}$ plant to flower in each treatment group, the number of flower buds and flowers were recorded. Due to the time constraint imposed by counting the very large number of flower buds and flowers present, number of flower buds and flowers were counted on 4$^{th}$, 5$^{th}$ and 6$^{th}$ plants to flower, in an attempt to measure the "average" potential of the flowering treatment group.

Days to flower, rate of progress to flowering, thermal time to flower, percent reproductive laterals, number of open flowers and number of buds and flowers were analyzed using SAS's (SAS Institute, Cary, N.C.) PROC MIXED and the least significant difference procedure was used for paired comparisons with $P$=0.05 as a maximum value for significance. The experiment was completely randomized with temperature and duration as treatment factors in a factorial arrangement. Pooled data from both replications were analyzed for all response variables except number of open flowers. Number of open flowers differed significantly between the two replications and hence was analyzed separately.

**Results**

*Survival and Leaf Development During Vernalization.* All plants treated at ≥0 °C survived and showed no visual symptoms of injury during their subsequent

growth. Following exposure to −2.5 °C, 36% and 27% *Campanula* 'Birch Hybrid' plants died during replications 1 and 2, respectively, and several surviving plants exhibited varying degrees of visual symptoms of freezing injury such as water-soaked areas and necrosis. Therefore, all data from −2.5 °C treatments were eliminated from analyses.

During temperature treatments, time to develop one node decreased in a non-linear fashion with increasing temperature (Fig. 1A) and averaged 50.8 and 7.5 d at 0 and 20 °C, respectively. The rate of node development fit a linear regression model ($r^2$=0.63, significant at $P$=0.001) (Fig. 1B) and based on the model, the base temperature and the thermal time (°C d) for node development were estimated to be −2.9 °C and 151.5 °C d, respectively.

*Flowering Response*. No plants flowered without temperature treatment (data not shown) and only 1 out of 90 plants in each of the 17.5 and 20 °C treatments flowered following vernalization (Fig. 2A and B). Based on flowering percentage, *Campanula* 'Birch Hybrid' vernalized between 0 to 15 °C and the response was quantitative with duration. For example, flowering percentage at 15 °C increased from 0 to 39% following treatment for 5 and 7 weeks, respectively and further increased to 94% after 12 weeks. Complete (100%) flowering of *Campanula* 'Birch Hybrid' was achieved following 5 weeks at 2.5 to 7.5 °C, 7 weeks at 0 to 7.5 and 12.5 °C, and 9 and 12 weeks at 0 to 12.5 °C. Incomplete flowering occurred after shorter durations at 0 to 15 °C.

*Rate of Progress to Flowering, Time to Flower and Total Time to Flower.* All parameters relating to flowering time were significantly affected by treatment temperature, duration and their interaction. The fastest rate of flowering following treatment for 5 to 9 weeks was achieved at 5 or 7.5 °C, and after 12-week treatment at 7.5 °C (Fig. 3A). One plant each treated at 17.5 or 20 °C flowered, but flowering was considerably delayed and the rate of progress to flowering was close to zero. Also, rate of progress to flowering following 3-week treatment was significantly lower than flowering rates of all treatments that induced complete flowering. Following treatment at 0 to 15 °C, the rate of progress to flowering continued to increase with durations up to 12 weeks, the longest duration tested (Fig. 3B). The minimum time to flower and total time to flower within each duration occurred after 3 weeks at 5 °C, 5 weeks at 5 and 7.5 °C, 7 weeks at 2.5 to 7.5 °C, 9 weeks at 2.5 to 10 °C and 12 weeks at 2.5 to 12.5 °C (Fig. 3C and E). Increasing the treatment durations at 0 °C and 5 to 15 °C for up to 12 weeks reduced time to flower (Fig. 3D). Following treatment at 2.5 °C, the average time to flower decreased for up to 9 weeks. The average total time to flower varied between 88 to 164 d, depending on the temperature and duration of treatment. Minimum total time to flower of treatments eliciting ≥75% flowering was after 5 weeks at 0 to 7.5 °C and 7 weeks at 10 and 12.5 °C (Fig. 3 F).

*Thermal Time to Flower.* Average thermal time to flower varied between 1226 to 3599 °C d, depending on treatment temperature and duration (Fig. 4A and B). Minimum thermal time to flower within each duration was recorded following

treatment for 5 weeks at 5 °C, 7 and 9 weeks at 0 to 5 °C and 12 weeks at 0 and 2.5 °C. Plants flowering after treatment at 17.5 and 20 °C required significantly greater thermal time compared to plants treated at 0 to 12.5 °C at the respective durations. Increasing treatment duration significantly decreased thermal time to flower following treatment at 0 to 15 °C for 7 weeks.

*Percent Reproductive Laterals.* Treatment temperature, duration and their interaction significantly affected the percent reproductive laterals. Percent reproductive laterals of all plants treated at 0 to 15 °C increased following up to 9 weeks treatment, while extending treatments to 12 weeks further increased reproductive laterals on plants treated at 0, 5, 7.5, and 15 °C (Fig. 5A and B). Highest percent reproductive laterals within each duration were produced following treatment for 5 weeks at 7.5 °C, 7 weeks at 0 to 12.5 °C, 9 weeks at 2.5 °C and 12 weeks at 0 to 10 °C. The average percent reproductive laterals of the two plants that flowered following 17.5 and 20 °C treatments were 0.13 and 0.31, respectively and were statistically similar to vegetative plants having no reproductive laterals. Following treatments at 0 to 12.5 °C for ≥7 weeks, all plants had >50% reproductive laterals. Although several individual plants produced 100% reproductive laterals, no treatment combination elicited in 100% reproductive laterals on all plants.

*Number of Open Flowers.* The number of open flowers counted one week following first open flower differed between the two replications, so data from the

two replications were analyzed separately. During both replications, treatment temperature and duration and the interaction between them were significant. In both replications, plants that flowered after 3 weeks of temperature treatments produced significantly fewer open flowers compared to plants treated for ≥5 weeks (Fig. 6A). Overall, in both replications the number of open flowers after 5-week treatment at 0 to 12.5 °C was similar (Fig. 6B) and following 7-week treatment, more flowers were produced by plants treated at 7.5 to 12.5 °C compared to the other temperatures (Fig. 6C). Following 9 and 12-week treatment, maximum number of flowers primarily occurred at 7.5 to 12.5 °C in replication 1 whereas, in replication 2, plants treated at 5 to 12.5 °C generally produced more flowers (Fig. 6D and E). In replication 1 and 2, the number of open flowers following treatment at 0 °C did not increase with additional treatment durations after 7 and 5 weeks, respectively. Number of open flowers after treatment at 2.5 to 7.5 °C increased as the treatment duration increased to 12 weeks in replication 1, while in replication 2, number of open flowers after treatment at 2.5, 5 and 7.5 °C did not increase after 9, 12 and 7 weeks, respectively. The average number of open flowers did not increase significantly after 7 and 9 weeks at 10 °C in replication 1 and 2, respectively. In both replications, maximum open flowers were produced after 9 weeks at 12.5 and 15 °C, with no further increase following treatment for 12 weeks.

*Number of Flower buds and flowers.* Treatment temperature, duration and their interaction had a significant effect on number of flower buds and flowers

produced one week after first open flower on the 4th, 5th and 6th plant to flower within each treatment (Fig. 7A and B). The effect of treatment temperature on the number of flower buds and flowers was highly variable and there was no clear temperature optimum (Fig. 7A). Generally, increasing treatment duration from 5 to 7 weeks increased the number of flower buds and flowers, while further increases to 9 or 12 weeks had little to no effect on the number of flower buds and flowers (Fig. 7B).

**Discussion**

*Campanula* 'Birch Hybrid' has been previously reported to have an obligate vernalization requirement (Enfield, 2002; Niu et al., 2004). In the current study only two out of 180 plants flowered following exposure to 17.5 and 20 °C. However, flowering of these plants was delayed and their average rate of progress to flowering approached zero and was similar to vegetative plants. Additionally, these two flowering plants had few reproductive laterals and open flowers. These findings indicate that *Campanula* 'Birch Hybrid' has botanically a near-obligate vernalization requirement and horticulturally, it can be characterized as an obligate requirement necessary for complete, uniform, rapid and profuse flowering (Runkle et al., 2001). A similar vernalization response has been reported in *Oenothera fruticosa* 'Youngii-lapsley' where only one out of 180 plants flowered in four different experiments, with considerably delayed flowering (Clough et al., 2001).

In this study, we established that, based on flowering percentage, $T_{min}$ for vernalizing *Campanula* 'Birch Hybrid' was <0 °C and $T_{max}$ was between 15 and 17.5 °C. The $T_{min}$ and $T_{max}$ for vernalization are species-specific and the reported $T_{min}$ and $T_{max}$ of select species are presented in Table 1. Although some plants exposed to −2.5 °C died or exhibited freezing injury, those that survived flowered. Based on this observation, we postulate that the $T_{min}$ of *Campanula* 'Birch Hybrid' is <−2.5 °C. The USDA Cold Hardiness Zones of the parents of *Campanula* 'Birch Hybrid', *Campanula portenschlagiana* Schult. and *Campanula poscharskyana* Degen. are 4 and 3, respectively (Griffiths, 1994) and *Campanula* 'Birch Hybrid' is hardy at least to Zone 5 (personal observation). Direct transfer of plants previously grown at 20 °C to −2.5 °C caused freezing injury and death of some plants. It is likely that acclimation at 0 or 5 °C for two weeks prior to treatment at −2.5 °C may have improved the survival of *Campanula* 'Birch Hybrid' as reported by Engle (1994) for 20 species of herbaceous perennials including *Campanula carpatica*.

The $T_{opt}$ for vernalization is species-specific and may differ depending on the duration of temperature treatment and response being assessed (Lang, 1965). Additionally, the duration of low temperature treatment affects the kinetics of the vernalization response, with increasing duration enhancing the vernalization response until it saturates (Lang, 1965; Thomas and Vince-Prue, 1984). In the current study, we investigated the vernalization response based on the number of plants flowering in a population, rate of progress to flowering, time to flower from the end of temperature treatments, total time to flower and thermal

time to flower from start of temperature treatments to flowering, percent reproductive laterals, number of flowers and number of flower buds and flowers. When assessed as percentage of plants flowering in the population, the $T_{opt}$ was 2.5 to 10 °C after 5 weeks and 0 to 12.5 °C after ≥7 weeks. Thus, there was an interaction between treatment temperature and duration, and prolonging the temperature treatments broadened the vernalization temperature optima of *Campanula* 'Birch Hybrid'. Since no treatment duration at 15 °C resulted in 100% flowering, based on flowering percentage, 12 weeks were insufficient to saturate the flowering response at 15 °C.

The quantitative nature of the vernalization response is often illustrated by flowering time or leaf number at flowering. Since the growthhabit of *Campanula* 'Birch Hybrid' did not permit monitoring leaf number at flowering, only flowering time will be discussed. Time to flower measured from the end of temperature treatments at 0 °C and 5 to 15 °C decreased as treatment duration increased to 12 weeks. $T_{opt}$ for shortest flowering time measured from end of treatments was 5 to 7.5 °C after 5 weeks, 2.5 to 7.5 °C after 7 weeks , 2.5 to 10 °C after 9 weeks and 2.5 to 12.5 °C after 12 weeks. Hence, similar to flowering percentage, prolonging durations of treatments broadened the temperature optima of flowering response assessed as time to flower, although the $T_{opt}$ for flowering percentage and time to flower differed. The effect of thermoinductive temperature and duration on total flowering time measured from start of temperature treatments was also evaluated to account for growing time during the treatments. The $T_{opt}$ for total time to flower and time to flower was the same

but the duration at which the response saturated differed. Total time to flower did not decrease with additional treatment after 3 weeks at 0 °C, 7 weeks at 2.5 °C, 5 weeks at 5 and 7.5 °C and 7 weeks at 10 to 15 °C. It is noteworthy that 12-week treatments at 0 to 15 °C significantly increased total time to flower compared to 5- to 9-week treatments, although time to flower measured after treatments decreased at 0 °C and 5 to 15 °C after 12 weeks. Thus, an additional 3 weeks of treatment decreased time to flower by <3 weeks.

The flowering times reported in this study are up to 3 weeks longer than those reported by Niu et al. (2004) and up to 3 weeks shorter than those reported by Enfield (2001). Differences in starting material and environmental conditions prior to and following temperature treatments may have caused these differences. Additionally, Niu et al. (2004) reported that temperature did not affect flowering time following treatment at 0 to 10 °C for 6 and 8 weeks, which is contradictory to our findings and we can not explain this discrepancy.

The growth rate of plants is temperature-dependant and therefore, to account for differences in plant development during temperature treatments, we also evaluated flowering response based on the thermal time to flower, which was computed from the start of temperature treatments. Thermal time to flower decreased following treatment at 0 to 15 °C for up to 7 weeks. The $T_{opt}$ based on thermal time to flower was 5 °C after 5 weeks, 0 to 5 °C after 7 and 9 weeks and 0 and 2.5 °C after 12 weeks. However, thermal time to flower should theoretically begin when a plant has made a developmental transition to flowering. Due to the lack of a physiological marker for this developmental switch,

thermal time to flower is calculated from the start of temperature treatments and therefore, may be overestimated. To evaluate the effect of this overestimation on flowering response, we chose 5 and 7 weeks as times when the switch to flowering may be made at all vernalizing temperatures and calculated the thermal time after 5 and 7 weeks of temperature treatments. The effect of treatment temperature and duration on thermal time to flower computed after 5- and 7-week treatments was similar (Fig. 8A and B). Following treatment at 2.5 to 12.5 °C, the effect of treatment duration on thermal time to flower computed after 5 and 7-week treatment was similar to thermal time to flower from the start of treatments. However, at 0 °C the minimum thermal time to flower after 5- and 7-week treatments was achieved after 12-week treatment. $T_{opt}$ at each treatment duration varied slightly and was 5 or 7.5 °C after 5 weeks, 2.5 to 7.5 °C after 7 and 9 weeks and 0 to 7.5 °C after 12 weeks.

The effect of temperature treatments and durations on flowering is often evaluated based on flowering time and flowering time has been used to develop vernalization models in crops including wheat and Easter lily (Lange and Heins, 1990; Rawson et al, 1998; Streck amd Schuh, 2005). Changes in vernalization temperature optima based on the response variable being assessed were reported in wheat cultivars by Rawson et al. (1998). The response assessed altered the $T_{opt}$ of *Campanula* 'Birch Hybrid' in this study and therefore, has significance in developing and interpreting vernalization models.

We also evaluated the effect of temperature treatments on percent reproductive laterals, flower number and number of flower buds and flowers. All

data were collected one week after the first open flower, which was arbitrarily chosen as a time to account for gradual flower opening. The $T_{opt}$ for percent reproductive laterals were somewhat variable. Overall, $T_{opt}$ was 0 to 7.5 °C following 5-week treatment and 0 to 12.5 °C following 7 to 12-week treatments. Increasing treatment duration to 9 weeks typically increased percent reproductive laterals. Overall, ≥7 weeks at 0 to 12.5 °C resulted in >60% reproductive laterals. We expect that additional laterals were formed during the treatment at warmer temperatures, and depending on the temperature and duration, only some of these new laterals vernalized during the treatment. However, the specific relationship of treatment temperature and duration with formation and vernalization of laterals is unknown. Also, when flowering was delayed, additional vegetative laterals may have developed in the greenhouse, affecting percent reproductive laterals reported. However, we found no correlation between percent reproductive laterals and rate of progress to flowering (data not presented), indicating that their relationship is complex.

The number of open flowers varied to even a greater extent with significant differences in flower number between the two replications of the study. Flowering response assessed as the number of open flowers typically saturated after ≥7 weeks of treatment depending on the temperature and replication. Following a 7-week treatment at 0 to 12.5 °C, >25 open flowers were present on all flowering plants. There was no consistent trend associated with the highest number of flower buds and flowers at different treatment temperatures and durations. This was at least in part due to high variability which could partly be

attributed to the small sample size. It is notable that ≥7 weeks at 0 to 12.5 °C resulted in an average of 583 to 1261 flower buds and flowers. Unexpectedly, there was no correlation between average number of flower buds and flowers and average number of open flowers (data not presented). The number of open flowers and flower buds and flowers may have been affected by percent reproductive laterals and development of plants during temperature treatments, especially at warmer temperatures although these relationships were unclear. Number of open flowers and flower buds and flowers influence marketability of potted plants, however unlike edible crops, higher numbers do not always translate into greater financial returns. Following ≥7-week treatment at 0 to 12.5 °C *Campanula* 'Birch Hybrid' plants were marketable since the average number of flower buds and flowers and open flowers exceeded 500 and 25, respectively.

The complexity of vernalization is not only limited to its physiological aspects, but also extends to the analysis and interpretation of the flowering responses. The effective treatment temperature and duration for optimum flowering response have been reported based on many responses including flowering percentage, node development, rate of node development, time to visible bud or flower, thermal time to flower, number of flower buds and flowers at flowering and percent reproductive lateral nodes. In the present study we conclusively demonstrate that the optimum temperature and duration for vernalizing *Campanula* 'Birch Hybrid' vary significantly based on the response being assessed (Table 2 and 3). In general, temperatures between 0 and 12.5 °C successfully vernalized *Campanula* 'Birch Hybrid' when given for ≥7 weeks,

although extending durations for up to 12 weeks improved many flowering responses. We did not see any adverse effects of long-term storage of *Campanula* 'Birch Hybrid' at 0 to 12.5 °C and therefore, when resources permit, we recommend that commercial growers use longer durations.

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Table 1. The minimum ($T_{min}$), maximum ($T_{max}$) and optimum ($T_{opt}$) cardinal temperatures for vernalization of select herbaceous biennial and perennial species.

| Species | $T_{min}$ (°C) | $T_{opt}$ (°C) | $T_{max}$ (°C) | Response assessed | Reference |
|---|---|---|---|---|---|
| *Allium cepa* | <8[z] | ---[y] | >17[x] | ---[w] | cited by Lang, 1965 |
| *Beta vulgaris altissima* | <−4[z] | 2-4 | >12[x] | ---[w] | cited by Lang, 1965 |
| *Daucus carota* | −1 | 6.5 | 16 | Rate of flowering, bolting | Atherton et al., 1990 |
| *Dianthus gratianopolitanus* 'Bath's Pink' | <0 | 5 | >10 | Flowering percentage, time to flower, nodes at flowering, buds and flowers | Chapter III |
| *Hyoscyamus niger* | <3[z] | ---[y] | ≥17[x] | ---[w] | cited by Lang, 1965 |
| *Isotoma axillaris* | <−2.5 | 5-10 | 15 | Percent flowering | Fausey, 2005 |
| *Miltoniopsis* Augres 'Trinity' | 8 | 11-14 | 17 | Percent flowering | Lopez and Runkle, 2006 |
| *Secale cereale* 'Petkus' | <−5[z] | 1-7 | >15[x] | ---[w] | cited by Lang, 1965 |
| *Rhapsalidopsis xgraeseri* | <5 | ---[y] | ≥15 | ---[w] | cited by Lang, 1965 |
| *Senecio cineraria* | −0.3 | 5.9 | 15.8 | 1/leaf number below flower | Yeh et al., 1997 |
| *Triticum aestivum* | −1.3±1.5[v] | 4.9±1.1[v] | 15.7±2.6[v] | Various | Porter and Gawith, 1999 |
| *Veronica spicata* 'Red Fox' | <−2.5 | −2.5 | 12.5 | Flowering percentage, percent lateral nodes flowering | Fausey, 2005 |

[z]lowest effective temperature cited by Lang, 1965.
[y]$T_{opt}$ was not cited by Lang, 1965.
[x]highest effective temperature cited by Lang, 1965.
[w]response assessed was not cited by Lang, 1965.
[v]mean±SE of cardinal temperatures for vernalization reported in over 6 papers assessing different parameters relating to vernalization.

