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Roberto Gerardo Lopez

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ABSTRACT

EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON GROWTH AND FLOWERING OF SIX ORCHID HYBRIDS

By

Roberto Gerardo Lopez

Orchids are currently the second most valuable flowering potted floriculture crop in the United States. However, the commercial potential of the vast majority of orchids has not been explored primarily due to insufficient knowledge of how to regulate growth and flowering. A series of experiments was performed to determine the effects of photoperiod and temperature on growth and flowering of six orchid hybrids. *Brassia* Rex 'Sakata', *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch', and *Odontocidium* Tiger Crow 'Golden Girl' did not flower in response to a range of photoperiods (10 to 24 hours) or cool temperature (8 to 23 °C) treatments. In *Miltoniopsis* Augres 'Trinity', flowering percentage was greatest (≥90%) and was most rapid when plants were exposed to 9-h photoperiods for 4 to 8 weeks at 20 °C and then vernalized under short days at 14 °C for 8 to 12 weeks. Similarly, flowering of *Zygopetalum* Redvale 'Fire Kiss' was promoted most when exposed to short days at 23 °C for eight weeks followed by eight weeks of vernalization at 11 to 14 °C. Additional studies were performed to quantify the effects of temperature on pseudobulb development, time to flowering, and flower longevity. Thermal time models were developed for these processes and are presented.

DEDICATION

In memory of my grandmother, Mrs. Alicia C. Toledo (March 3, 1997) who inspired my interest in horticulture.

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SECTION I

LITERATURE REVIEW

Vernalization

The physiology of flowering in higher plants is an extraordinarily complex process influenced by both autonomous and environmental regulation. In certain plants, flowering is independent of the surrounding environmental conditions, which is known as autonomous flowering. In the majority of plants, the transition from vegetative growth (juvenility) to flowering is a process determined by the interaction of developmental regulation and environmental cues such as changes in daylength (photoperiodism) light quantity (photon flux density), light quality (spectral composition), nutrient and water availability, and extended periods of low temperatures (vernalization).

Some plants have an absolute requirement for specific environmental cues for flowering, which is referred to as an obligate or qualitative response. In other plants, flowering is promoted by certain environmental cues but plants will eventually flower in the absence of such a cue. These plants have a facultative, or quantitative, response to such a cue. Horticulturally, vernalization and photoperiod are the two most common mechanisms that control flower initiation.

Vernalization is the promotion of flowering of imbibed seeds or vegetative plants by low temperatures. The word "vernalization" is the translation of *yarovizatzya,* which combines the Russian root for spring with the suffix meaning "to make," and was derived from the observation that a cold treatment made strains of winter wheat (*Triticum aestivum* L.) behave like spring strains (Thomas and Vince-Prue, 1984). Vernalization was further defined by Chouard (1960) as "the acquisition or acceleration of the ability to flower by a chilling treatment".

A vernalization response is often observed in regions with low winter temperatures, which are unfavorable for growth and reproduction. A cold requirement prevents plants from flowering until the spring when environmental conditions are more favorable. A combined vernalization and photoperiod requirement can ensure that flowering occurs later in the spring rather than at the end of the winter. Low temperature requirements for flower induction have also been documented in tropical orchids. They may vary from more distinct elevation-dependant temperature fluctuations required by *Phalaenopsis schilleriana* to subtle rain-induced cooling, which initiates flowering in *Dendrobium crumenatum* (Goh et al., 1982).

Initiation of flower primordia generally does not occur at vernalizing temperatures. After exposure to low temperatures, plants are in a vernalized state (thermoinduced) and have the ability to flower under favorable conditions (Thomas and Vince-Prue, 1997). Depending on the species, these favorable conditions can be long days, short days or simply higher temperatures. However, a vernalization response is mainly observed in long-day plants, which have extended periods of vegetative growth and flower in the early summer at high latitudes (Vince- Prue, 1975). These species may be perennials, biennials, or winter annuals. Some well-documented examples of plants that exhibit this behavior are winter wheat, the biennial form of henbane (*Hyoscyamus niger* L.) (Chouard, 1960), *Coreopsis grandiflora* (Hogg ex Sweet) 'Sunray', *Aquilegia xhybrida* Sims, and *Achillea filipendulina* Lam. 'Cloth of Gold' (Heins et al., 1997).

Effects of Low Temperature

Plants respond to low temperature in ways other than flowering. Often, specific temperature regimens initiate critical steps in the life cycle: induction and breaking of dormancy, and seed germination. Dormancy is often overcome following exposure to cool temperatures for a particular duration, which removes growth inhibitors, and allows the emergence of pre-formed buds in woody perennials (Salisbury and Ross, 1992). The breaking of seed dormancy by exposure of moist seeds to low temperatures is often referred to as stratification or prechilling. Many seeds require a period of cold (0 to 10 °C) while in a fully imbibed state to germinate. This ensures that seeds do not germinate in the autumn, but rather the following spring. Generally, dry seeds do not respond to cold treatments.

Low temperature can directly affect floral initiation and development of brussel sprouts (*Brassica oleracea gemmifera* L.), *Iris* sp. 'Wedgewood', stock (*Matthiola incana* R. Br.), honesty (*Lunaria biennis* L.) and onion (*Allium cepa* L.) (Chouard, 1960; Thomas and Vince-Prue, 1997). These direct effects of low temperatures on flowering can be distinguished from vernalization, which is an inductive phenomenon.

Plants of tropical origin may show signs of chilling injury if they are exposed to cool, above-freezing temperatures. "Chilling injury is a physiological response of plants and their products that results in reduced quality and loss of product utilization following exposure to low temperatures" (Parkin et al., 1989). Several symptoms are observed when plants are maintained at or below low

temperatures for some period of time (Lyons, 1973). For example, exposure to temperatures of 2, 4, and 7 °C for 1, 2, 4, and 8 h resulted in spotting and pitting of *Phalaenopsis* leaves (McConnell and Sheehan, 1978). The greatest amount of injury was observed in plants subjected to 2 °C for 8 h. Symptoms ranged from small water-soaked spots visible 0.5 h after removal from the chilling temperatures, to pale green areas visible after 48 h. The areas then became yellow, collapsed and were prominent after two weeks. Within three weeks, the pitted areas began to darken and resembled virus symptoms. McConnell and Sheehan (1978) found that hypertrophied mesophyll cells that became collapsed between the main vascular bundles caused the pitted areas on leaves. Severity of the response depended on the physiological age of the leaf and the duration of the exposure to the chilling temperature. Chilling injury was confined to younger leaves between 50 to 75 % of their mature length.

Requirements for Vernalization

Plants must be mature, or beyond the juvenility phase, before they can respond to vernalization. In most woody plants, the juvenility period may last several years and can last up to 30 to 40 years in some forest trees (Thomas and Vince-Prue, 1984). Herbaceous plants have juvenile periods ranging from a few days to many weeks in duration. The terms "ripe-to-flower" and "capacity to flower" are used for plants that have completed the juvenile phase of their life cycle, but have not received the appropriate environmental conditions for flowering.

In all plant tissues studied, oxygen, water and carbohydrates must be present for vernalization to occur (Chouard, 1960; Lang, 1965). Treating imbibed or germinating seeds that are metabolically active satisfies the cold requirement of winter annuals. Seeds can be vernalized on the parent plant, but they must have a water content of about 40 to 50% of their dry weight. The time required for complete vernalization of winter annuals is about 40 to 45 d.

Some biennials such as beet (*Beta vulgaris* L.) can be vernalized as seeds. The vast majority germinate in the spring, forming vegetative plants that grow as rosettes during their first year. With the coming of the second spring, new leaves form and there is a rapid elongation of the flowering shoot. A cold treatment is only effective after the plant has reached a certain size.

Flowering of several perennial grasses is also promoted by cold. Some of these grasses have a subsequent long-day requirement. *Poa alpina* L. and *Poa alpigena* require three to four weeks of exposure to 3 to 6 °C under 8-h photoperiods for primary induction to (initiate inflorescence primorida) and a secondary induction requirement for long-days (for culm elongation, inflorescence development and anthesis) (Heide, 1994). The vernalization requirement for perennials is re-established during sexual reproduction.

Site of Perception

Perception of vernalization is believed to occur in the meristematic regions of growing apices. In addition, all actively dividing cells may be capable of responding to low temperatures (Levy and Dean, 1998). Experiments on celery

(*Apium graveolens* L.), radish (*Raphanus sativus* L.), sugar beet, and *chrysanthemum* indicate that low temperatures are perceived by cells in the shoot apex (Crosthwaite and Jenkins, 1993). Chilling the roots of field pennycress (*Thlaspi arvense* L.) at 4 °C for four weeks was ineffective, whereas chilling only the shoot tips initiated reproductive development when plants were transferred to long days and 21 °C (Metzger, 1988). In *Lunaria biennis* L., meristematic cells in the leaf petiole could be vernalized at 5 °C under a 12-h photoperiod, and the vernalized state was transferred to plants regenerated from those cells (Wellensiek, 1965).

However, in winter rye (*Secale cereale* L.), and *Cheiranthus allionii* seeds, low temperature treatments to non-meristematic tissue promoted flowering (Thomas and Vince-Prue, 1997). In another experiment, shoots regenerated from leaf cuttings of vernalized pennycress plants, which were fully expanded prior to low temperatures, developed reproductively (Metzger, 1988). These studies collectively suggest that vernalization requires cells undergoing mitosis. However, the vernalization requirement is reestablished during meiosis and progeny must be exposed to low temperatures to flower.

Effective Temperatures

Vernalization is a quantitative process that increases in magnitude until, with prolonged exposure, a saturation response is obtained. Horticulturally, the effectiveness of vernalization can be evaluated as a reduction in the days to flower, increased uniformity in flowering, higher flowering percentage, increased

flower production and vigor (Runkle et al., 1998). It is dependent on the cooling temperature and its duration. The length and effective temperature range for vernalization varies by species. The consistency of the cold treatment can also impact the effectiveness of vernalization. In general, plants require several weeks of cold to saturate the vernalization response, with 4 to 12 weeks being typical (Michaels and Amasino, 2000). Eight days of cold causes a substantial increase in flowering of celery with maximal promotion after four weeks (Michaels and Amasino, 2000).

The effective temperature range for vernalization is between -5 to 15 °C with a broad optimum between 1 and 7 °C (Lang, 1965). Usually the lower limit is set by the formation of ice crystals within the tissues. In certain plants native to tropical regions, such as *Phalaenopsis* orchids, four to five weeks of temperatures ≤ 20 to 22 °C are effective (Wang, 2001). Yet in some cereals, a response to vernalization is achieved at temperatures as low as -6 °C. In some plants that respond to vernalization, short days can substitute partially or entirely for low temperatures (Thomas and Vince-Prue, 1997).

Obligate or Facultative Requirements

Plants that will not flower unless exposed to low temperatures have a qualitative, absolute, or obligate cold requirement. Most plants that possess this requirement are biennials or herbaceous perennials. Annual sugar beets (*Beta vulgaris* L.) are known to have a facultative response and biennials often have an obligate requirement for vernalization (Lexander, 1985; Crosthwaite and Jenkins,

1993). In *Oenothera fruticosa* 'Youngii-lapsley', flowered after receiving ≥ 3 weeks of cold (at 5 °C) and only one non-vernalized plant flowered (Clough et al., 2001). Whitman et al. (1996) showed that exposure to 5 °C for 10 to 15 weeks was the primary factor promoting complete flowering of *Lavandula angustifolia* Mill. 'Munstead.'

Plants in which flowering is hastened after exposure to low temperatures, but will eventually flower without cold, have a quantitative, facultative, or cold-stimulated requirement. Many winter annuals and herbaceous perennials posses this response. For example, in *Rudbeckia fulgida* Ait. 'Goldsturm', 15 weeks of 5 °C under photoperiods ≥ 13 hastened flowering by 25 to 30 d (Runkle et al., 1999). Flowering of *Phlox paniculata* Lyon ex Pursh 'Eva Cullum' was hastened from (114 to 64 d) by vernalization as photoperiod increased from 10 to 24 hours when exposed to 15 weeks of 5 °C and flowering percentages never reached 100% without cold (Runkle et al., 1998).

Facultative and obligate requirements can vary between cultivars and species. There are three types of vernalization responses in wheat (*Triticum aestivum* L.) cultivars: 1) qualitative, 2) quantitative, and 3) unresponsive (Gardner and Barnett, 1990). Another interesting example of both facultative and obligate vernalization requirements is that of the *Oenothera* species. Many species of *Oenothera*, including *O. suaveolens* Pers., *O. longiflora* L., and *O. stricta* Lebed. ex Link, have facultative requirements for vernalization followed by obligate requirements for long-days (Chouard, 1960). In contrast, *Oenothera*

fruticosa 'Youngii-lapsley' is a facultative long-day plant with an obligate vernalization requirement (Clough et al., 2001).

Induced State

Flower development typically begins several days or weeks after the end of the cold treatment. This induced state can persist several weeks or months, varying by species. Certain cereal seeds, for example, can be moistened (to 40 percent water, which is inadequate for germination to occur), vernalized, and then dried and maintained for months or even years without loss of the vernalized state (Salisbury and Ross, 1992). Henbane, which requires long days after vernalization, can be placed under short days to delay flowering. No loss of the vernalization stimulus appears in such plants after 190 short days, even after all the original leaves exposed to the cold had died. Chrysanthemums must be exposed to cold temperatures before they can respond to short days, and are commonly propagated by cuttings that retain their initial vernalized condition.

Devernalization

The reversal of the vernalized state, or devernalization, can occur during or immediately following vernalization. The most common method for devernalization is by exposure to high temperatures of 18 to 40 °C immediately following exposure to vernalizing temperatures (Lang, 1965) and anerobic conditions (Chouard, 1960). If low temperature treatments have been sub-optimal, susceptibility to devernalization increases. Devernalizing temperatures for winter rye are ≥ 30 °C and must be applied for a few days and within four or

five days after the low temperature period (Salisbury and Ross, 1992). Exposure to daytime temperatures of 30 °C for 2 d during or immediately after vernalization did not devernalize *Campanula* 'Birch Hybrid' plants, while a 4 d exposure decreased flowering percentage and delayed flowering by 7 to 10 d (G. Niu, personal communication). After devernalization, many species can be revernalized with another cold treatment.

It has also been reported that short days (SD) can lead to devernalization in *Oenothera biennis* and *Cheiranthus allionii* (Wellensiek, 1965). However, once the cold requirement has become saturated, the vernalized condition is extremely stable and persists throughout the vegetative phase until the plants finally flower (Thomas and Vince-Prue, 1997). For example, in *Hyoscyamus,* a plant with an absolute requirement for cold and long days, the vernalized state is retained for over seven months when placed under short days (Lang, 1965).

Genetic and Molecular Components

While the physiology of vernalization has been studied extensively in some species, the genetic and molecular mechanisms remain largely unknown. Recent studies have revealed some of the molecular events that create the requirement for vernalization. The appearance of early-flowering ecotypes in the evolution of *Arabidopsis thaliana* suggests that there has been strong selection in some environments for ecotypes that do not require vernalization due to milder winters (Johanson et al., 2000). Genetic analysis of early- and late-flowering ecotypes of *Arabidopsis* has identified two loci that account for most of the

differences in time to flowering. The products of these two loci, *Flowering Locus C* (*FLC*), and *FRIGIDA* (*FRI*) appear to act synergistically to cause late flowering (Napp-Zinn, 1987; Lee et al., 1993; Koorneef et al., 1994). Vernalization is believed to suppress both *FRI* and *FLC*; both alleles interact to repress flowering, with the level of *FLC* and *FRI* expression determining time to flower (Koorneef et al., 1994; Lee and Amasino, 1995; Johanson et al., 2000). The repression of flowering by *FLC* and *FRI* ensures that flowering in the various ecotypes will occur under favorable conditions in the spring.

VERNALIZATION (VRN) gene

Plants with a mutation in the *VERNALIZATION* (*VRN*) gene were thought to be impaired in their ability to perceive cold or in the transduction of the cold signal by the vernalization pathway (Levy and Dean, 1998). To test this hypothesis, *vrn*1 and *vrn*2 *Arabidopsis* mutants were isolated based on their reduced vernalization response in the late-flowering vernalization-response *fca-1* mutant. Neither *vrn1* nor *vrn2* were impaired in their ability to acclimate to low temperatures. However, neither mutant was able to reduce *FLC* mRNA in response to low temperatures (Simpson et al., 1999). This indicates that the defect in these genes is specific to the vernalization pathway and not to low-temperature responses in general. Both *fca / vrn1* and *fca / vrn2* double mutants show *FLC* messenger RNA (mRNA) accumulation and an increase in time to flower. This suggests that the *VRN1* and *VRN2* genes may mediate the vernalization-induced down-regulation of the *FLC* gene (Sheldon et al., 1999;

Sheldon et al., 2000). The recent cloning of *VRN2* indicates that the repression of *FLC* by *VRN2* is stably maintained and serves as a cellular memory of vernalization (Gendall et al., 2002). It is also speculated that *VRN1* and *VRN2* are involved with the perception of light quality during vernalization (Levy and Dean, 1998).

FLOWERING LOCUS C (FLC)

The *FLOWERING LOCUS C* (*FLC*) gene is thought to act as a strong floral repressor by negatively regulating the expression of genes that promote the transition to flowering (Michaels and Amasino, 1999b). In *Arabidopsis*, *FLC* can be found mainly in roots and vegetative shoot apices, to a lesser extent in the leaves and stems, and is undetectable in the inflorescence. Exposure of germinating seeds to low temperatures reduces levels of *FLC* transcript mRNA and its protein product. Furthermore, the longer the period of exposure to cold, the less *FLC* mRNA is produced and the earlier flowering occurs (Michaels and Amasino, 1999b). Levels of *FLC* mRNA are not affected by photoperiod or plant age (Michaels and Amasino, 1999b).

