

PHOTOPERIODIC RESPONSE OF COMMERCIAL CALIBRACHOA CULTIVARS

By

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This document is dedicated to the graduate students of the University of Florida.

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The photoperiodic response of *Calibrachoa* × *hybrida* cultivars was screened in three experiments (Chapter 3). Experiment 1 tested the flowering response of 27 cultivars under 11, 12, 13, or 14-h photoperiods. Some cultivars (MiniFamous Lemon and Million Bells Terra Cotta) were less sensitive to photoperiod and flowered at 11 h, and other cultivars (Superbells White and Superbells Red) were more sensitive while requiring more than 12 h to flower. Under an inductive 14-h photoperiod, MiniFamous Light Blue and Superbells Red took longer to flower (32 to 39 days). MiniFamous Lemon and Million Bells Yellow flowered in 21 to 24 days.

Experiment 2A and 2B examined the time to flower under cool and warm greenhouse conditions, respectively. Nine cultivars were given 11, 12, and 13-h photoperiods and grown in a cool greenhouse (18C night (N)/24C day (D)) or a warm greenhouse (24/29C (N/D)). Heat delay affects cultivars under a marginal photoperiod (e.g., flowering is delayed at 11-h photoperiod for MiniFamous Dark Red in a cool greenhouse and it does not flower at 11 h in a warm greenhouse).

Comparison of time to flower for cultivars under natural-day photoperiods was made in experiment 3 while observing nine cultivars planted at different times during the year. On 24 Jan, natural-day photoperiod was only 10 h and 37 min. Some cultivars including Million Bells Cherry Pink and Million Bells Terra Cotta were less sensitive to photoperiod and flowered in <45 days, while Superbells White and Velvet Rose with Yellow Center were more sensitive and took >66 days to flower. The photoperiod on 2 Jun was 13 h 56 min (long enough to flower all cultivars) and differences in time to flower varied from 11 days for Million Bells Terra Cotta to 24 days for Superbells Red.

Chapter 4 examines the flowering response of *Calibrachoa*×*hybrida* cultivars in more detail. Time to stop flowering under short days for three and five cultivars was determined in experiments 4A and 4B, respectively. Plants were flowered under long days (14 h) and moved to short days (8 h) until they stopped flowering. Three flowering responses were observed including 1. those that took a longer time to start flowering and went out of flower quickly (Million Bells Trailing White and Velvet Rose with Yellow Center); 2. those that flowered and stopped flowering in about the same time (Million Bells Cherry Pink); and 3. those that flowered faster and took longer to or did not stop flowering (Million Bells Terra Cotta and MiniFamous White Pink Star)(Figure 5 and 6).

In experiments 5A and 5B, the number of inductive photoperiods needed to induce flowering were determined. Million Bells Cherry Pink and Million Bells Terra Cotta required less than 1 week of long-day photoperiods (14 h) to induce flowering whereas Million Bells Trailing White required more than 4 weeks (Figure 7). MiniFamous White Pink Star and Million Bells Cherry Pink did not flower with 3 days of inductive photoperiod but did flower with 6 days (experiment 5B).

CHAPTER 1 INTRODUCTION

The current trend for something new and different has pushed plant breeder companies in the ornamental horticulture industry to introduce novel, vegetatively propagated plant material. On the leading end of this development, a plant known as ‘wild petunia,’ ‘seaside petunia,’ and/or ‘Million Bells’ was introduced (Kartesz and Gandhi, 1989). Today this plant is better known by its genus name, *Calibrachoa*, and has become one of the most important vegetatively propagated annuals.

In 1992, the first *Calibrachoa* cultivar was released by Suntory Ltd (Smith, 2002). Since then, there have been at least eight major breeding companies that have introduced hybrid *Calibrachoa* to the North American market which include: Goldsmith Plants, Ball FloraPlant, Bodger Botanicals, Suntory Ltd., Selecta First Class, Danziger, Twyford International, Inc., and Sakata, Inc. These companies have rapidly flooded the market with plants differing in flower color, plant habit, and photoperiod requirements. Flower color and plant habit are visible characteristics that can be easily determined. Photoperiod is more difficult to select for, because it is only expressed under specific environmental conditions. Since *Calibrachoa* has been described as a facultative long-day plant (Michel et al., 1999; Starman et al. 2001), it would be considered a distinct market advantage to select for less day-length sensitive *Calibrachoa*×*hybrida*.

Researchers have briefly studied the photoperiodic response of *Calibrachoa*. These studies have selected a few cultivars and examined them under broad photoperiod ranges. Starman et al. (2001) gave *C. hybrid* ‘Cherry Pink’ 8, 10, 12, 14, and 16-h photoperiods,

but only reported that *Calibrachoa* is a facultative long-day plant. There are currently well over 100 cultivars being sold in the United States and the published research has barely identified the basic flowering response of *Calibrachoa*.

The floriculture crop category that *Calibrachoa* falls into is spring or fall annual. This plant responds better to cooler environmental conditions and stops flowering in the summer heat and humidity (Armitage, 2001). Commercial *Calibrachoa* production is timed for early spring and fall sales when the natural photoperiod is short and may not induce flowering at the time of sale or during the in-ground growing season, respectively. To overcome short-day photoperiod, growers often light the crop for part of the production.

In preliminary research it has been identified that *Calibrachoa* cultivars have demonstrated variability in their sensitivity to photoperiod. The following research was designed to screen several commercial *Calibrachoa* cultivars from different plant breeding companies for their response to day length under more specific photoperiod treatments; to evaluate how a flowering *Calibrachoa* responds to short days; and to identify the photoperiod requirement for flower initiation and development.

CHAPTER 2 LITERATURE REVIEW

Calibrachoa Classification and History

Classification

Calibrachoa×*hybrida* classification includes the following:

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Solanales
Family: Solanaceae
Genus: *Calibrachoa* Llave & Lex
(USDA, NRCS, 2004)

Nomenclature History

The genus *Calibrachoa* was established by La Llave and Lexarza in 1825 (Wijsman and de Jong, 1985) as a monotypic genus with the type species *Calibrachoa procumbens*. In 1911, Fries found it identical to *Petunia parviflora* Juss. and treated *Calibrachoa* as a synonym of the genus *Petunia* defined by Jussieu (Wijsman and de Jong, 1985). Wijsman and de Jong studied the taxonomy of *Petunia* and recognized two groups of species differentiating in several morphological characters that gave reason for generic separation. Two groups had been previously identified by Wijsman et al. (1983) where group 1 included two *Petunia* species with 14 chromosomes and group 2 included 3 *Petunia* species with 18 chromosomes. The $2n=18$ plants were further described as: small shrubs, woody stems; leaves linear or linear-spatheolate, obtuse, sessile; flower

limb white or purple with yellow or at least pale tube, one valve formed by two petals covering three petals (conduplicate aestivation); telomeric heterochromatin present; calyx lobes less deeply incised (pentafid); seed-coat walls with straight anticlinal walls (Wijsman and de Jong, 1985; Stehmann and Semir, 1997).

It was not until 1990, that Wijsman resurrected the genus *Calibrachoa*, and transferred 15 species of *Petunia* sharing morphological characters similar to that of *P. parviflora* ($2n=18$ plants) to *Calibrachoa*. Thereafter, Stehmann and Semir (1997) transferred 10 more species of *Petunia* to *Calibrachoa*. The 25 described species included: *C. calycina*, *C. caesia*, *C. dusenii*, *C. eglandulata*, *C. elegans*, *C. ericaefolia*, *C. excellens*, *C. hassleriana*, *C. heterophylla*, *C. humilis*, *C. linearis*, *C. linoides*, *C. macrodactylon*, *C. micrantha*, *C. ovalifolia*, *C. paranensis*, *C. parviflora*, *C. pygmaea*, *C. regnellii*, *C. reptensis*, *C. sellowiana*, *C. sendtneriana*, *C. serrulata*, *C. spathulata*, *C. thymifolia* (Wijsman, 1990; Stehman and Semir, 1997).

Plant History

Except for one, *Calibrachoa* species occur in subtropical and temperate regions of eastern South America, from the Minas Gerais state of Brazil southwards to Uruguay, with the maximum abundance in the Santa Catarina and Rio Grande do Sul states of Brazil. The exception is *Calibrachoa parviflora*, the type for the genus, occurring both in North and South America (Wijsman and de Jong, 1985).

All species in *Calibrachoa*, except *Calibrachoa parviflora*, exhibit self-incompatibility (Tsukamoto et al., 2002). *Calibrachoa* is not homogenous and comprises two distinct subgroups of species: subgroup 1, *C. parviflora* plus *C. pygmaea*, and subgroup 2, the rest of the species, based on interspecific cross-compatibility (Watanabe et al., 1997), seed morphology (Watanabe et al., 1999), and nuclear DNA content

(Mishiba et al., 2000). *C. pygmaea* is the sole species that can be crossed with *C. parviflora* (Watanabe et al., 1997). *C. parviflora* is a unique species exhibiting autogamy in the principally xenogamous genus *Calibrachoa* (Tsukamoto et al., 2002).

In 2001, Ando et al. identified that at least one species (*Calibrachoa heterophylla*) is bee pollinated. They also observed that *C. heterophylla* and *C. parviflora* exhibit diurnal opening and closing movements of the corolla lobes, and that the corolla lobes temporarily close when ambient temperatures reached 40C.

Photoperiod

Calibrachoa is a facultative long-day plant (Michel et al., 1999; Starman et al., 2001). Much of the terminology used in describing the flowering response to day length originally came from the early studies of Garner and Allard (1920). They understood that sexual reproduction in plants can be attained when the day length was favorable thus naming plants that flowered under day length conditions greater than 12 h “long day” plants (LDP), and “short day” plants (SDP) were ones that flowered with less than 12-h day lengths. Today, SDP are described as ones that only flower or flower more rapidly when the hours of light are less than a certain amount, and LDP are ones that only flower or flower more rapidly when the hours of light are greater than a certain amount (Thomas and Vince-Peru, 1997).

Photoperiod as described by Garner and Allard (1920) is the favorable length of day for each organism, and photoperiodism is the response of an organism to the relative length of day and night. In their studies, Garner and Allard observed the flowering response by exposing several annuals and perennials to a minimum of 5 h of light per day to a maximum of 12 h per day. In addition to the flowering response, Garner and Allard (1923) demonstrated several other ways that plants respond to photoperiod.

Apogeotropism (increase in stature) was observed in cosmos, bidens, and tobacco; enlarged roots as seen in radish; leaf resetting in beets; formation of bulbs and tubers in onion, potatoes, and artichoke; root growth observed in Biloxi soybean; pubescence on *Amaranthus* leaves; and pigment formation in poinsettia.

Day-length responses are much less precise for long-day plants and also much less well understood (Thomas and Vince-Peru, 1997). There are certain mechanisms and plant sensors that are involved in both LDP and SDP. Photoperiodic timekeeping is based on a circadian oscillator. Circadian clocks are roughly 24-hour cycles (dependant on ambient temperature), which reset spontaneously after completing each cycle. The timing mechanism can be signaled by several environmental changes including temperature, but the daily light/dark transitions at dawn and dusk seem to control the rhythm for day-length sensing. The periodicity and phase control of the oscillator may be regulated by the pigment phytochrome.

Circadian rhythms in LDP have been observed in the promotion of flowering by far-red light (FR). One example is demonstrated by Vince-Peru (1975) where they observed the response of *Lolium*. In this study, they gave *Lolium* plants an 8 h natural day with a 40 h day length extension with red light (R). Four hours of FR was added at various times during the day length extension. They saw that the plant response to added FR varied in the form of a circadian rhythm with maxima response occurring at 8-10 and 35 h from the start of the photoperiod (Vince-Peru, 1975).

Another rhythm was seen in the response to a night-break. *Lolium* was observed by Perilleux et al. (1994). After an 8 h day, the plants were given a 40 or 64 h dark period that was interrupted by 8 h R night-break at different intervals. Two peaks in

response were observed under both dark periods, but light at 56 h into the 64 h dark period had a strong effect on flower promotion. The results indicate that the night-break rhythm may interact with both a rhythm of light sensitivity and the following photoperiod (Thomas and Vince-Peru, 1997).