Table 2. Summary of optimum treatment temperatures for maximum flowering response at different durations for various flowering responses assessed in *Campanula* 'Birch Hybrid'.

| Flowering response | Optimum treatment temperatures (°C) for maximum response at duration (weeks) | | | |
|---|---|---|---|---|
| | **5** | **7** | **9** | **12** |
| Flowering percentage | 2.5-7.5 | 0-7.5, 12.5 | 0-12.5 | 0-12.5 |
| Rate of progress to flowering | 5-7.5 | 5-7.5 | 5-7.5 | 7.5 |
| Time to flower | 5-7.5 | 2.5-7.5 | 2.5-10 | 2.5-12.5 |
| Total time to flower | 5-7.5 | 2.5-7.5 | 2.5-10 | 2.5-12.5 |
| Thermal time to flower | 5 | 0-5 | 0-5 | 0-2.5 |
| Thermal time to flower computed after treatment for 5 weeks | 5-7.5 | 2.5-7.5 | 2.5-7.5 | 0-7.5 |
| Thermal time to flower computed after treatment for 7 weeks | 5-7.5 | 2.5-7.5 | 2.5-7.5 | 0-7.5 |
| Percent reproductive laterals | 7.5 | 0-12.5 | 2.5 | 0-10 |
| Number of open flowers | 0-12.5 | 7.5-12.5 | 7.5-12.5 | 7.5-12.5 |

Table 3. Summary of minimum treatment durations for maximum flowering response at different temperatures for various flowering responses assessed in *Campanula* 'Birch Hybrid'.

| Flowering response | Minimum treatment duration (weeks) for maximum response at temperature (°C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **2.5** | **5** | **7.5** | **10** | **12.5** | **15** |
| Flowering percentage | 7 | 5 | 5 | 5 | 9 | 7 | 12 |
| Rate of progress to flowering | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| Time to flower | 12 | 9 | 12 | 12 | 12 | 12 | 12 |
| Total time to flower | 3[z] | 7 | 5 | 5 | 7 | 7 | 7 |
| Thermal time to flower | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Thermal time to flower computed after treatment for 5 weeks | 12 | 7 | 7 | 7 | 7 | 7 | 7 |
| Thermal time to flower computed after treatment for 7 weeks | 12 | 7 | 7 | 7 | 7 | 7 | 7 |
| Percent reproductive laterals | 12 | 9 | 12 | 12 | 9 | 9 | 12 |
| Number of open flowers | 7[y] | 12[y] | 12[y] | 12[y] | 7[y] | 9[y] | 9[y] |
| | 5[x] | 9[x] | 12[x] | 7[x] | 9[x] | 9[x] | 9[x] |

[z]only a few plants in the populations flowered at this duration.
[y]data from replication 1.
[x]data from replication 2.

Figure 1. Influence of temperature on node development of *Campanula* 'Birch Hybrid' rooted cuttings. Open circles represent mean±SE and solid lines represent the regression equations generated using 722 observations from two replications. The intercept and the slope of linear regression analysis for the rate of node development were 0.0193±0.0023 and 0.0066± 0.0002, respectively.

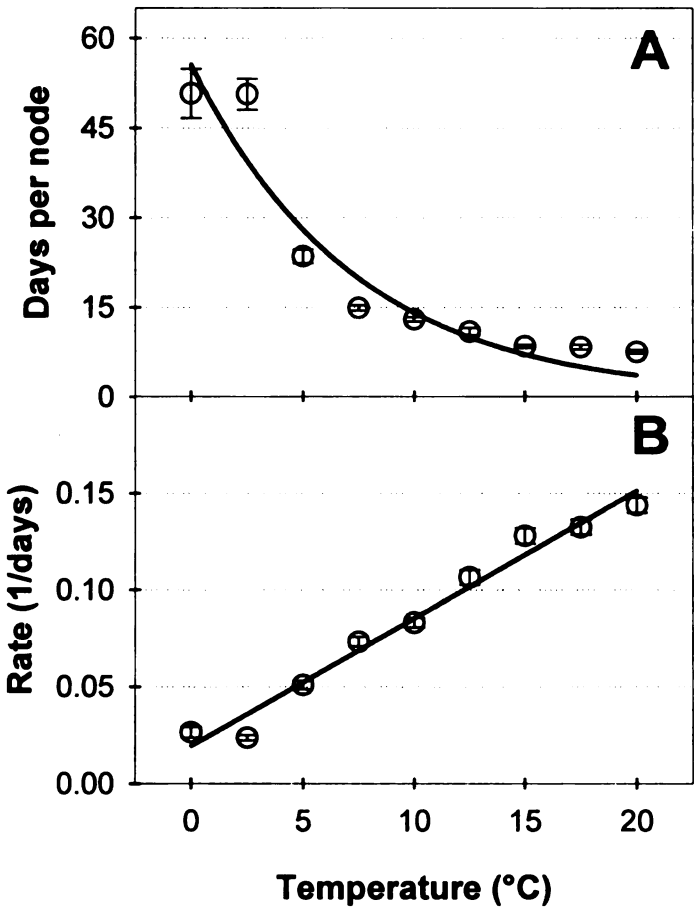


Figure 2. Flowering percentage of *Campanula* 'Birch Hybrid' as a function of treatment temperature (A) and duration (B). Flowering percentage was computed after growing plants in a glass greenhouse set at 20 °C. Plants without open flowers after 105 d in the 20 °C greenhouse were considered vegetative. Averages of pooled data from two replications are presented.

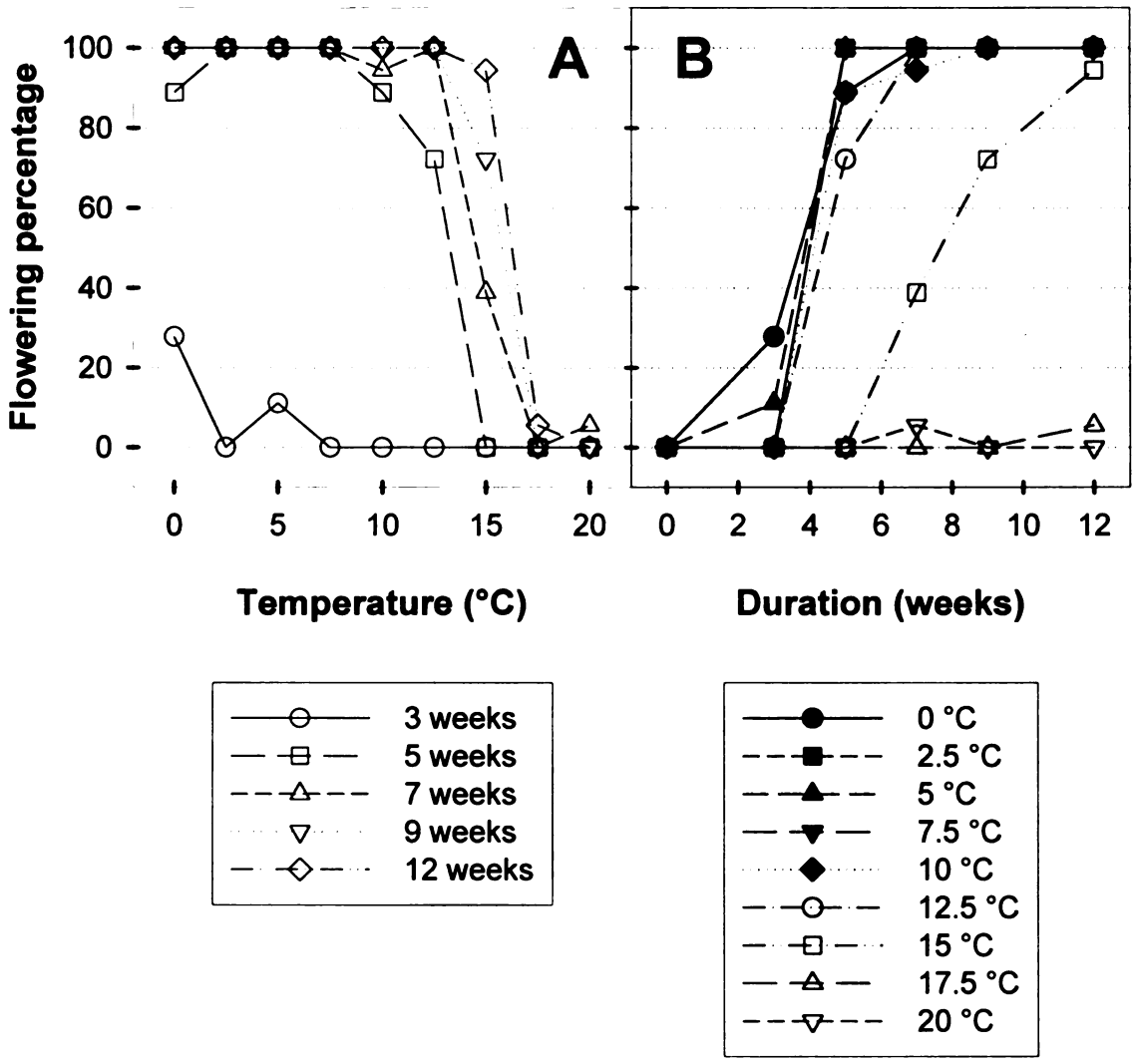


Figure 3. Influence of temperature treatment and its duration on rate of progress to flowering (A and B), time to flower (C and D) and total time to flower (E and F) of *Campanula* 'Birch Hybrid'. Rate of progress to flowering was computed as 1/days to first open flower from end of temperature treatment. Time to flower was computed starting from end of temperature treatment and total time to flower was computed starting at the beginning of temperature treatments. Means±SE data pooled from two replications are presented.

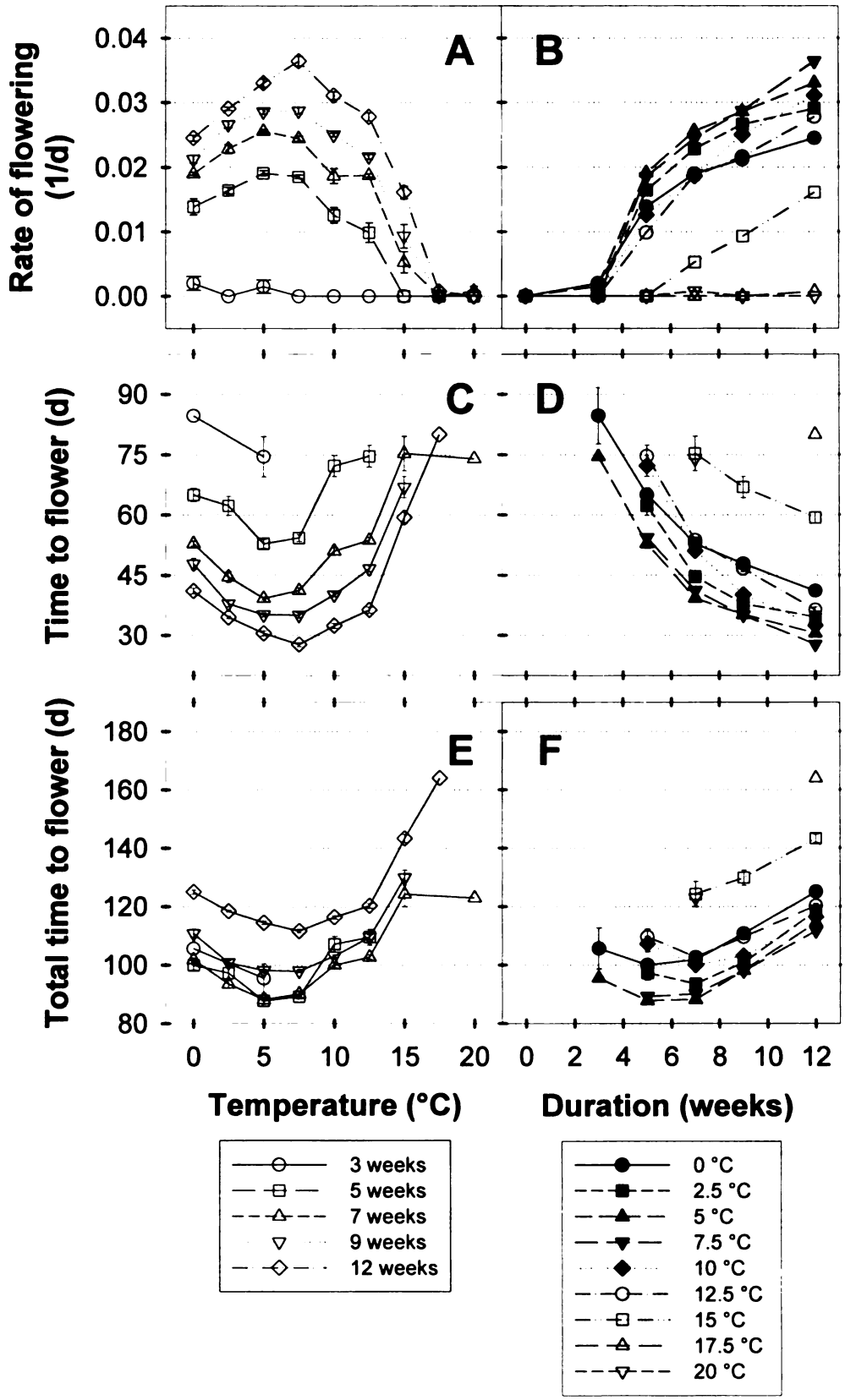

Rate of flowering (1/d)
0.04
0.03
0.02
0.01
0.00
A
B
Time to flower (d)
90
75
60
45
30
C
D
Total time to flower (d)
180
160
140
120
100
80
E
F
0
5
10
15
20
0
2
4
6
8
10
12
Temperature (°C)
Duration (weeks)
3 weeks
5 weeks
7 weeks
9 weeks
12 weeks
0 °C
2.5 °C
5 °C
7.5 °C
10 °C
12.5 °C
15 °C
17.5 °C
20 °C

Figure 4. Influence of temperature treatment (A) and its duration (B) on thermal time to flower from beginning of treatment. Thermal time was calculated as average daily temperature minus estimated leaf unfolding base temperature of −2.9 °C and expressed in °C d. Means±SE of data pooled from two replications are presented.

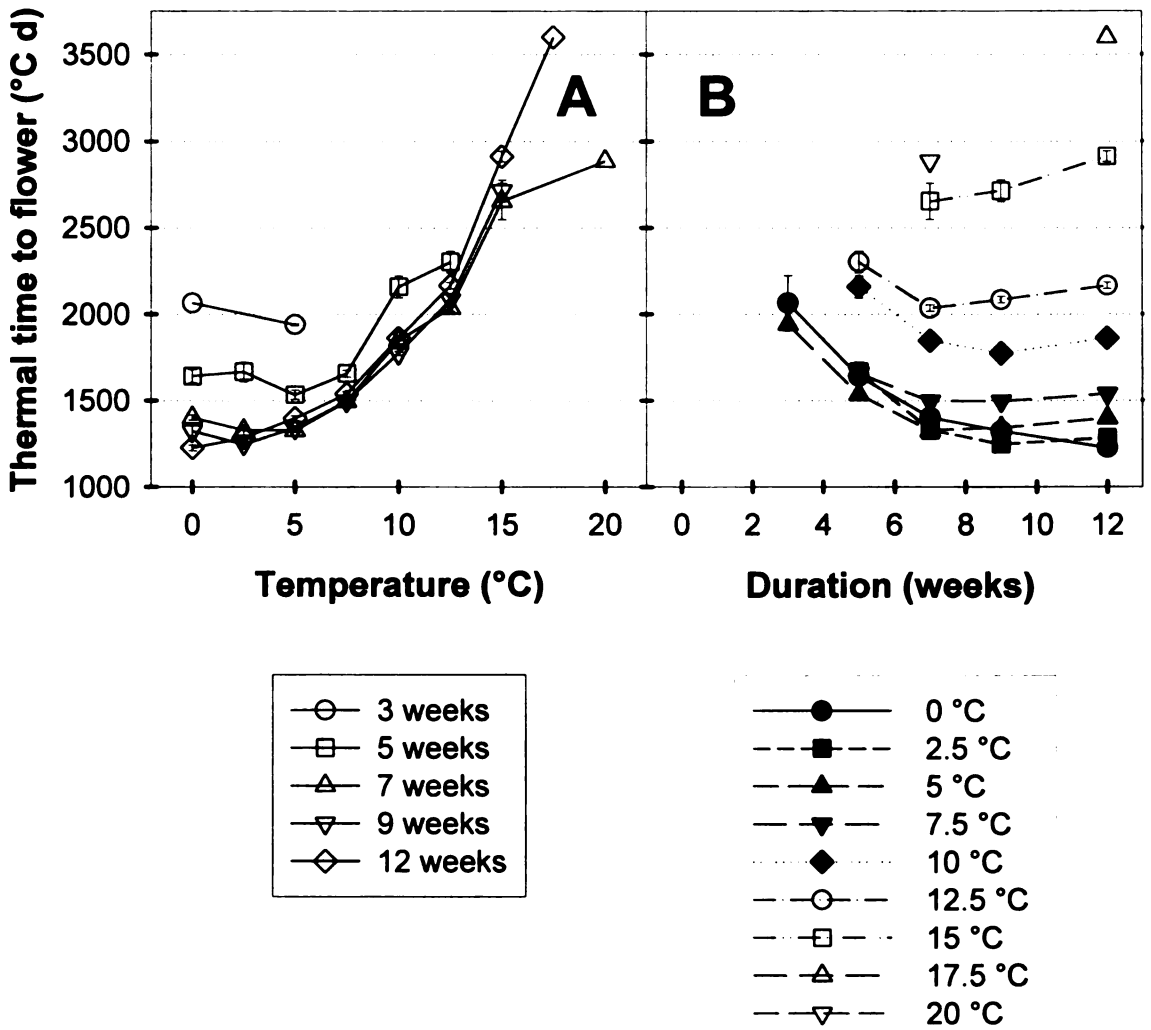


Figure 5. Percent reproductive laterals one week after first open flower of *Campanula* 'Birch Hybrid' as a function of treatment temperature (A) and duration (B). Means±SE (A) and means (B) of 18 observations pooled from two replications are presented.