Landsberg *erecta* (L*er*) is an early flowering ecotype of *Arabidopsis*. Its late flowering mutants exhibit very high levels of *FLC* transcript, which is reduced after vernalization (Sheldon et al., 2000). A vernalization treatment also reduces *FLC* transcript in other late flowering ecotypes, which suggests *FLC* is a common repressor of flowering. When *FLC* is over-expressed, flowering is delayed, whereas in a loss-of-function mutant, flowering occurs early (Koorneef et al.,

1994; Michaels and Amasino, 1999b). *Arabidopsis* lines with additional copies of *FLC* never flower in the absence of cold and respond as true biennials (Michaels and Amasino, 2000). However, even when there is a complete loss-of-function of *FLC*, plants possess the ability to respond to vernalization (Michaels and Amasino, 2000). These findings suggest that there are two or more pathways that lead to a vernalization response, with at least one that is *FLC*-independent.

One component of the *FLC*-independent pathway may be *VRN1* and *VRN2* (Sheldon et al., 2000). These two genes are thought to be active in both the *FLC*-dependent, and *FLC*-independent pathways. Mutants *vrn1* and *vrn2* have been isolated and identified as having a small vernalization response (Chandler et al., 1996).

There are two types of *FLC* alleles: late flowering and suppressor (Sanda and Amasino, 1996; Sheldon et al., 2000). Late flowering types are most common and promote vegetative growth in *FRI* mutants. Suppressor *FLC* alleles inhibit late flowering in *FRI* mutants. Despite differences in their mode of action, *Arabidopsis* ecotypes C24, Col and L*er* have the same nucleotide sequence of the coding region (Sheldon et al., 2000). Thus, it is likely that the *FLC* alleles in these ecotypes differ in some aspect of their regulation, perhaps as a result of the sequence differences in the promoter region of the *FLC* gene.

FLC codes for a MADS-box type transcription factor (Michaels and Amasino, 1999b). It has been identified as a "semidominant repressor of floral induction" and is believed to be the central regulator of the transition to flowering

in response to vernalization in *Arabidopsis*. Homologue MAF1 (MADS Affecting Flowering 1) encodes a protein very similar to *FLC* and has a 62% amino acid sequence identity with *FLC* (Ratcliffe et al., 2001). Studies with this homologue have shown similar results to *FLC* when overexpressed with the cauliflower mosaic virus 35S promoter. However, late flowering MAF1 were not overcome by vernalization and were thought to act independently of *FLC*. Ratciffe et al. (2001) proposed a gene family of six *FLC*-like genes that regulate time to flower downstream of *FLC*.

FRIGIDA (FRI)

FRIGIDA (*FRI*) gene activity is also associated with late flowering in *Arabidopsis*. *FRI* encodes a protein that promotes the accumulation of *FLC* mRNA (Michaels and Amasino, 1999b; Simpson and Dean, 2002). The predicted protein is not similar to other proteins with known functions (Johanson et al., 2000). By promoting the accumulation of *FLC* mRNA, *FRI* can repress floral transition to a degree where it overrides favorable conditions.

To test whether *FRI* acts solely through *FLC*, Michaels and Amasino (2001) analyzed the flowering behavior of isogenic lines containing dominant and recessive *FRI* alleles in an *flc-3* mutant. As has been shown in previous experiments, *FRI* strongly delays flowering in the wild type. In the *flc-3* mutant, the effect of *FRI* is eliminated under both long and short day conditions. Thus, the late-flowering phenotype is likely to result from an upregulation of *FLC* activity.

DNA demethylation

Low temperature (vernalization) has been linked with the demethylation of DNA (Burn et al., 1993). In this process, demethylation occurs in the promoter region of a gene or genes whose expression is critical for the induction of flowering. Vernalization of four to eight weeks reduced DNA methylation in *Arabidopsis* by 15% compared with seedlings that were not vernalized (Finnegan et al., 1998).

Treatments with the demethylation agent 5-azacytidine (5-azaC) can mimic the vernalization response and thus accelerate flowering. With no prior vernalization treatment, germinating *Arabidopsis* seeds treated with 5-azaC showed earlier floral initiation and a reduction in the number of rosette leaves formed before flowering (Burn et al., 1993). This process is only observed in late flowering ecotypes of *Arabidopsis* that require vernalization; early flowering ecotypes showed no response to 5-azaC. In addition, vernalized plants flowered earlier than those treated with the demethylation agent. This suggests that demethylation alone does not regulate the vernalization response.

In addition to causing demethylation, 5-azaC is a general inhibitor of transcription; therefore, it is possible that the promotion of flowering by 5-azaC results from effects other than demethylation of DNA. To test this hypothesis, Finnegan et al. (1998) used *Arabidopsis* plants that had reduced levels of DNA methylation due to the presence of a methyltransferase (*METI*) antisense transgene. They found that the demethylation of DNA is sufficient to cause early

flowering and that the promotion of flowering was directly proportional to the decrease in methylation in the *METI* antisense lines.

Plants that have reduced levels of methylation show a number of pleiotropic effects including expression of floral organ-identity genes such as *AGAMOUS*, which is known to induce flowering when ectopically expressed (Michaels and Amasino, 2000). Thus, changing patterns of DNA methylation may contribute to the epigenetic changes in gene expression that are likely responsible for the vernalized state. However, more research is needed to establish the molecular basis of this event.

Photoperiod

Photoperiodism refers to the response of plants to the length of day. The word is derived from the Greek roots for "light" and "duration of time." Hillman (1969) defined photoperiodism as a response to the timing of light and darkness. Plant responses influenced by daylength include bud dormancy, formation of storage organs, asexual reproduction, leaf development, stem elongation, germination, and flower initiation and development (Thomas, and Vince-Prue, 1984).

The classification of plants according to their photoperiodic response is usually made on the basis of flowering. With respect to flower initiation, most species' responses to daylength can be classified into three groups. Day-neutral plants (DNP) flower regardless of the photoperiod to which they are exposed. In short-day plants (SDP), flowering occurs only in, or is accelerated by daylengths

shorter than a particular duration known as the "critical daylength". Flowering in long-day plants (LDP) occurs only in, or is accelerated by daylengths exceeding a particular duration. Less commonly, some species have a dual daylength requirement, i.e. a period of short days followed by a period of long days, or vice versa (SLDP and LSDP) (Thomas and Vince-Prue, 1997). Responses to daylength can be further divided into two categories: qualitative or quantitative.

Some tropical plants of equatorial origin are believed to be more sensitive to small differences in daylength than those from temperate regions (Sanford, 1974). An example of a tropical plant influenced by photoperiod is the epiphytic cacti *Hatiora* spp, which is classified botanically as a SLDP for flowering at 15-20 °C (Boyle, 1991). In its native habitat of Panará and Santa Catarina, Brazil (~26 °S lat.) the shortest civil daylength (the duration of time when the sun is 6° below the horizon before sunrise to 6° below the horizon after sunset) is 11 h 28 min and the critical photoperiod for photoinduction in *Hatiora* is 11-12 h (Boyle, 1991).

Schlumbergera truncata and *Cattleya* spp are SD tropical epiphytes native to the Organ Mountains north of Rio Janeiro, Brazil (~22 °S lat.) where the photoperiod ranges from 11 to 13.5 h (Morrison, 2000). Commercially, *Schlumbergera* is placed under 8 to 11 h short day photoperiods and night temperatures of 13 to 15 °C for flower initiation (Dole and Wilkins, 1999). In *Cattleya warscewiczii, C. gaskelliana,* and C. *mossiae,* flower induction occurs under continuous 9-h short days at 13 °C, while flowering is inhibited under 16-h long days at 13 °C (Rotor, 1952; Rotor 1959).

Rohwer (2002) reported that a 10-h short day treatment followed by a vernalization treatment at 12.5 or 15 °C increased flowering percentage and apical phylloclades flowering, bud number per flowering apical phylloclade of *Hatoria gaertneri*. The most uniform flowering occurred when plants were exposed to a sequence of six weeks of short days, vernalization at 8 to 15 °C for eight weeks, followed by long-days at warmer temperatures.

Orchids

The *Orchidaceae* is the largest and most diverse of all plant families. Approximately ten percent of all flowering plant species are orchids, which comprise 20,000 to 25,000 species grouped into 725 genera (Griesbach, 1985). In addition to the natural abundance of orchids, there are > 100,000 man-made hybrids. Orchids are indigenous to a wide array of mesic and xeric habitats, including tropical and temperate forests, prairies, tundra and even deserts (Morrison, 2000). In the tropics, orchids are distributed according to elevation gradients. Diversity is highest in the montane cloud forests between 1000 to 2000 m above sea level (Morrison, 2000). The greatest abundance is found in regions with annual rainfall of 250 cm or more.

Orchids are terrestrial, lithophytic or epiphytic as they are found growing in soils, on rocks or on trees. Most terrestrial orchids have storage organs in the form of corms, rhizomes, or tuberoids, while epiphytes have enlarged stems referred to as pseudobulbs and aerial roots (Ng and Hew, 2000). The vast

majority of tropical orchids are epiphytic and water availability is the limiting factor to their distribution (Fu and Hew, 1982).

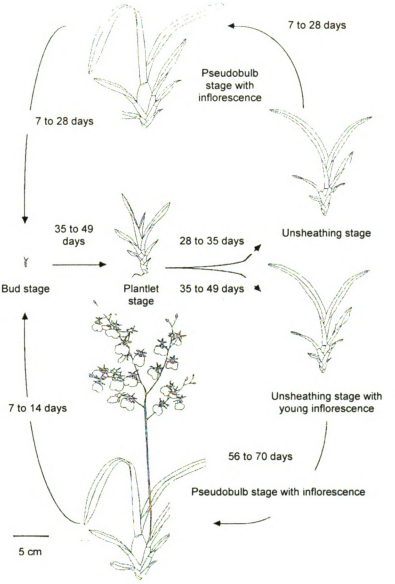

Figure 1. The growth cycle of *Oncidium* Goldiana under greenhouse conditions (Hew and Yong, 1997)

In monopodial orchids such as *Phalaenopsis*, the stem apex grows indefinitely resulting in a single upright shoot. New leaves develop from the end of the apical stem. As a rule, monopodials produce lateral inflorescences.

Sympodial orchids such as *Cattleya* have repeated growth cycles producing clumps of pseudobulbs and axillary buds. The axillary buds are present at the base of the pseudobulb and are not normally visible because leaves cover them. Most sympodial orchids produce terminal, lateral, or both types of inflorescences (Figure 1).

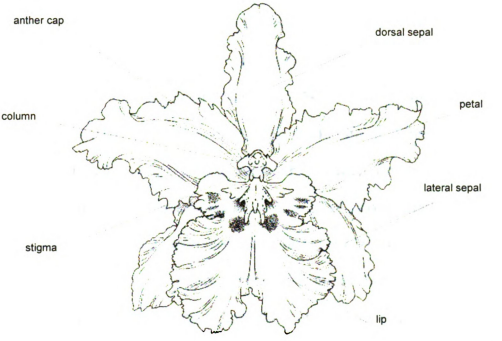

Figure 2. Orchid flower anatomy (Rittershausen and Rittershausen, 2001)

Orchids are considered the most evolutionarily advanced of all monocotyledons, having specializations for pollination, water uptake and storage in pseudobulbs, seed germination and associations with fungi. One feature defines the orchid from all other flowering plants: their stamens and pistils are fused into a structure termed the column (gynostemium) (Figure 2). Such monandrous orchids have a single fertile stamen with pollen massed into packets

termed pollinia. The flowers are zygomorphic, meaning they are symmetrical about a single plane (Morrison, 2000).

Orchid Nomenclature

Following Linnaeus's binomial system, orchids have two Latin names for their species, e.g., *Cattleya skinneri*. Closely related genera are grouped into subtribes, names ending in -inae. For example, *Cattleya* is in the subtribe Laeliinae along with its relatives such as Laelia and Encyclia. Related subtribes are then grouped into larger taxonomic units referred to as tribes; names of tribes end in -eae. Subtribe Laeliinae is in the tribe Epidendreae. Finally, related tribes are grouped into a subfamily, the names of which always end in -oideae. The tribe Epidendreae is in the subfamily Epidendroideae. For hybridizations or awards, specific plants are given clonal or cultivar names, following species names and enclosed in single quotes such as *Cattleya* skinneri 'Many'. An award that a clone receives then follows the clonal name, e.g., *Cattleya* skinneri 'Many' CCM/AOS (certificate of cultural merit) (Morrison, 2000). There are several national orchid societies that grant awards for flower quality or cultural excellence: the American Orchid Society (AOS), the Royal Horticultural Society (RHS), Detsche Orchideen-Gesellschaft (DOG).

Commercial Genera

Brassia. Approximately 25 to 30 species, mainly epiphytes, make up the genus *Brassia* (or the spider orchid). They are distributed from the warm forests

of Costa Rica and islands of the West Indies at low elevations of 200 m, to the mountains of Brazil and Peru at elevations of 2000 to 3000 m (Rentoul, 1982). They are characterized by compressed, oblong and cylindrical pseudobulbs surrounded by up to three leaves at their apex and the lower portions are sheathed by basal leaves. The inflorescence develops from the side of the pseudobulb and is enclosed by the longer of the sheathing leaves or bracts. Although closely related to the *Oncidium*, the flowers of *Brassia* are distinct due to their musky fragrance and long tail-like sepals, which range in color from pale green to golden yellow with black markings. The genus is named in honor of the 18th century botanical artist William Brass, who collected orchids in South America and South Africa (Stewart and Griffiths, 1995).

Cattleya. Cattleya is a genus composed of 60 species originating throughout tropical regions of Central and South America. The epiphytic plant is found growing atop trees of moist and wet forests from sea level to 1500 m in elevation. They are characterized by the presence of cylindrical pseudobulbs of several nodes with thick apical leaves, and at the apex is a bud primordium capable of developing into an inflorescence (Morrison, 2000). There are often other bud primordia at the basal part of the pseudobulb that give rise to vegetative shoots (Rotor, 1952). The racemose inflorescence has large and showy flowers. The discovery of *Cattleya* (known as the corsage orchid) is credited to William Cattley. In 1818, pseudobulbs of *C. labiata* were sent from the Organ Mountains near Rio de Janeiro, Brazil, as packing material for other

ornamental plants. Cattley had the plants moved to his greenhouse where they later flowered (Morrison, 2000).

Cymbidium. Cymbidium is a genus of approximately 50 species native from tropical Asia to Australia. They are terrestrial, epiphytic, lithophytic and semi-epiphytic plants. A characteristic shared by most *Cymbidium* is the large globose-conical or oval compressed pseudobulbs, sheathed by the base with long and narrow leaves. The inflorescence is produced form the axils of the lower leaves. The large and showy flowers bear a three-lobed labellum with a central ridged callus (Morrison, 2000).

Degarmoara. Degarmoara is an epiphytic hybrid between *Miltassia* cartagena x *Odontoglossum* gledhow that produces large pseudobulbs and leaves that grow to 40 cm or more in height. This star shaped orchid produces large 10 to 15 cm flowers with small purple spots and yellow streaks towards the center of the petals and sepals. The flower spikes appear from large and swollen pseudobulbs.

Dendrobium. Dendrobium (or the spray orchid) is one of the largest genera of the orchid family, with some 900 species that are native to tropical and subtropical Asia, Australia and various Pacific Islands (Eigeldinger and Murphy, 1972). Its epiphytic origin is identified with the Latin name of the genus: dendron, "a tree" and bios "life". A few species are fragrant, but the scent of some can be unpleasant. Most *Dendrobium* form tall cylindrical stem-like pseudobulbs with long spikes that grow from the apex. An exception is *D. nobile*, where the flowers are produced in clusters of two or three from the nodes along the stem-

like pseudobulb. *Dendrobium moniliforme*, possibly the first *Dendrobium* in cultivation, is mentioned in Chinese literature as early as the Chin Dynasty (290-307 AD). It was not until 1799 that Olaf Swartz established the genus. Early attempts in Europe of cultivating *Dendrobium* failed, as most gardeners did not understand the conditions of tropical environments.

Miltassia. *Miltassia* are epiphytic hybrid between *Brassia* verrucosa x *Miltonia* spectabilis. Their growth is characterized by the presence of two apical light green leaves on large pseudobulbs. *Miltassia* have deep plum purple, star shaped flowers, with pale green halos. The flower spikes appear from the base of mature and immature pseudobulbs. Flowers can last from four to six weeks.

Miltoniopsis. *Miltoniopsis* is an epiphytic or lithophytic genus of six species distributed from the extremely wet cloud forest regions (610 to 2,100 m) of Costa Rica to Peru (Baker and Baker, 1993b; Morrison, 2000). Their sympodial growth habit is characterized by the presence of one gray-green apical leafed pseudobulb surrounded by leaf-like sheaths. The pseudobulb is capable of producing one or more inflorescences only when it is mature. The fragrant flowers are flat, large and showy, ranging in color from cream to pink to magenta to scarlet. The flowers can last four to eight weeks on the plant (Baker and Baker, 1993a). John Lindley named the genus in honor of the orchid enthusiast Lord Fitz-William Milton (1786-1857) (Berliocchi, 1996). In 1889, Godefroy-Lebeuf separated *Miltonia* into two groups, *Miltonia* and *Miltoniopsis*. However, this distinction was not officially recognized until 1976 (Rentoul, 1982), and in some literature the two groups are treated as one genus for registration

purposes. *Miltoniopsis* has one-leafed pseudobulbs, while *Miltonia* has two-leaved pseudobulbs. *Miltoniopsis* are the fifth most valuable potted orchid commercially produced in the Netherlands, with 797,000 pots sold in 2001 (Barendse, 2002). Often, *Miltoniopsis* are referred to as the cool-growing, Colombian miltonias or the pansy orchid.