Early experiments attempting to determine the relationship of flowering and plant hormones was conducted by Hamner and Bonner (1938) using the short-day plant (SDP) *Xanthium pennsylvanicum* as the principal plant material. First, they placed defoliated and un-defoliated plants under short photoperiods. They observed that the un-defoliated plants had large flower buds and the defoliated plants remained vegetative. Next, they placed un-defoliated plants under long photoperiod (in excess of 18.5 h) and gave one leaf of these plants a short photoperiod (9 h). The results showed that one leaf under short days was enough to cause flowering. Hamner and Bonner also looked at two branched plants where one branch was defoliated and put under long photoperiods, and the other branch was left intact under short periods. It was observed that the entire plant initiated flower buds. They then performed experiments to determine the leaf stage of development required to sense photoperiod. It was found that mature leaves are much more effective than young leaves in perceiving photoperiod. Lastly, two *Xanthium* plants were approach grafted together and of one of the plants was given a short photoperiod while the other remained under a long photoperiod. Both plants flowered indicating that the floral stimulus is capable of moving from one plant to another. All of these experiments helped determine that mature leaves are required for photoperiod perception, the stimulus for floral initiation is translocated throughout the plant, and the floral stimulus can be transferred from one plant to another.

Flower initiation and time to flower in long-day plant (LDP) is related to light quality and the timing of light treatments in the photoperiod (Thomas and Vince-Peru, 1997). Pringer and Cathey (1960) examined the effects of photoperiod and the kind of light on *Petunia* flowering. After an 8-h natural-day-length, they extended the day length given to two *Petunia* varieties with incandescent light (containing R+FR light) or fluorescent light (only R light). Both varieties flowered 2 to 3 weeks faster under the incandescent day light extension treatment.

Vince (1965) studied the LDP *Lolium temulentum* and the promoting effect of far-red light by giving light treatments at different times in the photoperiod. In one experiment, red light was used as an 8-h day length extension at the beginning or end of an 8-h natural day. Both treatments resulted in some induction, but the red light extension at the beginning of the 8-h natural day resulted in strong flowering. Vince also observed that red light extension interrupted by far-red light after the 8-h natural day noticeably promoted flowering. This study and others support the conclusion that red light appears to be effective during the early part of long photoperiods and far-red light is important in the flower inductive response later in the photoperiod for long-day plants, which is a different response as compared to short day plants (Thomas and Vince-Peru, 1997).

Two terms were presented to explain the different plant behaviors to light quality. Light dominant describes a plant where the change of spectral distribution during the light period influences flowering, and dark dominant refers to floral induction related to uninterrupted dark period. Light and dark dominant correspond to long- and short-day plants in most cases (Thomas and Vince-Peru, 1997).

Long-day plants respond to both day extension as well as night-break treatments by flowering (Thomas and Vince-Peru, 1997). When the days are longer than a critical length, flowering is induced. Facultative long-day plants such as *Calibrachoa* and *Petunia* will flower faster as the day gets longer until the time reaches a particular day length where a longer day will not hasten flowering (Adams et al., 1998; Starman et al., 2001). This was shown by Adams et al. (1998) when they looked at the effect of photoperiod on the rate of flowering in petunia. 'Express Blush Pink' petunia responded by flowering quicker as the photoperiod increased until a critical photoperiod of 14.3 h/d, and extending the day-length beyond this time did not further hasten flowering.

The photoperiod above or below which the time to flower is minimal in facultative LDP is called the critical day length (Thomas and Vince-Peru, 1997). Critical day length is different for each plant species and sometimes different for cultivars within a species.

Phytochrome

The first action spectra for photoperiodic plants were obtained by using a spectrograph at the USDA laboratories in Beltsville, MD (Thomas and Vince-Peru, 1997). SDP *Glycine max* cv. Biloxi and *Xanthium strumarium* (Parker et al., 1946), and LDP *Hordeum vulgare* (Borthwick et al., 1948) and *Hyoscyamus niger* (Parker et al., 1950) were given night-break treatments with very high intensity light during the middle of the night length for less than 25 minutes. The results showed that the action spectra for the four plants was very similar with the cutoff wavelengths longer than 720 nm, maximum effect on flowering (by initiation in LDP and prevention in SDP) in the red region between 600 and 660 nm, rapid change in sensitivity between 500 and 560, and minimum effect at 480 nm (Thomas and Vince-Peru, 1997). Absolute amount of energy required for a response between 600 and 660 nm was also very similar in all four plants

which indicated that the same pigment was responsible in both SDP and LDP. This pigment was called phytochrome (Thomas and Vince-Peru, 1997).

It was later discovered that the floral induction effects of red light can be photoreversed by radiation in the 720-745 nm region given as the final exposure before darkness in the LDP *Hordeum* and *Hyoscyamus* (Downs, 1956). Phytochrome exists in two forms called Pr and Pfr. Red light is absorbed by the Pr form of phytochrome and is consequently converted to Pfr (Thomas and Vince-Peru, 1997). The Pfr form is the active form that controls the photoperiodic response and promotes flowering in long-day plants. Far-red light, on the other hand, is absorbed by Pfr and consequently then converted to Pr. Phytochrome is synthesized in darkness as Pr which was the biologically inactive form.

In addition to photoperiodic induction of flowering, phytochrome regulates almost every aspect of plant development from seed germination to flowering and senescence (Thomas and Vince-Peru, 1997). Phytochrome responses can be grouped under two categories including inductive responses and high-irradiance responses. Inductive responses are named because Pfr continues in darkness after the red light treatment has ceased, they are R/FR reversible, and they can be fulfilled by either light intensity or duration of exposure (Thomas and Vince-Peru, 1997). High-irradiance responses include irradiance dependency, no reciprocity between light intensity and duration, and they react to light in the blue and FR parts of the spectrum (Mancinelli and Rabino, 1978).

Phytochrome gene studies with *Arabidopsis* have identified five gene sequences and designated the sequences as: *phyA*, *phyB*, *phyC*, *phyD*, and *phyE* (Sharrock and Quail, 1989; Clack et al., 1994; Thomas and Vince-Peru, 1997). The discovery of these 5

genes has led scientist to consider that these phytochromes may have different properties and functions (Thomas and Vince-Peru, 1997). It is possible that more than one phytochrome can co-regulate the same photoperiodic response).

Photoperiod and *Calibrachoa*

The photoperiodic response of commercial *Calibrachoa* varieties has been briefly examined to date. Most of the research published has been information in trade journal magazines or abstracts from talks or posters presented at conferences. The earliest information on photoperiod and commercial *Calibrachoa* cultivars was presented at the Annual Conference of the American Society for Horticultural Science. Cutlan et al. (1997) gave photoperiod treatments of 9 h, ambient daylight (≈ 8 h) plus night interruption lighting (2200 to 0200 h), or ambient daylight plus continuous light to *C. x hybrida* 'Cherry Pink'. They only reported that the continuous lighting resulted in the least days to anthesis.

A study presented in a German journal by Michel et al. (1999) evaluated the influence of day length and quantity on flower induction for three *Calibrachoa* hybrids. The abstract described Million Bells Trailing Blue, Carillon Blue, and Carillon Rose as quantitative long-day plants that flowered in the shortest amount of time under 16 h daylight supplemented with fluorescent light at 500–800 Lux.

In 2001, Starman et al. examined the response of *C. hybrid* 'Cherry Pink' to different photoperiod treatments. Cherry Pink was grown under 8 h, 10 h, 12 h, 14 h, and 16-h photoperiods. They reported in the abstract that the cultivar flowered faster with increased day length and was described as a facultative long-day plant.

Lastly, Colorburst Violet and Liricashowers Rose were propagated under short (8 h) days or long (8 h with 4 h of night-interruption lighting) days with either ambient or

ambient plus supplemental HID lighting and transplanted to 10.2-cm pots (J.M. Dole, unpublished data). After transplant, they were moved to 16-h photoperiods. Long-day photoperiods reduced the number of days to flower for Colorburst Violet by 6 to 16 days and 2 to 7 days for Liricashowers Rose compared to the 8 h photoperiod. Both cultivars were described as long-day plants.

Effects of Photoperiod and Temperature

It has been demonstrated clearly in SDP poinsettia and chrysanthemum that temperature influences the critical day length (Cathy, 1963; Langhans and Larson, 1960; Langhans and Miller, 1960; Larson and Langhans, 1963; Parker et al., 1950; Whealy et al., 1987). The temperature effect on LDP flowering has been briefly considered in *Petunia* and *Lupinus* (Adams et al., 1998; Keeve et al., 1999; Pirnger and Cathey, 1960). For photoperiodic plants, the night temperature is more important than the day temperature. As the night temperature increases, the critical day length gets longer.

There has not been any work published on the effects of photoperiod with high temperature on the time to flower in *Calibrachoa*. However, research on this topic has been conducted with *Petunia* (another member of Solanaceae). Pirnger and Cathey (1960) studied the effect of photoperiod, kind of supplemental light, and temperature on the growth and flowering of petunia plants. ‘Ballerina’ was given photoperiod treatments of 8, 9, 10, 12, 14, or 16 h. After 8-h natural daylight, incandescent light was given to extend each photoperiod, or fluorescent light was given to extend one photoperiod to 16 h. They reported that petunia flowering can be hastened by long days with incandescent light. Day extension to 16 h with fluorescent light had less of an effect on time to flower than 12-h extension with incandescent light. The same variety was used for another part of the study where they considered the effects of four night temperatures (10, 16, 21, or

27C) on flowering under 16-h photoperiods. 'Ballerina' flowered 5 days earlier when grown at 27C night temperature compared to 21C night temperature.

Adams et al. (1998) took the petunia research one step further and attempted to combine photoperiod, temperature, and *PPF* into a model to support production growers. In this research, *Petunia × hybrida* 'Express Blush Pink' grown under 8, 11, 14, or 17-h photoperiods and six air temperature regimes (minimum temperatures of 4, 10, 14, 18, 22, and 26C). These results also demonstrated that flowering was hastened with increasing photoperiod up to a critical photoperiod estimated at 14.3 h. Increasing temperature up to the optimum temperature of 24.3C, increased the rate of progress to flowering. Temperatures above the optimum temperature declined the rate of progress to flowering linearly. The figure describing the relationship between temperature, photoperiod, and the rate of progress to flowering is difficult to read making it problematical to determine the exact effect of high temperatures.

The response of other long-day crops to photoperiod and temperature has been studied. *Lupinus albus* has been described as a long-day plant (Keeve et al., 1999). The influences of photoperiod, temperature, and genotype were investigated using the cultivars 'Tifwhite', 'Esta', and 'Kiev.' These three cultivars were subjected to 8 or 16-h photoperiods at three temperature treatments of 10/20C (N/D), 18/28C (N/D), and 20C continuously. In the cold environment, all cultivars flowered earlier under the longer photoperiod. In the warm environment, Tifwhite flowered faster under 16-h photoperiod, but Esta and Kiev flowered more quickly under 8-h photoperiod. One possible explanation that Esta and Kiev flowered more quickly under 8-h photoperiod may be that they are more sensitive to high temperature treatments and therefore exhibit heat delay.

Under 20C continuous treatment, all cultivars flowered earlier under 16-h photoperiod compared to 8-h.

Besides long day plants, research on short day ornamental crops is helpful. The influence of temperature on flowering of the short-day-plant poinsettia (*Euphorbia pulcherrima*) has been examined carefully. Poinsettia cultivar 'Oak Leaf' was transferred from 8-h photoperiod (after initiation) to a 12-h photoperiod and continued to flower normally where the same plants failed to continue to develop at 16-h photoperiods (Parker et al., 1950). Langhans and Larson (1960) studied 'Barbara Ecke Supreme' and the effects of various combinations of N/D temperatures. The treatments included four temperatures (10, 16, 21, or 27C) and two photoperiods of 9-h and natural photoperiod (12 h and 15 minutes at the start of the experiment on 10 Oct, and 10 h and 25 minutes two months later). The plants under the 9-h photoperiod had visible buds before plants under natural days, except for three treatments. Plants given 16/27, 16/16, and 16/10C (N/D) had the same days to visible bud for both 9 h and natural photoperiod. In this study, the night temperature had more of an effect on the number of days to visible bud than the day temperatures. Twenty one and 27C night temperatures and natural photoperiod prevented or delayed flowering of Barbara Ecke Supreme. 10 and 16C night temperatures had less difference in flowering at natural day length. The 9 h inductive photoperiod reduced the effect of the night temperature (Langhans and Larson, 1960).