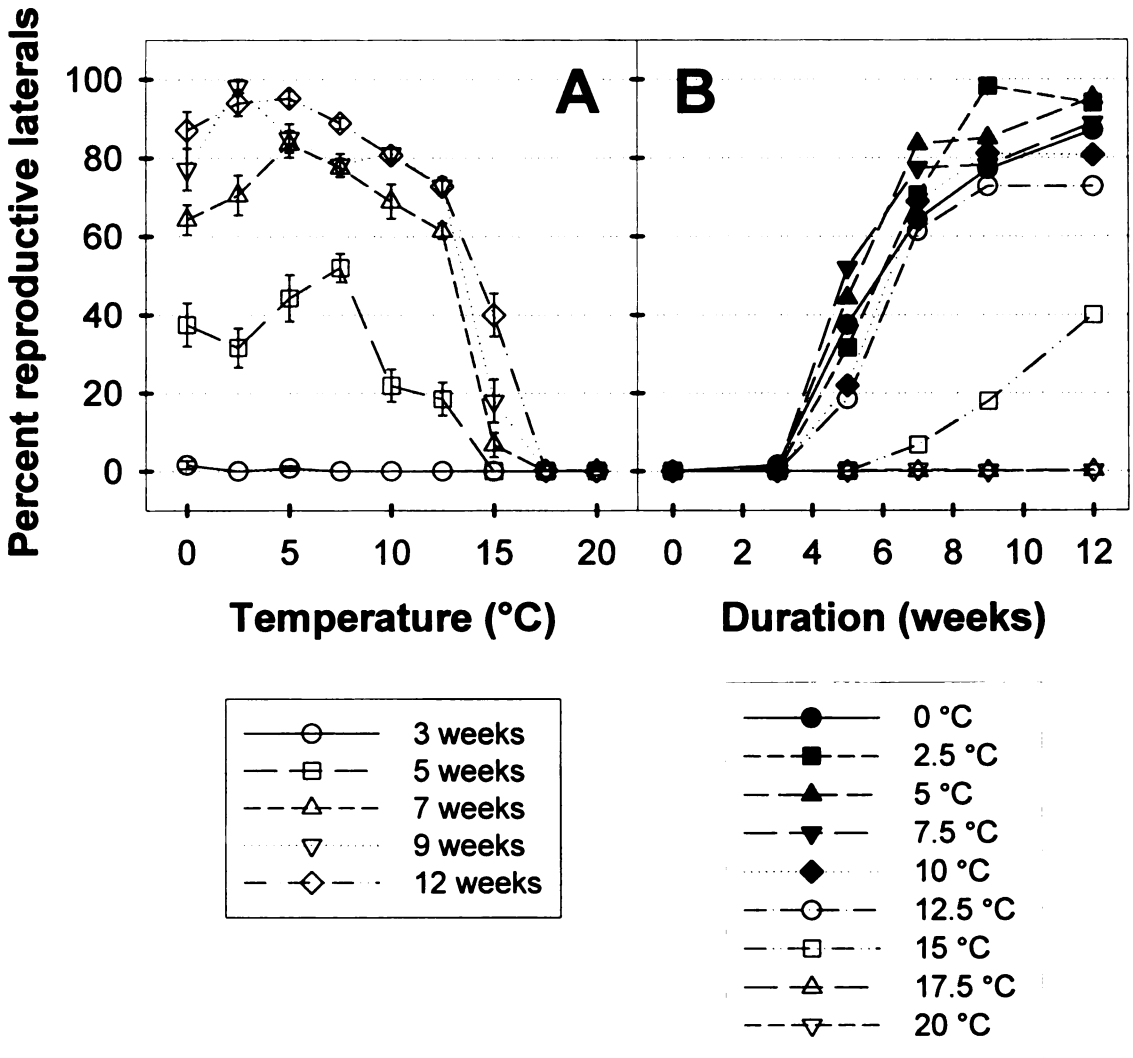


Figure 6. Influence of vernalization temperature on number of open flowers counted one week following first open flower in *Campanula* 'Birch Hybrid' vernalized for 3 weeks (A), 5 weeks (B), 7 weeks (C), 9 weeks (D) and 12 weeks (E). Squares represent mean±SE in replication 1 and diamonds represent mean±SE in replication 2.

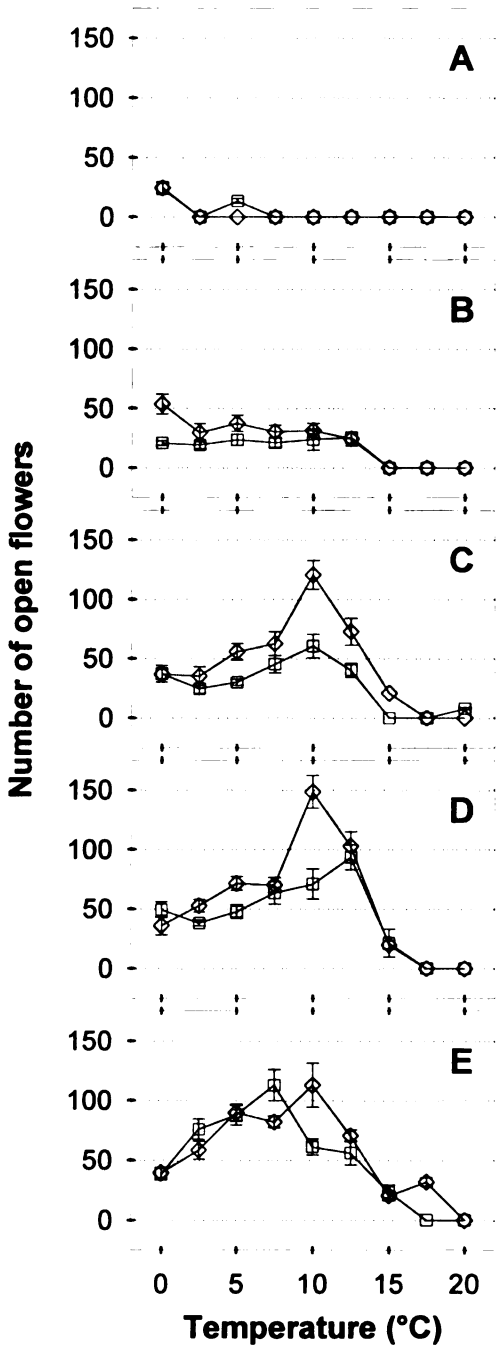


Figure 7. Number of flower buds and flowers on 4th, 5th and 6th plant to flower after first open flower, counted one week following the first open flower on *Campanula* 'Birch Hybrid' plants as a function of (A) treatment temperature and (B) treatment duration. Means±SE (A) and means (B) of 3 observations are presented. Treatments resulting in <4 flowering plants were considered to have 0 flower buds and flowers.

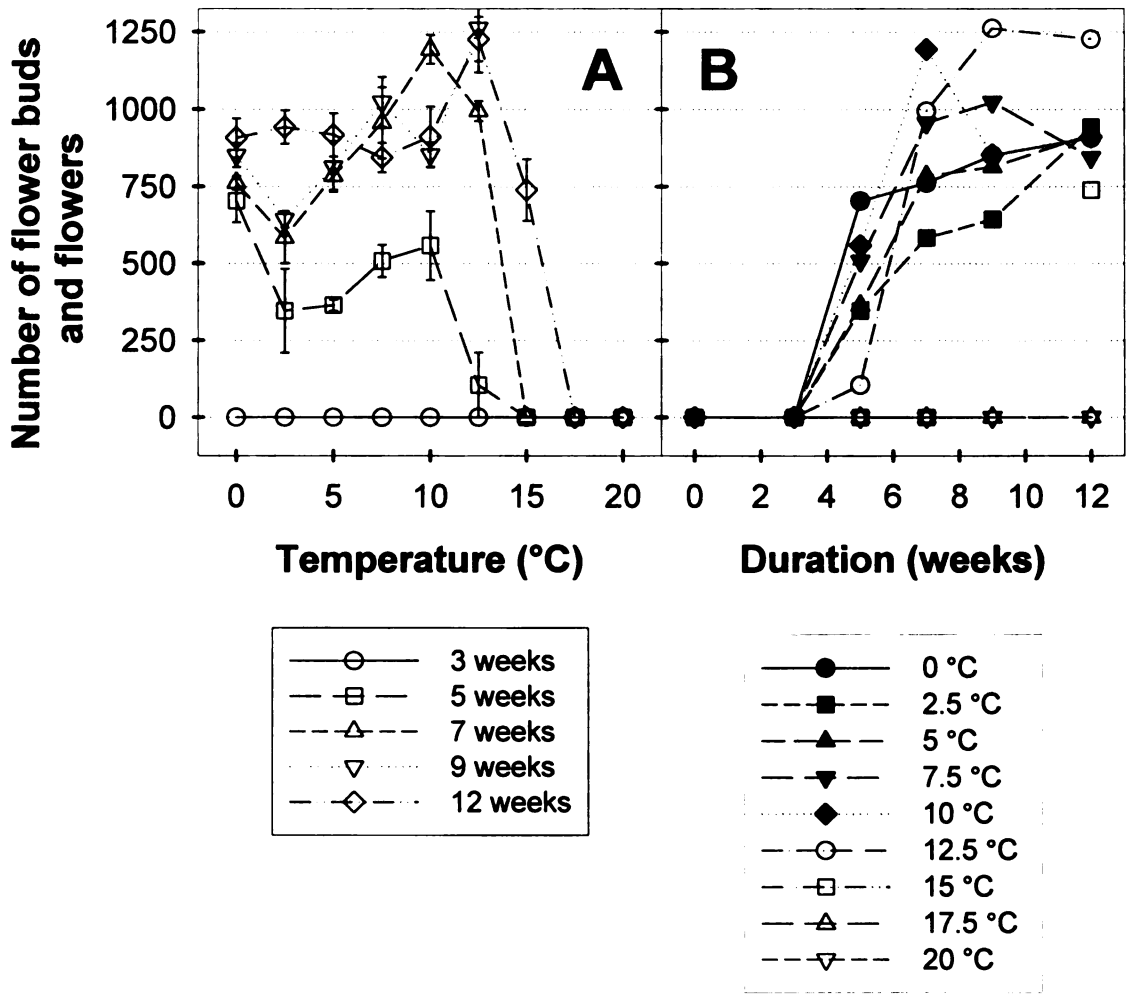
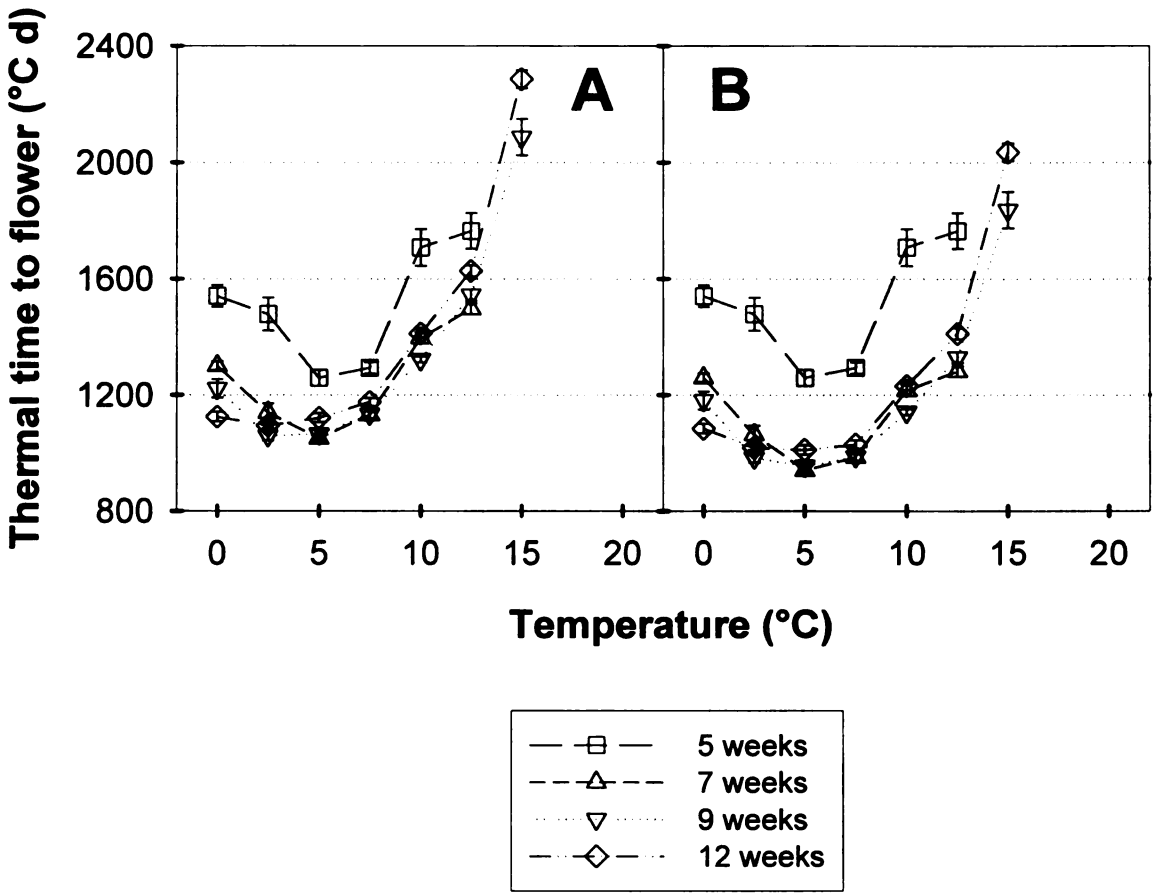


Figure 8. Thermal time to flower from 5 weeks (A) and 7 weeks (B) after beginning of temperature treatment as a function of treatment temperature. Thermal time was calculated 5 or 7 weeks from the beginning of temperature treatment to the day of first open flower as average daily temperature minus estimated leaf unfolding base temperature of −2.9 °C and expressed in °C d. Means±SE of data pooled from two replications are presented for treatment combinations resulting in >70% flowering.



# CHAPTER III:

# *DIANTHUS GRATIANOPOLITANUS* 'BATH'S PINK' HAS A NEAR-OBLIGATE VERNALIZATION REQUIREMENT

*Dianthus gratianopolitanus* 'Bath's Pink' has a Near-Obligate Vernalization Requirement

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

**Abstract**

The flowering response of *Dianthus gratianopolitanus* 'Bath's Pink' was characterized following varying durations at vernalizing temperatures. Clonally propagated plants were treated at 5 °C for 3, 6, 9, 12 or 15 weeks in experiment I, at 0, 5 or 10 °C for 2, 4, 6 or 8 weeks in experiment II and at 0, 5, 10 or 15 °C for 1, 2, 4, 6 or 8 weeks in experiment III. *Dianthus* 'Bath's Pink' exhibited a quantitative vernalization response following treatment at 0 to 10 °C and did not vernalize at 15 °C in 8 weeks, the longest duration tested. Complete flowering was achieved following ≥4 weeks at 0 °C, ≥3 weeks at 5 °C and 8 weeks at 10 °C. Based on time to anthesis and node number at anthesis, the flowering response was saturated following vernalization treatment at 0 °C for ≥4 weeks and 5 °C for ≥3 weeks. However, maximum flower buds and flowers at anthesis were recorded following 8 weeks at 0 °C and ≥6 weeks at 5 °C. Plants took

significantly longer to reach anthesis following 8-week treatment at 10 °C than ≥6 weeks at 0 °C and ≥4 weeks at 5 °C. Based on the minimum vernalization duration required to achieve maximum flowering response, the order of efficacy of vernalizing temperatures was 5 °C>0°C>>10°C.

**Introduction**

The wholesale value of herbaceous perennials has grown by 63% over the last five years, reaching over $708 million in 2005 (U.S. Department of Agriculture, 2006). This increase in sales of herbaceous perennials can in part be attributed to the growing ability of producers to force and market perennials in flower at scheduled times. Forcing perennials in flower requires an understanding of the regulation of flowering and subsequent manipulation of environmental factors including photoperiod and vernalization to promote flower induction.

Flowering of many winter annuals, biennials and perennials is promoted following exposure to low temperatures. This phenomenon, known as vernalization, has been defined as "the acquisition or acceleration of the ability to flower by a chilling treatment" (Chouard, 1960). Cold treatment that induces or accelerates flowering is also referred to as vernalization treatment (Napp-Zinn, 1987). An important aspect of vernalization is the temporal separation of the thermoinductive treatment and the flowering response. Therefore, at the end of thermoinductive treatment, the only indicator of completion of vernalization requirement is the subsequent flowering after exposure to warm temperatures.

Insufficient vernalization can result in incomplete and/or delayed flowering, which can cause considerable monetary losses to commercial producers of herbaceous perennials.

Vernalization has been extensively studied in many winter annuals and biennials and select perennials and the physiology of vernalization was comprehensively reviewed decades ago (Chouard, 1960; Lang, 1965; Thomas and Vince-Prue, 1984; Vince-Prue, 1975). Vernalization responses of plants can be categorized as qualitative (obligate) or quantitative (facultative) (Lang, 1965). Plants exhibiting a qualitative vernalization response require a cold treatment to acquire the ability to flower, whereas a cold treatment accelerates flowering and/or improves flowering characteristics of plants with a quantitative vernalization response. Vernalization has been shown to be a quantitative process; the flowering response increases with vernalization duration until saturation (Lang, 1965). The effective ranges of thermoinductive temperatures and durations that elicit a maximum vernalization response are species-specific. For example, the thermoinductive temperatures producing the greatest flowering response of *Veronica spicata* L. 'Red Fox' and *Isotoma axillaris* Lindl. were −2.5 °C and 5 to 10 °C, respectively (Fausey, 2005). Therefore, selection of appropriate thermoinductive temperatures is important for commercial production of herbaceous perennials. Even within a species, the optimum temperature range may change with changing durations (Lang, 1965). For example, complete flowering of *Campanula* 'Birch Hybrid' was achieved following a 5-week vernalization treatment at 2.5 to 7.5 °C, however, all plants flowered following a

9-week treatment at 0 to 12.5 °C (Chapter II). Hence, scheduling herbaceous perennials in flower necessitates empirical studies investigating the vernalization temperatures and durations most effective for flowering.

*Dianthus gratianopolitanus* is an herbaceous perennial native to western and central Europe and is hardy to USDA Cold Hardiness Zone 3 (Griffiths, 1994). *Dianthus* 'Bath's Pink' is a selection of *Dianthus gratianopolitanus* popular for its outstanding garden performance. *Dianthus* 'Bath's Pink' flowers in mid-spring in gardens in Michigan and is then largely vegetative through the summer and autumn (personal observation). In a preliminary experiment, *Dianthus gratianopolitanus* 'Bath's Pink' did not flower without a vernalization treatment when grown for 15 weeks in a greenhouse under 9- or 16-h photoperiods, whereas all plants flowered under 9- and 16-h photoperiods following a 15-week treatment at 5 °C (Cameron et al., unpublished data). The objectives of this study were to 1) characterize the flowering response of *Dianthus* 'Bath's Pink' to a range of thermoinductive temperatures and durations, and 2) determine the effective vernalization temperatures and durations for complete, rapid and uniform flowering of *Dianthus* 'Bath's Pink'.

**Materials and Methods**

*Propagation and Propagule Culture.* Clonally propagated *Dianthus* 'Bath's Pink' stockplants were maintained in a glass greenhouse set at 20 °C under a 16-h photoperiod from 0600 to 2200 HR provided by a combination of sunlight and high-pressure sodium (HPS) lamps. HPS lamps turned on when the ambient

light was below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and turned off when ambient light was above 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and provided an additional 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (*PPF*). Plants were hand watered when necessary with reverse osmosis water containing nutrients (125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe, 0.5 Mn, 1.0 Cu, 0.3 B, 0.1 Mo $mg \cdot L^{-1}$; MSU Special, Greencare Fertilizers, Chicago). Shoot-tip cuttings with 6 to 8 nodes were taken from stockplants and dipped in a commercial rooting hormone (Dip 'N Grow; Clackamas, Ore.) containing 1 $g \cdot L^{-1}$ indole-3-butyric acid and 0.5 $g \cdot L^{-1}$ naphthalene acetic acid, and rooted in 72-cell trays (50-mL cell volume; Landmark Plastic Corporation, Akron, Ohio) containing 50% commercial peat-perlite media (Sure-Mix, Michigan Growers Products, Galesburg, Mich.) and 50% coarse perlite (Therm-O-Rock; East Inc., New Eagle, Pa.). Cuttings were rooted under mist for ~2 weeks in a propagation facility set at air and medium temperatures of 23 and 26 °C, respectively and 0.3 kPa vapor pressure deficit generated by injecting water vapor in the air. After rooting, propagules were grown in 72-cell trays under the same environmental conditions and nutritional regimen as stockplants until the vernalization experiments were initiated.