Phalaenopsis. The genus *Phalaenopsis* is made up of ≈50 species originating from tropical and subtropical areas of the South Pacific Islands and Asia (Baker and Baker, 1991). Their distribution in the South Pacific extends from Southern India to Australia. In Asia, they are found in China, Taiwan and the Philippines. The greatest concentration, and the species that have had the greatest influence on hybridizing, have originated from the Philippine Islands (Rittershausen and Rittershausen, 2000). All *Phalaenopsis* are monopodial, and the vast majority are epiphytic. The first *Phalaenopsis* plants were discovered by Rumphius in 1750, however they were not classified until 1825 when Karl Ludwig Blume placed them into the genius *Phalaenopsis*. The word *Phalaenopsis* was derived from two Greek words: *phalaina*, meaning "moth" and *opsis*, denoting "resemblance". The common name for *Phalaenopsis* is the "moth orchid". Today's *Phalaenopsis* cultivars range in color from pure white, lavender, purple, red, and yellow. Most flowers are long-lasting and can stay in bloom two to four months under favorable conditions (Wang, 1994).

Zygopetalum. Zygopetalum or the ladybird orchid is a sympodial, terrestrial, and epiphytic South American genus made up of ≈20 species. They are native to neotropic mid-elevation mountains (1,300 to 1,700 m) of Brazil,

Guiana, Venezuela and Peru (Rose, 1993). *Zygopetalum* Redvale 'Fire Kiss' is moderately compact (25- to 40-cm tall) and produces two to three, broad and leathery leaves atop mature green pseudobulbs. Four or more leaf-like sheathing bracts, which grow from the base, protect the immature pseudobulbs but these bracts become dry and fibrous with age (Baker and Baker, 1991). The erect inflorescences emerge from the third, forth or fifth sheathing bract of immature pseudobulbs. Lime-green and dark maroon sepals and petals, featuring broad three-lobed labellums in deep magenta and white, characterize the exotic, fragrant and waxy flowers. William Jackson Hooker named the genus in 1827, referencing the Greek word *zygon* (yoke) and *petalon* (petal or sepal), which pertains to the thickened callus at the base of the labellum that appears to hold together (or yoke) the petals (Monkhouse, 1994).

Commercial Production

In 1957, James Shoemaker, an economist stated, "orchid growing has not fully achieved the transition from a hobby to an industry" (Griesbach, 2002). Today, orchid cultivation is still in its infancy, as it is evolving from a hobbyists' market into a highly commercial market and international business. An example of the developing international trade can be traced with *Phalaenopsis* production, when breeding occurs in the United States (Griesbach, 2002). Selected clones are sent to Japan for tissue culture propagation. The successful cultures are then mass proliferated in China and sent as in vitro plantlets to the Netherlands

for greenhouse production. Plants are finally sent to the United States for flowering and sold to consumers.

Orchids such as *Cymbidium*, *Dendrobium*, *Phalaenopsis*, and *Oncidium* are marketed globally as cut flowers for corsages and floral arrangements, as potted flowering plants, and as bedding or aerial plants in tropical regions. In 1995, the world demand for potted orchids was 1.22 billion units of plant stock with an estimated increase of four percent over the next five years (Hew and Yong, 1997). According to the American Orchid Society, over 75% of all orchids sold in the U.S. are *Phalaenopsis* (Griesbach, 2002).

In the early 1990's, the production of potted blooming orchids began to rise in the United States. The United States Department of Agriculture (USDA) National Agricultural Statistical Service considered orchids to be a minor crop and did not collect production information until 1997 (USDA, 1998). In wholesale value, orchids have become the second most valuable flowering potted crop in the United States (USDA, 2003). In the past six years, production value has increased 147%, with an average annual increase of 23%, and in 2002 the estimated wholesale value was $105.6 million. The value of potted orchids produced in Japan has experienced an increase of 1125% in 26 years. Production in 1965 was valued at $44,000 and $49 million in 1991 (Ichihashi, 1997). The overall market value of imports and domestic production in Japan was estimated to be $261 million in 1993 (Hew and Yong, 1997). In 1993, *Cymbidium*, *Phalaenopsis* and *nobile*-type *Dendrobium* were the most widely grown species in Japan. *Phalaenopsis* are currently the most valuable potted

crop in the Netherlands. From 1983 to 1994, the number sold through the auction at Aalsmeer increased from 50,000 to 3,150,000 plants, and in 1994 was valued at $62 million (Barendse, 2002; Hew and Yong, 1997). Large scale potted production is also taking place throughout the world, including China, Germany, and Taiwan (Griesbach, 2000).

Worldwide potted orchid production is continually increasing and is driven by: 1) increasing popularity; 2) improvements in propagation; 3) grower acceptance of orchids as profitable crops; 4) improved plant vigor, especially of hybrids; and 5) segmentation of the supply chain (Britt, 2000). Many growers in the United States have expanded their production beyond the traditional orchid hobbyists and now target mass marketers such as Frank's, Lowe's, Home Depot, Target, Kmart and Wal-Mart. The largest demand for potted blooming orchids at these retail outlets is for traditional holidays such as Valentine's Day, Easter and Mother's Day. However, year-round production and sales are expanding beyond those of traditional potted plants such as chrysanthemums and African violets (USDA, 2003).

No official standards exist for the commercial production of orchids, but the industry consensus for producing *Phalaenopsis* is in 15-cm pots and with six or more flower buds per plant (Wang and Lee, 1994). However, many growers produce plants in 10- or 11.5-cm pots with fewer flowers for lower prices. Until recently, *Phalaenopsis* with multiple spikes and well-branched spikes are often sold at a higher price than plants with only one spike and no lateral branches.

The wholesale price of a 15-cm pot is $8 to $12, with a retail price of $15 to $25 on the mass market (Wang, 2001).

Similar large-scale production is in the early stages of development for most other orchid species, as there have been few scientific studies investigating the relationships between environmental parameters such as temperature, light quantity, and photoperiod on flower induction. For controlled flower induction to be commercially viable, the following conditions must be satisfied: 1) the methods must be simple, economical and give reproducible results for growers; 2) the quantity and quality of flowers must not be adversely affected by the treatment; 3) there should be no adverse affects on the plant in size or quality (Hew and Yong, 1997).

Propagation

Asexual mericlonal tissue culture and sexual seed propagation are both widely utilized as sources of orchid plantlets. Mass rapid clonal propagation (MRCP), or the mass propagation of cuttings or stem segments, was first commercially utilized for propagation of *Cymbidium* in the early 1960s, and is now commonly used with other orchids such as the *nobile*-type *Dendrobium* (Ichihashi, 1997). Seed production is somewhat more difficult than tissue culture, as seeds are extremely small, have no endosperm and have a rudimentary seed coat (Morrison, 2000). Seeds are often grown in flasks on agar medium at 21 to 24 °C for one year, and transferred to community flats for 6 to 12 months. Mortality is often high when seedlings are transferred to community trays.

Sexually produced plantlets are often cheaper than clones, but pose challenges for growers due to the genetic variability.

Konow and Wang (2001), showed that an in vitro photosynthetic photon flux (*PPF*) of 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ produced *Phalaenopsis* seedlings with 4.0 cm roots, compared to 2.8 cm at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Increased *PPF* also increased fresh weight, which can lead to an earlier transfer time out of flasks.

Tissue culture is one of the most rapid methods of multiplying vegetative orchid plants from shoot tip, root tip, pseudobulb cuttings or pollen grains. The excised tissue is sterilized, placed in flasks containing liquid nutrient medium, sealed with aluminum foil and placed on an orbital shaker. The cultured flask is then provided with illumination of about 10 to 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 8 to 16 h a day at a constant temperature of 25 °C. Thousands or even millions of genetically identical and uniform plants can be produced from the tissue in one to two years, depending on the hybrid or species. Both sexual and asexual methods require aseptic conditions, with specific nutrients and environmental conditions (Anonymous, 1998).

Media, Nutrition and Irrigation

One of the most challenging aspects of commercial orchid production is selecting an appropriate media. The medium must not rapidly degrade, its water holding capacity must not be excessive, it must support the plant within the container and have a pH of 5.0 to 6.0 (Griesbach, 1985). Consequently, orchids do not perform well in traditional peat- and perlite-based potting media often used

for other floricultural crops. These medias do not provide adequate drainage or aeration and the roots can become waterlogged and rot.

Commercial orchid growers often use fresh douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] bark. However, fresh bark does not usually retain adequate amounts of moisture or nitrate-nitrogen and decomposes quickly (Wang, 2000). Wang and Gregg (1994) reported that a mix containing perlite, Metro Mix 250 (containing peatmoss, perlite, vermiculite, ground-charred bark, and granite sand with a balanced pH and nutrient charge), horticultural charcoal and composted pine bark can be used to grow excellent *Phalaenopsis.* As a result, growers are now using mixtures of fir bark with various other materials such as charcoal, sphagnum moss, peat, volcanic rock, rockwool, vermiculite, and perlite. An alternative to fir bark is chopped coconut coir (*Coco nucifera* L.) husks of various sizes. A risk of using chopped coconut is root injury from high salt concentrations if the coir has not been properly leached.

Studies by Wang and Gregg (1994) have illustrated the importance of providing epiphytic orchids such as *Phalaenopsis* with sufficient fertility throughout greenhouse production to promote vegetative growth and flowering. Increased fertility from 0.25 to 1.0 g·liter^{-1} of a 20-8.6-16.6 fertilizer (50 to 200 ppm N) increased flower counts from 6.7 to 8.0, the number of inflorescences from 1.5 to 2.1 and leaf production from 2.2 to 3.4. Wang (1994) recommends two hundred ppm nitrogen from a 20-20-20 fertilizer at each irrigation for most orchids, and 10-30-20 for mature plants.

Orchid roots are sensitive to high salinity, and water high in dissolved salts tends to injury roots (Wang, 1998b). An electrical conductivity (EC) of 1.3 to 1.5 $dS \cdot m^{-1}$ or less is recommended (Wang, 1994). Root injury can occur when the EC exceeds 3 $dS \cdot m^{-1}$. *Cymbidium* plants produced more spikes per square meter of growing area as E.C. was increased from 0.6 to 1.4 $dS \cdot m^{-1}$. The flower count on those spikes was unaffected (de Kreij and van der Berg, 1990). These results may be a function of increasing fertility since the change in EC was solely due to increased fertilizer concentration. Roots of *Phalaenopsis* 'TAM Butterfly' hybrid plants in a 100% fine-grade fir bark were more tolerant of high salinity (1.4 $dS \cdot m^{-1}$) when compared to those grown in a 4:1 bark:peat medium (Wang, 1998b).

Most orchids require infrequent watering compared to other floricultural crops. Growing medium as well as temperature, humidity and light are prime dictators of water need. Water stress can cause decreased growth rates and flower bud or flower abortion. Plants should be irrigated in the morning to allow leaves to dry before nightfall, since moist or wet conditions on the plant can promote incidence of diseases.

Irradiance, Temperature and Photosynthesis

Many orchids such as *Phalaenopsis* and *Miltoniopsis* require relatively low light levels for growth and flowering. Maximum recommended light levels are 300 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Cattleya*, 360 to 480 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Cymbidium,* 480 to 720 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Dendrobium,* 200 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Miltoniopsis,* 240

to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Phalaenopsis*, and 400 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Zygopetalum* (Baker and Baker, 1991, 1993a; Griesbach, 1985).

Wang (1995) reported that *Phalaenopsis* must be exposed to light above a certain threshold level to induce VI. Plants receiving 0 or 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12-h did not respond to inductive low temperatures (20 ºC day/15 ºC night) and remained vegetative if kept under low light. Those plants receiving 60 or 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12 h perceived the low temperature treatments and flowered in an average of 28 or 34 d.

Recommended temperatures for vegetative growth of *Cattleya* orchids are 16 to 18 ºC day and 16 ºC night, due to their montane origin. However, greenhouse studies have shown that *Cattleya* grown for 17 weeks in day/ night temperatures of 32/29 ºC and a light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had 4.5 times the leaf elongation than those grown at 25/20 ºC. Those under the 32/29 ºC conditions had an average of two to three lateral shoots after 24 weeks compared to only one for those in the 25/20 ºC greenhouse treatment (Krizek and Lawson, 1974). According to Rotor (1952), 9-h short days at 18 ºC stimulated vegetative growth, as plants produced two successive growths under this daylength than under long days or natural photoperiods.

Cymbidium require long days and day/night temperatures of 30/ 25 ºC for optimal growth and pseudobulb maturity. In the hot summer months, a process known as 'Yama-age' or 'the mountain technique' of shifting cultivation from production areas in the lowlands to the highlands is employed in Japan. This method ensures that cold-requiring orchids such as *Cattleya*, *Cymbidium*, and

Miltonia receive temperatures that accelerate vegetative growth during the summer (Ichihashi, 1997).

For most *Dendrobium* orchids, optimal vegetative growth is achieved at temperatures between 24 and 30 °C. *Dendrobium phalaenopsis* grown at 13 and 18 °C produced one pseudobulb per year, however those at 13 °C were short and spindly. Vegetative growth was also delayed by two months at 13 °C. Short days at 18 °C induced earlier development of vegetative buds and the new growth matured at least one month earlier than those grown under natural daylengths (Rotor, 1952). It is reported that temperatures below 10 °C may cause leaf drop in some species.

Phalaenopsis orchids remain vegetative at temperatures above 27 to 29 °C (Sakanishi et al., 1980) and can tolerate temperatures as high as 32 to 35 °C (Baker and Baker, 1991). Baker and Baker (1991) suggest that 17 to 18 °C is a minimum growing temperature and that 21 to 30 °C is ideal. The base temperature for *Phalaenopsis* is similar for all growth stages and ranges from 10.8 to 11.2 °C (Robinson, 2002). The thermal time to flower is similar when a model relating time from spiking to flower is used (769 degree-days; T_b=10.8) or when two models predicting 1) days to visible bud and 2) visible bud to flower (787 degree-days, T_b=11.2 and 11.0 °C) are used.

Approximately 36% of all orchid species photosynthesize via the crassulacean acid metabolism (CAM) pathway (Hietz et al., 1999). Most thin-leaved *Cymbidium* and *Dendrobium nobile* photosynthesize via the Calvin-Benson cycle (C_3 pathway). Due to the epiphytic nature of *Phalaenopsis*, the

efficient CAM pathway can often be affected by environmental growing conditions (Goh et al., 1983). Carbon dioxide (CO_2) uptake is maximized when day/night temperatures are kept at a constant 20 °C in *Phalaenopsis*. However, higher day and lower night temperatures of 25/15 °C are more favorable to CO_2 assimilation by CAM photosynthesis. Day and night CO_2 uptake increases as light intensity increases to a saturation point of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Ota et al., 1991). According to Lootens and Heursel (1998), at 20 °C day/15 °C night, CO_2 uptake increases as light increases to a *PPF* saturation point of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in *Phalaenopsis* cultivars '70' and 'L'. Compared with other plants, this is a relatively low saturation point (Ota et al., 1991).

The green aerial roots of *Phalaenopsis, Cattleya gigas, Epidendrum xanthium,* and *Vanda suavis* also photosynthesize via CAM. This can pose challenges for commercial growers. When grown in opaque pots, the roots of these plants grow out of their pot in search of light. Many growers avoid this problem by growing their plants in clear or translucent pots, which allow the roots to photosynthesize within the pot.

Flower Induction

With the exception of Vanilla, orchids are grown for the aesthetic beauty of their flowers and foliage. However, research on flower initiation, induction, development and physiology have only recently been initiated. The first known studies with orchid flower induction were with *Dendrobium crumenatum* at the Bogor Botanical Gardens in 1887 to 1898 by Treub, Massart, and Went.

Subsequent studies on other orchid species were performed from 1950 to 1970 in the United States by Curtis, Rotor, and Vacin; in West Africa by Sanford; in Venezuela by Dunsterville and Dunsterville; in the Philippines by Quisumbing; in Poland by Żotkiewicz and in Singapore by Goh (Goh et al., 1982). Research was not conducted to determine the flowering requirements of *Phalaenopsis* until the 1950s, even though they were first grown commercially in Europe in the early 19th century during the "orchid craze." One of the first studies was performed by De Vries (1950), in which he determined that if mature plants were exposed to night temperatures below 21 °C for two to three weeks, they would eventually flower.

Similar to other flowering plants, a juvenile phase exists in orchids and they must reach a certain stage of growth before attaining the capacity to flower. Thus, sexual reproduction is delayed until plants reach a size sufficient to maintain the energetic demands of flowering and seed production. This period of juvenility varies among species and cultivars. Four to seven years are required for many orchids to flower from seed (Goh and Arditti, 1985), but many commercially important hybrids flower after 12 to 36 months of growth (Hew and Yong, 1997). After the juvenile period has passed, environmental or other factors can be employed to induce flowering. If conditions are unfavorable, initiated inflorescence buds may not all develop to maturity and bloom. However, once buds have developed to a certain size (e.g., approximately 3 mm in *Aranda* hybrids), they usually continue to develop to anthesis (Goh and Arditti, 1985).

Effects of Vernalization and Photoperiod

The majority of published studies indicate that *Cattleya* species and hybrids require short days and low temperatures to flower. In *C. warscewiczii, C. gaskelliana,* and C. *mossiae,* flower induction occurs under continuous 9-h short days at 13 °C, while flowering is inhibited under 16-h long days at 13 °C (Rotor, 1952; Rotor, 1959). The only difference being that *C. warscewiczii* apparently requires short days, low temperature conditions while the pseudobulb is developing. Flowering of *C. warscewiczii* under short days at 18 °C was reduced and delayed by two to three months compared with plants under short days at 13 °C.

Goh and Arditti (1985) developed a schedule for flowering *C. gaskelliana* for Christmas or New Years in the northeastern United States. They suggest growing plants warm (≥ 16 °C) during the previous November, December and January when growth normally begins. Plants should begin receiving short days from February through September, and in the autumn, night temperatures should be reduced to 13 °C. Flowering occurs approximately three to four months after bud initiation. In *C. labiata* and *C. schilleriana*, 16-h long days at 18 °C prevents flowering. *Cattleya labiata* and *C. schilleriana* will flower under short days regardless of temperature and under long days if the temperature is maintained below 16 °C (Rotor, 1952; Rotor, 1959). *Cattleya bowringiana* grown under 8-h photoperiods flowered in 273 d, and flowering did not occur under 16-h photoperiods (Goh and Arditti, 1985).