In another experiment, 'Barbara Ecke Supreme', 'Eckes White', and 'Pink' were given constant temperatures of 10, 16, 21, and 27C (Langhans and Miller, 1960). The standard photoperiods used were 8, 10, or 12 h for 20, 30, 40, 50, or 70 days. Additional temperatures and photoperiods were looked at including: 27C at 8.5, 9, and 9.5-h

photoperiods; 21C at 10.5 and 11.5-h photoperiods; 16C at 11.5 h photoperiod; and 10C at 11.5 h photoperiod. The data indicated that there are two distinct phases in flowering poinsettia (initiation and development). Barbara Ecke Supreme produced a bud but no flower (no stamens appeared) when grown at 21C for 70 days with a 12-h photoperiod. Here the critical photoperiod and the number of short days required for flower initiation were fewer than for development.

This same study demonstrates the effect of photoperiod on the number of short days required for flowering. When grown at 21C, Barbara Ecke Supreme flowered in 30 days under 8-h and 10-h photoperiod, 50 days under 11.5-h photoperiod, and no flowering occurred at 12-h photoperiod.

The idea of flower bud initiation in poinsettia was further explored looking at the influence of temperature by Larson and Langhans (1963). A 9-h photoperiod was used for flower initiation at constant temperatures of 10, 16, 18, 21, and 27C. The shoot apices were examined microscopically. Under constant temperatures 27 and 10C, flower initiation is retarded. Initiation occurred in 16 days at 21C, 18 days at 65 and 16C, 24 days at 10C, and 30 days at 27C.

Another short day crop that has been observed for temperature affects on flowering is chrysanthemum. Using a 9-h photoperiod and factorial experiment of 4, 10, 16, 21, and 27C of both day and night temperatures, Cathey (1963) found that flowering time is influenced by the night temperature. This agrees with conclusions made by Langhans and Larson (1960) with poinsettias. A 21C night temperature resulted in plants flowering within 11 days of each other for 4, 10, 16, 21, and 27C days. A 21C day temperature resulted in plants flowering within 36 days of each other for 4, 10, 16, 21, and 27C

nights. The higher the night and day temperature, the less time necessary for visible bud, but continuous high temperature lengthened the period between visual bud expansion and the flower opening.

Floral development in chrysanthemum has been looked at more closely by Whealy et al. (1987) in relation to high temperature. “Heat delay” is the occurrence of delayed flowering attributed to high temperatures during production. Two cultivars were used in this study to represent a high temperature sensitive plant (Orange Bowl) and a high temperature tolerant plant (Surf). The plants were grown under 9-h photoperiods in a cool chamber at 18/22C and a warm chamber 26/30C (N/D). Flower development was observed from the start of short days using a scanning electron microscope. High temperatures delayed flower initiation and differentiation in Orange Bowl. As the high temperature exposure increased, the number of days to color and open flower increased. The experiment conclusions state that high temperatures during the photoinductive period enhanced vegetative growth and retarded floral development.

Calibrachoa Culture and Landscape Importance

Information for growing and propagating *Calibrachoa* are well documented. *Calibrachoa* is vegetatively propagated by cuttings (Ball FloraPlant, 2004; Smith, 2002). When rooting, the soil temperature should be sustained at 20–23C and fertilized with N at 75–100 mg·L⁻¹ as roots begin to develop. *Calibrachoa* is sensitive to iron deficiency. Maintaining a soilless medium pH of 5.2 and 5.8 will maximize the availability of iron to the plants. The media should dry slightly between watering to avoid root diseases (Ball FloraPlant, 2004).

Smith (2002) recommends that rooted cuttings can be planted placing 3 liners per 20.3 or 25.4-cm container, or one liner per 10.2 to 15.24-cm pot. A soft pinch at planting

can create better branching. *Calibrachoa* requires high light during production and N at 200 mg·L⁻¹ for fertilization that can be administered through constant liquid feed.

Calibrachoa is a good host for aphids and susceptible to pythium.. A fungicide drench at planting can help prevent pythium.

Temperature during production should be 10-14C/21-24C (N/D) (Ball FloraPlant, 2004). Higher temperatures will cause poor branching, unwanted stem stretch, and reduced flowering. Ball FloraPlant suggests N at 250 to 300 mg·L⁻¹ while growing on, pinching 1 to 2 weeks after transplant, and one plant per pot for 10.2-cm, one to three plants per pot for 15.2-cm, and four to five plants per pot for 25.4 to 30.5-cm containers which is higher, later, and more plants per pot than the Smith (2002) recommendations.

Calibrachoa is a groundcover by nature and usually doesn't grow much taller than 6 inches tall (Armitage, 2001). They can be used in the landscape or in baskets/containers where they trail down the sides. *Calibrachoa* is cold tolerant and can survive the winter in USDA zone 7 and warmer. High temperatures and humidity can delay flowering in the hot summer months of July and August (Armitage, 2001).

CHAPTER 3 CALIBRACHOA×HYBRIDA CULTIVAR SCREEN

Calibrachoa, a vegetatively propagated annual/perennial, is a relatively new floriculture crop. The photoperiodic responses for *Calibrachoa* has not been thoroughly reported. Cutlan et al. (1997), with only one cultivar, used photoperiods of 9 h, ambient daylight (~8 h) plus night interruption lighting (2200-0200 h), or ambient daylight with continuous light and reported that time to anthesis was the shortest for plants grown under the continuous lighting treatments. Starman et al. (2001) studied the response of one cultivar under 8, 10, 12, 14, or 16 h and reported *C. hybrid* 'Cherry Pink' as a facultative long-day plant. These studies only scratch the surface of the photoperiod response of *Calibrachoa* because there are over 100 cultivars currently on the market that have not been evaluated and the photoperiods used in the research are not very specific (the photoperiods are 2 h or more different).

Calibrachoa cultivars have demonstrated some variability in sensitivity to photoperiod (unpublished research). Information on a crop's sensitivity to day length is helpful to greenhouse growers when scheduling crop time to insure plants are in flower at the appropriate time for marketing. The following research was designed to screen numerous commercial *Calibrachoa* cultivars from different plant breeding companies for their response to day length under more specific photoperiod treatments.

Materials and Methods

Experiment 1. Comparison of time to flower for different cultivars under varying photoperiods. Rooted cuttings of 27 cultivars of *Calibrachoa* were obtained from four

plant breeding companies between 7 Jun and 14 Jun 2002. The cuttings were planted in 11.4-cm pots using sphagnum peat based Fafard No. 2 soilless growing medium (Agawam, MA), and given a hard pinch. These plants were placed in a glass greenhouse with temperature range of 24/31C (N/D) and given 8-h photoperiods (covering with black cloth from 1630 to 0830 hours) to promote vegetative growth. They were fertilized at every irrigation with Peters Professional 'Florida Special' water soluble fertilizer 20N-4.7P-16.6K (Scotts Co., Marysville, OH) with N at 150 mg·L⁻¹. On 27 Jun 2002, the plants were pinched a second time.

Day length treatments of 11 h, 12 h, 13 h, or 14 h were started on 11 Jul 2002. Photoperiods were provided by covering plants with black cloth from 1630 until 0830 and lighting with 60-W incandescent light bulbs which provided not less than 1 μ Em⁻²s⁻¹ from 1630 until 1930, 2030, 2130, and 2230 hours, respectively. The number of days to first open flower was recorded. An open flower was counted when the petals opened to expose the tube. There were eight plants per treatment. This experiment was terminated on 10 Sep 2002. Data were analyzed by taking the mean days to flower and calculating the standard errors.

Experiment 2A and 2B. Time to flower in cool (2A) or warm (2B) greenhouse conditions. Cultivars for this experiment were selected from Experiment 1 based on their photoperiodic response to provide one or two representatives from each of the six response groups. Nine cultivars of *Calibrachoa* (MiniFamous Dark Red, MiniFamous White Pink Star, Million Bells Cherry Pink, Million Bells Terra Cotta, Superbells Pink, Million Bells Trailing Blue, Superbells White, Superbells Red, and Velvet Rose with Yellow Center) were established as stock plants and grown under 8-h day-lengths.

Cuttings from these plants were also rooted under 8-h day lengths. Rooted cuttings were planted on 7 Jan 2003 in 11.4-cm pots using sphagnum peat based Fafard No. 2 (Agawam, MA) and hard pinched on 15 Jan 2003. These plants were placed in a glass greenhouse with temperature range of 20-22/23-25C (N/D) and given 8-h photoperiods (covering with black cloth from 1630 until 0830 hours) to promote vegetative growth. They were fertilized at every irrigation with Peters Professional 'Florida Special' water soluble fertilizer 20N-4.7P-16.6K (Scotts Co., Marysville, OH) with N at 150 mg·L⁻¹ and hard pinched on 15 Jan 2003.

Day-length treatments of 11 h, 12 h, or 13 h were started on 23 Jan 2003.

Photoperiods were provided by covering with black cloth from 1630 until 0830 and lighting with 60-W incandescent light bulbs which provided not less than 1μEm⁻²s⁻¹ from 1630 until 1930, 2030, and 2130 hours, respectively. Plants for experiment 2A were placed in a greenhouse where the temperatures were 18/24C (N/D) on 23 Jan 2003. Plants for experiment 2B were placed in a greenhouse where the temperatures were 24/29C (N/D) on 23 Jan 2003. Each temperature was a separate experiment with three photoperiod treatments and twelve plants per treatment. The number of days to first open flower was recorded. The experiments were terminated on 4 Apr 2003. Data were analyzed by taking the mean days to flower and calculating the standard errors.

Experiment 3. Comparison of time to flower for different cultivars under natural day lengths. Nine cultivars of *Calibrachoa* (MiniFamous Dark Red, MiniFamous White Pink Star, Million Bells Cherry Pink, Million Bells Terra Cotta, Superbells Pink, Million Bells Trailing Blue, Superbells White, Superbells Red, and Velvet Rose with Yellow Center) were established as stock plants and grown under 8-h day-lengths. Cuttings were

also rooted under 8-h day-lengths. Rooted cuttings were planted on 7 Jan, 21 Mar, and 19 May 2003 in 11.4-cm pots using sphagnum peat based Fafard No. 2 (Agawam, MA). These plants were placed in a glass greenhouse with temperature range of 22/27C from 7 Jan to 14 May and 23/30C (N/D) from 19 May to 27 Jun 2003, and given 8-h photoperiods (covering with black cloth from 1630 until 0830 hours) to promote vegetative growth. They were fertilized at every irrigation with Peters Professional 'Florida Special' water soluble fertilizer 20N-4.7P-16.6K (Scotts Co., Marysville, OH) with N at 150 mg·L⁻¹ and hard pinched on 15 Jan, 28 Mar, or 26 May 2003, respectively.

Natural day-length treatments were started on 24 Jan, 3 Apr, and 2 Jun 2003. Natural-day photoperiods at the start of the treatments determined by sunrise to sunset time or civil twilight beginning to end time were 10 h 37 min. or 11 h 27 min., 12 h 32 min. or 13 h 8 min., and 13 h 56 min or 14 h 56 min. respectively. The number of days to first open flower was recorded. The research was terminated on 22 Apr, 14 May, and 27 Jun 2003, respectively. Data were analyzed by taking the mean days to flower and calculating the standard errors.

Results and Discussion

Experiment 1. Comparison of time to flower for different cultivars under varying photoperiods. There were differences in how the cultivars responded to the four photoperiod treatments (Figure 1) and the cultivars can be grouped into six different types of responses. For MiniFamous Yellow Lilac, MiniFamous Lemon, and Million Bells Terra Cotta the number of days to flower was not affected by the four photoperiod treatments. For a second group (MiniFamous White Pink Star, Superbells Salmon Coral, Superbells Pink, and Superbells 11), time to flower was not affected by the 12-h, 13-h, and 14-h photoperiods, but they took longer to flower under the 11-h photoperiod.