*Experiment I. Dianthus* 'Bath's Pink' cuttings were taken on 14 December 2003, rooted for ~2 weeks and grown in 72-cell trays for ~1 month. The 72-cell trays containing propagules were placed in a cooler set at 5 °C on 3 February 2004 for 3, 6, 9, 12 or 15 weeks. Cool-white fluorescent lamps provided ~10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* from 0800 to 1700 HR in the cooler. During vernalization treatments, plants

were hand watered when necessary with acidified well water ($H_2SO_4$ to a titratable alkalinity of ~140 mg·L$^{-1}$ $CaCO_3$) containing nutrients (40 N, 4 P, 40 K, 5 Ca, 0.3 Fe, 0.2 Mn, 0.2 Cu, 0.03 B, 0.03 Mo, 0.2 Zn mg·L$^{-1}$; MSU Special, Greencare Fertilizers, Chicago). Ten additional plants were maintained in the greenhouse as non-vernalized controls under the same conditions as stockplants.

*Experiment II and III. Dianthus* 'Bath's Pink' cuttings were propagated on 27 April 2004, rooted for ~2 weeks in the propagation house and grown in 72-cell trays for ~1 month in experiment II. On 11 June 2004, 72-cell trays containing the propagules were placed in coolers set at 0, 5, or 10 °C for 2, 4, 6 or 8 weeks. In experiment III, cuttings were taken on 27 October, 2004 and 1, 2, 4 and 6 weeks thereafter. Propagules were rooted for ~2 weeks, grown for 2 weeks and placed in coolers set at 0, 5, 10 or 15 °C for 1, 2, 4, 6, or 8 weeks. The light and nutritional conditions in the coolers were the same in all three experiments. An additional 10 and 40 plants were maintained as non-vernalized controls in the greenhouse in experiments II and III, respectively.

*Growing Conditions and Environmental Control.* In all three experiments, following vernalization treatments, plants were transplanted into 13-cm square plastic containers (1.1-L volume) filled with commercial soil-less medium (Sure-Mix; Michigan Grower Products, Galesburg, Mich.) and grown in a glass greenhouse under the same light and nutritional regimens as stockplants.

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

*Data Collection and Analysis.* At transplant, number of nodes was recorded and at anthesis of first flower, date and number of nodes on the main axis were recorded in all experiments. Additionally, number of flower buds and flowers at anthesis were recorded in experiments II and III and number of vegetative and reproductive laterals were recorded and percent reproductive laterals were computed in experiment III. Plants without a visible flower bud 15 weeks after transplant were considered vegetative. In all experiments, 10 plants were subjected to each treatment combination and were arranged in a completely

randomized design. Data from experiment I were subjected to analysis of variance using SAS's PROC MIXED (SAS Institute, Cary, N.C.). Data from experiments II and III were pooled for analysis as a factorial experiment with vernalization temperature and duration as treatment factors using SAS's PROC MIXED. Least significant difference procedure was used for paired comparisons with $P$=0.05 as a maximum value for significance.

**Results and Discussion**

*Flowering response*. In experiment I, 1 out of 10 non-vernalized control plants flowered and in subsequent experiments an average of 21% of non-vernalized control plants flowered (Fig. 1A and 2A). Thus, *Dianthus* 'Bath's Pink' exhibited a facultative vernalization response. Complete flowering of *Dianthus* 'Bath's Pink' was achieved following vernalization at 0 °C for ≥4 weeks, 5 °C for ≥3 weeks and 10 °C for 8 weeks. Only 1 out of 40 plants flowered following vernalization treatment at 15 °C for ≤8 weeks. The order of most effective vernalization temperatures based on the shortest duration of vernalization treatment required for achieving complete flowering was 5 °C>0 °C>>10 °C.

The effective duration of vernalization for promotion of flowering is species-specific (Lang, 1965). For example, *Tulipa* species require a 14.5 to 23.5-week vernalization treatment (Dole and Wilkins, 2005), whereas radish can be vernalized in 4 to 8 d (Erwin et al., 2002). However, many herbaceous perennials including *Campanula* 'Birch Hybrid', *Isotoma axillaris* and *Veronica spicata* 'Red Fox' require relatively longer (4 to 8 weeks) exposure to vernalizing

temperatures for complete flowering (Fausey, 2005; Chapter II). In this study we found that *Dianthus* 'Bath's Pink' flowered completely after comparatively short exposure to vernalizing temperatures and only 1, 2 and ≥3 weeks at 5 °C resulted in 70, 95 and 100% flowering, respectively (Fig. 2A). Similarly, *Oenothera fruticosa* 'Youngii-lapsley' was completely vernalized following a 3-week exposure to 5 °C (Clough et al., 2001).

*Flowering Time*. Vernalization promoted and synchronized flowering of *Dianthus* 'Bath's Pink'. The time to reach anthesis of non-vernalized control plants was highly variable. In experiment I, after 15 weeks in the greenhouse, only 1 out of 10 non-vernalized plants reached anthesis and in 114 d (Fig. 1B). In the subsequent experiments, 21% of non-vernalized plants reached anthesis, in an average of 58 d (Fig. 2B). Many species with a facultative vernalization response flower sporadically without vernalization treatment and the sporadic nature of flowering of *Dianthus* 'Bath's Pink' may explain the differences in flowering times of non-vernalized plants between the different experiments. We postulate that plants that remained vegetative for 15 weeks in all experiments would have subsequently flowered at variable times and considerably increased the average time to reach visible flower bud and anthesis for the entire population.

In experiment I, compared to the non-vernalized controls, 3 weeks at 5 °C accelerated flowering significantly and average time to subsequently reach anthesis was 41 d. An additional 3 weeks of vernalization treatment hastened flowering time by approximately one week but vernalization treatment for up to 15

weeks did not further hasten flowering. At 5 °C, the standard errors associated with average time to anthesis were low after ≥3 weeks of vernalization treatment, indicating that vernalization synchronized flowering. Overall, the average number of nodes on vernalized plants at anthesis did not vary from 3 to 15 weeks of vernalization at 5 °C (Fig. 1C). Based on node number at planting and anthesis, the reported 1 week decrease in flowering time after 3- and ≥6-week vernalization treatment was not due to developmental acceleration of flowering. Hence, this 1 week decrease in time to flower can not be explained as the direct effect of additional vernalization treatment. In experiment II and III, the flowering time of *Dianthus* 'Bath's Pink' decreased as vernalization duration at 5 °C increased from 1 to 2 weeks, with no further reduction in time to flower following up to 8 weeks at 5 °C (Fig. 2B). The number of nodes at anthesis followed a trend similar to flowering time (Fig. 2C). Overall, the results from all three experiments indicate that complete and rapid flowering of *Dianthus* 'Bath's Pink' was achieved following vernalization treatment at 5 °C for ≥3 weeks.

Average time to reach anthesis and the average number of nodes at anthesis significantly decreased as the duration of vernalization treatment at 0 °C increased to 4 weeks. Hence, complete and rapid flowering of *Dianthus* 'Bath's Pink' occurred following ≥4-week vernalization treatment at 0 °C. *Dianthus* 'Bath's Pink' flowered completely and earlier following 4 weeks at 5 °C compared to 4 weeks at 0 °C. However, ≥6 weeks at 0 °C or 5 °C were equally effective in promoting flowering based on flowering percentage and flowering time.

Vernalization treatment at 10 °C for ≤6 weeks resulted in incomplete flowering after 15 weeks of growing in the greenhouse, when we chose to terminate the experiment. Given more time, it is possible that remaining plants in these treatments may have flowered, which would have increased average flowering time and node number than those reported here. An 8-week treatment at 10 °C induced complete flowering, however plants took significantly longer to reach anthesis and developed more nodes before anthesis compared to plants vernalized at 0 °C for ≥6 weeks or 5 °C for ≥4 weeks. Hence, 0 and 5 °C were more effective than 10 °C in promoting flowering of *Dianthus* 'Bath's Pink'. Based on flowering percentage, flowering time and node number at anthesis, *Dianthus* 'Bath's Pink' did not vernalize at 15 °C in 8 weeks.

*Number of Flower Buds and Flowers and Percent Reproductive Laterals at Anthesis.* All treatments except 8 weeks at 0 °C and ≥6 weeks at 5 °C resulted in similar numbers of flower buds and flowers at anthesis, and averaged between 3 to 9 (Fig. 2D). Plants averaged 16 to 21 flower buds and flowers following 8 weeks at 0 °C and ≥6 weeks at 5 °C. Since the DLIs received by plants under all treatment combinations were similar, the increased flower number was mostly a result of vernalization response. Percent reproductive laterals at anthesis averaged between 6 to 33 and followed the overall general trend of number of flower buds and flowers at anthesis (Fig. 2E). Hence, these additional vernalization durations contributed to improving the visual appeal of plants at flowering. Even after 8-week vernalization treatments at 0 or 5 °C, flowering was

sparse and plants were of low quality. Since *Dianthus* 'Bath's Pink' produces flowers on the terminal and laterals that are developed at the onset of vernalization, growing plants for a longer time prior to the initiation of vernalization treatment could allow development of additional laterals prior to vernalization and increase the number of flowers produced.

*Summary*. Based on our results, *Dianthus* 'Bath's Pink' exhibited a near-obligate vernalization response. The shortest vernalization duration resulting in complete flowering was 3 weeks at 5 °C although plants produced fewer flower buds and flowers at anthesis compared to ≥6-week treatment. Following vernalization for 2 and 4 weeks, 5 °C was the most effective vernalization temperature based on flowering percentage and flowering time, however after ≥6 weeks, 0 and 5 °C were similarly effective. *Dianthus* 'Bath's Pink' flowered completely after vernalization at 10 °C only after 8-week treatment although flowering was delayed and fewer flower buds and flowers were produced at anthesis compared to 8 weeks at 0 and 5 °C. Plants did not vernalize at 15 °C in 8 weeks and therefore, the maximum temperature for vernalizing *Dianthus* 'Bath's Pink' is ≥10 °C. Compared to other treatments, more flower buds and flowers were produced following 8-week vernalization at 0 °C and ≥6-week vernalization at 5 °C. Additional growing of *Dianthus* 'Bath's Pink' prior to vernalization treatment could improve final plant quality.

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Table 1. Planting date, average daily temperature (ADT) and average photosynthetic daily light integral (DLI) of *Dianthus gratianopolitanus* 'Bath's Pink' vernalized for different durations. ADT and DLI were calculated for each flowering plant from day of transplant to anthesis and for vegetative plants, 105 d after transplant. Mean±SE of ADT and DLI of 10, 30 and 240 plants are reported for experiment 1, 2 and 3, respectively.

| Experiment | Vernalization duration (week) | Planting date | ADT (°C) | Avg. DLI ($mol \cdot m^{-2} \cdot d^{-1}$) |
|---|---|---|---|---|
| 1 | 0 | 2/03/2004 | 20.9 ± 0.0 | 13.3 ± 0.0 |
| | 3 | 2/24/2004 | 20.7 ± 0.0 | 13.6 ± 0.2 |
| | 6 | 3/16/2004 | 20.2 ± 0.0 | 17.5 ± 0.0 |
| | 9 | 4/06/2004 | 20.8 ± 0.1 | 14.4 ± 0.5 |
| | 12 | 4/27/2004 | 21.8 ± 0.0 | 9.7 ± 0.0 |
| | 15 | 5/18/2004 | 22.4 ± 0.0 | 10.5 ± 0.0 |
| 2 | 0 | 6/11/2004 | 23.0 ± 0.0 | 11.6 ± 0.1 |
| | 2 | 6/25/2004 | 23.0 ± 0.1 | 12.0 ± 0.1 |
| | 4 | 7/09/2004 | 23.4 ± 0.1 | 11.6 ± 0.1 |
| | 6 | 7/23/2004 | 22.3 ± 0.0 | 10.7 ± 0.0 |
| | 8 | 8/06/2004 | 22.6 ± 0.0 | 10.4 ± 0.0 |
| 3 | 0-8 | 1/08/2005 | 18.5 ± 0.3 | 11.7 ± 0.4 |

Figure 1. Flowering response of *Dianthus gratianopolitanus* 'Bath's Pink' in experiment I following vernalization at 5 °C at increasing durations. Open symbols represent the averages for flowering plants and vertical bars represent standard errors. In A, flowering percentage was computed 105 d after transplant.

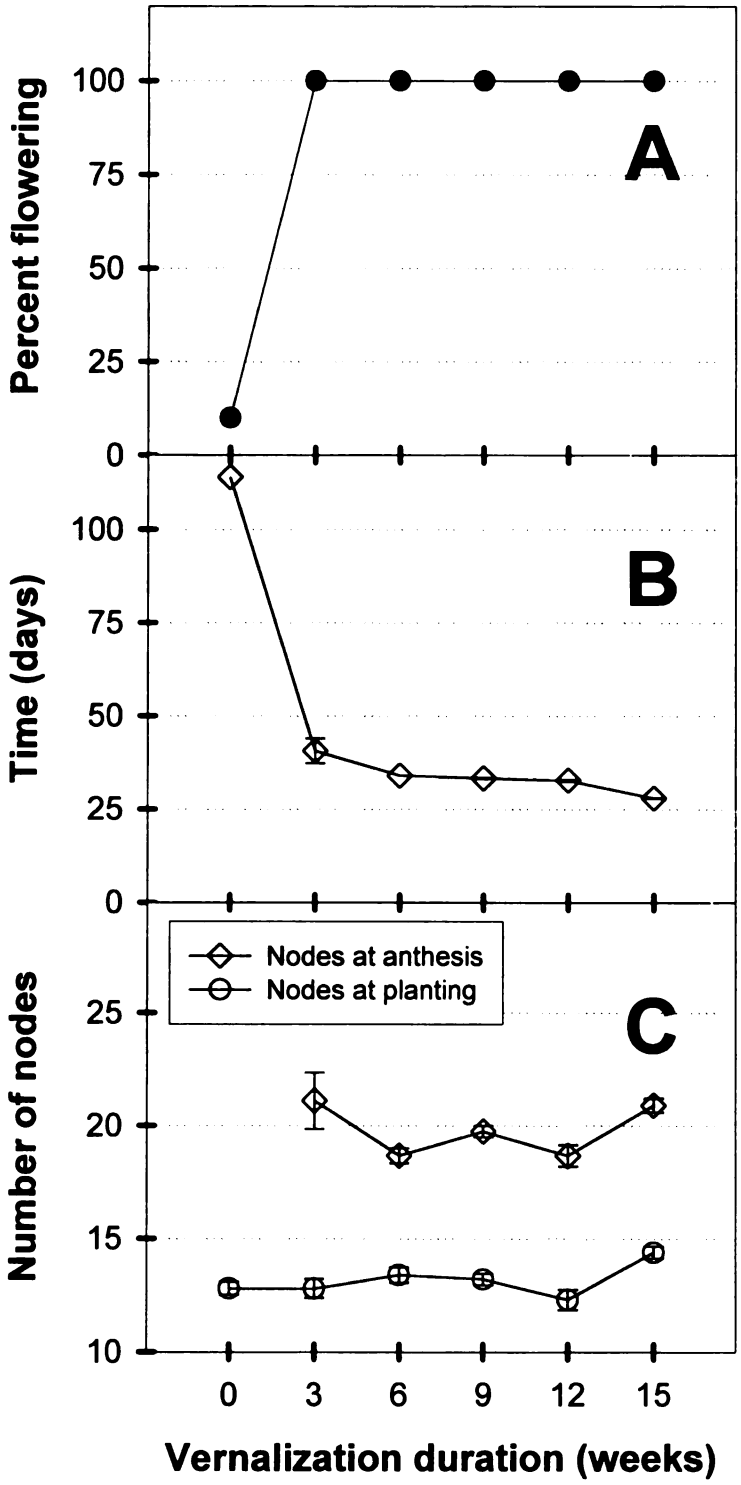
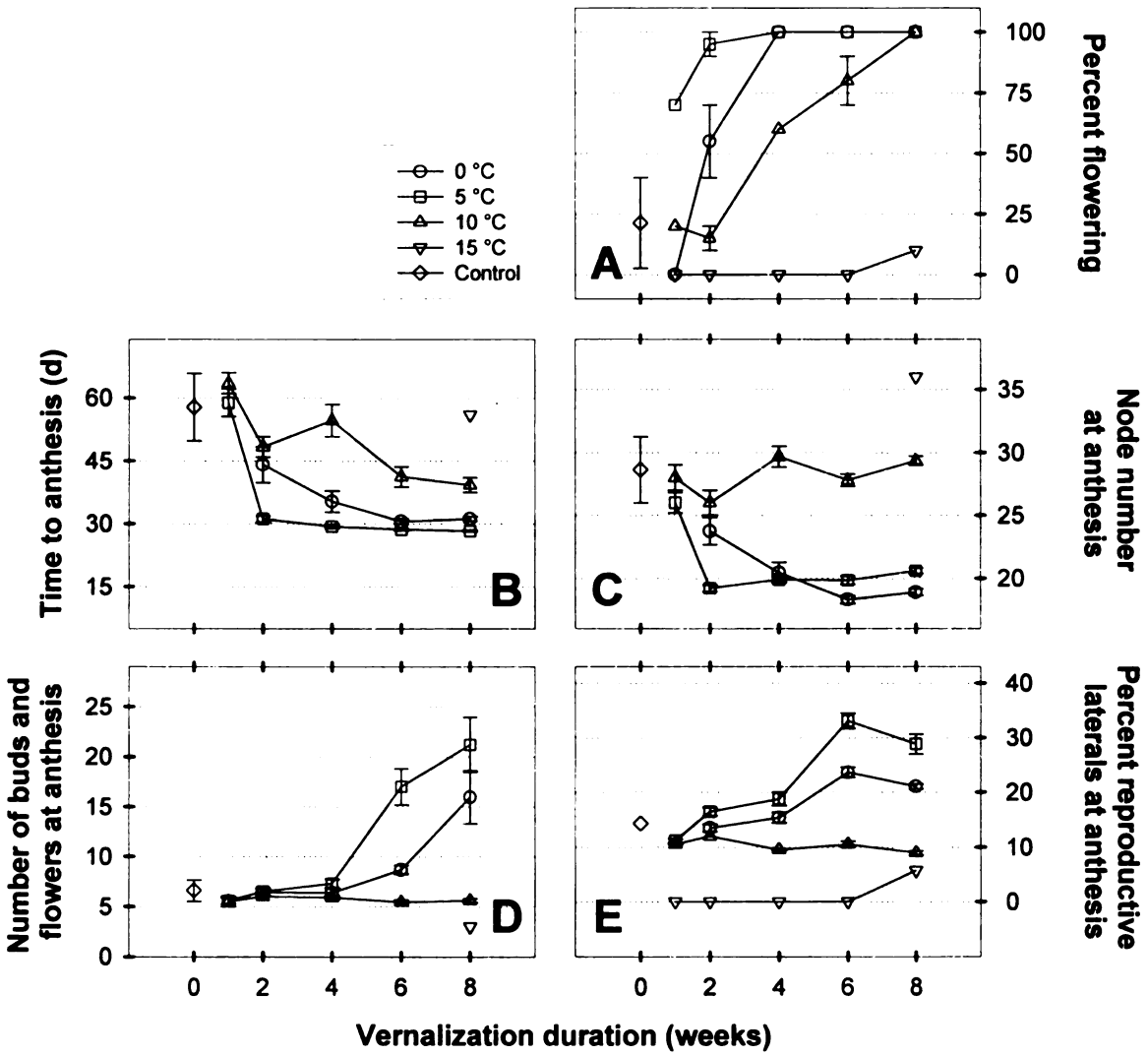


Figure 2. Flowering response of *Dianthus gratianopolitanus* 'Bath's Pink' in experiment II and III following vernalization at 0, 5, 10 or 15 °C at increasing durations. Open symbols represent the averages for flowering plants and vertical bars represent standard errors. In A, flowering percentage was computed 105 d after transplant. In D and E, data were collected at anthesis.