Cymbidium are induced to flower by warm days and cold night temperatures (i.e., with large diurnal fluctuations). No studies have found photoperiod to have an effect on flower induction (Goh et al., 1982, Goh and Arditti, 1985). Many temperate *Cymbidium* orchids derived from the "Asiatic *Cymbidium* Belt" are reported to require large diurnal temperature fluctuations of 10 to 14 °C to initiate flowers (Powell et al , 1988). According to Dole and Wilkins (1999), temperatures of 5 to 8 °C should be maintained for optimal flower induction, then subsequently raised to 25 °C when flower spikes become visible. *Cymbidium* growers in California provide relatively high light intensities (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and night temperatures of 10 to 13 °C for flower bud initiation (Goh and Arditti, 1985). In Japan, the temperatures commonly utilized for flower induction are between 10 to 16 °C and the cumulative temperature required is 34,000 °C hours (calculated by multiplying the hours under 10 to 16 °C by the temperature) (Ichihashi, 1997). Powell et al. (1988) reported that *C.* Astronaut 'Rajah' exposed to day/night temperatures of 26/12 °C under 14-h photoperiods for up to six months resulted in 5.9 inflorescences per plant. At 20/12 °C and 26/18 °C, only 0.8 and 1.7 inflorescences per plant developed, respectively. Went (1957) found that *Cymbidium* did not flower when grown for one to two months at 26/14 °C or 23/14 °C; inflorescences developed at 26/7 °C and 26/10 °C, and the best floral production occurred at 20/10 °C and 20/14 °C.

In vitro flowering of *Cymbidium niveo-marginatum* has been achieved with a combined treatment of cytokinin (6-benzylaminopurine), restricted nitrogen supply with phosphorus enrichment, and root excision (pruning). In vivo, *C.*

niveo-marginatum requires four to seven years before it can flower (Kostenyuk, et al., 1999).

The optimum temperature for flower induction differs among *Dendrobium* cultivars. In *Dendrobium nobile,* dormant buds on upper nodes of mature pseudobulbs flower in response to low temperatures. Buds on immature pseudobulbs stay dormant and do not respond to low temperatures. Flower buds grow up to 3 mm long with five or six scales after their induction (Ichihashi, 1997). Temperatures for induction vary among cultivars. In *Dendrobium nobile,* plants exposed to 13 °C produced flowers regardless of photoperiod, whereas plants held at 18 °C remained vegetative (Rotor 1952; Goh and Arditti, 1985) Under long days flowering is hastened by one to four weeks (Rotor, 1952). In Japan, plants are moved to the mountains after the summer to provide cool temperatures (< 15 °C) for early flower induction (Ichihashi, 1997). Optimal flower induction in *D.* Snowflake 'Red Star' is achieved by providing 25/10 °C (day/night temperature) for 40 to 60 d. Lower day temperatures can cause leaf yellowing, defoliation and reduced growth rates and higher temperatures can delay flower bud development (Ichihashi, 1997). In *D. phalaenopsis,* short days at 18 °C hastened flower bud development and flowering by ≈6 weeks compared to plants under long or natural daylengths at 18 °C (Rotor, 1952). At 13 °C, this same tendency was observed, but flower bud development was very slow and it was recommended that plants be placed at 18 °C for normal opening of flowers. Long days, at either 13 or 18 °C retarded flower bud initiation and development and the delay was accentuated by lower temperatures (Rotor, 1952).

According to Goh and Arditti (1985), photoperiodic and low temperature treatments can affect the levels of endogenous growth regulators. Low temperatures and short day induction of flowering in the sympodial orchids could result from changes in the endogenous levels of growth regulators. Sakai et al. (2000) reported on the that the injection of 100 mM 6-benzyladenine (BA) into *Dendrobium* Jaquelyn Thomas 'Uniwai Princess' (cut flower) significantly increased the number of flowering inflorescences on first year pseudobulbs compared to untreated plants, 8.9 vs. 0.5, respectively. The injection of 100 mM or 10 mM BA into second year pseudobulbs significantly increased floral spray production (6.3 and 4.0, respectively) compared with untreated plants (0.2). The high concentrations of 100 mM BA reduced inflorescence length and caused the development of abnormally formed flowers. The addition of 100 mM gibberellic acid (GA) to the100 mM BA injection solution significantly increased - inflorescence length and reduced the percentage of abnormally formed flowers.

Most *Phalaenopsis* species and hybrids require a period of exposure to relatively cool temperatures (< 28 °C) to trigger the elongation of the inflorescence (Lee and Lin, 1984, 1987; Sakanishi et al., 1980; Yoneda et al., 1992; Wang, 1995). This is often referred to as spiking (Wang, 1998). Flower bud initiation occurs after the inflorescence has reached a size of ≈5 cm when environmental conditions are favorable. Lin and Lee (1984) showed that uniform spiking can be achieved when plants are grown at day/night temperatures of 25/20 °C or 20/15 °C for four to five weeks. Although temperatures below 25 °C can initiate VI, they do not necessarily initiate flowering immediately. Once

spiked, plants grown at 20 °C can flower almost a full month after those grown at 25 °C (Sakanishi et al., 1980). Temperatures above 25 °C can also affect flowering. When placed at 28 °C, a spike can form a vegetative air plantlet known as a keiki instead of flower buds, or buds may abort. Abortion of buds can be avoided if the air temperature around a plant is kept below 28 °C until the spike is approximately 5 cm in height. At this point, the spike will develop at temperatures above the base temperature (11 °C) but not > 25 °C, because the floral primordia have initiated and differentiated (Sakanishi et al., 1980). Temperature has little or no effect on spike height or flower size (Sakanishi et al., 1980).

Photoperiod does not influence flower induction of *Phalaenopsis* (Baker and Baker, 1991; Sakanishi et al., 1980). However, a few studies have reported that short days enhance spiking and long days promote vegetative growth or keikis (DeVries, 1950; Rotor, 1952; Griesbach, 1985). However, the short day enhancement is thought to be a result of the extension of cool night temperatures and not the day length itself (Sakanishi et al., 1980).

Robinson (2002) conducted studies to quantify the effects of temperature (14, 17, 20, 23, 26 or 29 °C) on time from spike emergence to flowering and on plant quality. The most important factor in scheduling *Phalaenopsis* was proper temperature control; daylength and light intensity had minimal effects. The optimum temperature for floral promotion once spiking had occurred was 26 °C. As temperature was increased from 20 to 23 °C, days to flower decreased from 50 to 35 d. The rate of flower, node and bud development for all the cultivars of

Phalaenopsis used in the investigation increased linearly as temperature increased from 14 to 26 °C. Robinson (2002) also found that keiki formation occurred at temperatures above 28 °C, in agreement with Sakanishi et al. (1980).

Flower Longevity

The life span of orchid flowers varies considerably among genera. *Phalaenopsis* flowers may last for months, while other orchid flowers are ephemeral, lasting only one day or less. The full bloom period of an inflorescence depends on the number of flowers and the longevity of individual flowers. Factors such as air pollution, physical damage to the plant or flower, slight disturbances to the pollinia or anther caps, pollination and temperature can considerably accelerate the senescence of flowers (Goh, and Arditti, 1985).

Orchid flowers are extremely sensitive to very low levels of ethylene gas (C_2H_4). Ethylene at 0.05 ppm can cause flowers and buds to drop within a few days after exposure. Studies by Wang (2002) have shown that blooming *Phalaenopsis* plants fumigated with 0.1 to 1.0 µL/L of the organic gas 1-methylcyclopropene (1-MCP) for 6 h, exposed to C_2H_4 for 24 h and evaluated under continuous 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* at 23 to 25 °C were equally protected compared to the non-MCP treated plants. Flowers on untreated flowers wilted within 2 d of exposure to C_2H_4 and those treated with 1-MCP lasted 27 to 34 d. In another experiment, flowers treated with 0.4 µL/L 1-MCP and then exposed to C_2H_4 after 7 or 14 d wilted within 4.5 d.

Conclusion

The physiology, biochemistry and genetics of flowering in orchids is an extraordinarily complex process influenced by both internal (juvenility) and external factors (daylength and temperature). In *Arabidopsis*, the genetic repression of flowering via *FLC* and *FRI* genes ensures that sexual reproduction occurs under favorable conditions. The range and complexity of flowering allows great diversity in plant responses to their given environments. These interactions pose challenges for today's orchid growers as consumers increasingly demand a diversity of orchids from which to choose. As more controlled research is performed, additional knowledge on the flowering requirements will become known, which will facilitate production of additional genera at desired sales dates.

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SECTION II

FLOWERING AND VEGETATIVE GROWTH RESPONSES OF BRASSIA, DEGARMOARA, MILTASSIA, ODONTOCIDIUM AND ZYGOPETALUM ORCHIDS TO PHOTOPERIOD

Flowering and Vegetative Growth Responses of *Brassia*, *Degarmoara*, *Miltassia*, *Odontocidium* and *Zygopetalum* Orchids to Photoperiod

Roberto G. Lopez[1] and Erik S. Runkle[2]

Department of Horticulture, Michigan State University, East Lansing, MI 48824

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[1]Graduate Student.

[2]Assistant Professor and Extension Specialist, to whom reprint requests should be addressed (E-mail: runkleer@msu.edu).

Introduction

In the early 1990's, the production of potted blooming orchids began to rise significantly in the United States. The United States Department of Agriculture (USDA) National Agricultural Statistical Service considered orchids to be a minor crop and therefore did not collect production information until 1997 (USDA, 1998). Since then, many growers began expanding their sales beyond the traditional orchid hobbyists and now target mass marketers such as Frank's, Lowe's, Home Depot, Target, Kmart and Wal-Mart. The largest demand for potted blooming orchids at these retail outlets is for traditional holidays such as Valentine's Day, Easter and Mother's Day. However, year-round production and sales are expanding beyond those of traditional potted plants such as chrysanthemums and African violets (USDA, 2003). As a result, orchids have become the second most valuable flowering potted crop in the United States in wholesale value. In the past six years, production value has increased 147%, and in 2002 the estimated wholesale value was $105.6 million. According to the American Orchid Society, over 75% of all orchids sold in the U.S. are *Phalaenopsis* (Griesbach, 2002).

The production of orchid plants has also increased in other countries, particularly in Japan and the Netherlands. The value of potted orchids produced in Japan experienced an increase of 1125% in a recent 26 year span: production in 1965 was valued at $44,000 and ≈ $49 million in 1991 (Ichihashi, 1997). The overall market value of imports and domestic production in Japan was estimated to be $261 million in 1993 (Hew and Yong, 1997). In 1993, *Cymbidium*,

Phalaenopsis and *nobile*-type *Dendrobium* were the most widely grown orchid species in Japan. In the Netherlands, *Phalaenopsis* is currently the most valuable potted crop. From 1983 to 1994, the number of plants sold through the Aalsmeer flower auction increased from 50,000 to 3,150,000 plants, and in 1994 was valued at $62 million (Barendse, 2002; Hew and Yong, 1997). Large-scale potted production has also taken place in Germany and Taiwan, among others (Griesbach, 2000). The world demand for potted orchids in 1995 was 1.22 billion units of plant stock and was estimated to increase four percent from 1995 to 2000 (Hew and Yong, 1997).

Britt (2000) identified five reasons for the dramatic increase in worldwide potted orchid production: 1) increasing consumer popularity; 2) improvements in propagation; 3) grower acceptance of orchids as profitable crops; 4) improved plant vigor, especially of hybrids; and 5) segmentation of the supply chain. However, of the 20,000 to 25,000 species that comprise the *Orchidaceae* family, a small number (e.g., *Cattleya, Cymbidium*, *Dendrobium*, *Phalaenopsis*, and *Oncidium*) are marketed globally as cut flowers for corsages and floral arrangements, as potted flowering plants, and as bedding or aerial plants in tropical regions. The commercial potential of the vast majority of other orchids has not yet been explored partly due to insufficient knowledge of their growth and flowering responses to controlled environments.

For controlled flower production to be commercially viable, the following conditions must be satisfied: 1) the methods must be simple, economical and give reproducible results for growers; 2) the quantity and quality of flowers must

not be adversely affected by the treatment; and 3) there should be no adverse affects on the plant in size or quality (Hew and Yong, 1997

Photoperiodism refers to the response of plants to the length of day. The word is derived from the Greek roots for "light" and "duration of time." Hillman (1969) defined photoperiodism as a response to the timing of light and darkness. Plant responses influenced by daylength include bud dormancy, formation of storage organs, asexual reproduction, leaf development, stem elongation, germination, and flower initiation and development (Thomas and Vince-Prue, 1984). Some factors that may be influenced by daylength, such as flowering percentage and flower number, are important horticulturally, yet botanically they are often not considered photoperiodic responses.

The classification of plants according to their photoperiodic responses is usually made on the basis of flowering. With respect to flower initiation, most plant responses to daylength can be classified into three categories. Day-neutral plants (DNP) flower regardless of the photoperiod to which they are exposed. In short-day plants (SDP), flowering occurs only under, or is accelerated by, daylengths shorter than a particular duration known as the "critical daylength". Flowering in long-day plants (LDP) occurs only under, or is accelerated by, daylengths exceeding a particular duration. Less commonly, some species have a dual daylength requirement, i.e., a period of short days followed by a period of long days or vice versa (Thomas and Vince-Prue, 1997).

Some tropical plants of equatorial origin are believed to be more sensitive to small differences in daylength than those from temperate regions (Sanford,

1974). An example of a tropical plant influenced by photoperiod is the epiphytic cacti *Hatiora* spp., which is classified botanically as a short-day long-day plant (SLDP) for flowering at 15 to 20 °C (Boyle, 1991). In its native habitat of Panará and Santa Catarina, Brazil (~26 °S lat.) the shortest civil daylength (the duration of time when the sun is 6° below the horizon before sunrise to 6° below the horizon after sunset) is 11 h 28 min and the critical photoperiod for photoinduction in *Hatiora* is between 11 and 12 h (Boyle, 1991). Other examples of epiphytes that respond to photoperiod include *Schlumbergera truncata* and *Cattleya* spp., which are SD tropical epiphytes native to the Organ Mountains north of Rio Janeiro, Brazil (~22 °S lat.) where the photoperiod ranges from 11 to 13.5 h (Morrison, 2000). Commercially, *Schlumbergera* is placed under 8 to 11 h short-day photoperiods and night temperatures of 13 to 15 °C for flower initiation (Dole and Wilkins, 1999). In *Cattleya warscewiczii, C. gaskelliana*, and C. *mossiae,* flower induction occurred under continuous 9-h short days at 13 °C, while flowering was inhibited under 16-h long days at 13 °C (Rotor, 1952; Rotor 1959).

Since photoperiod influences flowering of some tropical epiphytes, the objective of this experiment was to determine how photoperiod influences growth and flowering of *Brassia* Rex 'Sakata', *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch,' *Odontocidium* Tiger Crow 'Golden Girl' and *Zygopetalum* Redvale 'Fire Kiss'. The orchid hybrids were chosen based on greenhouse grower interests and suitability as potted flowering plants based on fragrance, size and floral characteristics.

Materials and Methods

Plant material. Plants were grown by a commercial greenhouse (Calif.) at 16 to 26 °C under natural photoperiods (37 °N lat.) with a maximum photosynthetic photon flux (*PPF*) of ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Propagation dates and container sizes are provided in Table 1. Five hundred *Brassia* Rex 'Sakata', *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch', *Odontocidium* Tiger Crow 'Golden Girl', and *Zygopetalum* Redvale 'Fire Kiss' planted in a bark and perlite-based media (10-cm pots) were received in East Lansing, Mich. on 6 May 2001 (Year 1) and 22 July 2002 (Year 2). *Zygopetalum* received in Year 2 were transplanted into 13-cm pots. All plants were maintained at ≈23 °C in a glass-glazed greenhouse until experiments began. The photoperiod was a constant 16 h, consisting of natural daylengths (42 °N lat.) with day-extension lighting from high-pressure sodium (HPS) lamps, which delivered a supplemental *PPF* of ≈50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height [as measured with a with a light quantum sensor (Apogee Instruments, Inc., Logan, Utah)].

Photoperiod treatments. The experiment was replicated in time, beginning on 31 May 2001 (Year 1) and 24 July 2002 (Year 2). Experimental treatments were identical between years unless otherwise noted. Ten plants of each species were randomly assigned to greenhouse benches each year and were placed under each of seven photoperiods: 10, 12, 13, 14, 16 or 24 h of continuous light or 9 h with a 4-h (2200 to 0200 HR) night interruption (NI).

Continuous photoperiods consisted of 9-h days completed by day extension lighting; lamps were turned on at 1700 HR and turned off after each photoperiod was completed. Day-extension and NI lighting (≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) was provided by incandescent lamps. Opaque black cloth was pulled at 1700 HR and opened at 0800 HR everyday on all benches so plants received a similar daily light integral (Table 2). From 0800 to 1700 HR, HPS lamps provided a supplemental *PPF* of ≈70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level when the ambient greenhouse *PPF* was <120 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Greenhouse temperature and irradiance control. Plants were grown in a glass greenhouse with a constant temperature setpoint of 20 °C. Temperatures on each bench were measured by a thermocouple in an aspirated chamber every 10 s, and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, Utah). To help provide uniform night temperatures of 20 °C, a data logger controlled a 1500-W electric heater, which provided supplemental heat under each bench as needed. Average daily air temperature from the beginning of forcing until the end of treatments under every photoperiod each year were calculated (Table 2). Light transmission through the greenhouses was reduced using permanent woven shade curtains that reduced light by ≈55% (OLS 50; Ludvig Svensson, Charlotte, N.C.) and by applying whitewash (up to 50%) to the glass as needed so that the maximum *PPF* was ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Plant culture. Plants in Year 1 were irrigated as necessary with well water (containing 95, 34, and 29 $mg \cdot L^{-1}$ Ca, Mg, and S, respectively) supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 125 K,

15 Ca, 1.0 Fe, 0.1 B, and Mo and 0.5 Mn, Zn, and Cu. (MSU Special, Greencare Fertilizers, Chicago, IL). Water was acidified with H_2SO_4 to a titratable alkalinity of 140 $mg \cdot L^{-1}$ $CaCO_3$. In Year 2 plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo.