MiniFamous Rose Pink, MiniFamous Dark Red, and Million Bells Trailing Blue are a third group where they did not flower under 11-h photoperiod in the duration of this experiment. Time to flower for these cultivars was not affected by the 12-h, 13-h, and 14-h photoperiod treatments. A fourth group, MiniFamous Light Blue, Superbells 51, Million Bells Trailing Pink, and Velvet Rose with Yellow Center, did not flower under 11-h photoperiod, took longer to flower under 12-h day length, and were not affected by the 13-h and 14-h photoperiod.

Million Bells Trailing White and Superbells White make up a fifth group. They did not flower under 11-h or 12-h photoperiods, but time to flower was not different under 13-h and 14-h photoperiod. Superbells Red exhibited a sixth response. It did not flower at 11-h or 12-h photoperiod and flowered faster under 14-h than under 13-h photoperiod. These data support the conclusions of Michel et al. (1999) and Starman et al. (2001) that *Calibrachoa*×*hybrida* is a facultative long-day plant which was clearly confirmed by response groups 2, 4, and 6 which take longer to flower under one photoperiod compared to another.

Figure 1 demonstrates the variability of flowering for different cultivars. The four photoperiod treatments show that some cultivars are less sensitive (those that flower at 11 h) and some cultivars are more sensitive (those that need more than 12 h to flower). The critical photoperiod (shortest day length required for the fastest flowering (Thomas and Vince-Peru, 1997)) for *Calibrachoa* cultivars as seen here can be between <11 h to \geq 13 h or possibly 14 h.

All the *Calibrachoa* cultivars flowered at 14-h photoperiod, but there was a 19-day difference in the time to flower at that photoperiod (Figure 1). MiniFamous Lemon was

the first to flower taking only 21 days to flower. MiniFamous Yellow Lilac, MiniFamous Light Pink, and Million Bells Yellow also flowered quickly in 22 to 24 days from the start of long day treatment. Million Bells Trailing White took the longest to flower at 39 days, while MiniFamous Light Blue, Superbells Red, and Liricashower Pink at 32 to 34 days were also slow to flower.

Differences in the time to flower are also shown in Figure 1. Some cultivars took longer and flowered at 32 to 39 days under 14-h photoperiod where as others only took 20 to 24 days. Fourteen hours met the photoperiod requirement for flowering, so the response can be attributed to differences in the time requirement for flower initiation, flower development, or both.

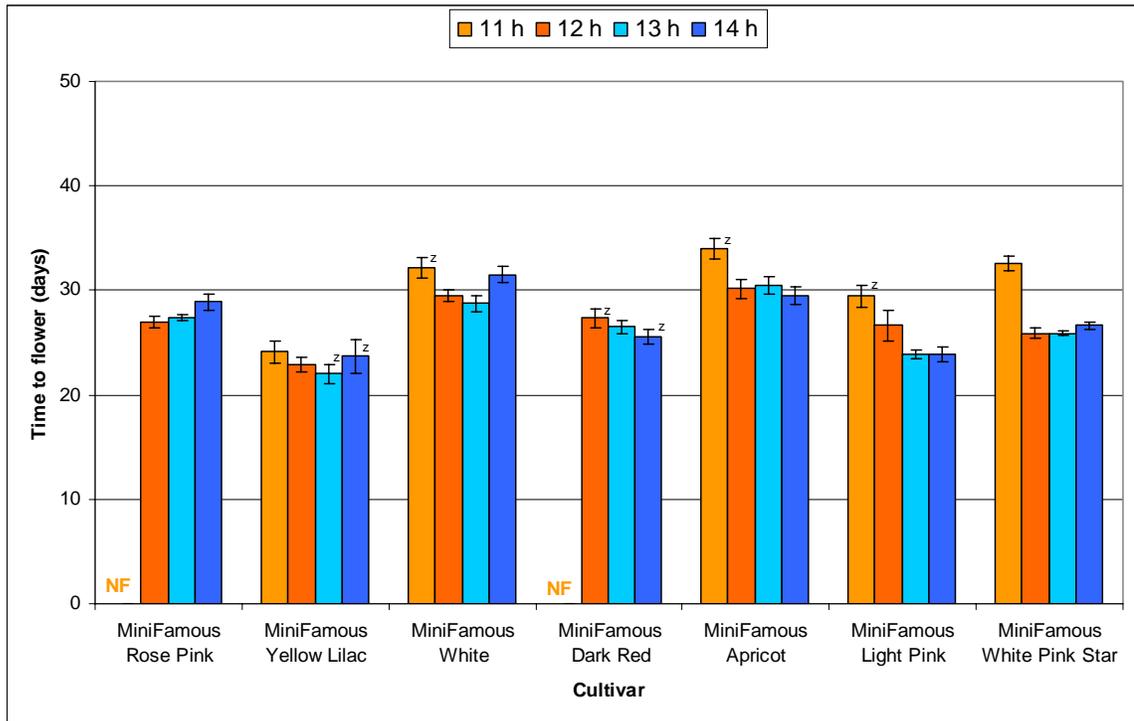


Figure 1. Time to flower (days) from start of photoperiod treatments under 11, 12, 13, or 14-h (Expt. 1). A column replaced by NF indicates that the cultivar did not flower at the given photoperiod. ^z indicates not all plants flowered: MiniFamous Yellow Lilac had 7 of 8 plants flower for 13-h and 14-h photoperiods: MiniFamous White had 7 of 8 plants flower for 11-h photoperiod: MiniFamous Dark Red had 6 of 8 plants flower for 12-h and 7 of 8 plants flower for 14-h photoperiods: MiniFamous Apricot had 7 of 8 plants flower for 11-h photoperiod: and MiniFamous Light Pink had 7 of 8 plants flower for 11-h photoperiod.

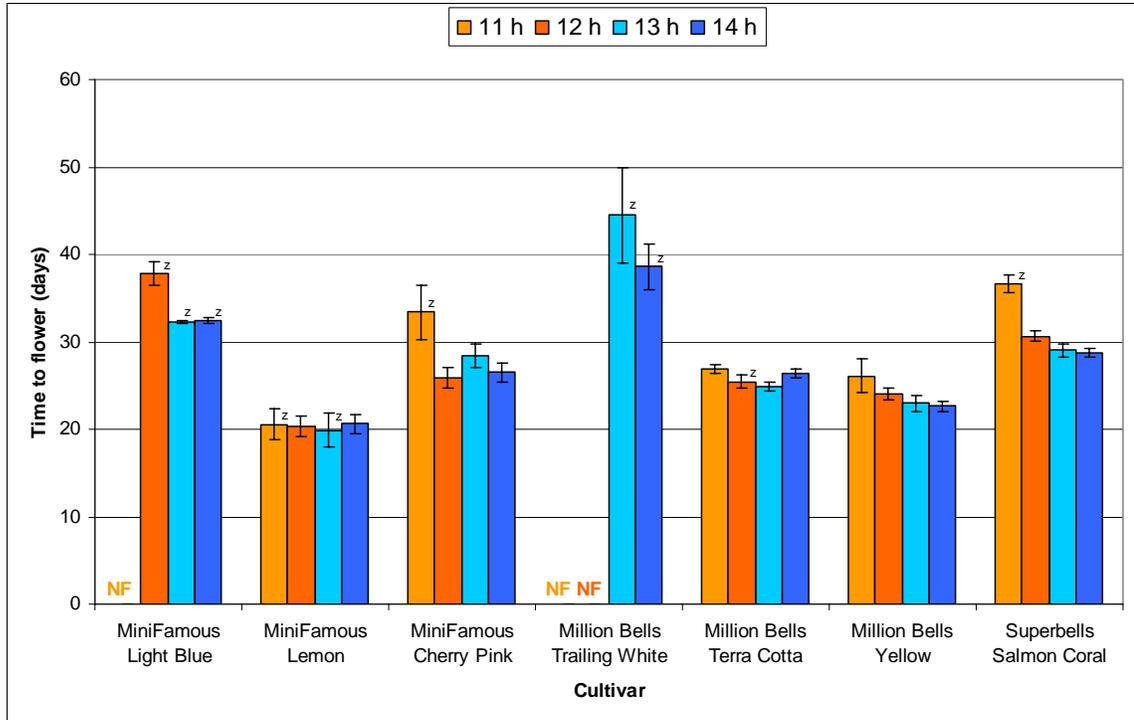


Figure 1. (continued). ^z indicates not all plants flowered: MiniFamous Light Blue had 5 of 8 plants flower for 12-h, 6 of 8 plants flower for 13-h, and 6 of 8 plants flower for 14-h photoperiods: MiniFamous Lemon had 7 of 8 plants flower for 11-h and 7 of 8 plants flower for 13-h photoperiods: MiniFamous Cherry Pink had 5 of 8 plants flower for 11-h photoperiod: Million Bells Trailing White had 4 of 8 plants flower for 13-h and 7 of 8 plants flower for 14-h photoperiods: Million Bells Terra Cotta had 7 of 8 plants flower for 12-h photoperiod: and Superbells Salmon Coral had 5 of 7 plants flower for 11-h photoperiod.

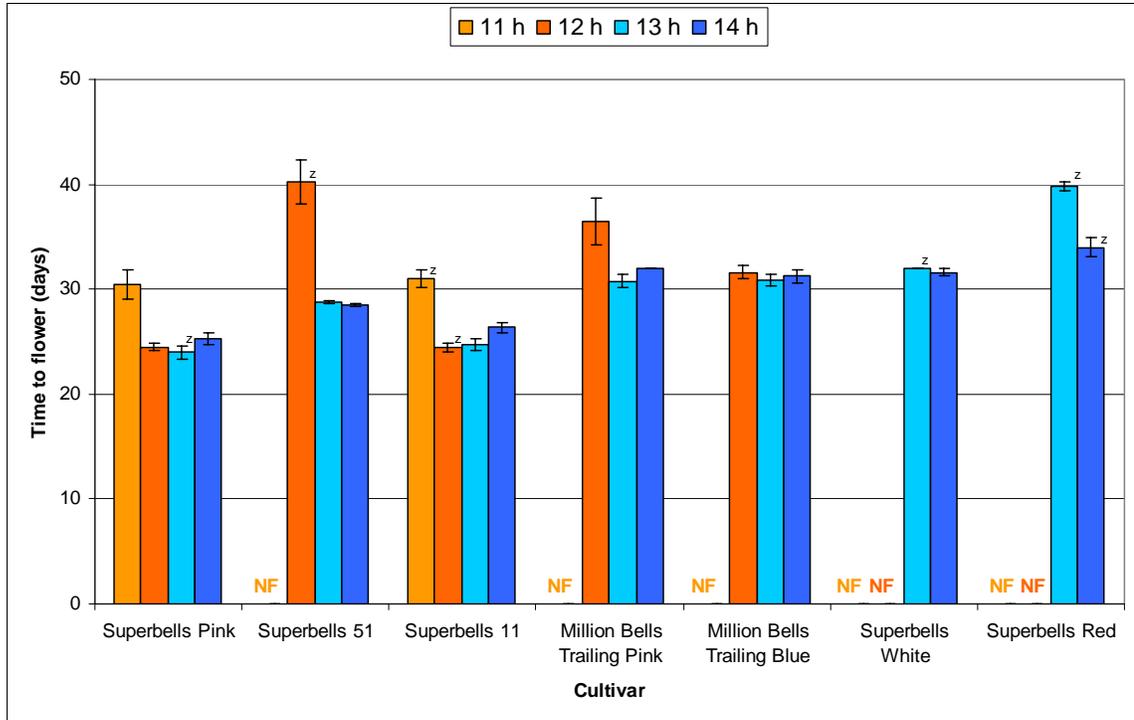


Figure 1. (continued). ^z indicates not all plants flowered: Superbells Pink had 7 of 8 plants flower for 13-h photoperiod: Superbells 51 had 4 of 8 plants flower for 12-h photoperiod: Superbells 11 had 7 of 8 plants flower for 11-h and 12-h photoperiods: Superbells White had 7 of 8 plants flower for 13-h photoperiod: and Superbells Red had 6 of 8 plants flower for 13-h and 7 of 8 plants flower for 14-h photoperiods.