# CHAPTER IV:

# DAILY LIGHT INTEGRAL DURING SECONDARY INDUCTION AFFECTS FLORAL EVOCATION IN *COREOPSIS GRANDIFLORA* 'SUNRAY' FOLLOWING PRIMARY INDUCTION TREATMENTS OF VERNALIZATION OR SHORT-DAY PHOTOPERIOD

Daily Light Integral During Secondary Induction Affects Floral Evocation in *Coreopsis grandiflora* 'Sunray' Following Primary Induction Treatments of Vernalization or Short-day Photoperiod

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*Additional index words.* dual induction, flowering, herbaceous perennial, photosynthetic photon flux, short long day plant

**Abstract**

The influence of vernalization, photoperiod and photosynthetic daily light integral (DLI) on floral evocation of *Coreopsis grandiflora* 'Sunray' was determined. Primary induction treatments of vernalization or short days (SD) were provided to clonally propagated *Coreopsis* 'Sunray' by either vernalizing plants at 5 °C under long days (LD; 16-h photoperiod) or growing them under SD (9-h photoperiod) at 20 °C for 0, 1, 2, 3, 4, 5, 6 or 8 weeks. Following the primary induction treatments, plants were given secondary induction treatments of LD under a high (~15 $mol \cdot m^{-2} \cdot d^{-1}$) or low (~6 $mol \cdot m^{-2} \cdot d^{-1}$) DLI. *Coreopsis* 'Sunray' exhibited a dual induction requirement for floral evocation, with primary induction by vernalization or SD and secondary induction by LD. Vernalization was more effective than SD in promoting floral evocation when their durations were sub-

optimal, as indicated by a higher flowering percentage and accelerated flowering. In addition, following sub-optimal durations of primary induction treatment, floral evocation of terminal and lateral buds was promoted under a high DLI in secondary induction environment. Plants vernalized for sub-optimal durations reached anthesis at an earlier developmental stage under high DLI. Hence, high DLI partially substituted for the primary induction treatment of vernalization or SD. Plant quality at anthesis was improved under high DLI by increased number of inflorescences and reduction in plant height.

**Introduction**

Flowering of many species from temperate origins is regulated by environmental cues of daylength (photoperiod) and low temperature to synchronize reproductive development with favorable environmental conditions and to maximize reproductive success. Flowering of photoperiodic species occurs, or is accelerated when the photoperiod exceeds (long day (LD) plants) or is below (short day (SD) plants) a critical value (Thomas and Vince-Prue, 1997). In some species, floral evocation is promoted following exposure to low temperatures, a phenomenon known as vernalization (Chouard, 1960). A low temperature treatment can also be referred to as a vernalization treatment (Napp-Zinn, 1987). In some species, a dual induction process regulates flowering, which includes a primary and a secondary induction phase (Heide, 1994). Flowering in several temperate grasses and a few herbaceous perennial species is under dual induction regulation; the primary induction is exposure to SD or vernalization and

the secondary induction is exposure to LD (Heide, 1994; Heide, 1995; Vince-Prue, 1975; Wellensiek, 1960). Dual-induction requiring species with primary induction by vernalization or SD have been categorized as vernalization-requiring LD plants or short-long-day (SLD) plants, where vernalization can be replaced by SD or vice versa (Vince-Prue, 1975).

*Coreopsis grandiflora* 'Single Mayfield Giant' was reported to have a dual induction regulation of flowering, with primary induction by SD or vernalization and secondary induction by LD (Ketellapper and Barbaro, 1966). Damann and Lyons (1996) reported that the primary induction in *Coreopsis grandiflora* 'Sunray' occurred only by vernalization, and not by a SD treatment. However, Runkle et al. (unpublished) found that *Coreopsis* 'Sunray' seedlings flowered in response to primary induction treatments of SD or vernalization.

The effect of DLI on floral evocation of herbaceous perennials is species-specific. For example, when non-vernalized and sub-optimally vernalized *Salvia* x*superba* Stapf 'Blaukönigin' was grown under a high DLI (14.4 $mol \cdot m^{-2} \cdot d^{-1}$), a greater percentage of plants flowered and flowering was hastened compared to plants grown under a low DLI (3.6 $mol \cdot m^{-2} \cdot d^{-1}$; Waaseth et al., 2006). However, after a saturating vernalization treatment, a higher DLI had no effect on flowering percentage and only slightly hastened flowering (Waaseth et al., 2006). In contrast, flowering percentage and time to anthesis were not influenced by DLI (from 5 to 20 $mol \cdot m^{-2} \cdot d^{-1}$) in *Achillea* x*millefolium* L. 'Red Velvet', *Gaura lindheimeri* Engelm. & Gray 'Siskiyou Pink' and *Lavandula angustifolia* Mill. 'Hidcote Blue' (Fausey et al., 2005).

In our preliminary studies, following sub-optimal exposure durations of vernalization or SD treatments, floral evocation was reduced under low photosynthetic daily light integral (DLI) in the secondary induction environment (Appendix A). The objectives of this study were to: 1) establish and characterize the influence of primary induction treatments of SD or vernalization on floral evocation in *Coreopsis* 'Sunray', and 2) evaluate the effect of DLI during secondary LD-induction on floral evocation and subsequent flowering characteristics.

**Materials and Methods**

*Stockplant Management and Propagule Culture*. *Coreopsis* 'Sunray' stockplants were developed by harvesting vegetative cuttings from a single seedling selected for high vigor from a 50-seedling population. The cloned stockplants were grown in 13-cm square plastic containers (1.1-L volume) filled with commercial soil-less medium (Sure-Mix; Michigan Grower Products, Galesburg, Mich.) in a controlled environment chamber set at constant 20 °C under a 16-h photoperiod (0600 to 2200 HR) provided by fluorescent and incandescent lamps that delivered ~150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (*PPF*) at plant height. Stockplants were watered when necessary with acidified well water ($H_2SO_4$ to a titratable alkalinity of ~140 $mg \cdot L^{-1}$ $CaCO_3$) containing 40 N, 4 P, 40 K, 5 Ca, 0.3 Fe, 0.2 Mn, 0.2 Cu, 0.03 B, 0.03 Mo, 0.2 Zn $mg \cdot L^{-1}$ (MSU Special, Greencare Fertilizers, Chicago). Shoot-tip cuttings with 3-4 nodes were harvested from stockplants 0, 1, 2, 3, 4, 5, 6 and 8 weeks prior to 1 March 2006.

The cuttings were dipped in a commercial rooting hormone (Dip 'n Grow; ; Clackamas, Ore.) containing 1 $g \cdot L^{-1}$ indole-3-butyric acid and 0.5 $g \cdot L^{-1}$ naphthalene acetic acid and rooted in 72-cell trays (50-mL cell volume; Landmark Plastic Corporation, Akron, Ohio) containing 50% commercial peat-perlite media (Sure-Mix, Michigan Growers Products, Galesburg, Mich.) and 50% coarse perlite (Therm-O-Rock, East Inc., New Eagle, Pa.). Cuttings were rooted under mist for 11 d and then weaned for 3 d by hand watering in a propagation facility. During propagation, a 16-h photoperiod was provided by sunlight from 0600 to 1700 HR and incandescent lamps from 1700 to 2200 HR. The propagation facility was set at air and medium temperatures of 23 and 26 °C, respectively and a 0.3 kPa vapor pressure deficit was maintained by injecting water vapor in the air. After rooting, propagules were grown in 72-cell trays for 2 weeks in a controlled environment chamber under the same environmental and nutritional conditions as stockplants until primary induction treatments were initiated.

*Primary Induction Treatments.* For vernalization treatments, plants in 72-cell trays were placed in a controlled environment chamber set at 5 °C under 16-h photoperiod provided by a combination of fluorescent and incandescent lamps (~50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from 0600 to 2200 HR. During vernalization, plants were watered when necessary with the same nutrients as stockplants. Plants receiving SD treatment were potted in filled 13-cm containers (as previously described) and grown in a greenhouse under a 9-h photoperiod maintained by a

blackout system that opened between 0800 to 1700 HR. The photoperiod was provided by sunlight supplemented by high-pressure sodium (HPS) lamps that automatically turned on when ambient light was below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* and ceased when ambient light exceeded 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*. HPS lamps provided an additional 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*. In the greenhouse, plants were watered using reverse osmosis water containing 125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe, 0.5 Mn, 1.0 Cu, 0.3 B, 0.1 Mo $mg \cdot L^{-1}$ (MSU Special, Greencare Fertilizers, Chicago). Induction treatments were imposed every 1, 2, 3, 4, 5, 6 and 8 weeks and constant LD (0-week primary induction treatment) and SD controls were planted such that all ended on 1 March 2006.

*DLI Treatments during Secondary Induction.* At the end of vernalization treatments, plants were transplanted into 13-cm containers and maintained under the same nutritional regimen as plants under SD. Plants from each treatment combination were randomly selected and placed on greenhouse benches with a high or low DLI. Based on preliminary experiments, 15 $mol \cdot m^{-2} \cdot d^{-1}$ and 6 $mol \cdot m^{-2} \cdot d^{-1}$ were selected as target high and low DLIs for secondary induction, respectively. The high DLI environment received 16-h photoperiod by sunlight supplemented with HPS lamps (at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*) when ambient light was below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* and ceasing when ambient light exceeded 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*. The low DLI environment was created by hanging permanent woven shade curtain that reduced light transmission by 50% (OLS 50; Ludvig Svensson, Charlotte, N.C.) and applying a thin layer of whitewash on the glass

glazing. Irradiance at plant level was measured under high and low DLI environments by line quantum sensors containing 10 photodiodes (Apogee Instruments, Logan, Utah) connected to a CR10 datalogger (Campbell Scientific, Logan, Utah). Light data were collected every 10 s and hourly averages were recorded in a computer. Average DLI was computed for each plant from the day of transplant until anthesis of first inflorescence and 105 d from transplant for flowering and vegetative plants, respectively. Based on the measured DLI, shading and lighting was manipulated such that average DLI received by all plants under high and low DLI environments ranged between 14.8 to 15.7 and 5.7 to 6.1 $mol \cdot m^{-2} \cdot d^{-1}$, respectively. The highest standard deviations for average DLI received by a single plant were 5.3 and 2.1 under high and low DLI environments, respectively.

Air temperature was measured every 10 s at plant height on each greenhouse bench using Type E thermocouples (TT-E-40; Omega engineering, Stamford, Conn.) placed in aspirated tubes and hourly averages were recorded in a computer. Average daily temperature (ADT) was computed for each plant for the same durations as average DLI. ADT of plants under high and low DLI environments ranged between 20.4 to 21.6 and 20.3 to 21.3 °C, respectively, and the highest standard deviations for ADT received by a single plant was 1.7 and 2.0 °C under high and low DLI environments, respectively.

*Data Collection and Analysis.* The number of nodes on each plant was recorded prior to primary and secondary induction treatments. At the appearance of first visible inflorescence and anthesis of first inflorescence, dates

were recorded and time to visible inflorescence and anthesis were computed from the initiation of secondary induction treatments. Plants without a visible inflorescence 105 d after the start of secondary induction were considered vegetative and flowering percentage was computed for each treatment combination. Rate of progress to anthesis was computed by taking the reciprocal of time to anthesis; the rate of anthesis of vegetative plants was reported as zero. The number of nodes below the terminal inflorescence was also recorded at anthesis. At anthesis, the number of vegetative and reproductive lateral shoots was counted and percent reproductive laterals was calculated. Also, at anthesis, the number of inflorescences was recorded and plant height from the plant base to the highest point was measured. Ten plants were subjected to each treatment combination in a completely randomized design. Response variables for each primary and secondary induction treatment were individually subjected to regression analysis using Sigma Plot version 8.0 (SPSS Inc., Chicago) when significant at $P$=0.0005.

**Results**

*Flowering Response.* No plants flowered without a primary induction treatment (Fig. 1A, E) or under continuous SD (data not shown). As primary induction treatment duration increased, an increasingly greater percentage of plants flowered until all plants flowered (Table 1). Under high DLI, ≥2 and ≥3-week primary induction treatments of vernalization and SD induced complete flowering, respectively. Under low DLI, 0, 40, 70 and 100% plants flowered following 1, 2, 3

and ≥4-week vernalization treatments, respectively. When treated with SD for 1, 2, 3 and ≥4 weeks, 0, 0, 90 and 100% plants flowered under low DLI, respectively. Thus, following sub-optimal durations of primary induction treatments, floral evocation was promoted under high DLI during secondary induction. In addition, sub-optimal durations of vernalization were generally more effective in promoting floral evocation than SD of the same duration for primary induction.

*Flowering Time*. The rate of progress to anthesis increased with primary induction treatment durations in similar patterns as did flowering percentage (Fig. 1B, F). Following sub-optimal durations of either primary induction treatment, high DLI reduced time to anthesis (Fig, 1C, G), and hence increased the rate of anthesis. Vernalization was generally more effective in promoting floral evocation than SD treatment following short durations irrespective of DLI during secondary induction. Increasing the duration of vernalization treatment beyond 2 or 4 weeks did not further increase flowering percentage under high and low DLIs in the secondary induction environment, respectively, whereas, time to anthesis decreased with increasing vernalization treatment duration up to 8 weeks. Vernalization and SD treatment of ≥3 weeks caused similar decreases in time to anthesis under both DLIs . Following 8 weeks of primary induction by vernalization or SD, plants flowered in about 50 d. Average time to visible inflorescence and anthesis of first inflorescence was highly correlated for all

treatment combinations (Fig. 2A) and the time from visible inflorescence to anthesis was ~23 d.

To evaluate the effect of induction treatments based on plant developmental age, the number of nodes that developed during secondary induction treatment to anthesis were assessed. Node number at anthesis decreased with increasing primary induction treatment and followed a trend similar to time to anthesis (Fig. 1D, H); there was a significant correlation between time to anthesis and node number at anthesis (Fig. 2B). This suggests that differences in time to anthesis could be primarily attributed to floral evocation that occurred at an earlier developmental stage. On average, 7 nodes were formed below the terminal flower during secondary induction under both DLIs after 8-week primary induction treatment.

*Flowering Characteristics*. Total laterals, percent reproductive laterals, number of inflorescences and plant height at anthesis were measured to assess the influence of induction treatments on the subsequent flowering characteristics. DLI during secondary induction did not affect the number of total laterals produced (Fig. 3A, E). Vernalized flowering plants produced 23 to 33 laterals and there was no apparent relationship between total laterals and duration of vernalization treatment. SD-treated plants produced 32 to 44 laterals on average, and for treatments that induced complete flowering, total lateral number increased with exposure to SD.

Regression analyses of percent reproductive laterals yielded sigmoidal response curves similar to flowering percentage and rate of anthesis for all induction treatment combinations (Fig. 3B, F). Under high DLI, vernalized plants produced more reproductive laterals than SD-treated plants irrespective of treatment duration. Under low DLI, plants vernalized for ≥5 weeks produced a higher percentage of reproductive laterals than SD-treated plants. Most plants had 100% reproductive laterals following vernalization treatments of ≥4 and ≥6 weeks under high and low DLIs, respectively. However, with the exception of one plant, SD treatments resulted in <100% reproductive laterals. Overall, following SD treatment for 4 weeks, percent reproductive laterals averaged 77 and 66% under a high and low DLI, respectively.

The number of inflorescences increased with exposure duration of either primary induction treatment (Fig. 3C, G). With longer primary induction treatment durations, the number of inflorescences saturated after 2 and 4 weeks of vernalization under a high and low DLI, respectively and after 4 weeks of SD under low DLI. However, under a high DLI, the number of inflorescences on SD-treated plants increased following primary treatment for up to 8 weeks, the longest duration tested. Typically, following either primary induction treatment, more inflorescences were produced under a high than low DLI.

With few exceptions, plants under the high DLI during secondary induction were shorter at anthesis compared to plants grown under the low DLI (Fig. 3D, H). Generally, duration of either primary treatment did not affect plant height at

anthesis and plants were of similar height following either primary induction treatment.

**Discussion**

*Coreopsis* 'Sunray' responded qualitatively to dual induction treatments and primary induction was achieved by vernalization or SD. These results contradict the finding of Damann and Lyons (1993) that SD can not substitute for vernalization as a primary induction treatment in *Coreopsis* 'Sunray'. Damann and Lyons (1993) transferred seedlings to LD (natural photoperiod with 4-h night interruption) after they had unfolded up to 12 nodes (according to Yuan et al. (1997), juvenility ends at ~8 nodes) under SD (9-h photoperiod) and observed no flowering. Although Damann and Lyons (1993) did not specify the duration of SD exposure, in our experiment, ~1 node was unfolded in 1 week under SD at 20 °C (data not shown). Hence, it is probable that the SD exposure provided by Damann and Lyons was suboptimal. Damann and Lyons (1933) did not indicate the DLI during the secondary induction and it is possible that secondary induction was under low DLI or that there were other limiting factors. Our results indicate that in *Coreopsis* 'Sunray', following sub-optimal durations of primary induction treatment, floral evocation can be reduced or inhibited under low DLI during the secondary induction and this may explain why our results differed from those of Damann and Lyons.

Niu et al. (2002) reported 0% flowering of *Coreopsis* 'Sunray' seedlings under LD (16-h photoperiod; ~3.7 $mol \cdot m^{-2} \cdot d^{-1}$ DLI) following a 5-week exposure

to SD (12-h photoperiod). We have observed 0% and 50% flowering following SD treatment at 12-h photoperiod, when secondary induction was under low (9.6 $mol \cdot m^{-2} \cdot d^{-1}$) and high (16.6 $mol \cdot m^{-2} \cdot d^{-1}$) DLIs, respectively (Appendix B). Therefore, the lack of flowering after the 5-week SD treatment reported by Niu et al. (2002) could be attributed to the photoperiod used.

Damann and Lyons (1996) reported 20% flowering under continuous SD (9-h photoperiod), whereas in the current study, no plants flowered under constant SD. We can not explain this discrepancy. Although our results are based on clonally propagated plants developed from a single seedling, Runkle et al. (unpublished) found a similar flowering response in *Coreopsis* 'Sunray' seedlings. Our results are consistent with the flowering response of *Coreopsis grandiflora* 'Single Mayfield Giant', which was reported to have a similar dual induction requirement (Ketellapper and Barbaro, 1966). For both *Coreopsis grandiflora* cultivars, the primary induction requirement could be met by either SD or vernalization, and the secondary induction requirement by LD.

Dual regulation of flowering by SD or vernalization treatment followed by LD has been reported in only a few herbaceous perennial species including *Campanula medium* L. and *Leucanthemum vulgare* Lam. (Heide, 1995; Wellensiek, 1960) and several grasses of temperate origin (Heide, 1994). Initially, it was proposed that in *Campanula medium*, primary induction via vernalization or SD occurs through distinct pathways (Wellensiek et al., 1960). Recently, Dubcovsky et al. (2006) reported that in dual induction-requiring *Triticum monococcum*, exposure to SD downregulated the vernalization

responsive flowering inhibitor *VRN2* and during secondary induction under LD, the meristem identity gene *VRN1* was upregulated. Hence, in *Triticum monococcum*, SD substituted for vernalization by regulating upstream vernalization responsive genes. Although the molecular regulation of floral evocation in response to primary induction via vernalization or SD in dual induction requiring herbaceous perennials including *Coreopsis* 'Sunray' remains unknown, a mechanism similar to *Triticum monococcum* may be responsible for the unique flowering requirement.