Data collection and analysis. Initial immature and mature pseudobulbs, leaves per pseudobulb, and leaf length were recorded for *Odontocidium* and *Zygopetalum* when forcing began (Table 3). The date at which the first inflorescence was visible (visible inflorescence, or VI) without dissection and the date the first flower opened were recorded for each plant. At flowering, the number of VI, flower buds, and nodes on immature or mature pseudobulbs below the inflorescence were counted and inflorescence length was measured. Plants that did not flower after 25 or 36 weeks were considered non-flowering. The percentage of plants that initiated flowers, the percentage of plants whose flowers developed normally (did not abort), days to VI, days from VI to flower, days to flower, leaf length, immature and mature pseudobulb number, and leaf-count increase from start of forcing were calculated. The few plants that died during the experiment were discarded and not included in the results.

A completely randomized design was used that included 10 observations for each photoperiod. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED). Data were pooled for all measured characteristics, except when there was a significant year x photoperiod

interaction, in which data were analyzed separately for each year. Data for *Degarmoara* and *Miltassia* was pooled for Year 1 and 2, and for flower bud count of *Zygopetalum*. Experiments for *Miltassia* and *Odontocidium* were not replicated in time.

Results

Brassia. Flowering was not influenced by any photoperiod at 20 °C and plants remained vegetative after 36 weeks under all photoperiods (Table 4). Leaf count increase after 36 weeks was not significantly influenced by daylength (Fig. 1A). As daylength increased from 10 to 14 h, the leaf length of pseudobulbs increased (linearly and quadratically) from 26.2 to 31.7 cm (Fig. 1B). The final number of immature and mature pseudobulbs produced after 36 weeks were not influenced by any photoperiod (Table 4).

Degarmoara. Twenty five to thirty five percent of plants initiated flowers under 12- to 16-h photoperiods and under a 4-h NI during the 25 week experiment (Table 5). Only ten and five percent of plants flowered under 10 and 24 h photoperiods, respectively, so data were not included in further analysis. Days to VI ranged from 60 to 83 d under 12- to 16-h photoperiods. Days from VI to flower were similar, ranging from 50 to 60 d under all photoperiods. The average number and length of inflorescences per pseudobulb were not significantly influenced by any daylength. In addition, flower number was highly variable under all photoperiods, and was not significantly different. Plants that produced terminal inflorescences produced fewer flowers and shorter

inflorescences compared to plants that developed lateral inflorescences (significantly different at $P \leq 0.001$; data not presented).

Miltassia. Flower initiation occurred in forty to sixty percent of plants under all photoperiods (Table 6). Many of the flower buds under 24-h of light did not develop and aborted while flowers in other photoperiods opened normally. Days to VI across all photoperiods were similar, ranging from 75 to 92 d. Days from VI to flower and days from start of forcing to flower were statistically similar under all photoperiods, ranging from 38 to 57 d and 118 to 144 d, respectively. The average number and length of inflorescences per pseudobulb was not significantly influenced by photoperiod. Flower number was highly variable under all photoperiods, and no significant differences existed. Plants that produced terminal inflorescences produced fewer flowers and shorter inflorescences compared to plants that developed lateral inflorescences (significantly different at $P \leq 0.001$; data not presented).

Odontocidium. Flower initiation was greatest under 13 and 16 h photoperiods, 100% and 80%, respectively, but there were no apparent trends in flowering with respect to flowering (Table 7). Days to VI were not significantly influenced by photoperiod (Fig. 2A). Mean days from VI to flower were similar among photoperiods, ranging from 57 to 63 d. Days from the start of forcing to flower were also similar, ranging from 160 to 190 d. A statistically quadratic relationship between photoperiod and inflorescence count was observed, with a maximum at 13 to 14 h (Fig. 2B). Inflorescence length per pseudobulb was not significantly influenced by daylength (Fig. 2C). Flower bud count decreased

linearly from 6.8 to 2.3 as photoperiod increased from 10 to 24 h (Fig. 2D). Plants that produced terminal inflorescences produced fewer flowers and shorter inflorescences compared to plants that developed lateral inflorescences (significantly different at $P \leq 0.001$; data not presented). Leaf count was significantly greater under NI than under any other photoperiod (Fig. 3A). Leaf length during the 36 weeks was not influenced by daylength (Fig. 3B). Daylength had no effect on the number of new immature or mature pseudobulbs formed (Fig. 3C and D).

Zygopetalum. Year 1. Flower initiation occurred in fifty to ninety percent of plants under all photoperiods (Table 8). Sixty to eighty percent of plants flowered when grown under every photoperiod except continual (24 h) light. Flowering occurred > 150 d after plants were placed under the various photoperiods. Plants reached VI earlier when grown under photoperiods ≤ 14 hours than under ≥ 16 h (Fig. 4A). Days from visible inflorescence to flower decreased linearly from 33 to 24 d as photoperiod increased from 10 to 24 h (Fig 4B). The average number of inflorescences produced per immature pseudobulb was not influenced by photoperiod (Fig. 4C). Mean inflorescence length decreased linearly as photoperiod increased from 10 to 24 (Fig. 4D). Mean flower bud count decreased linearly from 3.9 to 2.3 as photoperiod increased from 10 to 24 h (Fig. 4E).

As photoperiod increased from 10 to 24 h, the final number of leaves per pseudobulb decreased from 12.2 to 7.0 (Table 8). Leaf count during the 36 weeks decreased linearly from 8.0 to 2.9 as photoperiod increased from 10 to 24

h (Fig. 5A). Leaf length was not influenced by daylength (Fig. 5B). Photoperiod did not influence the development of immature pseudobulbs. Plants under continual light, however, developed more mature pseudobulbs per pot compared to those under shorter photoperiods (Table 8).

Year 2. Eighty to one hundred percent of plants flowered regardless of photoperiod; flowers only aborted under continuous light (Table 8). Average flowering occurred ≥ 97 d after plants were placed under the various photoperiods. Days to VI increased linearly or quadratically from 73 to 102 d with decreasing daylength from 24 to 10 h (Fig. 4A). Time from VI to flower and inflorescence count were not influenced by photoperiod. Average inflorescence length deceased linearly from 33 to 24 cm as photoperiod increased from 10 to 24 h (Fig. 4D).

Leaf count under all photoperiods was similar, ranging from 6.3 to 7.3, respectively (Fig. 5A). As photoperiod increased from 12 to 24 h, leaf length during the 36 weeks decreased linearly from 47 to 33 cm (Fig. 5B). The average number of immature pseudobulbs were similar among photoperiods (Fig 5C). The average number of new mature pseudobulbs formed was significantly influenced by photoperiod, increasing linearly from 1.0 to 2.7 as photoperiod increased from 10 to 24 h (Fig. 5D).

Discussion

Brassia. After thirty-six weeks of forcing, flowering of *Brassia* Rex 'Sakata' was not influenced by photoperiods ranging from 10 to 24 h of continuous light or

9 h with a 4-h night interruption (NI) at ≈20 °C. These photoperiods were chosen since they have been successfully utilized to elucidate the flowering responses of over two hundred herbaceous perennials and potted flowering plants at Michigan State University (Heins et al., 1997, Runkle, et al., 2001). Additionally, most orchids in their native environments are exposed to photoperiods > 10 and < 16, depending on their latitude. Juvenility cannot be readily attributed to the lack of flowering as plants had been out of tissue culture for over three years and most had two or more mature pseudobulbs at the beginning of forcing.

The only information currently available about the native distribution of *Brassia* is limited; it is an epiphyte found in the warm forests of Costa Rica and islands of the West Indies at low elevations (200 m) and in the mountains of Brazil and Peru (2000 to 3000 m) (Rentoul, 1982). *Brassia* Rex 'Sakata' is a heat-tolerant hybrid between *Brassia verrucosa* x *Brassia gireoudiana*. Their large geographic distribution does not readily provide clues for what environmental parameters may influence flowering.

Initial leaf length of immature pseudobulbs was similar among photoperiods. At the end of forcing, leaf length increase was significantly influenced by photoperiod. This information can be beneficial to commercial orchid growers because they can create and utilize different photoperiods to control the size of their plants. For example, by placing *Brassia* under a 4-h NI, growers can produce taller and fuller plants. Alternatively, if a grower prefers shorter and more compact plants, a short day (e.g. 10 h) would be ideal.

Most sympodial orchids such as *Brassia* that are grown for potted plant production are from tissue culture. However, they are often not uniform and are highly variable in their growth, and thus, uniform, complete and rapid flowering may be difficult to achieve. Further investigations are needed to reveal the flowering response of *Brassia* Rex 'Sakata.' They produce seven to fifteen large, exotic, spider-like, showy and fragrant flowers. The foliage however is not as attractive as the flowers; it often crinkles with age and is affected by water stress. Behe et al. (2004) indicated that consumers rated *Brassia* Rex 'Sakata' as their least favorite out of a sample of four orchids.

Degarmoara, Miltassia and *Odontocidium.* Photoperiod did not have a significant influence on flowering of *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch', or *Odontocidium* Tiger Crow 'Golden Girl'. Flower initiation percentage was nonuniform and incomplete across all photoperiods in both years. For *Degarmoara* the highest and lowest flower initiation, 35% and 5% occurred under 12- and 24-h photoperiods, respectively. Similar results were observed for *Miltassia* as the highest and lowest flower initiation were 55% under 12 and 14 h and 10% under 24-h photoperiods. *Odontocidium* showed the highest flower initiation, 100% under a 13 h daylength. The few plants that initiated inflorescences under continuous light (24 h) usually aborted in all three species.

Degarmoara Winter Wonderland 'White Fairy' is a hybrid between the hybrids *Miltassia* Cartagena and *Odontoglossum* Gledhow, which makes it difficult to establish what conditions induce flowering of its lineage. Further

investigation using diurnal temperature fluctuations and light quantity may elucidate flower induction.

Degarmoara has potential as a mass-produced potted flowering plant since it has large 7 to 10 cm, white, star-shaped flowers with small purple spots and yellow streaks towards the center of the petals and sepals. In an online survey, participants chose *Degarmoara* over *Brassia* as the third most likely orchid they would purchase (Behe et al., 2004).

Miltassia is a hybrid between the cool-growing *Miltonia spectabilis* and the intermediate-growing *Brassia verrucosa*, resulting in a cultivar that requires warm temperatures for optimal growth. This species may not be suitable for production in Northern climates as the potential for chilling injury is high (see Section III). They exhibit symptoms of chilling injury at temperatures at or below 17 ºC within two weeks. At temperatures of 11 ºC, *Miltassia* eventually die, lose all their leaves and pseudobulbs within eight weeks, however a few will send up new immature pseudobulbs.

Of the three previously mentioned orchids, *Odontocidium* Tiger Crow 'Golden Girl' has the most potential as a potted flowering plant. Some of its attributes include its large and multi branched inflorescences. The large and bright yellow flowers are speckled with maroon spots and can last up to a month in a home or office. Further investigations are needed into the flowering mechanisms of this species.

Zygopetalum. In year 1, *Zygopetalum* Redvale 'Fire Kiss' showed a quantitative short-day photoperiodic flowering response. Plants under

photoperiods ≤ 13 h reached VI nearly 21 d faster than those under NI or ≥ 14 daylengths. Time from VI to flower at ≈20 °C was relatively rapid (≈32 d) compared to other orchids such as *Phalaenopis,* where it takes ≈80 d (Robinson, 2002). However, regardless of photoperiod, time to VI was long (≥ 117 d) and relatively nonuniform. Inflorescence count was not influenced by photoperiod, but inflorescence length and flower bud count were. Under continuous light and a 4-h NI, plants produced shorter inflorescences with fewer flowers and as a result the number of days from VI to flower was ≈25.

Plants were uniform when they were placed under each photoperiod as initial leaf counts and lengths were similar in both years. Compared with plants grown under long days, those under short photoperiods (14 hours or less) developed fewer pseudobulbs per plant but each pseudobulb developed more leaves. Similar results have been observed in *Cymbidium,* as it requires long days and day/night temperatures of 30/ 25°C for optimal growth and pseudobulb maturity (Ichihashi, 1997). *Cattleya* and *Dendrobium phalaenopsis* respond differently, according to Rotor (1952). Short days (9-h) at 18 °C stimulated vegetative growth as plants produced 2 successive growths (pseudobulbs) under this daylength than under long days for *Cattleya.* In *Dendrobium phalaenopsis,* short days at 18 °C induced earlier development of vegetative buds and the new growth matured at least one month earlier than plants grown under natural daylengths (Rotor, 1952).

In year 2, *Zygopetalum* showed a quantitative long-day photoperiodic flowering response. Plants under photoperiods ≥ 16 h or a 4-h NI, reached VI

nearly ≈32 d faster than those under ≤ 14-h daylengths. Days from the start of forcing to flower occurred ≈48 d earlier under all photoperiods in year two compared to year one. We believe flower initiation had occurred before the plants were shipped to us. Mean days from VI to flower across photoperiods occurred in ≈36 d in year two, which was comparable to year one where it occurred in ≈30 d. Inflorescence count was influenced in year two, but not in year one.

Initial plant material (immature pseudobulb count, leaf count and length) was uniform, but pseudobulb count was significantly different among photoperiods. Plants received in year two were also nearly twice the size of those received in year one. Interestingly, leaf length was influenced by daylength in Year 2 as plants under continuous light or a 4-h NI had shorter leaves.

Zygopetalum is a South American genus of orchids that have fragrant flowers with lime-green and dark maroon flower petals and a white and magenta labellum. Their exotic, attractive flowers and naturally compact habit (≈25- to 40-cm tall) make *Zygopetalum* one of the most appealing orchids that we studied.

In all the orchids we investigated, flower abortion was high, and inflorescent and flower counts were reduced under a 24-h daylength. We speculated that orchids that photosynthesize via CAM are adversely affected by continuous light, as their stomata may remain closed for extended periods.

Brassia, *Degarmoara*, *Miltassia*, and *Odontocidium* all members of the *Odontoglossum* alliance, did not flower in response to photoperiod. In contrast, temperature and photoperiod influence flowering of *Phalaenopsis*, *Cattleya*,

Cymbidium and *Dendrobium.* For example, flower induction in *Phalaenopsis* follows exposure to temperatures below 25 ºC and may be promoted by short days (Sakanishi et al., 1980; Wang and Lee, 1994). Exposure to short photoperiods, sometimes in combination with low temperatures, induces flowering in a number of *Cattleya* species (Rotor, 1952). Diurnal temperature fluctuations that include cool nights induce flowering in the temperate *Cymbidium* Astronaut 'Rajah' (Powell et al., 1988) and in other hybrids of the genus (Ichihashi, 1997). Mature pseudobulbs of *Dendrobium* produce flower buds in response to low temperature, with effective temperatures varying between 10 and 20 ºC depending on cultivar (Ichihashi,1997). However to our knowledge, few scientific studies on the effects of photoperiod on growth and flowering have been performed on orchids until now. Consequently, further investigation is imperative for the establishment of production protocols for new species.

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Table 1. Propagation dates and container sizes for orchid hybrids.

Species	Year	*in vitro*	Out of flask	Transplant to 7.5-cm pot	Transplant to 10-cm pot
Brassia Rex 'Sakata'	1	June 1997	June 1998	June 1999	June 2000
Degarmoara Winter Wonderland 'White Fairy'	1	May 1997	May 1998	May 1999	May 2000
	2	-[z]	-	June 2000	June 2001
Miltassia Charles M. Fitch 'Dark Monarch'	1	June 1997	June 1998	June 1999	June 2000
	2	June 1997	June 1998	June 1999	June 2000
Odontocidium Tiger Crow 'Golden Girl'	1	-	-	June 2000	April 2001
Zygopetalum Redvale 'Fire Kiss'	1	June 1999	June 2000	--[y]	April 2001
	2	-	May 2001	--	May 2002

[z]Information not available.
[y]Plants were directly transplanted to 10-cm pots.

Table 2. Average air temperature and daily light integral (DLI) from the beginning of forcing to the end of the experiment under each photoperiod for *Brassia* Rex 'Sakata', *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch', *Odontocidium* Tiger Crow 'Golden Girl' and *Zygopetalum* Redvale 'Fire Kiss'.

Year	Experimental duration (weeks)	Species	Average DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Photoperiod (h)						
				10	12	13	14	16	24	NI[z]
				Average air temperature during experiment (°C)						
2001-02	25	*Degarmoara Miltassia*	7.2	22.0	23.1	23.0	22.7	21.8	22.6	23.2
	36	*Brassia Zygopetalum*	7.0	21.5	22.2	22.2	22.0	21.4	22.0	22.3
2002-03	25	*Degarmoara Miltassia*	6.8	20.8	20.9	20.8	21.0	20.9	21.3	20.8
	36	*Odontocidium Zygopetalum*	6.7	20.5	20.6	20.6	20.7	20.6	21.1	20.5

[z]NI = 9-h photoperiod plus 4-h night interruption.

Table 3. Initial leaf count, length and number of immature and mature pseudobulbs of *Odontocidium* Tiger Crow 'Golden Girl' and *Zygopetalum* Redvale 'Fire Kiss'.