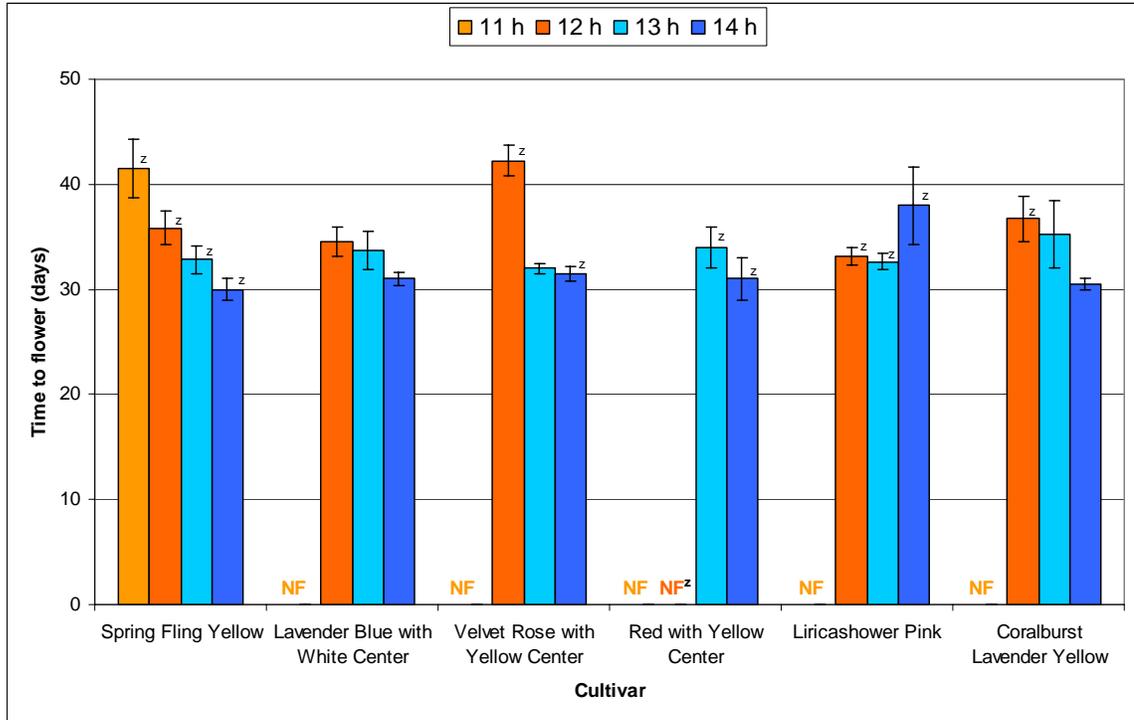


Figure 1. (continued). ^z indicates not all plants flowered: Spring Fling Yellow had 4 of 8 plants flower for 11-h and 14-h, and 6 of 8 plants flower for 12-h and 13-h photoperiod: Velvet Rose with Yellow Center had 4 of 8 plants flower for 12-h and 7 of 8 plants flower for 14-h photoperiod: Red with Yellow Center had 1 of 8 plants flower for 12-h, 7 of 8 plants flower for 13-h, and 5 of 8 plants flower for 14-h photoperiods: Liricashower Pink had 6 of 8 plants flower for 12-h and 13-h, and 7 of 8 plants flower for 14-h photoperiod: and Coralburst Lavender Yellow had 7 of 8 plants flower for 12-h photoperiod.

Experiment 2A . Time to flower in cool greenhouse conditions. Million Bells Cherry Pink and Million Bells Terra Cotta were not affected by the 11-h and 12-h photoperiods, and flowered slightly quicker at 13-h photoperiod (Figure 2). MiniFamous Dark Red and MiniFamous White Pink Star were delayed at 11-h and flowered quicker at 12-h and 13-h photoperiods. Superbells Pink and Million Bells Trailing Blue did not flower under 11-h, and flowered quicker under 13-h photoperiod than 12 h. Superbells White, Superbells Red, and Velvet Rose with Yellow only flowered at 13-h photoperiod.

Experiment 2B. Time to flower in warm greenhouse conditions. Million Bells Terra Cotta flowered at 11-h, faster at 12-h, and slightly faster at 13-h photoperiods (Figure 3). MiniFamous White Pink Star and Million Bells Cherry Pink also flowered at 11-h, faster at 12-h, and slightly faster at 13-h photoperiods, but the delay in flowering at 11 h was ≥ 14 days whereas Million Bells Terra Cotta was only delayed 1 or 2 days. MiniFamous Dark Red, Superbells Pink, and Million Bells Trailing Blue did not flower at 11-h photoperiod, and were not affected by 12-h and 13-h photoperiods. Superbells White, Superbells Red, and Velvet Rose with Yellow did not flower at 11-h or 12-h, and did flower at 13-h day lengths.

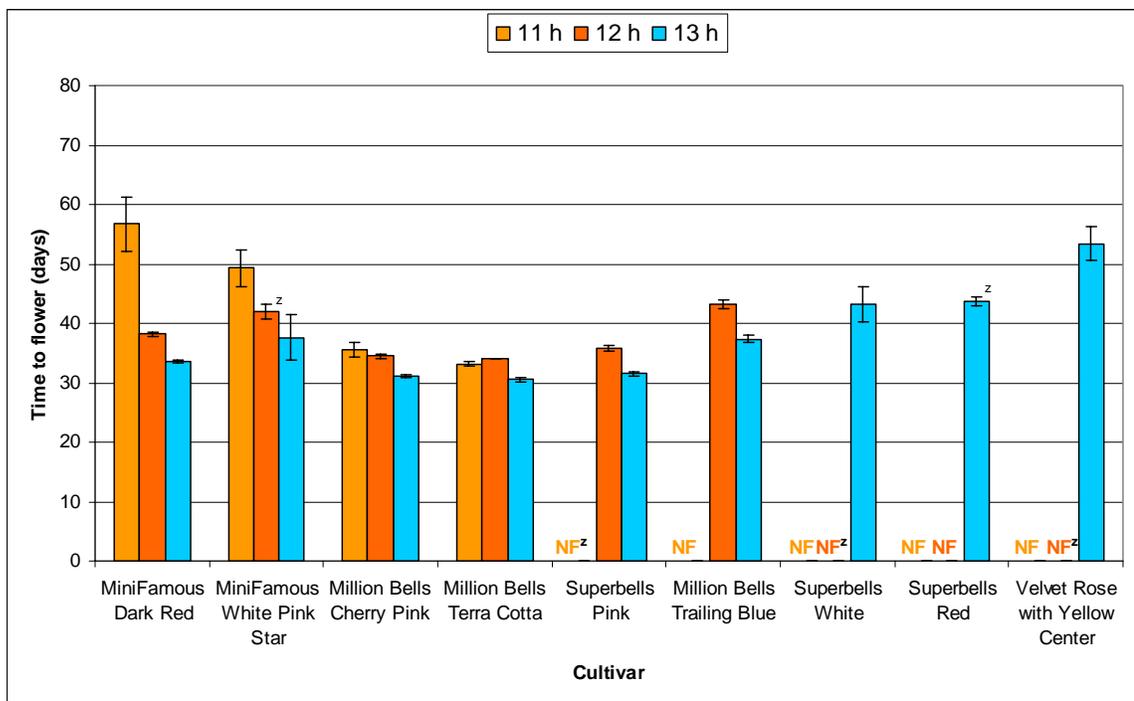


Figure 2. Time to flower (days) under 11, 12, or 13-h photoperiod in a cool greenhouse at 18/24C. A column replaced by NF indicates that the cultivar did not flower at the given photoperiod. ^z indicates not all plants flowered: MiniFamous White Pink Star had 10 of 12 plants flower for 13-h photoperiod: Superbells Pink had 3 of 12 plants flower for 11-h photoperiod: Superbells White had 1 of 12 plants flower for 12-h photoperiod: Superbells Red had 8 of 12 plants flower for 13-h photoperiod: and Velvet Rose with Yellow had 1 of 12 plants flower for 12-h photoperiod.

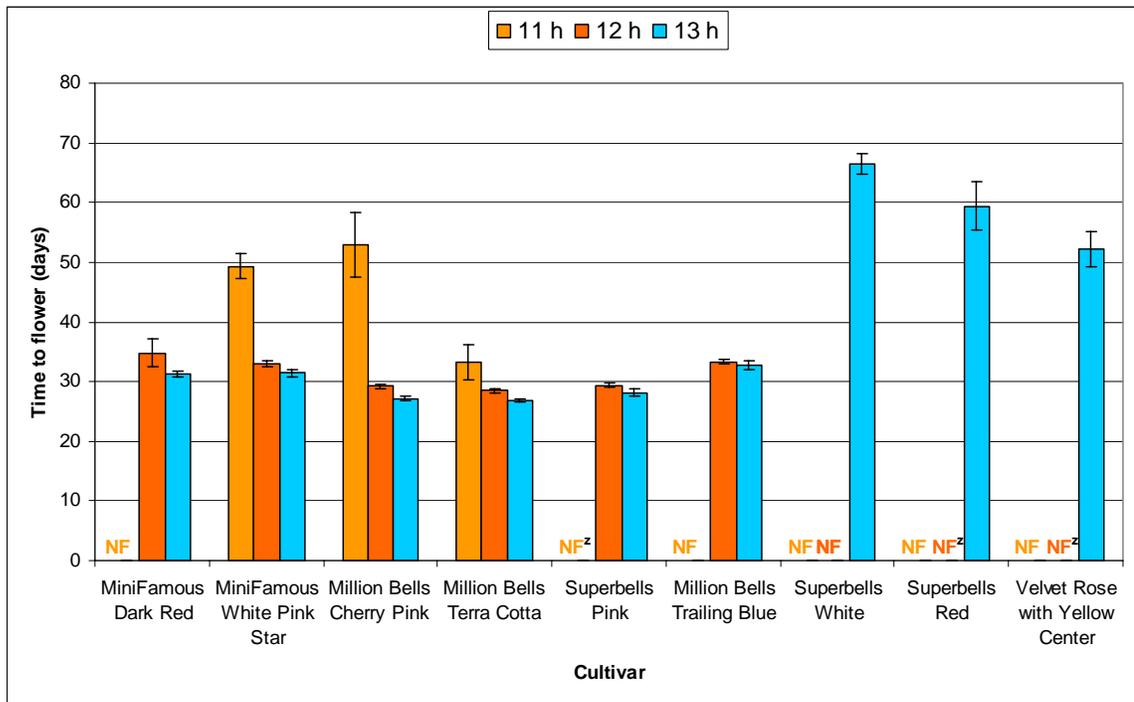


Figure 3. Time to flower (days) under 11, 12, or 13-h photoperiod in a warm greenhouse at 24/29C. A column replaced by NF indicates that the cultivar did not flower at the given photoperiod. ^z indicates not all plants flowered: Superbells Pink had 4 of 12 plants flower for 11-h photoperiod; Superbells Red had 1 of 12 plants flower for 12-h photoperiod; and Velvet Rose with Yellow Center had 1 of 12 plants flower for 12-h photoperiod.

Cultivars were selected for experiments 2A and 2B based on their photoperiodic responses to experiment 1. The results for experiment 2A and 2B are similar with regards to flowering under the photoperiod treatment to those seen in experiment 1, except when a cultivar had delayed flowering at a certain photoperiod. Figure 2 and 2B show that Superbells Pink and Velvet Rose with Yellow Center did not flower at 12-h photoperiod where Figure 1 shows that they did flower at 12 h, but they were delayed.

The results from experiments 2A and 2B show differences in the cultivars that can be attributed to the greenhouse temperatures. The term “heat delay” refers to the phenomenon of high temperatures delaying flowering. In poinsettias (Larson and

Langhans, 1963) and chrysanthemums (Whealy et al., 1987), this response is primarily due to an increase in the time to flower initiation. MiniFamous Dark Red flowered at 11-h photoperiod in the cool greenhouse (Figure 2) but did not flower at 11-h photoperiod in the high temperature house (Figure 3). The idea of heat delay appears most clearly under a marginal photoperiod (e.g. flowering is delayed at 11 h day length for MiniFamous Dark Red in a cool greenhouse). The temperature effect is seen in Superbells White and Superbells Red where the plants flowered in 40 to 46 days under 13-h in the cool greenhouse compared to 55 to 68 days in the warm greenhouse.