Following sub-optimal durations of primary induction treatment, vernalization at 5 °C under LD was typically more effective in promoting floral evocation in *Coreopsis* 'Sunray' than the same durations of SD treatment at 20 °C based on flowering percentage, flowering time, percent reproductive laterals and number of inflorescences at anthesis. Hence, it appears that *Coreopsis* 'Sunray' was more sensitive to primary induction via vernalization than SD treatment under our experimental conditions. Heide (1995) reported that in *Leucanthemum vulgare*, primary induction predominantly occurred through vernalization and SD treatment did not fully substitute for vernalization. Also, *Campanula medium* flowered completely when primary induction was by vernalization whereas, after primary induction by SD treatment, flowering percentage was rarely 100% (Wellensiek, 1960). These results indicate that vernalization has a stronger induction effect than SD treatment for plants with a dual induction flowering including *Coreopsis* 'Sunray', *Leucanthemum vulgare* and *Campanula medium*.

DLI before, during, and after induction treatments can influence floral evocation in some herbaceous perennial species. For example, low pre-vernalization DLI (4 $mol \cdot m^{-2} \cdot d^{-1}$) induced a higher flowering percentage in *Lavandula angustifolia* 'Hidcote' and hastened floral evocation in *Aquilegia xhybrida* Sims 'Remembrance' compared to high DLI (14 $mol \cdot m^{-2} \cdot d^{-1}$; Niu et al., 2002). Although the influence of DLI prior to primary induction treatments on floral evocation was not evaluated in this study, Niu et al. (2002) reported that pre-vernalization DLI did not affect floral evocation or flowering characteristics of *Coreopsis* 'Sunray'. In the current study, following ≤3-week exposure to either primary induction treatment, fewer plants of *Coreopsis* 'Sunray' flowered during secondary induction under a low DLI, compared to a high DLI.

We also assessed the influence of DLI during secondary induction on timing of floral evocation. The reported time to anthesis of *Coreopsis* 'Sunray' after saturating durations of either primary induction treatment in this study is consistent with previous reports (Damann and Lyons, 1996; Yuan et al., 1997; Yuan et al., 1998). Following sub-optimal vernalization treatment, flowering was hastened under a high DLI due to floral evocation at an earlier developmental stage as indicated by the formation of fewer nodes under the terminal flower compared to plants under the low DLI. However, for treatment durations that resulted in complete flowering, SD treatment resulted in a similar number of nodes below the terminal flower under both DLIs. Therefore, at least in the case of plants vernalized for sub-optimal durations, high DLI was a promoter of floral evocation.

In many determinate herbaceous perennial species including *Coreopsis* 'Sunray', the strength of the induction "signal" can be assessed by evaluating percent reproductive laterals (Fausey, 2005; Heide, 1995) since, in these species, the terminal bud is induced first, followed by the lateral buds, from apical to basipital (personal observation). *Coreopsis* 'Sunray' produced a similar number of total laterals at anthesis, although under high DLI, there were more reproductive laterals following vernalization treatment for ≤5 weeks and following all durations except 4 weeks of SD treatment. Hence, the observed decrease in percent reproductive laterals under low DLI was due to failure of some laterals to become reproductive. Overall, our results suggest that following sub-optimal exposure to primary induction treatments, floral evocation of apical and basipital buds was limited and floral evocation was delayed during secondary induction under low DLI.

A similar role of DLI has been reported in plants with a quantitative vernalization response including *Salvia* x*superba* 'Blaukönigin' (Waaseth et al., 2006) and *Digitalis purpurea* L. 'Foxy' (Fausey et al., 2001). Several physiological studies have investigated the mechanism involved in promotion of floral evocation by high DLI and it has been proposed that the increased concentration of assimilates under high DLI may promote floral evocation via a feed forward control and the timing of elevated assimilate supply may also be of significance (reviewed by Sachs, 1987). Waaseth et al. (2006) postulated that regulation of flowering occurred in *Salvia* x*superba* 'Blaukönigin' via a *PPF*-dependant flowering pathway and proposed that following vernalization the *PPF*-

dependant flowering pathway became redundant. However, in the current study, the lack of flowering without an induction treatment under high DLI does not support the existence of *PPF*-dependant flowering pathway operating parallel to a primary induction pathway in *Coreopsis* 'Sunray'. Alternatively, *Coreopsis* 'Sunray' requires at least some primary induction for *PPF*-dependant pathway to operate, implying that high DLI can partially substitute for primary induction treatments of vernalization or SD.

The observed increase in number of total laterals in *Coreopsis* 'Sunray' plants treated with SD for ≥3 weeks was probably due to the formation of additional laterals during SD treatment, and since some of these laterals did not receive sufficient SD exposure, percent reproductive laterals did not increase with increasing durations of SD treatment. Due to development of laterals under SD that were not induced by the end of SD treatment, percent reproductive laterals of SD-treated plants never reached 100%, unlike vernalized plants. Also, plants treated with 1 or 2 weeks of SD had more laterals than plants treated for 3 weeks as their flowering was delayed and therefore additional laterals may have been developed during secondary induction.

In addition to promoting floral evocation and its timing, high DLI may also impact various flowering characteristics including number of laterals and inflorescences produced and plant height at anthesis. As discussed earlier, DLI during secondary induction of *Coreopsis* 'Sunray' did not affect the number of laterals produced at anthesis. However, the number of laterals increased with DLI in three chrysanthemum cultivars (Schoellhorn et al., 1996) and in *Heliconia*

'Golden Torch' (Catley and Brooking, 1996). In this study, *Coreopsis* 'Sunray' typically had more inflorescences at anthesis under high DLI compared to low DLI. This is consistent with production of more inflorescences under higher DLI reported in several herbaceous annual and perennial species, presumably due to availability more assimilates for reproductive development (Fausey et al., 2005; Faust et al., 2005; Niu et al., 2001; Pramuk and Runkle, 2005). The effect of DLI on plant height is species-specific, with high DLI promoting, inhibiting or not affecting stem extension, depending on the species (Faust et al., 2005). Generally, under high DLI, stem extension of *Coreopsis* 'Sunray' plants was inhibited compared to low DLI.

In summary, our results demonstrate that flowering of *Coreopsis* 'Sunray' is regulated by a dual induction process, with primary induction via vernalization or SD treatment and the secondary induction via LD. Hence, *Coreopsis* 'Sunray' can be categorized as a short-long-day plant or vernalization-requiring LD plant. Vernalization was somewhat more effective in promoting floral evocation if induction treatment durations were sub-optimal. Floral evocation of *Coreopsis* 'Sunray' was promoted by high DLI during secondary induction following exposure to sub-saturating durations of either primary induction treatment. High DLI during secondary induction also improved plant quality: inflorescences produced at anthesis were greater and plants were shorter when grown under a high DLI.

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Table 1. Parameters of regression analyses (significant at $P$=0.0005) relating to flowering percentage, rate of anthesis, time to anthesis, nodes at anthesis, percent reproductive laterals and number of inflorescences at anthesis of *Coreopsis grandiflora* 'Sunray' following primary induction treatments of vernalization or short days (SD) and secondary induction under high or low daily light integrals (DLIs).

| Parameter | Treatment | DLI | Equation[z] | $r^2$ |
|---|---|---|---|---|
| Flowering percentage | Vernalization | High | y=100/(1+exp(−(x−0.06)/0.87)) | 1.00 |
| Flowering percentage | Vernalization | Low | y=100.9/(1+exp(−(x−2.39)/0.56)) | 0.99 |
| Flowering percentage | SD | High | y=100.7/(1+exp(−(x−1.63)/0.42)) | 1.00 |
| Flowering percentage | SD | Low | y=100/(1+exp(−(x−2.88)/0.06)) | 1.00 |
| Rate of anthesis | Vernalization | High | y=0.019/(1+exp(−(x−0.81)/0.18)) | 0.90 |
| Rate of anthesis | Vernalization | Low | y=0.019/(1+exp(−(x−2.84)/0.67)) | 0.84 |
| Rate of anthesis | SD | High | y=0.020/(1+exp(−(x−1.91)/0.43)) | 0.86 |
| Rate of anthesis | SD | Low | y=0.019/(1+exp(−(x−2.84)/0.14)) | 0.96 |
| Time to anthesis | Vernalization | High | $y=66.0+(-4.31)*x+0.25*x^2$ | 0.76 |
| Time to anthesis | Vernalization | Low | $y=105.0+(-14.71)*x+1*x^2$ | 0.79 |
| Time to anthesis | SD | High | y=123.4+(−75.5)*(1−exp(−0.8*x)) | 0.97 |
| Time to anthesis | SD | Low | y=6457.5+(−6406)*(1−exp(−2.1*x)) | 1.00 |
| Nodes at anthesis | Vernalization | High | $y=10.6+(-0.76)*x+0.03*x^2$ | 0.79 |
| Nodes at anthesis | Vernalization | Low | $y=15.2+(-2.04)*x+0.12*x^2$ | 0.79 |
| Nodes at anthesis | SD | High | y=17.6+(−10.9)*(1−exp(−0.67*x)) | 0.98 |
| Nodes at anthesis | SD | Low | y=417.3+(−410.3)*(1-exp(−1.9*x)) | 0.97 |
| Percent reproductive laterals | Vernalization | High | y=96.2/(1+exp(−(x−1.15)/0.51)) | 0.92 |
| Percent reproductive laterals | Vernalization | Low | y=93.4/(1+exp(−(x−3.31)/0.67)) | 0.90 |
| Percent reproductive laterals | SD | High | y=77.9/(1+exp(−(x−2.26)/0.48)) | 0.86 |
| Percent reproductive laterals | SD | Low | y=65.7/(1+exp(−(x−2.95)/0.11)) | 0.85 |
| Number of inflorescences | Vernalization | High | y=64.3/(1+exp(−(x−0.80)/0.2)) | 0.81 |
| Number of inflorescences | Vernalization | Low | y=121.5/(1+exp(−(x−2.85)/0.69)) | 0.71 |
| Number of inflorescences | SD | High | y=291/(1+exp(−(x−5.26)/2.27)) | 0.75 |
| Number of inflorescences | SD | Low | y=107.8/(1+exp(−(x−2.88)/0.26)) | 0.75 |

Figure 1. Flowering of *Coreopsis grandiflora* 'Sunray' in response to primary induction treatment of vernalization (◇/□) at 5 °C with 16-h long days (A-D) or short-days (SD; △/▽) under 9-h photoperiod at 20 °C (E-H) for varying durations and secondary induction treatments of long days under high (◇/△) or low (□/▽) daily light integrals (DLI). Flowering response was assessed as flowering percentage 105 d from initiation of secondary induction (A, E), rate of progress to flowering computed as reciprocal of time to anthesis with flowering rate of vegetative plants reported as zero (B, F), time to anthesis from initiation of secondary induction (C, G) and number of nodes from initiation of secondary induction until anthesis (D, H). Closed symbols are based on 10 observations and open symbols represent average±SE of 10 replicates for B, F and average±SE of flowering plants for C-D, G-H. Solid and dashed lines represent regression analyses for individual plants under high and low DLIs, respectively.

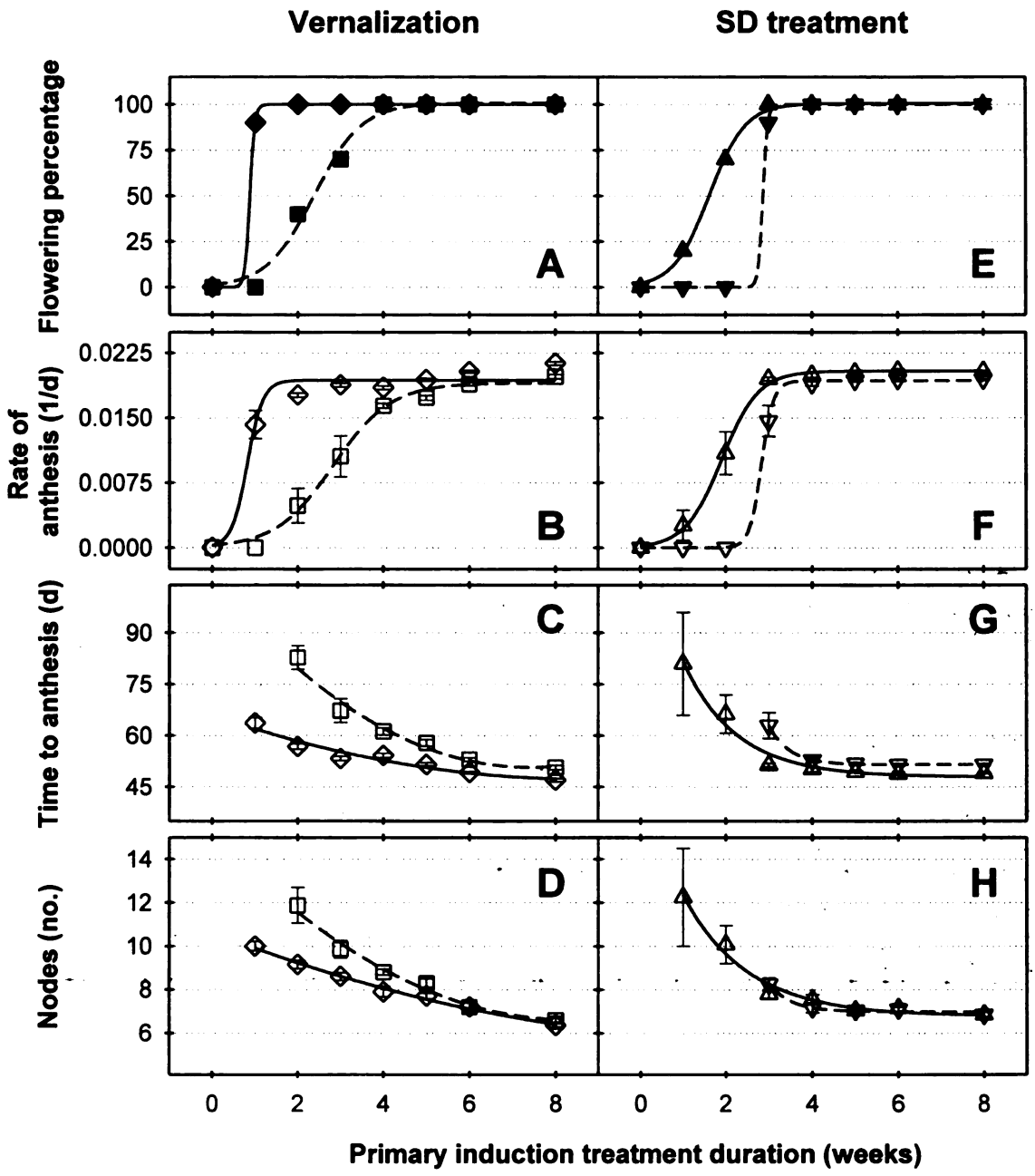

Vernalization
SD treatment
Flowering percentage
100
75
50
25
0
A
E
Rate of anthesis (1/d)
0.0225
0.0150
0.0075
0.0000
B
F
Time to anthesis (d)
90
75
60
45
C
G
Nodes (no.)
14
12
10
8
6
D
H
0
2
4
6
8
Primary induction treatment duration (weeks)

Figure 2. Correlation analysis between time to anthesis and time to visible inflorescence of *Coreopsis grandiflora* 'Sunray' plants computed from the start of secondary induction to anthesis and appearance of first visible inflorescence, respectively (A). Correlation analysis between time to anthesis and number of nodes developed from the start of secondary induction to anthesis (B). Data are pooled for all flowering plants that were previously given primary induction treatments of vernalization (16-h photoperiod at 5 °C) or short-days (9-h photoperiod at 20 °C). Each regression line is generated using over 225 observations for all flowering plants (significant at $P$=0.0001; $r^2$=0.96 and 0.79 for A and B, respectively).

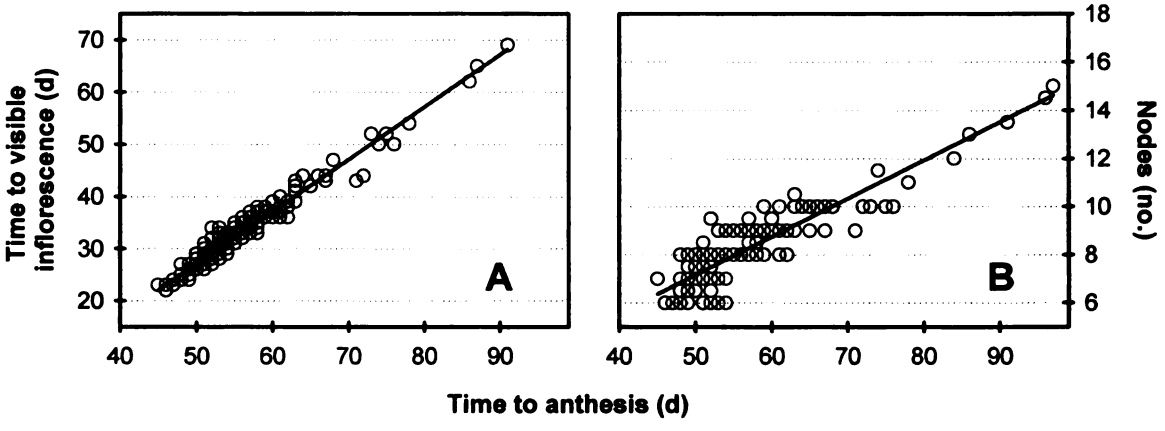
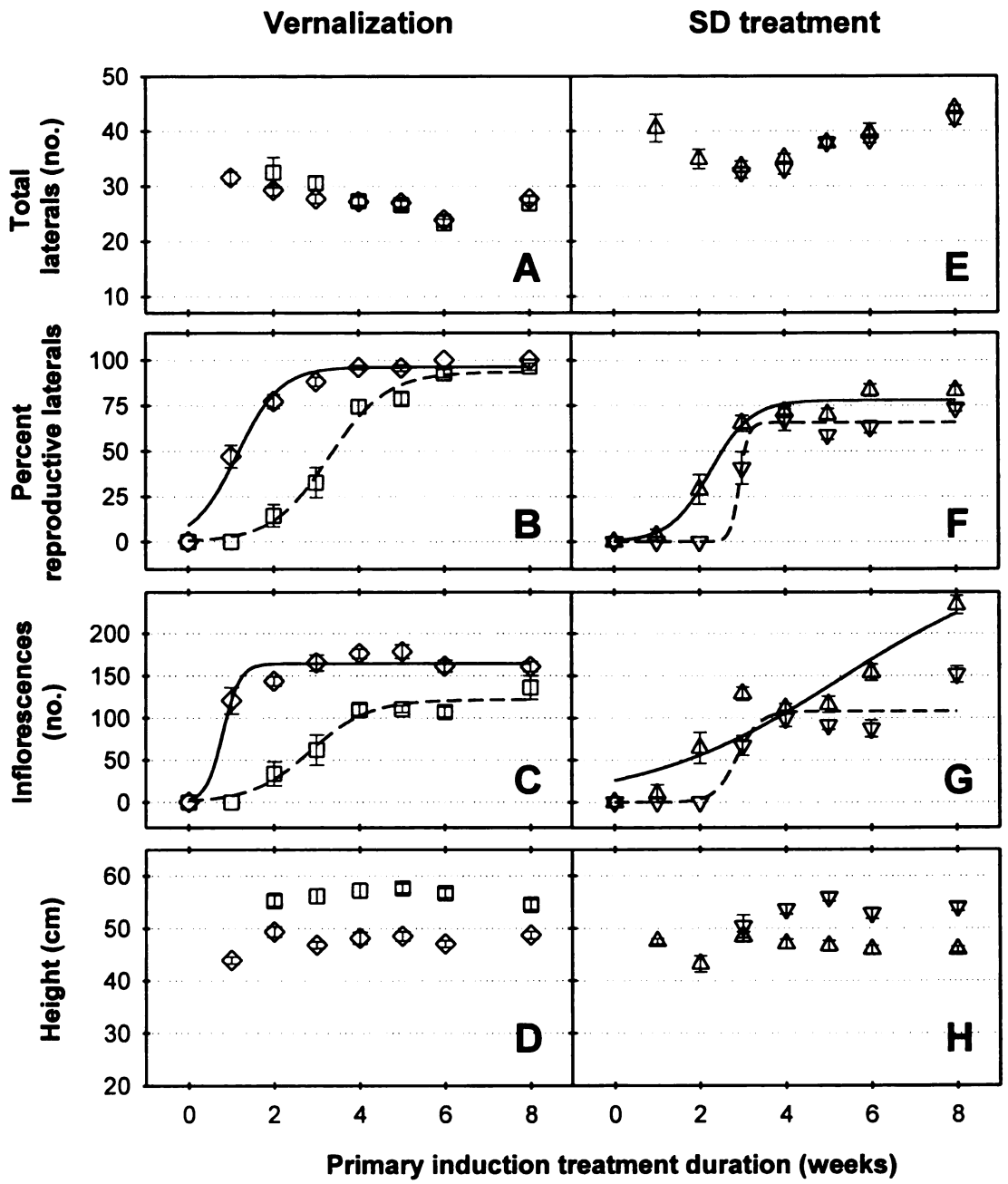


Figure 3. Flowering characteristics of *Coreopsis grandiflora* 'Sunray' in response to primary induction treatment of vernalization (◇/□) at 5 °C with 16-h long days (A-D) or short-days (SD; △/▽) under 9-h photoperiod at 20 °C (E-H) at varying durations and secondary induction treatments of long days under high (◇/△) or low (□/▽) daily light integrals (DLI). Flowering characteristics assessed included total laterals (A, E), percent reproductive laterals (B, F), number of inflorescences (C, G) and plant height (D, H) at anthesis. Plant height was measured from plant base to the highest point. Open symbols represent average±SE of 10 replicates for B-C, F-G and average±SE of flowering plants for A, D, E, H. Solid and dashed lines represent regression analyses for individual plants under high and low DLIs, respectively.