	Odontocidium				*Zygopetalum*			
	Leaf count[z]	Leaf length (cm)[z]	Immature pseudobulb no.[y]	Mature pseudobulb no.[y]	Leaf count	Leaf length (cm)	Immature pseudobulb no.	Mature pseudobulb no.
10	6.7	10.4	4.2	8.3	4.2	3.7	2.4	3.5
12	7.1	11.5	3.7	8.4	3.6	3.5	2.5	3.1
13	6.8	16.4	3.1	5.3	4.2	3.7	1.9	4.5
14	7.1	11.6	3.2	8.0	4.0	3.1	2.7	3.0
16	6.9	12.8	3.1	7.3	3.7	3.4	2.9	3.0
24	7.1	12.5	2.6	5.6	3.8	3.5	2.6	3.1
NI[x]	6.8	10.4	2.8	5.7	3.8	3.5	1.6	3.6

[z]Year 1 data.

[y]Pooled data.

[x]NI = 9-h photoperiod plus 4-h night interruption.

Table 4. The effect of photoperiod on final immature and mature pseudobulbs formed during forcing of *Brassia* Rex 'Sakata'.

Photoperiod (h)	Flower initiation (%)	Immature pseudobulb number			Mature pseudobulb number		
		Initial	Final	New	Initial	Final	New
10	0	--[z]	5.0	--	--	2.0	--
12	0	--	6.0	--	--	2.0	--
13	0	--	4.9	--	--	3.1	--
14	0	--	5.3	--	--	2.8	--
16	0	--	4.5	--	--	2.5	--
24	0	--	3.0	--	--	2.5	--
NI[y]	0	--	5.3	--	--	3.0	--
Significance							
Photoperiod		--	NS	--	--	NS	--

[z]Not measured.
[y]NI = 9-h photoperiod plus 4-h night interruption.
[NS]Nonsignificant.

Table 5. The effect of photoperiod on days to visible inflorescence (VI), days from visible inflorescence to flower (VI to FLW), and days to flower from the beginning of forcing (FLW); flower bud and inflorescence count; and inflorescence length at flowering of *Degarmoara* Winter Wonderland 'White Fairy'.

Photoperiod (h)	Flower initiation (%)	Flower development (%)	Days			Inflorescence		Flower bud no.
			VI	VI to FLW	FLW	Count	Length (cm)	
10	10	10	--[z]	--	--	--	--	--
12	35	35	81	50	131	1.9	41.6	6.7
13	35	30	83	50	133	1.6	53.4	6.6
14	25	25	73	59	132	1.3	38.7	4.8
16	25	25	60	54	114	1.4	49.9	8.4
24	15	5	--	--	--	--	--	--
NI[y]	25	25	101	60	161	1.2	35.5	4.8
Significance								
Photoperiod			*	NS	NS	NS	NS	NS

[z]Not included in analysis.

[y]NI = 9-h photoperiod plus 4-h night interruption.

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

Table 6. The effect of photoperiod on days to visible inflorescence (VI), days from visible inflorescence to flower (VI to FLW) and, days to flower from the beginning of forcing (FLW); flower bud and inflorescence count; and inflorescence length at flowering of *Miltassia* Charles M. Fitch 'Dark Monarch'.

Photoperiod	Flower initiation	Flower development	Days			Inflorescence		Flower
(h)	(%)	(%)	VI	VI to FLW	FLW	Count	Length (cm)	bud no.
10	40	40	92	38	130	2.8	30.9	4.0
12	55	55	81	48	129	2.6	36.0	4.6
13	45	45	77	41	118	2.0	35.9	5.0
14	60	55	76	45	121	1.3	35.5	4.6
16	45	45	87	57	144	2.1	50.0	7.2
24	50	10	75	57	132	2.5	41.5	3.0
NI[z]	50	50	81	42	123	1.9	36.1	4.5
Significance								
Photoperiod			NS	NS	NS	NS	NS	NS

[z]NI = 9-h photoperiod plus 4-h night interruption.
[NS]Nonsignificant.

Table 7. The effect of photoperiod on days from visible inflorescence to flower (VI to FLW) and days to flower from the beginning of forcing (FLW) of *Odontocidium* Tiger Crow 'Golden Girl'.

Photoperiod (h)	Flowering initiation (%)	Flower development (%)	Days VI to FLW	Days FLW
10	50	50	60	187
12	40	40	57	168
13	100	100	63	160
14	40	40	57	190
16	80	80	59	186
24	60	40	61	176
NI[z]	60	50	60	174
Significance				
Photoperiod			NS	NS

[z]NI = 9-h photoperiod plus 4-h night interruption.
[NS]Nonsignificant.

Table 8. The effect of photoperiod on days to flower from the beginning of forcing (FLW), final immature and mature pseudobulb number, and final leaf count of *Zygopetalum* Redvale 'Fire Kiss'.

Photoperiod (h)	Flower initiation (%)	Flower development (%)	Days to FLW	Final pseudobulb number		Final leave count
				Immature	Mature	
Year 1						
10	70	70	155	1.1	2.9	12.2
12	80	80	162	1.4	3.0	10.5
13	70	70	151	2.0	3.2	11.4
14	70	70	156	1.5	3.1	11.3
16	90	60	187	1.7	3.9	8.7
24	50	40	166	1.0	5.4	7.0
NI[z]	70	70	184	0.8	4.5	9.0
Significance						
Photoperiod			NS	NS	***	***
Year 2						
10	90	90	137	--[y]	--	--
12	90	90	138	--	--	--
13	100	100	133	--	--	--
14	100	100	116	--	--	--
16	90	90	97	--	--	--
24	100	80	108	--	--	--
NI[z]	100	100	100	--	--	--
Significance						
Photoperiod			***			

[z]NI = 9-h photoperiod plus 4-h night interruption.

[y]New immature and mature pseudobulbs and leave count increase data used (Fig 5A, C & D).

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

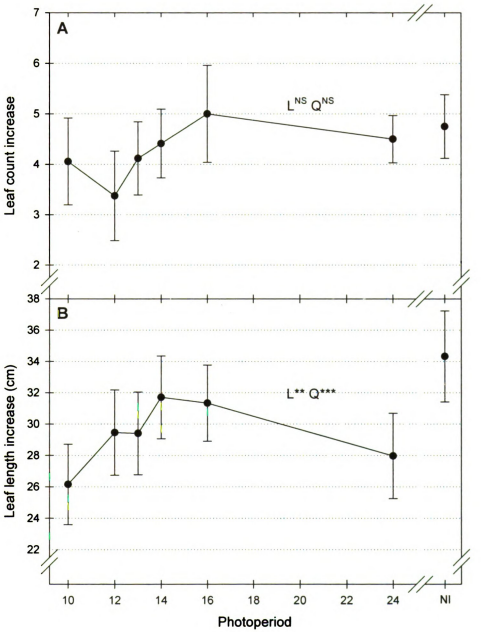

Figure 1. Growth responses of *Brassia* Rex 'Sakata' to various photoperiods (NI = 4-h night interruption). Error bars represent 95% confidence intervals. (L = linear, Q = quadratic trends; NS, **, *** nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively).

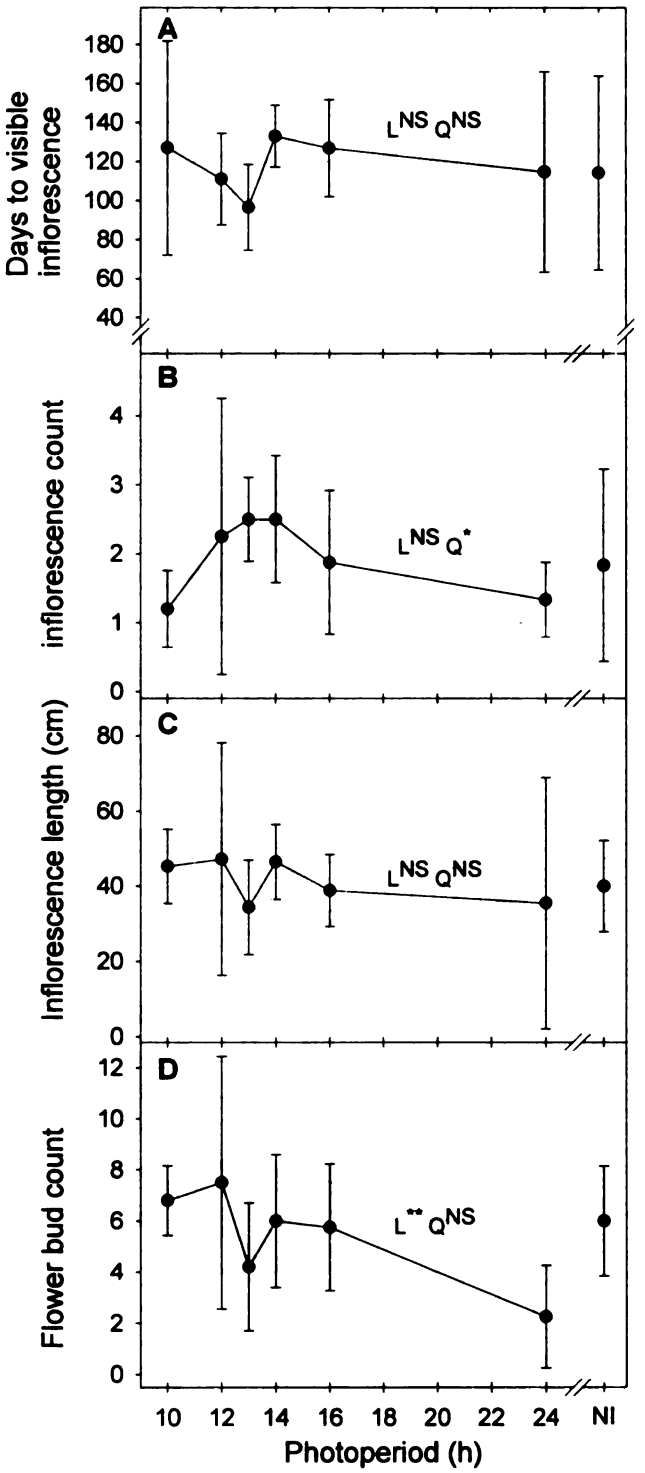

Figure 2. Flowering responses of *Odontocidium* Tiger Crow 'Golden Girl' to various photoperiods (NI = 4-h night interruption). Error bars represent 95% confidence intervals. (L = linear, Q = quadratic trends; NS, *, **nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively).

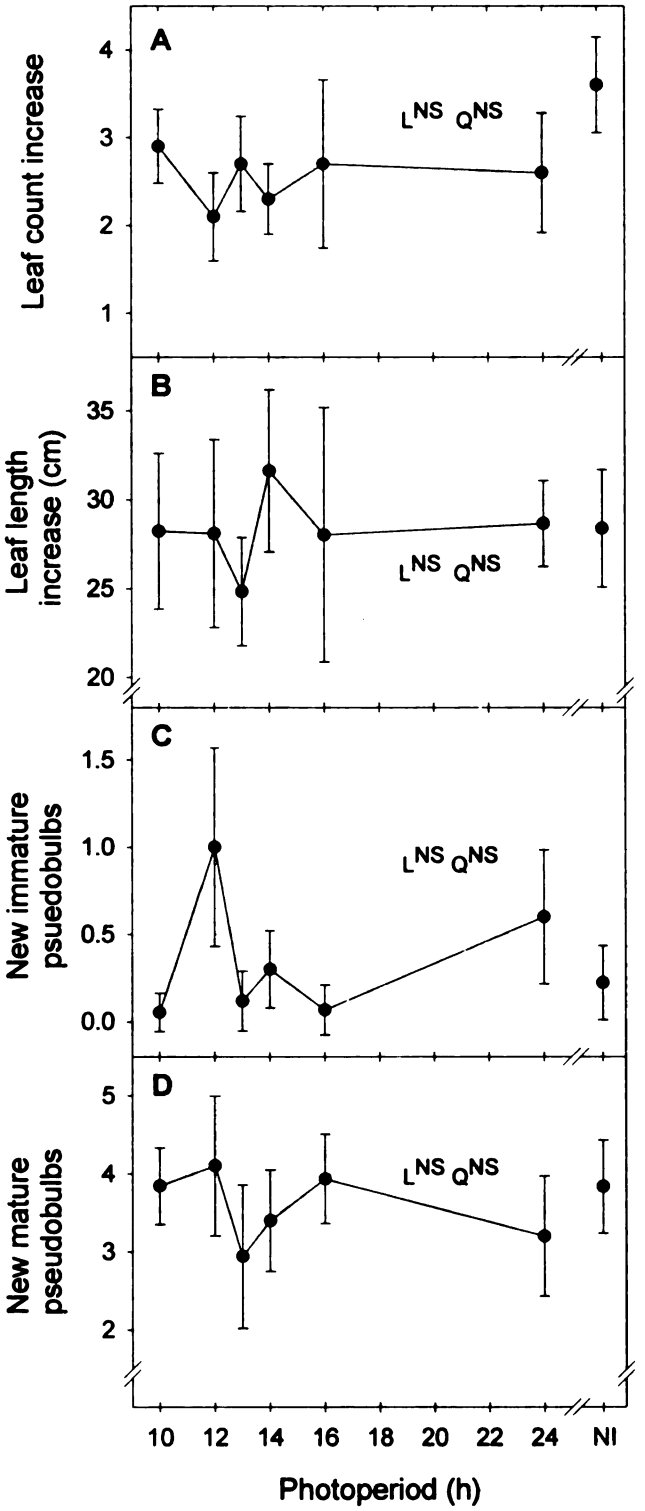

Figure 3. Growth responses of *Odontocidium* Tiger Crow 'Golden Girl' to various photoperiods (NI = 4-h night interruption). Error bars represent 95% confidence intervals. (L = linear, Q = quadratic trends; NS nonsignificant).

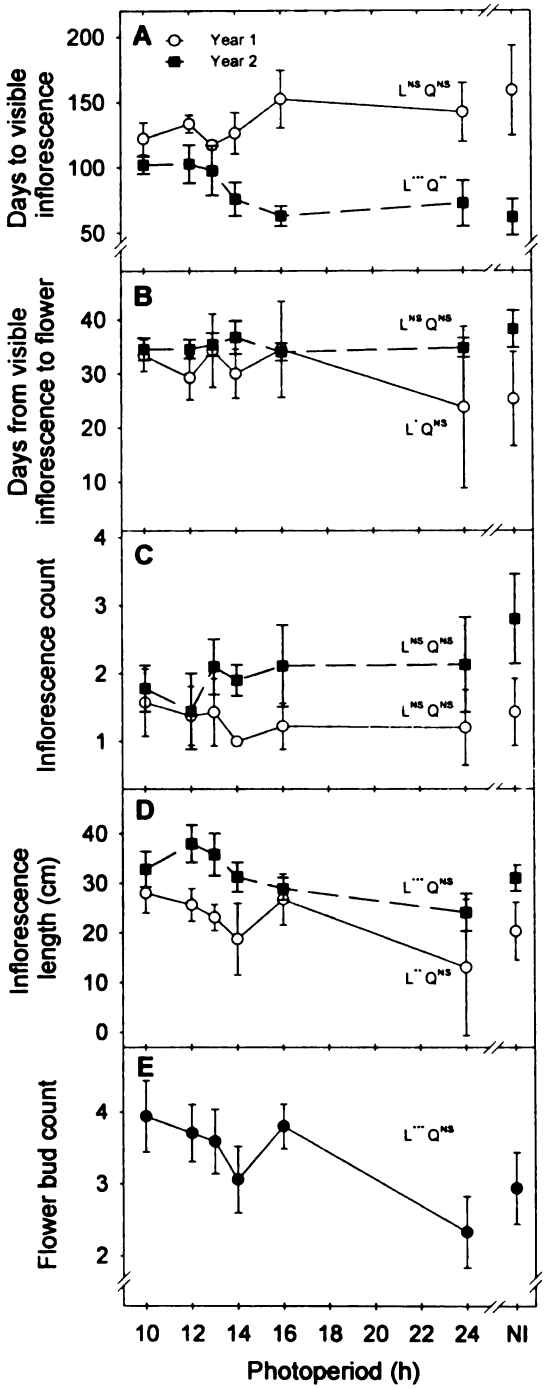

Figure 4. Flowering responses of *Zygopetalum* Redvale 'Fire Kiss' to various photoperiods (NI = 4-h night interruption). Error bars represent 95% confidence intervals. (L = linear, Q = quadratic trends; NS, *, **, ***nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively). Data were pooled in E.

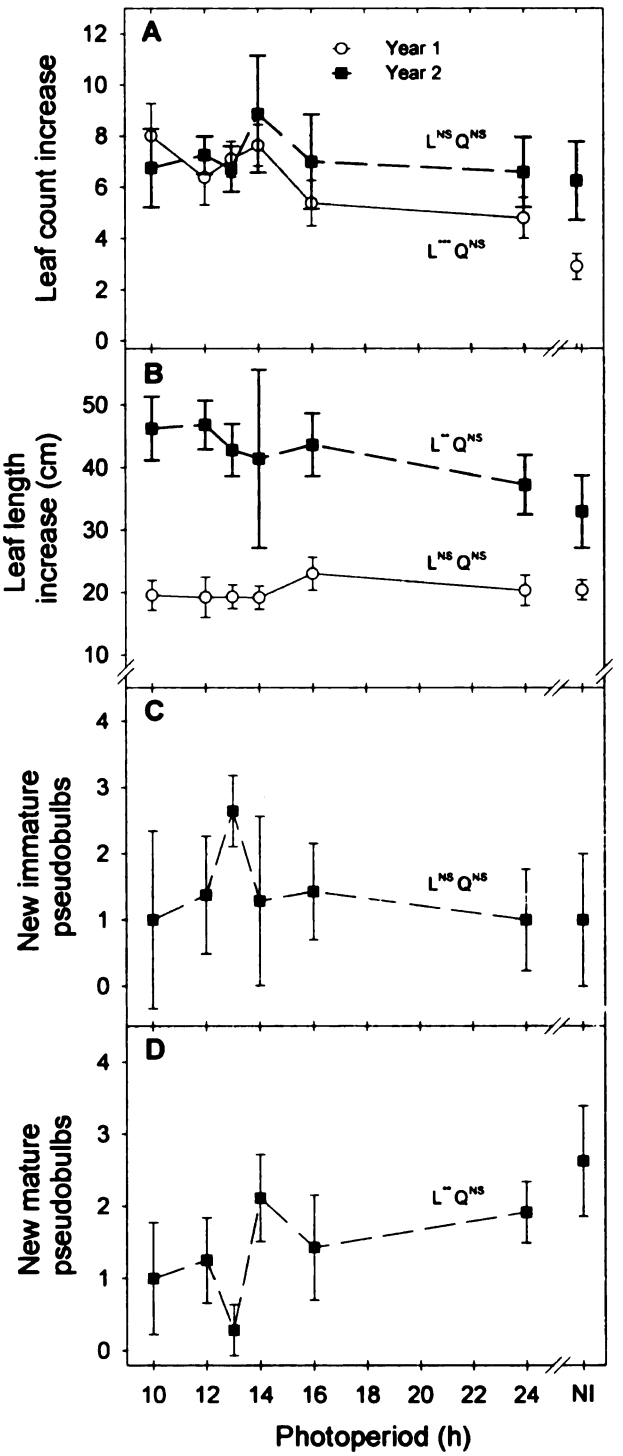

Figure 5. Growth responses of *Zygopetalum* Redvale 'Fire Kiss' to various photoperiods (NI = 4-h night interruption). Error bars represent 95% confidence intervals. (L = linear, Q = quadratic trends; NS, **, *** nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively). Data for Year 2 in C and D.