It has been shown in poinsettias that high temperature (27C) delays flower bud initiation (Larson and Langhans, 1963). Here they microscopically observed poinsettia apices to determine flower bud initiation. Heat effects on flower bud initiation was also observed in chrysanthemum by Whealy et al. (1987). Meristem transition to the reproductive state was reported to be delayed under high temperatures (26/30C N/D) when observed under an electron microscope.

Typically, a cultivar that is less sensitive to photoperiod such as MiniFamous White Pink Star, Million Bells Cherry Pink, Million Bells Terra Cotta, and Million Bells Trailing Blue flowered faster (Figure 3) under 14-h photoperiod and warm conditions. The same cultivars in a cool greenhouse (Figure 2) took longer to flower because plant development is slower at cool temperatures. This is shown in *Petunia* where flowering is hastened by increasing temperatures up to the optimum temperature (Adams et al., 1998).

Heat delay is an important concept for greenhouse growers in warm production climates. The cultivars that are sensitive to heat delay may not perform the same for a grower in a cool climate as for a grower in a warm climate. As example, Million Bells

Terra Cotta would be a better fit for Florida commercial operations than MiniFamous Dark Red.

Experiment 3. Comparison of time to flower for different cultivars under natural-day photoperiods. In commercial production, often growers do not control photoperiods for spring crops and early flowering cultivars under natural-day conditions is important. Million Bells Terra Cotta flowered the quickest at each of the three experiment dates (Figure 4) which is consistent with the flowering response in experiments 1, 2A, and 2B. Superbells White, Superbells Red, and Velvet Rose with Yellow Center took longer to flower than the other cultivars at more than 65 days in the 24 Jan experiment date, flowered in 33 days (similar to all the other cultivars) at the 3 Apr experiment date, and took only 23 days to flower at the 2 Jun experiment date which is slightly longer to flower compared to the other cultivars. In the 3 Apr treatment, all the cultivars flowered in 28 to 34 days and the differences between varieties was much less than the other two experiment times.

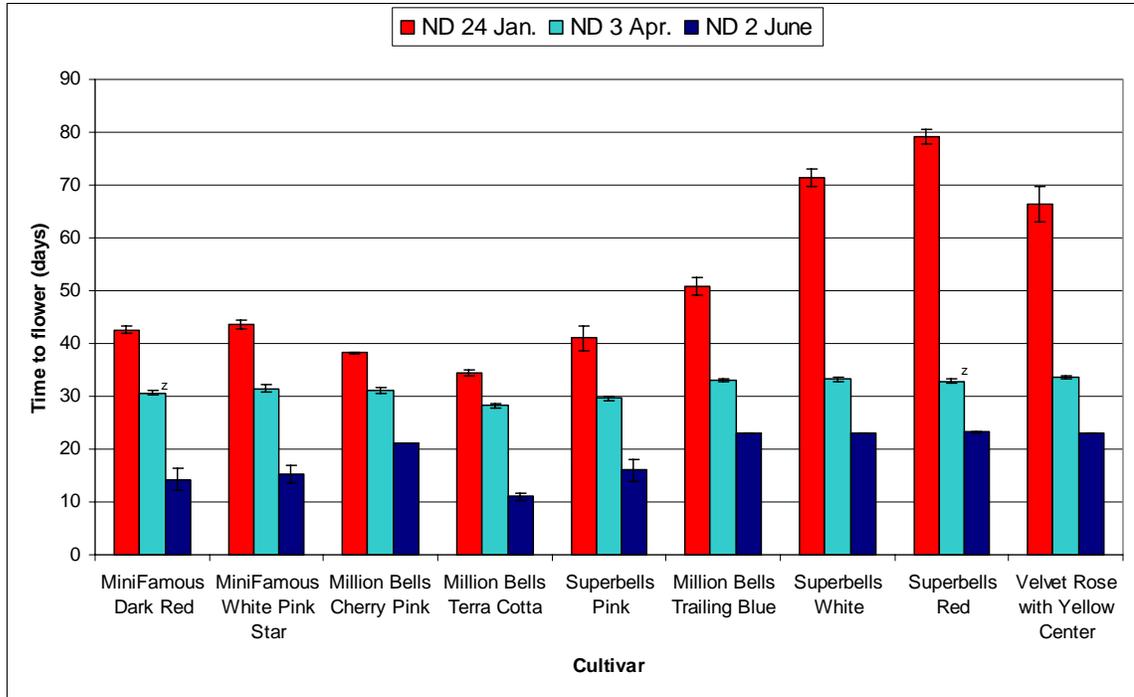


Figure 4. Time to flower under natural day conditions started on 24 Jan, 3 Apr, or 2 Jun. ^z indicates not all plants flowered: MiniFamous Dark Red and Superbells Red had 9 of 10 plants flower at 3 Apr experiment date.

Results from Experiment 1 are helpful in interpreting the information in Figure 4. MiniFamous Dark Red, MiniFamous White Pink Star, Million Bells Cherry Pink, Million Bells Terra Cotta, and Superbells Pink flowered in less than 45 days for the 24 Jan experiment date at 34 to 44 days (Figure 4) and they also flowered in less than 30 days at 14-h photoperiod in experiment 1 (Figure 1). The photoperiod during late January and February is between 11.5 h to 12 h and these cultivars are also ones that seem to have a critical photoperiod between 11 h and 12 h (Figure 1). Superbells White and Superbells Red have a longer development period (Figure 1) and also require a day length longer than 12 h to flower (Figure 1), which helps explain why they took longer to flower under the natural-day photoperiod for the 24 Jan experiment date. Greenhouse growers

producing plants for early spring and growing under natural days would be better off with the cultivars that flower the fastest under the shorter days of winter (Figure 4).

All the cultivars flowered between 28-32 days at the 3 Apr experiment date (Figure 4). At this time, the day length was 12 h 32 min. and getting longer to 13 h and 37 min. by the end of the experiment. The photoperiod at the start of the experiment was long enough or marginal for the critical photoperiod requirement of all cultivars. The plant responses for the 3 Apr experiment date do not demonstrate the variability in photoperiodic response or the differences in time to flower. According to the results shown in experiment 1, experiment 2, and the other two natural day photoperiod treatments, *Calibrachoa* has a variable flowering response. There is not a clear explanation for the un-variable response for the 3 Apr experiment date. These results could be attributed to greenhouse temperatures or light levels during the experiment that may be favorable to some cultivars (Superbells White, Superbells Red, or Velvet Rose with Yellow Center) and unfavorable to other cultivars (Million Bells Cherry Pink or Million Bells Terra Cotta) thus causing them to flower just 4 days apart from one another.

The 2 Jun experiment date confirmed the differences in the time to flower as seen in experiment 1 (Figure 1). At this date, the photoperiod was getting close to 14 h which fulfilled the photoperiod requirement and the plants were being pushed to grow with warm temperatures and high light. Million Bells Terra Cotta flowered quickly in just 10 to 12 days; MiniFamous Dark Red, MiniFamous White Pink Star, and Superbells Pink took longer at 12 to 18 days; and Million Bells Cherry Pink, Million Bells Trailing Blue, Superbells White, Superbells Red, and Velvet Rose with Yellow Center took even longer at 21 to 23 days.

The two plant flowering responses as seen in experiment 1 are also demonstrated in experiment 3. Results from the 24 Jan experiment date show that the natural-day photoperiod was not long enough for flowering Superbells White, Superbells Red, and Velvet Rose with Yellow Center because they took much longer (>20 days) to flower compared to the next slowest cultivar Million Bells Trailing Blue (Figure 4). When the day length was long enough to meet the photoperiod requirements for all cultivars at the 2 Jun experiment date, variability in the time required for flower development was shown. MiniFamous Dark Red, MiniFamous White Pink Star, Million Bells Terra Cotta, and Superbells Pink all took less than 18 days to flower where Million Bells Cherry Pink, Million Bells Trailing Blue, Superbells White, Superbells Red, and Velvet Rose with Yellow Center took longer (>21 days)(Figure 4).

CHAPTER 4 FLOWERING RESPONSE OF CALIBRACHOA×HYBRIDA CULTIVARS

Chapter 3 confirmed that *Calibrachoa* is a facultative long-day plant as stated by Starman et al. (2001). The research presented in Chapter 3 expounded on Starman's research by examining more cultivars and additional photoperiods and demonstrated that there are large differences in the critical photoperiod between cultivars. In commercial production often it is desired to provide long-day photoperiods for a short time to fulfill the photoperiod requirement and then go back to short or natural days. Cultivars are needed that stay in flower after going back under non-inductive photoperiods.

Day-length requirements for flower initiation and flower development appear to be different. This phenomenon has been looked at previously in poinsettias but not *Calibrachoa*. The poinsettia cultivar 'Oak Leaf' was transferred from 8-h photoperiod (after initiation) to a 12-h photoperiod and continued to flower normally where the same plants failed to continue to develop at 16-h photoperiod (Parker et al., 1950). 'Barbara Ecke Supreme' grown at 21C for 70 days under 12 h photoperiods, produced a bud and never flowered, which indicated that the critical day length was longer and the number of short days was fewer for initiation than development (Langhans and Miller, 1960).

These studies address the above mentioned plant responses for *Calibrachoa*. The following research was designed to evaluate how a flowering *Calibrachoa* responds to short days and to identify the photoperiod requirement required for flower initiation and development.

Materials and Methods

Experiment 4A. Time to stop flowering under short days. Three cultivars of *Calibrachoa* (Million Bells Trailing White, Million Bells Cherry Pink, and Million Bells Terra Cotta) were obtained from a commercial propagator on 10 Jun 2002, planted in 4.05 L containers using sphagnum peat based Fafard No. 2 (Agawam, MA), and given a hard pinch. These plants were placed in a glass greenhouse with temperature range of 24/32C and given 8-h photoperiods (covering with black cloth from 1630 until 0830 hours) to promote vegetative growth. They were fertilized at every irrigation with Peters Professional 'Florida Special' water soluble fertilizer 20N-4.7P-16.6K (Scotts Co., Marysville, OH) with N at 150 mg·L⁻¹. On 5 July, 2002, the plants were pinched again. Starting on 11 July, 2002, photoperiods were provided covering plants with black cloth from 1630 until 0830 and lighting with 60-W incandescent light bulbs which provided not less than 1 μ Em⁻²s⁻¹ from 1630 until 2230 hours. On 9 Sept., 2002 the photoperiod was changed to 8-h (covering with black cloth from 1630 until 0830 hours), and the average greenhouse temperature was lowered to 23/28C (N/D). There were eight plants per treatment. This experiment was terminated on 8 Nov 2002. The number of days to first open flower after the start of long days and time from start of short days to last flower was recorded. Data were analyzed by taking the mean days to flower and calculating the standard errors.

Experiment 4B. Repeat time to stop flowering under short days. Five cultivars of *Calibrachoa* (MiniFamous Dark Red, MiniFamous White Pink Star, Million Bells Cherry Pink, Superbells White, and Velvet Rose with Yellow Center) were established as stock plants and grown under 8-h day length. Cuttings were also rooted under 8-h day lengths. Rooted cuttings were planted on 20 Oct 2003 in 15.2-cm pots using sphagnum peat based

Fafard No. 2 (Agawam, MA) and these plants were placed in a glass greenhouse with temperature range of 17/23C (N/D) and given 8-h photoperiods (covering with black cloth from 1430 until 0830 hours) to promote vegetative growth. They were fertilized at every irrigation with Peters Professional 'Florida Special' water soluble fertilizer 20N-4.7P-16.6K (Scotts Co., Marysville, OH) with N at 200 mg·L⁻¹ and pinched on 3 Nov 2003.

Photoperiod of 14 h was started on 18 Nov 2003 provided by covering with black cloth from 1630 until 0830 and lighting with 60-W incandescent light bulbs which provided not less than 1μEm⁻²s⁻¹ from 1630 until 2230 hours. On 14 Jan 2004 the 14 h photoperiod treatment was stopped and the photoperiod was changed to 8-h (covering with black cloth from 1630 until 0830 hours). There were twelve plants per treatment. The number of days to first open flower after the start of long days and time from start of short days to last flower was recorded. This experiment was terminated on 10 Mar 2004. Data were analyzed by calculating means and standard errors.