Vernalization
SD treatment
Total laterals (no.)
Percent reproductive laterals
Inflorescences (no.)
Height (cm)
A
B
C
D
E
F
G
H
Primary induction treatment duration (weeks)

# APPENDIX A:

# DAILY LIGHT INTEGRAL AFFECTS THE FLOWERING RESPONSE OF *COREOPSIS GRANDIFLORA* 'SUNRAY' TO INDUCTIVE TREATMENTS OF VERNALIZATION OR SHORT-DAY PHOTOPERIOD

Daily Light Integral Affects the Flowering Response of *Coreopsis grandiflora* 'Sunray' to Inductive Treatments of Vernalization or Short-day Photoperiod

## Introduction

*Coreopsis grandiflora* 'Sunray' flowers under long-day (LD) photoperiods following vernalization (Yuan et al., 1998). Short-day (SD) photoperiods substituted for vernalization in *Coreopsis* 'Single Mayfield Giant' (Ketellapper and Barbaro, 1966). Damann and Lyons (1993) reported that *Coreopsis* 'Sunray' seedlings did not flower in response to 10 weeks of SD followed by LD. However, Runkle et al. (unpublished data) found that 10 weeks of SD followed by LD resulted in complete (100%) flowering of *Coreopsis* 'Sunray'. The objectives of this study were to: 1) establish *Coreopsis* 'Sunray' as a short-long-day (SLD) plant, 2) determine the effective duration of vernalization or SD treatment prior to LD for complete and uniform flowering, and 3) determine whether photoperiod during vernalization influences flowering.

## Materials and Methods

*Stockplant Management and Propagule Culture.* To minimize genetic variability, stockplants were developed from a single seedling and all plants used in subsequent experiments were clonally propagated. The plant selection for clonal propagation was made from a seedling population based on high vigor. Stockplants were maintained in 13-cm square pots (1.1 L) in commercial soil-less

media containing peat and perlite and grown under a 16-h photoperiod at a 20 °C temperature setpoint in a glass greenhouse and growth chamber in experiments I and II, respectively. In the greenhouse, the 16-h photoperiod was provided from 0600 to 2200 HR by a combination of sunlight and high-pressure sodium (HPS) lamps that turned on when ambient light levels were below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (*PPF*) and ceased when the light exceeded 400 $\mu mol \cdot m^{-1} \cdot s^{-1}$ *PPF*. The HPS lamps provided an additional 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*. In the growth chamber, the 16-h photoperiod was provided by a combination of fluorescent and incandescent lamps and the *PPF* was ~150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Stockplants grown in the greenhouse were watered with reverse osmosis water containing 125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe, 0.5 Mn, 1.0 Cu, 0.3 B, 0.1 Mo $mg \cdot L^{-1}$ (MSU Special, Greencare Fertilizers, Chicago). In the growth chamber, stockplants were watered with acidified well water ($H_2SO_4$ to a titratable alkalinity of ~140 $mg \cdot L^{-1}$ $CaCO_3$) containing nutrients (40 N, 4 P, 40 K, 5 Ca, 0.3 Fe, 0.2 Mn, 0.2 Cu, 0.03 B, 0.03 Mo, 0.2 Zn $mg \cdot L^{-1}$; MSU Special, Greencare Fertilizers, Chicago).

In experiment I, cuttings were taken on 27 February 2004, dipped in a commercial rooting hormone (Dip 'N Grow; Clackamas, Ore.) containing 1000 ppm indole-3-butyric acid and 500 ppm naphthalene acetic acid and stuck in 72-cell trays (50-mL cell volume; Landmark Plastic Corporation, Akron, Ohio) containing 50% commercial peat-perlite media (Sure-Mix, Michigan Growers Products, Galesburg, Mich.) and 50% coarse perlite (Therm-O-Rock; East Inc., New Eagle, Pa.). Cuttings were rooted under mist in a propagation house under

16-h photoperiod from 0600 to 2200 HR, provided by a combination of sunlight and incandescent lamps. The air and soil temperature setpoints in the propagation house were 23 and 26 °C, respectively and a 0.3 kPa vapor pressure deficit was generated by injecting water vapor in the air. Plants were rooted for 2 weeks and then grown in 72-cell trays in a growth chamber for 2 weeks under the same environmental and nutritional conditions as stockplants grown in the growth chamber. In experiment II, cuttings were taken 0, 4, 8, 16, 20 and 42 d prior to 6 December 2004 and were rooted and grown as in experiment I.

*Inductive Treatments.* In experiment I, on April 6 2004, a group of plants (4 weeks old from the stick date) was vernalized by placing cell trays in a cooler at 5 °C under ~100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* for 16-h provided by a combination of fluorescent and incandescent lamps from 0600 to 2200 HR. A second group of plants was potted in 13-cm square pots containing the same media as stockplants and was given a short-day treatment in the greenhouse set at 20 °C by placing them under a 9-h photoperiod provided by a blackout system that opened at 0800 HR and closed at 1700 HR. During the 9-h photoperiod, light intensity was controlled similar to the stockplants grown in the greenhouse. In experiment II, 4, 8, 12, 16, 20 and 42 d prior to 6 December 2004 plants were either given vernalization or short-day treatment as described above. In both experiments, one additional group of plants was maintained in the greenhouse in 13-cm square pots under a 16-h photoperiod as long-day controls and another

group of plants was maintained under a 9-h photoperiod as short-day controls. The inductive treatments were provided to nine plants per treatment combination for 14, 28, 42 or 56 d in experiment I and 10 plants per treatment combination for 4, 8, 12, 16, 20 or 42 d in experiment II.

*Plant Culture and Climate Control.* Following the vernalization treatments, plants were potted in 13-cm square pots and grown in the greenhouse at 20 °C setpoint temperature and 16-h photoperiod similar to the greenhouse-grown stockplants along with plants treated with SD. The greenhouse climate was controlled by a climate control computer (Priva, Model CD750; De Lier, The Netherlands). Air temperature was measured on each greenhouse bench by type E thermocouples (TT-E-40; Omega engineering, Stamford, Conn.) placed in aspirated tubes and *PPF* at plant height was measured at two locations by line quantum sensors containing 10 photodiodes (Apogee Instruments, Logan, Utah). Temperature and light sensors were connected to a CR10 datalogger (Campbell Scientific, Logan, Utah) and data were collected every 10 s and hourly averages were calculated and recorded in a computer.

*Data Collection.* The number of nodes on each plant was recorded prior to initiation of inductive treatments in both experiments and again at the end of inductive treatments in experiment II. On the day of first visible inflorescence and anthesis of first inflorescence, dates were recorded and time to visible inflorescence and anthesis was computed starting from the end of inductive

treatments. At anthesis, number of inflorescences and lateral shoots were counted and plant height was measured from the media surface to the highest point. Plants without inflorescene 105 d after the start of forcing were considered vegetative. Average daily temperature (ADT) and daily light integral (DLI) were computed for each plant from the start of forcing until anthesis and 105 d for flowering and vegetative plants, respectively, and are presented in Fig. 1.

**Results and Discussion**

*The Effect of Inductive Treatments on Flowering.* No SD control plants flowered in either experiment (data not shown) and no LD control plants flowered in experiment II (Fig. 2A and G). In experiment I, two of the nine LD control plants flowered, although the flowering plants were considerably delayed compared to plants that had received inductive treatments (Fig 2B, C, H, I). Additionally, these two flowering LD control plants developed an average of only 2 inflorescences (Fig. 2D and J) and were considerably shorter compared to plants that received inductive treatments (Fig. 2F and L). All plants flowered following exposure to 5 °C or SD for ≥14 d in experiment I, indicating that *Coreopsis* 'Sunray' has a near-obligate vernalization requirement that can be substituted by a SD treatment prior to exposure to LD. Therefore, *Coreopsis* 'Sunray' can be considered as a vernalization-requiring or SLD plant.

*Effective Durations of Inductive Treatments.* Fourteen d of vernalization or SD was sufficient to induce complete flowering in experiment I, but in experiment

II, complete flowering was achieved only after ≥16 d vernalization and 42 d SD treatment; shorter durations of inductive treatments induced incomplete flowering. The cultural conditions were identical in the two experiments, although the forcing environments were different as indicated by the differences in ADT and DLI between the two experiments (Fig. 1). The ADT difference of <2 °C could have altered the flowering time by a few days, but probably would not affect the process of floral evocation. However, the difference in DLI between the two experiments was significant (~5 $mol \cdot m^{-2} \cdot d^{-1}$). Additionally, during the initial 4 weeks of forcing, when most plants made the transition from vegetative to reproductive growth, the difference in DLI between the two experiments was even larger (~13 $mol \cdot m^{-2} \cdot d^{-1}$). Based on these differences, we postulate that following shorter durations of vernalization or SD treatment, a high DLI promoted flowering and/or a low DLI inhibited flowering.

During both experiments, increasing duration of either inductive treatment for up to 42 d decreased time to visible inflorescence and anthesis. The number of inflorescences produced also saturated after 42 d of vernalization or SD treatment in experiment I and after SD treatment in experiment II. However, all durations of vernalization induced similar number of inflorescences in experiment II. Due to significantly delayed flowering, LD control plants developed additional lateral shoots compared to vernalized plants in experiment I. In experiment II, delayed flowering appeared to correlate with lateral shoot development, although this was not evident in vernalized plants in experiment I. In experiment I, plants had more lateral shoots following increasing durations of SD and this may have

been due to formation of additional laterals during SD treatments. However, due to the large variability in the number of vegetative laterals produced following 20-d SD treatment in experiment II, we can not confirm this. In experiment I, the two LD control plants flowered on a lateral shoot rather than the terminal and hence, both plants were significantly shorter than the vernalized plants at anthesis. Overall, the inductive treatments and their durations did not consistently affect plant height at anthesis in both experiments.

*Comparing Efficacy of Vernalization Treatment With SD Treatment in Regulating Flowering Responses.* In experiment I, at each duration of inductive treatment from 14 to 56 d, vernalization and SD treatment were equally effective in promoting flowering and elicited similar flowering responses at anthesis except number of lateral shoots (Fig. 2A-L). As previously explained, SD-treated plants probably unfolded additional lateral shoots during the 20 °C treatment and hence had more laterals at anthesis compared to plants exposed to 5 °C. In contrast, in experiment II, under the low DLI, short durations of vernalization treatment were more effective in promoting flowering compared to SD treatment. For example, following 16 SD, no plants flowered, whereas all plants vernalized for 16 d flowered. Hence, it appeared that under low DLI, vernalization treatment was more effective in promoting flowering than SD treatment based on flowering percentage. Once floral evocation occurred, flowering time was similar for plants receiving either inductive treatment. Interestingly, under the lower DLI, SD-

treated plants produced more lateral shoots but formed considerably fewer inflorescences compared to vernalized plants.

*Photoperiod During Vernalization.* To determine if photoperiod during vernalization affects the flowering response of *Coreopsis* 'Sunray', plants were vernalized at 5 °C under SD for 2 or 8 weeks and compared with plants vernalized at 5 °C under a 16-h photoperiod in experiment I. The results indicate that photoperiod during vernalization did not affect any flowering response studied (data not presented). However, since the flowering response was saturated after 2-week inductive treatments based on flowering percentage, we can not speculate if vernalization under SD would have been more effective under shorter treatment durations.

**Summary**

*Coreopsis* 'Sunray' has an obligate requirement for vernalization or SD treatment prior to exposure to LD for complete, rapid, synchronized and profuse flowering. The DLI during forcing may significantly affect flowering when the inductive treatments are given for sub-optimal durations. Under the higher forcing DLI, 14 d of SD or vernalization was sufficient for complete flowering although longer treatment durations decreased flowering time and increased the number of inflrescences produced at anthesis. Overall, under the higher forcing DLI, vernalization and SD treatments were similarly effective in affecting all flowering responses measured. Under the lower DLI, complete flowering

occurred only following ≥16 d of vernalization or 42 d of SD treatment. Based on flowering percentage, short durations of vernalization treatment were more effective than SD treatment in promoting flowering under the lower forcing DLI.

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Figure 1. Average daily temperature (ADT; A, C) and daily light integral (DLI; B, D) of *Coreopsis grandiflora* 'Sunray' plants forced at a 20 °C greenhouse temperature setpoint and a 16-h photoperiod. Prior to forcing, plants were provided inductive treatments for varying durations either by vernalizing at 5 °C under 16-h photoperiod in 72-cell trays (A, B) or by growing under SD (9-h photoperiod) at 20 °C in 13-cm square pots (C, D). Diamonds represent data from experiment I and squares represent data from experiment II. Each symbol represents ADT or average DLI for an individual plant and was calculated from transplant until anthesis and 105 d from transplant for flowering and vegetative plants, respectively. Error bars representing SE not presented since smaller than the symbol size.

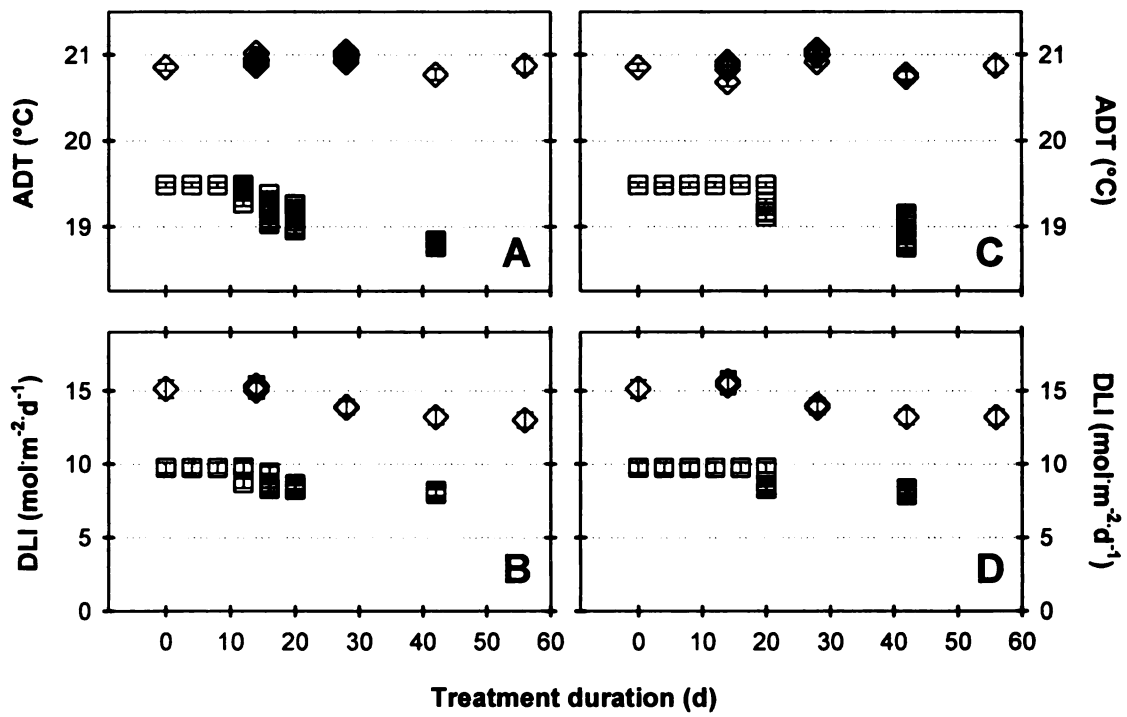
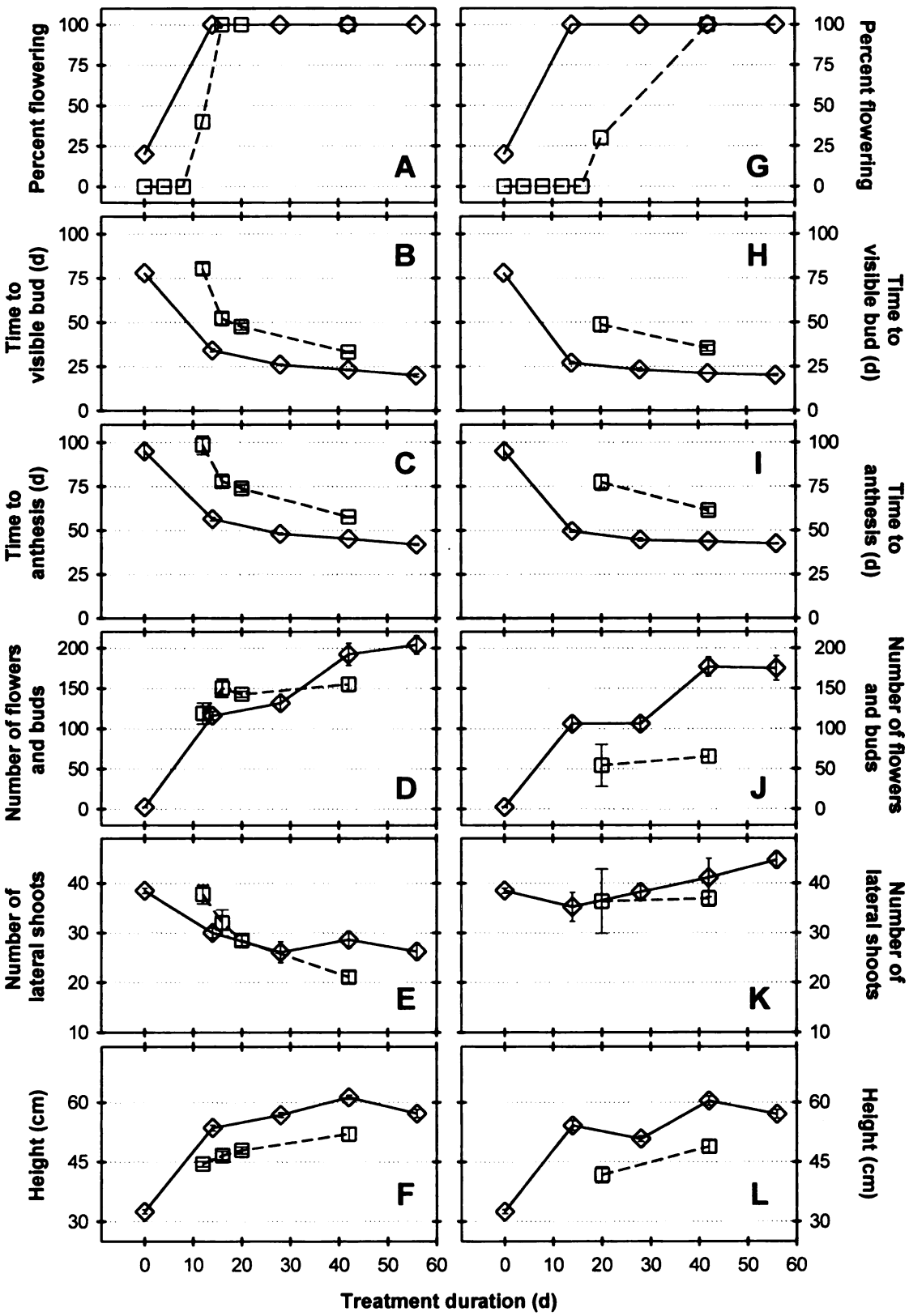


Figure 2. The influence of vernalization treatment (A-F) or SD treatment (G-L) on flowering percentage (A, G), time to visible inflorescence (B, H), time to anthesis (C-I), number of inflorescences (D-J), number of lateral shoots (E, K) and plant height (F, L) of *Coreopsis grandiflora* 'Sunray'. Plants were vernalized at 5 °C in 72-cell trays and exposed to short day (SD) treatment (9-h photoperiod at 20 °C) in 13-cm square pots prior to forcing under a 16-h photoperiod at 20 °C. Flowering percentage was computed 105 d after transplant. D-F and J-L were counted on the day of anthesis. Diamonds represent data from experiment I and squares represent data from experiment II. Mean±SE of 9 and 10 replicates are presented for experiment I and II, respectively.