SECTION III

THE EFFECT OF PREVERNALIZATION PHOTOPERIOD AND VERNALIZATION ON FLOWERING OF BRASSIA, DEGARMOARA, MILTASSIA, MILTONIOPSIS, ODONTOCIDIUM AND ZYGOPETALUM ORCHIDS

The Effect of Prevernalization Photoperiod and Vernalization on Flowering of *Brassia*, *Degarmoara*, *Miltassia*, *Miltoniopsis*, *Odontocidium* and *Zygopetalum* Orchids

Roberto G. Lopez[1] and Erik S. Runkle[2]

Department of Horticulture, Michigan State University, East Lansing, MI 48824

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[1]Graduate Student.

[2]Assistant Professor and Extension Specialist, to whom reprint requests should be addressed (E-mail: runkleer@msu.edu).

Introduction

In the early 1990's, the production of potted flowering orchids began to rise in the United States. Despite this, the United States Department of Agriculture (USDA) National Agricultural Statistical Service considered orchids to be a minor crop and did not collect production information until 1997 (USDA, 1998). In wholesale value, orchids have become the second most valuable flowering potted crop in the United States (USDA, 2003). In the past six years, production value has increased 147% and in 2002 the estimated wholesale value was $105.6 million. According to the American Orchid Society, over 75% of all orchids sold in the U.S. are *Phalaenopsis* (Griesbach, 2002).

As *Phalaenopsis* becomes widely available from home improvement stores to floral shops, retailers and consumers are demanding a wider assortment of species and colors. Unfortunately, the introduction of new floricultural crops research to eluciate production protocols. Important considerations for the introduction of orchid species as potted flowering plants include showiness of flower display, ease and length of production, crop uniformity and size, consumer appeal, and post-production flower longevity. The flowering requirements and commercial potential of other orchid species such as the fragrant and exotic *Brassia* Rex 'Sakata', *Miltoniopsis* Augres 'Trinity', and *Zygopetalum* Redvale 'Fire Kiss' or the showy *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch', and *Odontocidium* Tiger Crow 'Golden Girl' have not been identified.

With the exception of Vanilla, orchids are grown for the aesthetic beauty of their flowers and foliage. However, research on flower initiation, induction and, development of orchids has been limited. Controlled studies to determine the flowering requirements of *Phalaenopsis* were not conducted until the 1950s, even though they were first grown commercially in Europe in the early 19th century during the "orchid craze." One of the first published studies was performed by De Vries (1950), in which he determined that if mature plants were exposed to night temperatures below 21 °C for two to three weeks, they would eventually flower.

Some plants have an absolute requirement for specific environmental cues for flowering and in other plants, flowering is promoted by certain environmental cues but plants will eventually flower in the absence of such a cue. Horticulturally, vernalization and photoperiod are the two most common mechanisms for control of flower initiation.

Vernalization is the promotion of flowering of imbibed seeds or vegetative plants by low temperatures. Vernalization was further defined by Chouard (1960) as "the acquisition or acceleration of the ability to flower by a chilling treatment". A vernalization response is often observed with plants native to regions with low winter temperatures, during which conditions are unfavorable for growth and reproduction. A cold requirement prevents plants from flowering until the spring or summer, when environmental conditions are more favorable for reproduction and seed dispersal. A combined vernalization and photoperiod requirement can ensure that flowering occurs later in the year rather than at the end of the winter.

Low temperature requirements for flower induction have also been documented in tropical orchids. They may vary from distinct elevation-dependant temperature fluctuations required by *Phalaenopsis schilleriana* to subtle rain-induced cooling, which initiates flowering in *Dendrobium crumenatum* (Goh et al., 1982).

Initiation of flower primordia generally does not occur at vernalizing temperatures. After exposure to low temperatures, plants are in a vernalized state (thermoinduced) and have the ability to flower under favorable conditions for growth and reproduction (Thomas and Vince-Prue, 1997). Depending on the species, these favorable conditions can be long days, short days or simply higher temperatures. However, a vernalization response is primarily observed in long-day plants, which have extended periods of vegetative growth and flower in the early summer at high latitudes (Vince- Prue, 1975). These may be perennials, biennials, winter annuals and epiphytes.

Low temperatures, short photoperiods, or both, regulate the flowering process of *Phalaenopsis*, *Cattleya*, *Cymbidium*, and *Dendrobium* orchids (Rotor, 1952; Sakanishi et al., 1980). *Cymbidium* are induced to flower under warmer days than night temperatures (i.e., with large diurnal fluctuations). No studies have found photoperiod to have an effect on floral induction of *Cymbidium* (Goh et al., 1982, Goh and Arditti, 1985). Many temperate *Cymbidium* orchids derived from the "Asiatic *Cymbidium* Belt" are reported to require large diurnal temperature fluctuations of 10 to 14 °C to initiate flowers (Powell et al., 1988). Most *Phalaenopsis* species and hybrids require a period of (3 to 5 weeks)

exposure to temperatures < 28 °C to trigger the elongation of the inflorescence (Lee and Lin, 1984, 1987; Sakanishi et al., 1980; Yoneda et al., 1992; Wang, 1995). Lin and Lee (1984) showed that uniform spiking can be achieved when plants are grown at day/night temperatures of 25/20 °C or 20/15 °C for four to five weeks.

Orchids such as *Cattleya* and *Dendrobium nobile* require specific combinations of temperature and photoperiod for flower induction. A few published studies indicate that *Cattleya* species and hybrids require short days and low temperatures to flower (Rotor, 1952; Rotor, 1959). In *C. warscewiczii, C. gaskelliana,* and C. *mossiae,* flower induction occurs under continuous 9-h short days at 13 °C, while flowering is inhibited under 16-h long days at 13 °C. In *Dendrobium nobile,* plants exposed to 13 °C produced flowers regardless of photoperiod, whereas plants held at 18 °C remained vegetative (Rotor 1952; Goh and Arditti, 1985). Long days accelerated flowering by 1 to 4 weeks (Rotor, 1952). In *D. phalaenopsis,* short days at 18 °C hastened flower bud development and flowering by ≈6 weeks compared to plants under long or natural daylengths at 18 °C (Rotor, 1952).

We preformed an investigation using different combinations of photoperiod before vernalization (prevernalization photoperiods) and vernalization temperatures and photoperiods to determine if plants flowered in response to temperature, photoperiod, or both. *Brassia* Rex 'Sakata', *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch', *Miltoniopsis* Augres 'Trinity', *Odontocidium* Tiger Crow 'Golden Girl', and *Zygopetalum*

Redvale 'Fire Kiss', were chosen for study based on greenhouse grower interests and suitability as potted flowering plants based on fragrance, size and floral characteristics.

Materials and Methods

Plant material. Plants were grown by a commercial greenhouse (Calif.) at 16 to 26 °C under natural photoperiods (37 °N latitude) with a maximum photosynthetic photon flux (*PPF*) of ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Propagation dates and container sizes are provided in Table 1. All species were planted in a bark and perlite-based media (10-cm pots) and were received in East Lansing, Mich. on 6 May 2001 (Year 1) and 22 July 2002 (Year 2). *Brassia*, *Degarmoara*, and *Miltassia* received in Year 1 were transplanted into 15-cm pots. *Zygopetalum* received in Year 2 were transplanted into 13-cm pots. All plants were maintained at ≈23 °C in a glass-glazed greenhouse until experiments began. The photoperiod was a constant 16 h, consisting of natural daylengths (42 °N latitude) with day-extension lighting from high-pressure sodium (HPS) lamps, which delivered a supplemental *PPF* of ≈50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height [as measured with a line quantum sensor (Apogee Instruments, Inc., Logan, Utah)].

Prevernalization photoperiods. The experiment was replicated in time and experimental treatments were identical between years unless otherwise noted. Two hundred plants of each species were placed at 23 °C for eight weeks under 9-h short days (SD) or 16-h long days (LD) (pre-vernalization photoperiods) beginning on 1 September 2001 (Year 1) and 28 August 2002 (Year 2).

Photoperiods were created by pulling opaque blackout cloth over plants between 1700 and 0800 HR, and for the 16-h photoperiod, light from incandescent lamps (delivering ≈3 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was provided between 1700 and 2400 HR.

Vernalization temperatures and photoperiods. On 27 October 2001 (Year 1) and 23 October 2002 (Year 2), 20 plants of each species were transferred to greenhouses with temperature setpoints of 11, 14, 17, 20, and 23 °C (8 °C was also utilized in Year 2). At each temperature, ten plants were placed under SD and LD photoperiods (provided as described above with incandescent lamps), also for eight weeks. Vapor pressure deficit during the temperature treatments was maintained at ≈0.7 kPa by the injection of water vapor as needed. Following eight weeks at the various temperature treatments, plants were forced in a common greenhouse at 23 °C under LD using HPS lamps.

Greenhouse temperature and irradiance control. Air temperature in each greenhouse was measured by a thermocouple in an aspirated chamber every 10 s, and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, Utah). Average daily light integral and air temperature from the beginning of the pre-vernalization photoperiod treatments until the end of forcing were calculated (Table 2 and 3). Light transmission through the greenhouses was reduced using permanent woven shade curtains that reduced light by ≈55% (OLS 50; Ludvig Svensson, Charlotte, N.C.) and by applying whitewash (up to 50%) to the glass as needed so that the maximum *PPF* was ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Plant culture. In Year 1, plants were irrigated as necessary with well water (containing 95, 34, and 29 $mg \cdot L^{-1}$ Ca, Mg, and S, respectively) supplemented

with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 125 K, 15 Ca, 1.0 Fe, 0.1 B and Mo, and 0.5 Mn, Zn, and Cu (MSU Special, Greencare Fertilizers, Chicago, IL). Water was acidified with H_2SO_4 to a titratable alkalinity of 140 $mg \cdot L^{-1}$ $CaCO_3$. In Year 2, plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B and 0.1 Mo.

Data collection and analysis. Immature and mature pseudobulbs were counted before and after vernalization treatments. The date at which the first inflorescence was visible (visible inflorescence, or VI) without dissection and the date the first flower opened were recorded for each plant. At flowering, the number of VI, flower buds and pseudobulb node number at which the inflorescence appeared were counted and inflorescence length was measured. Plants that did not flower 150 d after the end of the vernalization treatment were considered non-flowering. The percentage of plants that initiated flowers, the percentage of plants whose flowers developed normally (did not abort), days to VI, days from VI to flower, and days to flower were calculated. The few plants that died during the experiments were discarded and not included in the results.

Data was analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED). Data were pooled for all measured characteristics, and when there was a significant year x treatment interaction, the comparisons were analyzed separately for each year. The experiment was replicated for

Brassia, *Degarmoara*, and *Zygopetalum* and was performed once for *Miltassia*, *Miltoniopsis* and *Odontocidium*.

Results

Brassia. The percentage of *Brassia* Rex 'Sakata' that initiated flowers was low and nonuniform regardless of pre-vernalization photoperiod or vernalization treatment. The highest percentage of plants that initiated flowers, 55%, were those placed under a 16-h pre-vernalization photoperiod and held at 23 °C with a LD photoperiod (Fig. 1A). Plants vernalized at 8 °C were discarded due to severe chilling injury and not included in further analysis. Flower development was not adversely affected by any treatment (Fig. 1B). Vernalization temperature alone influenced the number of days to VI after forcing (significantly different at $P \leq 0.001$) (Fig. 1C). On average, the few plants that flowered at 11 °C required ≈37 d to reach VI, while those at 23 °C required ≈78 d. The number of days from visible inflorescence to flower was fairly uniform (≈37 d) and was not influenced by any treatment (Fig. 1D). Flower bud count was significantly influenced by vernalization temperature and photoperiod; as temperature increased under LD, flower bud count generally increased (Table 4). Inflorescence count was not influenced by any treatment. However, inflorescence length of plants vernalized under LD was significantly greater than those under SD, but the magnitude varied with cooling temperature.

Degarmoara. Flower initiation was low and nonuniform in *Degarmoara* Winter Wonderland 'White Fairy' and no trends were observed among treatments

in Year 1 (Fig. 2A). In Year 2, only two plants initiated flowers so data was not included in further analysis. Flower development followed a similar pattern as flower initiation and all plants that initiated flowers developed to flower opening (Fig. 2B). Average days to VI occurred > 100 d after vernalization across all treatments, except in plants induced under the SD pre-vernalization photoperiod and cooled at 11 °C with SD, which averaged ≈72 d (Fig. 2C). The time from VI to open flower was similar among the twenty treatments ranging from 47 to 71 d (Fig. 2D). Flower bud count, inflorescence count and length were unaffected by pre-vernalization photoperiod or by vernalization temperature and vernalization photoperiod (Table 5).

Miltassia. Plants vernalized at 11 and 14 °C were discarded due to severe chilling injury and not included in further analysis. Flower initiation and development of *Miltassia* Charles M. Fitch 'Dark Monarch' did not reach 100 percent in any treatment. However, flower initiation and development was slightly higher in plants placed under a 9-h pre-vernalization photoperiod compared to those placed under a 16-h pre-veralization photoperiod (Fig. 3A and B). Days to VI and days from VI to flower were similar among treatments, ranging from 164 to 180 d, and 50 to 60 d, respectively (Fig. 3C and D). Pre-vernalization photoperiod, vernalization temperature and photoperiod did not influence flower bud or inflorescence count (Table 6). As cooling temperature increased from 17 to 23 °C, inflorescence length decreased from 40.4 to 28.2 cm. Plants that produced terminal inflorescences produced fewer flowers and shorter

inflorescences compared to plants that developed lateral inflorescences (significantly different at $P \leq 0.001$; data not presented).

Miltoniopsis. Flower initiation of *Miltoniopsis* Augres 'Trinity' was influenced by photoperiod before and during vernalization and by vernalization temperatures (Fig. 4). Flower initiation was greatest (100%) when plants were provided with a 9-h pre-vernalization photoperiod prior to being vernalized under 9-h photoperiods at 14 °C. Average days to visible inflorescence occurred > 160 days after plants were taken out of the temperature treatment. Inflorescence count was not influenced by any inductive treatment (data not presented).

Odontocidium. Flower initiation was high (up to 100%) in *Odontocidium* Tiger Crow 'Golden Girl', but nonuniform among treatments and no trends were apparent (Fig. 5A). Flower development was not adversely affected by any treatment (Fig. 5B). Plants began initiating flowers during the vernalization treatment, so days to VI were calculated from the end of the pre-vernalization treatment (Fig. 5C). Days to VI, days from VI to flower, flower bud count and inflorescence length were not significantly influenced by any treatment (Fig 5C and D, Table 7). Average days from VI to flower among treatments was ≈60. Inflorescence count was statistically greater when the pre-vernalization photoperiod was a LD (1.6) compared to a SD (1.4). Flower number and inflorescence length were significantly influenced by the position of the inflorescence (terminal and lateral) (significantly different at $P \leq 0.001$).

Zygopetalum. In Year 1, flowering of *Zygopetalum* Redvale 'Fire Kiss' was primarily influenced by photoperiod before vernalization and by cool

temperature, but not by photoperiod during the vernalization treatment (Fig. 6). In Year 2, no plants flowered under any treatment after 150 d as > 85% of plants flowered one month prior to the initiation of treatments. In Year 1, the percentage of plants that initiated and developed flowers was greatest when plants were vernalized at 11 °C. In particular, plants grown under SD before vernalization and then transferred to 11 or 14 °C for eight weeks had the highest flower initiation and development percentages and developed VI the quickest. None of the plants grown under a 9-h photoperiod followed by temperatures ≥ 17 °C flowered within the duration of the experiment. When plants were exposed to 16-h photoperiods before vernalization, flower initiation was generally reduced and flowering was delayed (Fig. 6A and C).

Several of the plants grown under LD then cooled at 14 °C or higher that initiated flowers did not develop to anthesis (Fig. 6B). In addition, the average time to VI when vernalized at 17 °C or higher was ≥ 50 d. Regardless of treatment, time from VI to flower was 30 to 39 d at ≈23 °C (Fig. 6D). Flower bud count was greatest (≥ 3.4) and inflorescence length was greatest (≥ 30.4 cm) when plants were cooled at 11 to 14 °C (Table 8). The length of inflorescences was generally shorter as vernalization temperature increased from 11 to 23 °C.