Experiment 5A. Number of inductive photoperiods needed to induce flowering. Rooted cuttings of three cultivars of *Calibrachoa* (Million Bells Trailing White, Million Bells Cherry Pink, and Million Bells Terra Cotta) were obtained from a commercial propagator on 10 Jun 2002, planted in 11.4-cm pots using sphagnum peat based Fafard No. 2 (Agawam, MA), and given a hard pinch. These plants were placed in a glass greenhouse with temperature range of 24/31C (N/D) and given 8-h photoperiods (covering with black cloth from 1430 until 0830 hours) to promote vegetative growth. They were fertilized every irrigation with Peters Professional 'Florida Special' water soluble fertilizer 20N-4.7P-16.6K (Scotts Co., Marysville, OH) with N at 150 mg·L⁻¹. On

5 July, 2002, the plants were pinched again. Photoperiod treatment of 14 h was started on 11 July, 2002. The photoperiod was provided by covering with black cloth from 1630 until 0830 and lighting with 60-W incandescent light bulbs which provided not less than $1\mu\text{Em}^{-2}\text{s}^{-1}$ from 1630 until 2230 hours. On 18 July, 25 July, 1 Aug, and 8 Aug 2002 the 14-h treatment was stopped and the photoperiod was changed to 8-h (covering with black cloth from 1630 until 0830 hours) to provide treatments with inductive photoperiods for 0, 1, 2, 3, or 4 weeks. There were four to eight plants of each cultivar in each group. The number of days to first open flower after the start of long days was recorded. This experiment was terminated on 10 Sept., 2002. Data was analyzed by taking the mean days to flower and calculating the standard errors.

Experiment 5B. Number of inductive photoperiods needed to induce flowering. Five cultivars of *Calibrachoa* (MiniFamous Dark Red, MiniFamous White Pink Star, Million Bells Cherry Pink, Superbells White, and Velvet Rose with Yellow Center) were established as stock plants and grown under 8-h day length. Cuttings were also rooted under 8-h day lengths. Rooted cuttings were planted on 20 Oct 2003 in 11.4-cm pots using sphagnum peat based Fafard No. 2 (Agawam, MA) and given a hard pinch on 3 Nov 2003. These plants were placed in a glass greenhouse with temperature range of 17/23C (N/D) and given 8-h photoperiods (covering with black cloth from 1430 until 0830 hours) to promote vegetative growth. They were fertilized every irrigation with Peters Professional 'Florida Special' water soluble fertilizer 20N-4.7P-16.6K (Scotts Co., Marysville, OH) with N at $200\text{ mg}\cdot\text{L}^{-1}$.

Photoperiod treatment of 14 h was started on 18 Nov 2003 provided by covering with black cloth from 1630 until 0830 and lighting with 60-W incandescent light bulbs

which provided not less than $1\mu\text{Em}^{-2}\text{s}^{-1}$ from 1630 until 2230 hours. Plants were given inductive photoperiods for 0, 3, 6, 9, or 12 days. On the appropriate date, the photoperiod was changed to 8-h (covering with black cloth from 1630 until 0830 hours). There were 12 plants of each cultivar in each group. The number of days to first open flower after the start of long days was recorded. The experiment was terminated on 14 Jan 2004. Data was analyzed by taking the mean days to flower and calculating the standard errors.

Results and Discussion

Experiment 4A. Time to stop flowering under short days. The number of days to first flower under an inductive 14 h day-length is one important plant response as seen in experiments 1, 2, and 3. The response of flowering plants to short-day conditions (8 h) is shown in experiment 4A (Figure 5). Of the three cultivars, Million Bells Trailing White took the longest time (about 45 days) to start flowering under 14-h photoperiods, and when moved to 8-h photoperiods, it stopped flowering in 28 days. Million Bells Cherry Pink had a different response: it started flowering in just 27 days and stopped flowering 31 days after being moved to the non-inductive photoperiod. Million Bells Terra Cotta had yet another response where it took 34 days to start flowering, but stayed in flower longer (53 days) than the other cultivars under 8-h photoperiods.

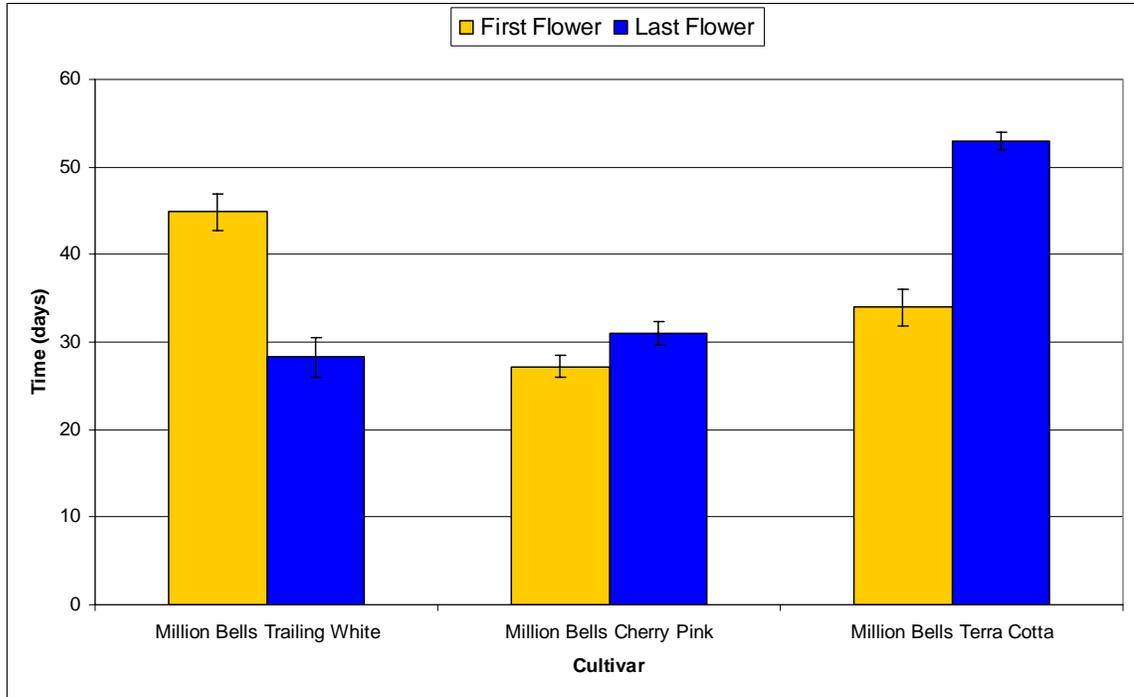


Figure 5. Days to flower under 14 h and to last flower under 8-h photoperiod.

Experiment 4B. Repeat Time to stop flowering under short days. The number of days under 8 h non-inductive photoperiods to stop flowering fluctuated from 24 days to >56 days (Figure 6). MiniFamous White Pink Star was still flowering when the experiment was terminated 56 days after the plants were shifted to the 8 h photoperiod. Superbells White, Velvet Rose with Yellow, and MiniFamous Dark Red were similar to Million Bells Trailing White in experiment 4. They took a relatively long time to start flowering (at least 42 days) and then stopped flowering quickly (24-25 days). Million Bells Cherry Pink was the only cultivar used in both experiment 4A and 4B and its response was similar in the two experiments.

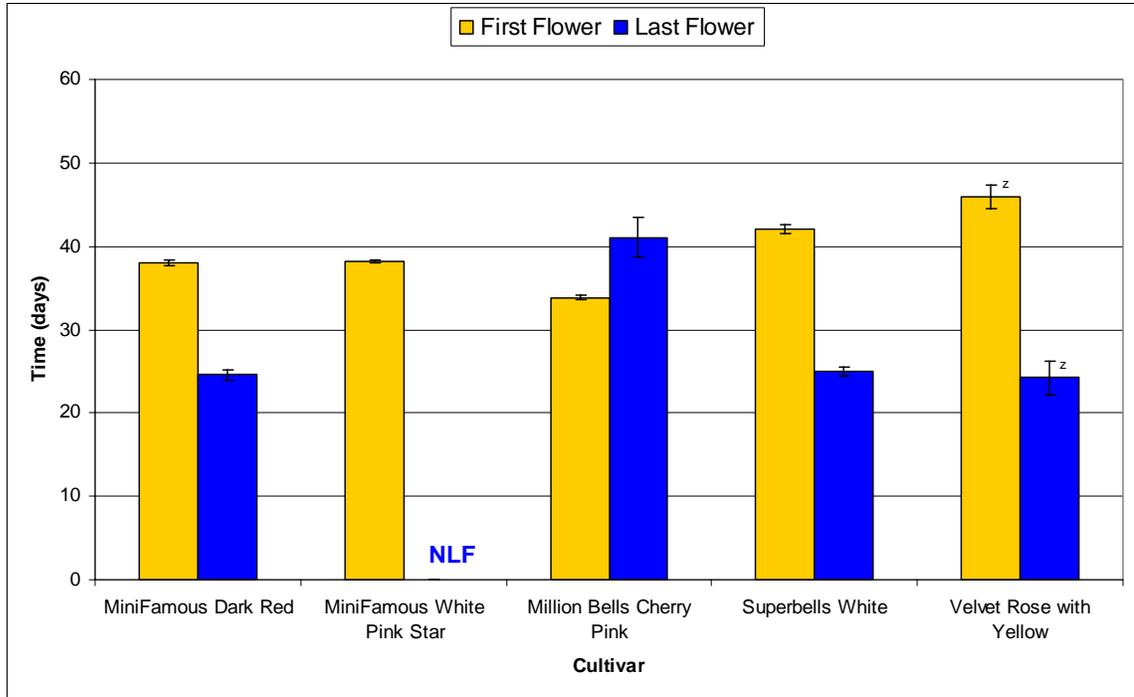


Figure 6. Repeat days to flower under 14 h and to last flower under 8-h photoperiod. A column replaced by NLF indicates that MiniFamous White Pink Star did not stop flowering before the experiment was terminated. ^z indicates not all plants flowered: Velvet Rose with Yellow had 9 of 12 plants flower and stop flowering.

Since many *Calibrachoa* do not naturally flower during the winter and early spring, many commercial operations use lights to provide long days during propagation, which may be about 4 weeks. Then the plants are often grown out to flower under natural days. Also, in warm climates *Calibrachoa* may be produced in the late summer or fall for use during the cooler months. These plants may be produced when the natural photoperiod is inductive and then planted in the landscape when natural photoperiods are too short. In this situation, a cultivar that stayed in flower for a long time without long days would be favorable. The three basic responses seen in experiment 4A and 4B are important for commercial operations where lighting is used to provide long days and where *Calibrachoa* is produced for fall sales. Million Bells Terra Cotta and MiniFamous

White Pink Star are examples of desirable *Calibrachoa* cultivars because the plants will stay in flower for longer durations if the natural photoperiod is non-inductive.

Experiment 5A. Number of inductive photoperiods needed to induce flowering. In commercial situations where lighting is used to provide long days, cultivars that require fewer inductive photoperiods for flowering are important. Million Bells Cherry Pink and Million Bells Terra Cotta plants given 1 week of long days flowered as quickly as did those given LD for longer periods (Figure 7). However, Million Bells Trailing White did not flower when given 4 weeks of LD. None of the cultivars flowered with zero weeks of LD.