Percent flowering
Time to visible bud (d)
Time to anthesis (d)
Number of flowers and buds
Number of lateral shoots
Height (cm)
Treatment duration (d)
A
B
C
D
E
F
G
H
I
J
K
L

# APPENDIX B:

# DAILY LIGHT INTEGRAL AFFECTS THE CRITICAL SHORT-DAY AND LONG-DAY PHOTOPERIODS OF THE SHORT-LONG-DAY PLANT *COREOPSIS GRANDIFLORA* 'SUNRAY'

Daily Light Integral Affects the Critical Short-Day and Long-Day Photoperiods of the Short-long-day Plant *Coreopsis grandiflora* 'Sunray'

## Introduction

*Coreopsis grandiflora* 'Sunray' flowers under a long-day (LD) photoperiod following an inductive treatment of either vernalization or short-day (SD) photoperiod (chapter IV; Appendix A). The critical photoperiods of *Coreopsis* 'Sunray' are unknown and therefore, the primary objectives of this study were to determine the critical SD and LD photoperiod for complete, rapid, synchronized and profuse flowering of *Coreopsis* 'Sunray'. Some short-long-day plants including *Echinacea purpurea* behave as intermediate photoperiod plants when the constant photoperiod is shorter than the critical SD and exceeds the critical LD photoperiod (Heide, 2004). Therefore, we also investigated the flowering responses of *Coreopsis* 'Sunray' under constant photoperiods.

## Materials and Methods

*Stockplant Management and Propagule Culture.* In experiment I and II, stockplants were developed and maintained as described in appendix A. Cuttings were taken on 13 October 2004 and 18 February 2005 in experiments I and II, respectively, and rooted for 2 weeks in 72-cell trays as described in appendix A. Rooted plants were grown for 2 weeks in 72-cell trays in a glass greenhouse under the same environmental and nutritional conditions as the

stockplants grown in the greenhouse. Subsequently, plants were transplanted in 13-cm square containers containing the same media used for the stockplants and eight plants were placed under each photoperiod treatment combination in the greenhouse.

*Critical Photoperiod Treatments.* To determine the critical SD photoperiod, plants were placed under a 10-, 11-, 12-, 13- or 14-h photoperiod for 3 weeks and then, forced under a 16-h photoperiod. To determine to critical LD photoperiod, plants were placed under a 9-h photoperiod for 3 weeks and then forced under a 12-, 13-, 14- or 15-h photoperiod. Additionally, plants were placed under constant 11, 12, 13, 14 or 15-h photoperiod during the entire course of the forcing.

The 9-h photoperiod was provided by a blackout system that opened from 0800 to 1700 HR. During the 9 h, plants received natural sunlight supplemented by high-pressure sodium (HPS) lamps that turned on when ambient light was below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (*PPF*) and ceased when ambient light exceeded 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*. HPS lamps provided an additional 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*. 10- to 15-h photoperiods were provided by a blackout system open from 0800 to 1700 HR and by extending the photoperiod after 1700 HR by incandescent lamps (at 1-3 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for the desired duration. For example, under the 10-h photoperiod, plants received a 9 h photoperiod by a combination of sunlight and HPS lamps and an additional 1 h of light by

incandescent lamps. The 16-h photoperiod was provided by a combination of sunlight and HPS lamps from 0600 to 2200 HR.

*Plant Culture and Climate Control.* During all photoperiod treatments and forcing, plants were in the greenhouse with a 20 °C temperature setpoint. The greenhouse climate was controlled by a climate control computer and air temperature was measured on each greenhouse bench and *PPF* was measured on 9-, 12- and 16-h photoperiod benches as described in appendix A.

*Data Collection.* The number of nodes on each plant was counted prior to initiation of inductive treatments and at the initiation of forcing in both experiments. On the day of first visible inflorescence and anthesis of first inflorescence, dates were recorded and time to visible inflorescence and anthesis was computed starting from the beginning of force At anthesis, number of flowering lateral shoots and inflorescences were counted. Plants not flowering 105 d after the start of forcing were considered vegetative. Average daily temperature (ADT) and average daily light integral (DLI) were computed from the start of inductive treatments until anthesis and 105 d for flowering and vegetative plants, respectively and are presented in Fig. 1. The ADT and average DLI for 126 d from the start of inductive treatments for experiments I and II were 19.9 and 21.8 °C and 9.1 and 16.6 $mol \cdot m^{-2} \cdot d^{-1}$, respectively.

**Results and Discussion**

*Critical Short-Day Photoperiod.* In experiment I and II, 88 and 100% of plants flowered following SD treatment at ≤11-h photoperiod, respectively (Fig. 2A). In experiment I (lower DLI), no plants flowered following treatment at ≥12-h photoperiod, but in experiment II (higher DLI), flowering percentage after exposure to 12-, 13- and 14-h photoperiod was 50, 75 and 75, respectively. In both experiments, following 10- and 11-h photoperiod treatments, flowering time and node number at anthesis were similar (Fig. 2B-D). In experiment II, flowering of plants treated at ≥12-h photoperiod appeared to be slightly delayed compared to plants treated at ≤11-h photoperiod. During experiment II, flowering was hastened compared to experiment I, which can be explained by elevated plant temperatures due to warmer ADT and higher DLI during experiment II. However, the difference in the node number at anthesis between the two experiments suggests that plants flowered at an earlier developmental stage under a high DLI. In both experiments, 10- and 11-h photoperiod treatments elicited similar number of flowering lateral shoots and number of inflorescences (Fig. 2E and F). In experiment II, ≤12-h photoperiod treatment resulted in more flowering lateral shoots and inflorescences compared to ≥11-h treatment.

Based on above data, the critical SD photoperiod for complete, rapid and synchronized flowering was 11 h. We postulate that the observed flowering following ≥12-h photoperiod in experiment II could be attributed to the higher DLI during experiment II. Since an unsaturating 3-week duration was used for inductive SD treatments, the flowering responses were highly sensitive to forcing

DLI (appendix A). Further studies are necessary to determine the effect of forcing DLI on the critical SD photoperiod following unsaturating and saturating durations of SD treatments.

*Critical Long-Day Photoperiod.* In experiment I, 100, 75 and 88% plants previously treated with SD at 9-h photoperiod flowered when forced under ≤13, 14 and 15-h photoperiods, respectively (Fig. 2G). All SD-treated plants flowered when forced under ≥12-h photoperiod in experiment II. Based on time to visible inflorescence and anthesis and number of nodes at anthesis, plants flowered at a similar time when forced under ≥13-h photoperiod in both experiments and flowering was delayed under the 12-h photoperiod (Fig. 2H-J). Plants flowered sooner in experiment II compared to experiment I, and this could have been due to elevated plant temperatures. However, the decreased number of nodes at anthesis in experiment II indicated that floral evocation was promoted under the higher DLI. When forced under a 12-h photoperiod, plants produced fewer flowering lateral shoots compared to plants forced under ≥13-h photoperiod in both experiments (Fig 2K). In both experiments, plants under a 12-h forcing photoperiod had fewer inflorescences compared to plants under ≥13-h photoperiod, and plants under 13-h photoperiod had fewer inflorescences compared to plants under ≥14-h photoperiod (Fig. 2L). Plants had similar number of inflorescences when forced under a 14- and 15-h photoperiod in both experiments.

Based on flowering percentage, the critical LD photoperiod of *Coreopsis* 'Sunray' was 12 h; based on flowering time and number of flowering lateral

shoots, the critical LD photoperiod was 13 h; and based on the number of inflorescences at anthesis, the critical LD photoperiod was 14 h. Our data also indicate that DLI only slightly affected the flowering responses at different critical LD photoperiods.

*Constant Photoperiods.* In experiment I, no plants flowered under constant photoperiods of 11 to 15 h (Fig. 2M), whereas in experiment II, flowering percentage was 0, 50, 88, 88 and 13 under 11-, 12-, 13-, 14- and 15-h photoperiod, respectively. Thus, flowering under constant photoperiods was incomplete and inconsistent and it is likely that flowering reported in experiment II was influenced by the DLI. Although average time to visible inflorescence and anthesis and number of nodes at anthesis indicate that flowering was hastened as forcing photoperiod increased from 11 to 15 h, due to incomplete flowering at all constant photoperiods, no conclusions can be made on the effect of constant photoperiods on flowering time (Fig. 2N-P). Similarly, due to incomplete flowering, no conclusions can be made on the effect of constant photoperiod on number of flowering shoots and number of inflorescences at anthesis (Fig. 2Q and R).

**Summary**

Based on the results from both experiments, the critical length of SD and LD photoperiods should be 11 and 12 h, respectively, for complete flowering of *Coreopsis* 'Sunray'. Increasing the LD photoperiod from 12 h to ≥14 h decreased

flowering time and increased the number of flowering shoots and number of inflorescences at anthesis. Incomplete and inconsistent flowering occurred at constant photoperiods. Overall, DLI affected flowering responses under all photoperiod treatment combinations tested.

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Figure 1. Average daily temperature (ADT; A) and average daily light integral (DLI; B) of *Coreopsis grandiflora* 'Sunray' plants grown in a 20 °C greenhouse. ADT and average DLI were computed from the beginning of the inductive treatments until anthesis and 105 d from initiation of forcing for flowering and vegetative plants, respectively. Dark lines represent the data from experiment I and grey lines represent data from experiment II.

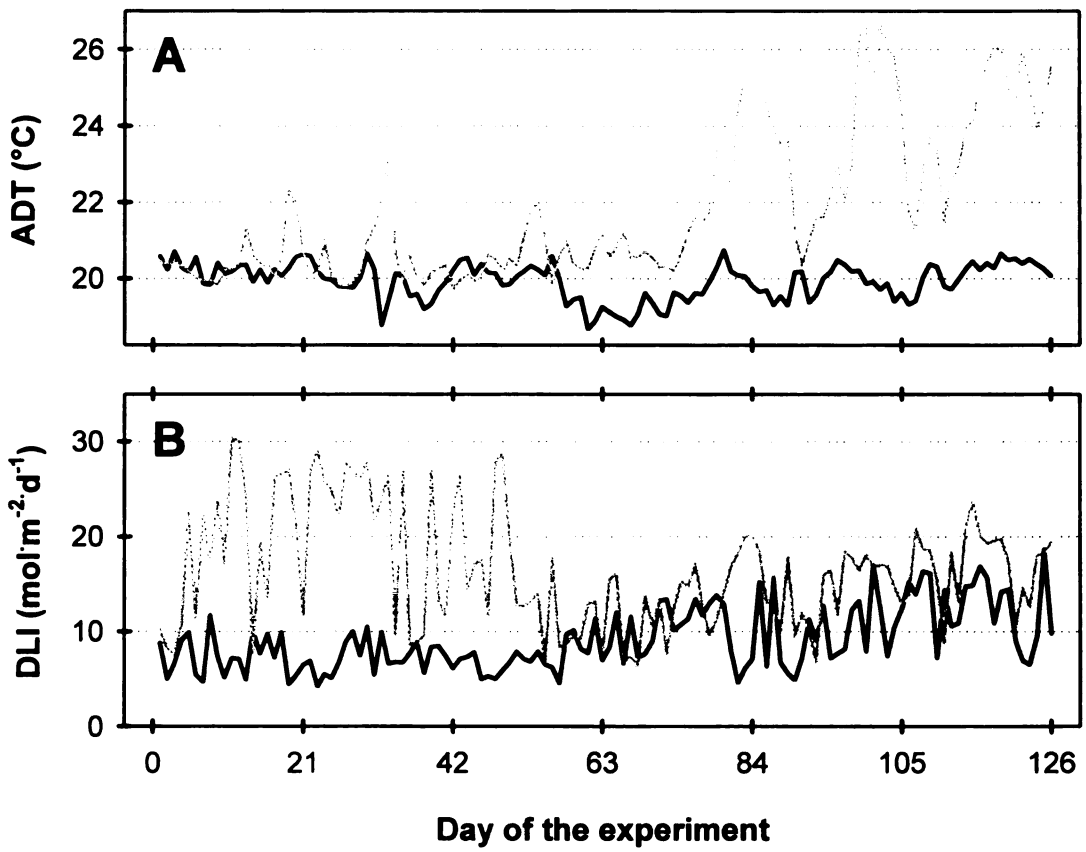
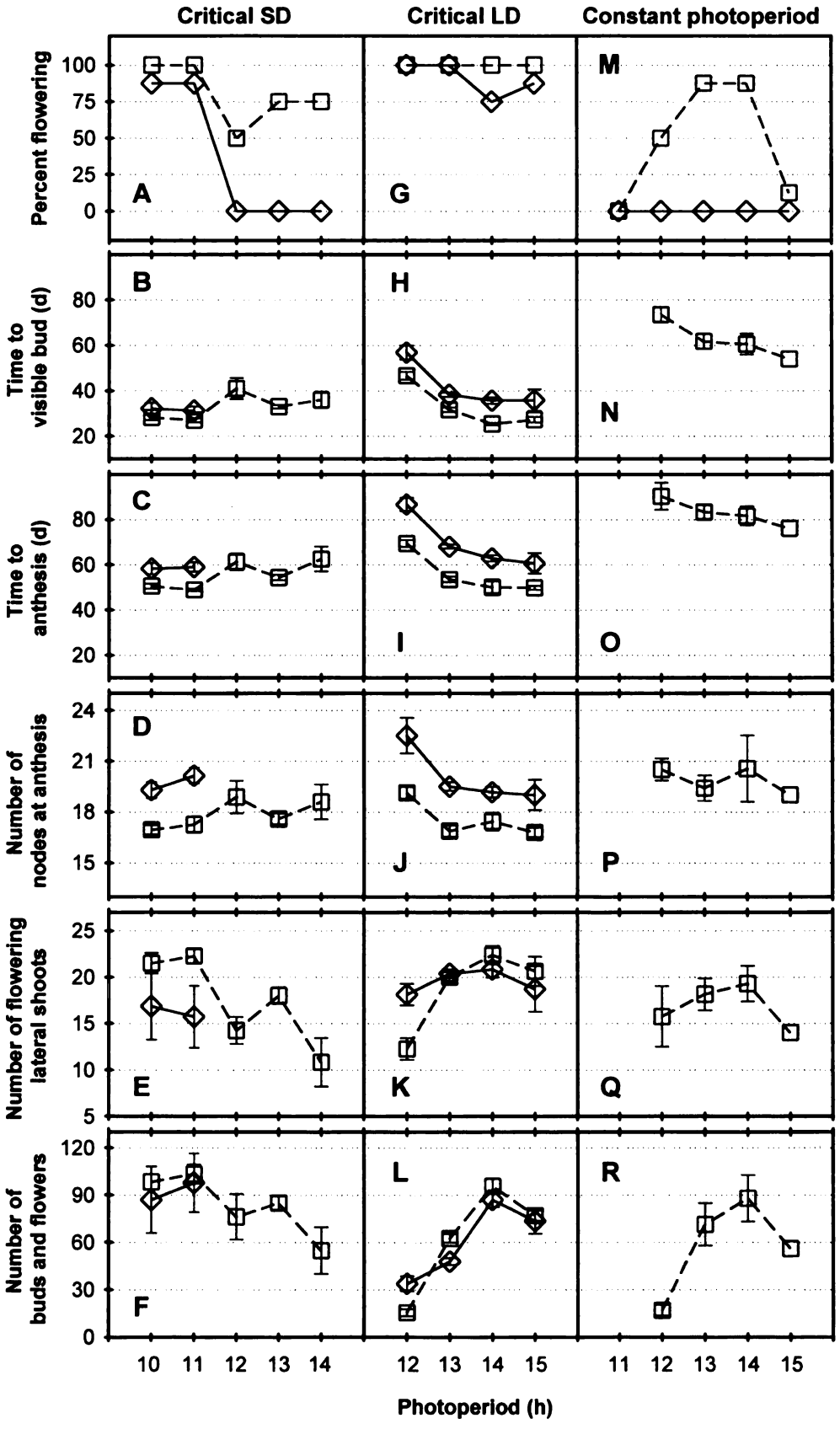


Figure 2. Flowering responses of *Coreopsis grandiflora* 'Sunray' plants following critical SD photoperiod of 10 to 14 h (A-F), critical LD photoperiod of 12 to 15 h (G-L) and constant photoperiod of 11 to 15 h (H-R). Following critical SD treatment for 3 weeks, all plants were forced under a 16-h photoperiod and prior to critical LD treatment, all plants were treated with a 9-h photoperiod for 3 weeks. The influence of photoperiod treatments on flowering of *Coreopsis grandiflora* 'Sunray' was assessed based on flowering percentage (A, G, M), time to visible inflorescence (B, H, N), time to anthesis (C, I, O), number of nodes at anthesis (D, J, P), number of flowering lateral shoots (E, K, Q) and number of inflorescences (F, L, R). Flowering percentage was computed 105 d after initiation of force. D-F, J-L and P-R were counted on the day of anthesis. Diamonds represent data from experiment I and squares represent data from experiment II. Mean±SE of 8 replicates are presented.


Critical SD
Critical LD
Constant photoperiod
Percent flowering
Time to visible bud (d)
Time to anthesis (d)
Number of nodes at anthesis
Number of flowering lateral shoots
Number of buds and flowers
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
Photoperiod (h)