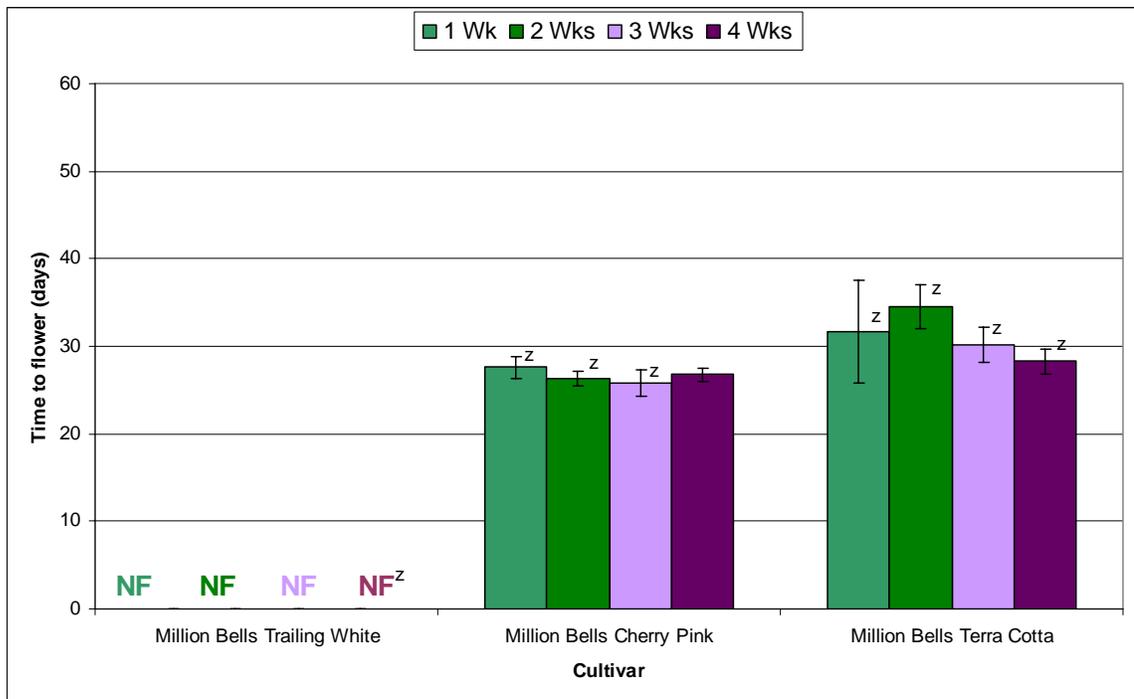


Figure 7. Weeks of long days (14 h) to initiate flowering. A column replaced by NF indicates that the cultivar did not flower at the given photoperiod. No plants flowered at 0 weeks of long days. ^z indicates not all plants flowered: Million Bells Trailing White had 1 of 8 plants flower at 4 weeks of long days. Million Bells Cherry Pink had 5 of 8 plants flower at 2 weeks, 6 of 8 plants flower at 3 and 4 weeks of long days. Million Bells Terra Cotta had 1 of 8 plants flower at 0 weeks, 5 of 8 plants flower at 1 week, 6 of 8 plants flower at 2 weeks, 7 of 8 plants flower at 3 weeks, and 4 of 8 plants flower at 4 weeks of long days.

Experiment 5B. Repeat number of inductive photoperiods needed to induce flowering. Since two of the three cultivars in experiment 4A flowered as quickly with 7 (one week) inductive photoperiods as with 28 inductive photoperiods, the number of inductive photoperiods given in this experiment were reduced. MiniFamous Dark Red, Superbells White, and Velvet Rose with Yellow did not flower under any of the LD conditions (Figure 8). For MiniFamous White Pink Star and Million Bells Cherry Pink six inductive cycles were enough to initiate flowering, but three were not. Million Bells Cherry Pink was in both experiments and once the number of long days photoperiods were adequate to induce flowering there is little effect increasing the number of long days. For Million Bells White Pink Star, 12 long days produced slightly faster flowering than six or nine long days.

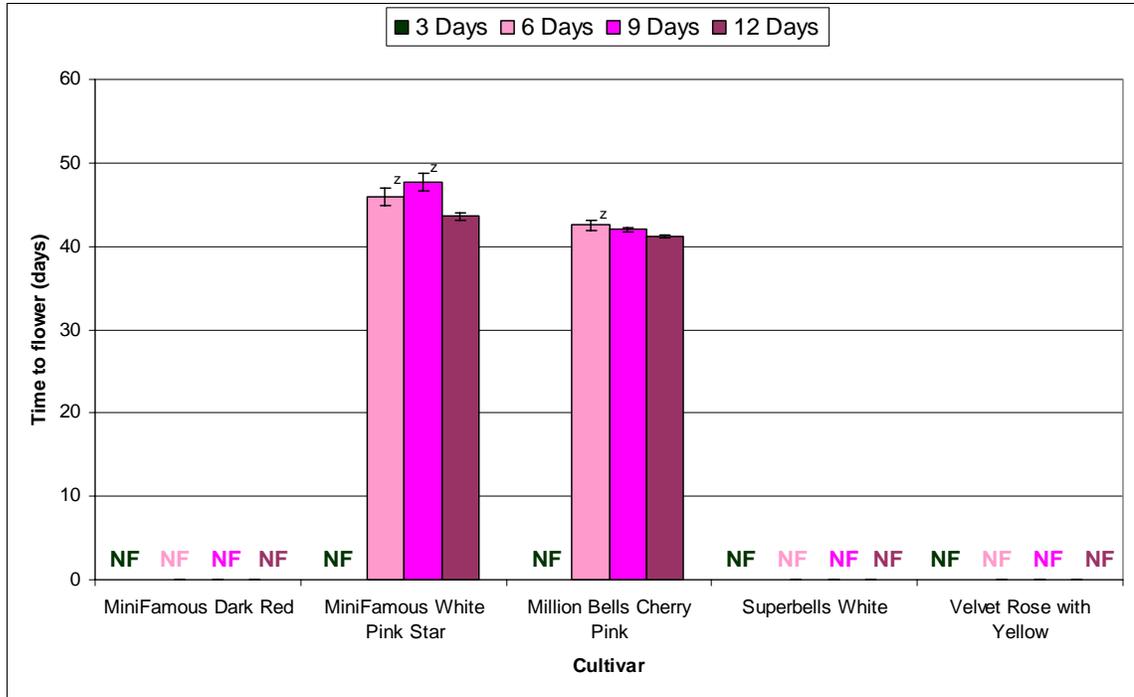


Figure 8. Days of long days (14 h) to initiate flowering. A column replaced by NF indicates that the cultivar did not flower at the given photoperiod. No plants flowered at 0 weeks of long days. ^z indicates not all plants flowered: MiniFamous White Pink Star had 11 of 12 plants flower at 6 and 9 days of long days. Million Bells Cherry Pink had 11 of 12 plants flower at 6 days of long days.

The results from experiments 5A and 5B demonstrate that some cultivars require fewer consecutive long days to promote flowering. MiniFamous White Pink Star, Million Bells Cherry Pink, and Million Bells Terra Cotta require 6 or 7 days under 14 h to develop flowers whereas MiniFamous Dark Red, Superbells White, and Velvet Rose with Yellow need more than 12 days (Figure 7 and Figure 8).

There was evidence in experiment 5B, that indicated there may be a long day requirement for flower initiation and also a long day requirement for continued flower development. MiniFamous Dark Red had visible buds that then aborted on plants that received 6, 9, and 12 long days. Likewise, Superbells White and Velvet Rose with Yellow had visible aborted buds on plants that received 12 long days. A similar response

was seen in poinsettias. 'Barbara Ecke Supreme' grown at 21C for 70 days under 12 h photoperiods, produced a bud and never flowered, which indicated that the critical day length was longer and the number of short days was fewer for initiation than development (Langhans and Miller, 1960).

Flowering *Calibrachoa* had a variable response to short days and also had different photoperiod requirements for the number of long days to cause flowering which depended on the cultivar. Experiments 4A and 4B showed that the number of short-day photoperiods can sometimes vary by more than 32 days to stop flowering. Million Bells Trailing White required more than 4 weeks of long-day photoperiods to initiate flowering whereas MiniFamous White Pink Star and Million Bells Cherry Pink required 6 days to cause flowering (Figure 7 and Figure 8).

CHAPTER 5 CONCLUSIONS

The popularity of *Calibrachoa* as a new floriculture crop has created a need for better production and crop culture information. Flowering is one of the most important physiological processes to understand because that is what drives commercial sales. This research was designed to screen numerous commercial *Calibrachoa* cultivars for their response to day length under more specific photoperiod treatments; to evaluate how a flowering *Calibrachoa* responds to short days; and to identify the photoperiod requirement for flower initiation and development.

Experiment 1 compared 27 commercial *Calibrachoa* cultivars to 11, 12, 13 or 14-h photoperiods and identified six different response types. Ideally, commercial cultivars would be more like group 1 where the days to flowering was not affected by the four photoperiod treatments and less like group 6 where Superbells Red did not flower at 11 or 12-h photoperiod and flowered slower under 13-h photoperiod than at 14 h (Figure 1). Besides differences in the critical photoperiod requirement, the diversity in days to flower under an inductive photoperiod was identified. Some cultivars flowered in 32 to 39 days whereas others took only 20 to 24 days (Figure 1).

Variable response time to day-length treatments makes scheduling difficult for commercial greenhouse growers. Someone in Florida trying to produce *Calibrachoa* for early spring sales would not be able to naturally flower varieties that require 12 h or 13 h day lengths. Flowers at the time of sale encourage consumers to buy plants and therefore, this grower would benefit from a variety that had a less sensitive photoperiod

requirement. Variable flowering response would also affect a grower in the north that is trying to ship plants south for the early spring season. This grower needs to supply long days with supplemental lighting in order to finish *Calibrachoa* plants with flowers. Lighting increases the cost of production and there is a need to identify those cultivars that either do not require artificial long days or that need less lighting.

Time to flower under an inductive photoperiod (14 h) is important for commercial production scheduling. Growers take into account the length of time needed to finish a crop in flower. Having all varieties flower in the same number of days under an inductive photoperiod makes production easier for greenhouse growers.

Differences in *Calibrachoa* cultivars attributed to greenhouse temperature is demonstrated in Experiments 2A and 2B. High temperatures can delay flowering as reported in poinsettia and chrysanthemum (Larson and Langhans, 1963; Whealy et al., 1987). Superbells White and Superbells Red exhibit heat delay by taking longer to flower in a warm greenhouse than a cool greenhouse. This is an especially important concept for commercial greenhouse growers in warm areas.

Early flowering cultivars under natural-day conditions are important for commercial producers that do not control photoperiods. Experiment 3 identifies quicker flowering cultivars for early production. When *Calibrachoa* is screened by plant breeders before plant introduction, they could select for early flowering and incorporate these plants into their breeding lines.

The response of a flowering plant when shifted to non-inductive, short-day photoperiods is explained in Experiment 4A and 4B. There are three ways that *Calibrachoa* cultivars tend to respond including: cultivars that come into flower and go

out of flower quickly; some that come into flower and stop flowering in the same amount of time or slightly longer time to go out of flower; and also cultivars that flower and stay in flower for a long time. The cultivars that stay in flower the longest would be beneficial to commercial operations that buy in induced liners and finish plants under non-inductive, natural-day photoperiods. These cultivars would also be good for production in the fall when photoperiods are becoming shorter.

Lastly, Experiments 5A and 5B demonstrate that some cultivars require fewer consecutive long days to promote flowering. The photoperiod requirements for MiniFamous White Pink Star and Million Bells Cherry Pink were met after just 6 inductive photoperiods for both flower initiation and flower development. Aborted flower buds gave evidence that 6, 9, or 12 days were long enough for flower initiation for MiniFamous Dark Red, but not for flower development.

Further research with *Calibrachoa* should be considered. Flower initiation and flower development would be interesting physiological responses to examine because *Calibrachoa* seems to be similar to poinsettia and chrysanthemum in that different cultivars have varying photoperiod requirements. Microscopic meristem observations would fill a gap in current floricultural crop research because there is little published information on long-day plants. With this, additional research on heat delay of plants grown in controlled temperature growth chambers could be studied. Again, the current research is somewhat limited and would benefit from close apical investigations.

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BIOGRAPHICAL SKETCH

Erika Mary Berghauer was born in Milwaukee, Wisconsin, in 1977. She attended the University of Minnesota and graduated with a Bachelor of Science in May, 2000. While working on her B.S. degree, she worked as an assistant gardener for a private home, a woody plant salesperson at a retail garden center, interned for a wholesale nursery, and worked as an assistant flower breeder. After the University of Minnesota, she interned as a cultural researcher for a plant breeding company and a plant science intern in Florida. Next, she was introduced to the University of Florida by accepting a research assistant position. Erika started working on her Master of Science degree in January, 2002.