Discussion

Brassia. Pre-vernalization photoperiod, vernalization temperature, and vernalization photoperiod did not influence flowering in *Brassia* Rex 'Sakata'. In a previous study (Section II), photoperiods ranging from 10 to 24 h of continuous

light or 9 h with a 4-h night interruption (NI) at ≈20 °C did not lead to any flowering response. *Brassia* Rex 'Sakata' is a hybrid (*Brassia* verrucosa x *Brassia* gireoudiana) bred in Hawaii for its heat tolerance with optimal vegetative growth at or above 20 °C; it can tolerate temperatures as low as 11 °C. At temperatures ≤ 8 °C, plants exhibited severe schilling injury symptoms and eventually died after eight weeks. An investigation with day and night temperature differentials could reveal a uniform flowering response for *Brassia*.

Degarmoara, Miltassia and *Odontocidium*. Photoperiod before or during vernalization and vernalization temperature did not have a significant influence on flowering or floral characteristics of *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch', or *Odontocidium* Tiger Crow 'Golden Girl.' Flowering percentages were nonuniform and incomplete across all treatments. In a previous experiment (Section II), flowering was not influenced by any of seven different photoperiods at ≈20 °C (mentioned above), in all three species. Unfortunately, the flowering response is still unknown in these hybrids and has proven to be more difficult than expected to elucidate. The complex hybrids created between the members of the *Odontoglossum* orchid "alliance" to create *Degarmoara, Miltassia* and *Odontocidium* pose a challenge in determining the environmental conditions that lead to complete, rapid and uniform flowering. However, we have gathered other important production information on these species, which could be useful for greenhouse growers and hobbyists.

In our vernalization studies, we observed that *Miltassia* (*Brassia verrucosa* x *Miltonia spectabilis*) is not a cold tolerant hybrid even though part of

its parentage is from the cool-growing *Miltonia*. At temperatures ≤ 17 °C, plants exhibited symptoms of chilling injury within two weeks of exposure; by eight weeks most plants had lost all their leaves and pseudobulbs at 11 to 14 °C, eventually died. In a separate study, optimal flower color, quality, and longevity of *Miltassia* were achieved at temperatures between 20 to 23 °C (Appendix A).

Degarmoara Winter Wonderland 'White Fairy' (*Miltassia* Cartagena x *Odontoglossum* Gledhow) and *Odontocidium* Tiger Crow 'Golden Girl' (*Odontocidium* Tiger Hambuhren × *Odontocidium* Crowborough) are intergeneric hybrids that can tolerate temperatures from 8 to 28 °C. However, the overall flower quality (size and color) of *Odontocidium* diminished at temperatures above 23 °C.

Miltoniopsis. Flower initiation of *Miltoniopsis* Augres 'Trinity' was uniform and complete only when plants were exposed to 9-h SD photoperiods before and during vernalization at 11 to 14 °C. A similar response is observed in *Cattleya warscewiczii, C. gaskelliana,* and C. *mossiae,* where flower induction occurred under continuous 9-h SD at 13 °C, while flowering was inhibited under 16-h LD at 13 °C (Rotor, 1952; Rotor, 1959). Both *Cattleya* and *Miltoniopsis* are epiphytes native to the moist and wet forests of Central and South America.

Time to visible inflorescence was long (> 160 d) in *Miltoniopsis* possibly due to pseudobulb maturity and a warm forcing temperature. Prior to pre-vernalization, most plants had one or two mature pseudobulbs, which we believe could no longer be induced to flower. Instead, these pseudobulbs had produced vegetative buds (immature pseudobulbs). The immature pseudobulbs were

small (< 10 cm), consequently taking over five months to develop into newly mature pseudobulbs that we believe were capable of flowering. Furthermore, after vernalization, plants were forced under 16-h LD at 23 °C, where overall plant quality was marginal and growth was slow. As a result, the forcing temperature was lowered to 20 °C on 7 May 2003 and a dramatic visual improvement in leaf color and turgor was seen within a few weeks.

Miltoniopsis Augres 'Trinity' (*Miltonia* Pam-pam x *Miltonia* Alger) is a cool growing hybrid with optimal growth at temperatures between 20 to 23 °C, although it can tolerate temperatures from 8 to 26 °C. Flower size and quality is adversely affected by high light, above 400 $\mu mol \cdot m^{-2} \cdot s^{1}$ and temperatures > 20 °C, and low humidity. Thus, *Miltoniopsis* is suited for potted flowering plant production in temperate climates for winter and spring holidays (Christmas, Valentine's Day, Easter and Mother's Day). Sales at these times would facilitate production schedules for greenhouse growers as they could utilize the natural short days and cool temperatures of the fall and winter for flower induction.

Other positive attributes of this genera include its compact nature (< 35 cm) and large, showy and fragrant flowers that resemble pansies. The flowers can last four to eight weeks on the plant (Baker and Baker, 1993). In an online survey of potted orchid buyers in the Midwest, participants between the ages of 20 to 62 years selected *Miltoniopsis* as their most preferred species among *Brassia, Degarmoara* and *Phalaenopsis* (Behe et al., 2004). Participants had the highest average purchase intention (5.5) on a scale of 1 to 7 (1= very unlikely and 7=very likely to purchase) for *Miltoniopsis* in a green plastic pot for $19.95.

Miltoniopsis in a bamboo pot for $29.95 (5.0) and *Phalaenopsis* in a black ceramic pot for $19.95 (5.0) were the second and third most popular choices, respectively.

For growers to predictably meet specific market dates for sales of flowering *Miltoniopsis* (e.g. Valentine's Day) several other investigations are necessary. An easy method of identifying pseudobulbs that are capable of being induced to flower is needed to reduce the time to VI. Possible methods include leaf or node count or pseudobulb characteristics such as width, diameter or circumference. For the establishment of production schedules, studies on the effects of temperature on flower and pseudobulb development would also produce valuable information.

Zygopetalum. The most rapid, complete, and uniform flowering occurred when plants were grown under SDs and then vernalized at 11 to 14 °C. Time to VI and time from VI to flower occurred relatively quickly within these treatments, 19 to 22 d and 35 to 37 d, respectively. In a previous study (Section II), *Zygopetalum* responded as a quantitative short-day plant (SDP) without vernalization. Photoperiod before vernalization also influences flowering in the tropical epiphyte *Hatiora* spp. (Easter cactus) (Rünger, 1960; Rohwer, 2002). In both *Hatiora* and *Zygopetalum*, flowering is hastened under SD, and a period of short days before vernalization increases flowering percentage and uniformity. Another similarity between these genera is that the maximum effective temperature for induction is around 14 to 15 °C (Rohwer, 2002).

In Year 2, no plants flowered after any treatment. One month prior to experimentation, > 85% of plants flowered and thus flower initiation had occurred before the plants were shipped to us. We believe that we did not provide an adequate "rest period" before the onset of treatments, and consequently the plants did not flower.

With the preliminary production information presented here, greenhouse growers can induce both *Zygopetalum* and *Miltoniopsis* into flower with similar protocols. Given that a SD photoperiod prior to vernalization and temperatures of 11 to 14 °C induced *Zygopetalum*, both species could be induced if also given SD during vernalization. However, future studies are needed to further understand and quantify the flowering responses of both hybrids.

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Table 1. Propagation dates and container sizes for orchid hybrids.

Species	Year	*in vitro*	Out of flask	Transplant to 7.5-cm pot	Transplant to 10-cm pot
Brassia Rex 'Sakata'	1	June 1997	June 1998	June 1999	June 2000
	2	June 1997	June 1998	June 1999	June 2000
Degarmoara Winter Wonderland 'White Fairy'	1	May 1997	May 1998	May 1999	May 2000
	2	-[z]	-	June 2000	June 2001
Miltassia Charles M.Fitch 'Dark Monarch'	1	June 1997	June 1998	June 1999	June 2000
Miltoniopsis Augres 'Trinity'	1	-	-	June 2001	May 2002
Odontocidium Tiger Crow 'Golden Girl'	1	-	-	June 2001	April 2002
Zygopetalum Redvale 'Fire Kiss'	1	June 1999	June 2000	--[y]	April 2001
	2	-	May 2001	--	May 2002

[z]Information not available.

[y]Plants were directly transplanted to 10-cm pots.

Table 2. Average daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$) during the pre-vernalization and vernalization treatments and for each month during subsequent forcing.

			Forcing month							
Year	Pre-vernalization	Vernalization	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
2001-02	5.4	2.9	5.2	5.9	7.3	12.0	20.1	10.0	10.7	10.5
2002-03	6.6	2.5	7.3	8.1	9.4	6.7	8.5	5.9	7.5	7.4

Table 3. Actual average air temperature (°C) during the pre-vernalization and vernalization treatments and for each month during subsequent forcing.

	Pre-vernalization setpoint	Vernalization setpoint						Forcing setpoint							
Year	23	8	11	14	17	20	23	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
2001-02	22.5	-[z]	12.0	14.2	17.2	20.7	23.4	23.2	23.6	24.6	24.2	25.3	24.1	25.9	25.4
2002-03	25.5	8.9	11.3	14.1	16.8	20.0	24.0	23.7	23.2	23.0	25.1	25.0	22.3	22.7	23.9

[z]Vernalization temperature not used.

Table 4. The effect of pre-vernalization photoperiod, vernalization temperature, and vernalization photoperiod on flower bud count, inflorescence length and count of *Brassia* Rex 'Sakata'. Plants were forced at 23 °C under a 16-h photoperiod.

Pre-vernalization photoperiod (h)	Vernalization		Flower bud count	Inflorescence	
	Temperature (°C)	Photoperiod (h)		Count	Length (cm)
9			9.9	1.2	42.3
16			11.5	1.2	40.3
	8		-[z]	-	-
	11		8.9	1.1	45.6
	14		8.8	1.0	42.5
	17		10.3	1.1	41.6
	20		11.3	1.2	40.7
	23		11.9	1.2	40.0
		9	9.8	1.2	38.9
		16	11.5	1.2	43.4
9	8	9	-	-	-
	8	16	-	-	-
	11	9	9.7	1.0	49.2
	11	16	8.3	1.3	43.5
	14	9	7.5	1.0	33.9
	14	16	9.0	1.0	50.0
	17	9	6.8	1.0	37.5
	17	16	12.0	1.3	48.0
	20	9	10.6	1.2	41.2
	20	16	11.0	1.3	42.3
	23	9	9.2	1.3	33.3
	23	16	13.3	1.0	49.3
16	8	9	-	-	-
	8	16	-	-	-
	11	9	—[y]	—	—
	11	16	8.0	1.0	41.3
	14	9	6.5	1.0	35.1
	14	16	11.7	1.0	43.8
	17	9	10.8	1.1	41.2
	17	16	11.1	1.0	41.6
	20	9	11.3	1.1	38.5
	20	16	12.4	1.3	40.3
	23	9	11.0	1.3	37.5
	23	16	13.3	1.2	41.2
Significance					
	Pre-vernalization photoperiod (PVP)		NS	NS	NS
	Vernalization temperature (VT)		***	NS	NS
	Vernalization photoperiod (VP)		**	NS	**
	PVP x VT		NS	NS	NS
	PVP x VP		NS	NS	*
	VT x VP		*	NS	**
	PVP x VT x VP		*	NS	NS

[z]Plants were discarded due to severe chilling injury.

[y]No plants showed visible inflorescence within 150 d of forcing.

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

Table 5. The effect of pre-vernalization photoperiod, vernalization temperature, and vernalization photoperiod on flower bud count, inflorescence length and count of *Degarmoara* Winter Wonderland 'White Fairy'. Plants were forced at 23 °C under a 16-h photoperiod.

Pre-vernalization photoperiod (h)	Vernalization		Flower bud count	Inflorescence	
	Temperature (°C)	Photoperiod (h)		Count	Length (cm)
9			7.1	1.5	48.4
16			7.8	1.3	49.1
	8		-[z]	-	-
	11		7.2	1.4	51.8
	14		7.6	1.3	48.1
	17		8.8	1.5	52.3
	20		5.7	1.3	37.2
	23		8.0	1.3	50.9
		9	7.2	1.5	48.1
		16	8.0	1.3	49.7
9	8	9	-	-	-
	8	16	-	-	-
	11	9	4.2	1.4	39.6
	11	16	7.7	1.8	57.4
	14	9	7.0	1.0	46.1
	14	16	10.3	1.3	54.3
	17	9	7.8	1.8	55.8
	17	16	10.0	1.0	47.6
	20	9	4.0	2.0	36.9
	20	16	6.0	1.0	35.7
	23	9	7.0	1.0	35.4
	23	16	9.0	2.0	70.5
16	8	9	-	-	-
	8	16	-	-	-
	11	9	9.0	1.5	57.9
	11	16	8.6	1.2	55.9
	14	9	7.2	1.6	48.3
	14	16	6.8	1.3	46.1
	17	9	9.0	1.8	49.2
	17	16	10.0	1.0	52.8
	20	9	6.0	1.3	35.5
	20	16	6.3	1.0	39.6
	23	9	8.8	1.7	55.0
	23	16	6.8	1.3	43.7
Significance					
	Pre-vernalization photoperiod (PVP)		NS	NS	NS
	Vernalization temperature (VT)		NS	NS	NS
	Vernalization photoperiod (VP)		NS	NS	NS
	PVP x VT		NS	NS	NS
	PVP x VP		NS	NS	NS
	VT x VP		NS	NS	NS
	PVP x VT x VP		NS	NS	NS

[z]No plants showed visible inflorescence within 150 d of forcing.
[NS]Nonsignificant.

Table 6. The effect of pre-vernalization photoperiod, vernalization temperature, and vernalization photoperiod on flower bud count, inflorescence length and count of *Miltassia* Charles M. Fitch 'Dark Monarch'. Plants were forced at 23 °C under a 16-h photoperiod.

Pre-vernalization photoperiod (h)	Vernalization		Flower bud count	Inflorescence	
	Temperature (°C)	Photoperiod (h)		Count	Length (cm)
9			4.9	1.4	35.2
16			4.6	1.6	35.5
	11		--[z]	--	--
	14		--	--	--
	17		5.0	1.7	40.4
	20		4.7	1.4	34.9
	23		4.6	1.4	28.2
		9	4.6	1.4	35.7
		16	5.0	1.6	34.9
9	11	9	--	--	--
	11	16	--	--	--
	14	9	--	--	--
	14	16	--	--	--
	17	9	4.4	1.7	35.4
	17	16	5.0	1.6	41.6
	20	9	4.4	1.3	35.9
	20	16	6.2	1.4	36.8
	23	9	4.8	1.0	35.6
	23	16	5.2	1.4	25.8
16	11	9	--	--	--
	11	16	--	--	--
	14	9	--	--	--
	14	16	--	--	--
	17	9	5.8	1.4	45.7
	17	16	4.9	2.0	40.7
	20	9	5.0	1.3	36.1
	20	16	2.0	1.5	15.0
	23	9	3.5	1.8	23.6
	23	16	4.7	1.7	28.1
Significance					
	Pre-vernalization photoperiod (PVP)		NS	NS	NS
	Vernalization temperature (VT)		NS	NS	***
	Vernalization photoperiod (VP)		NS	NS	NS
	PVP x VT		*	NS	NS
	PVP x VP		*	NS	NS
	VT x VP		NS	NS	NS
	PVP x VT x VP		*	NS	*

[z]Plants were discarded due to severe chilling injury.

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

Table 7. The effect of pre-vernalization photoperiod, vernalization temperature, and vernalization photoperiod on flower bud count, inflorescence length and count of *Odontocidium* Tiger Crow 'Golden Girl'. Plants were forced at 23 °C under a 16-h photoperiod.

Pre-vernalization photoperiod (h)	Vernalization		Flower bud count	Inflorescence	
	Temperature (°C)	Photoperiod (h)		Count	Length (cm)
9			5.8	1.4	44.0
16			5.9	1.6	42.3
	8		5.0	1.3	36.8
	11		5.5	1.7	40.7
	14		5.9	1.5	42.0
	17		5.2	1.4	40.9
	20		7.4	1.5	51.2
	23		6.3	1.6	47.9
		9	5.6	1.5	40.9
		16	6.0	1.5	45.5
9	8	9	5.5	1.2	36.9
	8	16	5.3	1.0	37.4
	11	9	5.2	1.5	39.5
	11	16	5.5	1.3	42.1
	14	9	4.0	1.0	32.1
	14	16	5.0	1.2	40.7
	17	9	4.5	1.0	40.2
	17	16	5.0	1.4	39.0
	20	9	6.4	1.7	40.6
	20	16	8.0	1.2	61.0
	23	9	6.6	1.3	45.9
	23	16	6.4	1.9	56.5
16	8	9	2.3	1.2	26.4
	8	16	5.7	1.6	40.7
	11	9	6.0	1.6	42.7
	11	16	5.5	2.3	39.7
	14	9	5.4	2.0	37.6
	14	16	9.0	1.2	58.8
	17	9	5.8	1.4	43.5
	17	16	5.6	1.7	40.9
	20	9	8.0	1.8	53.9
	20	16	7.7	1.5	54.1
	23	9	6.8	2.0	45.9
	23	16	5.2	1.3	41.1
Significance					
	Pre-vernalization photoperiod (PVP)		NS	**	NS
	Vernalization temperature (VT)		NS	NS	NS
	Vernalization photoperiod (VP)		NS	NS	NS
	PVP x VT		NS	NS	NS
	PVP x VP		NS	NS	NS
	VT x VP		NS	NS	NS
	PVP x VT x VP		NS	NS	NS

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

Table 8. The effect of pre-vernalization photoperiod, vernalization temperature, and vernalization photoperiod on flower bud count, inflorescence length and count of *Zygopetalum* Redvale 'Fire Kiss'. Plants were forced at 23 °C under a 16-h photoperiod.

Pre-vernalization photoperiod (h)	Vernalization		Flower bud count	Inflorescence	
	Temperature (°C)	Photoperiod (h)		Count	Length (cm)
9			3.6	1.5	32.5
16			3.4	1.3	29.1
	8		-[z]	-	-
	11		3.9	1.4	30.6
	14		3.4	1.4	34.7
	17		3.0	1.3	24.3
	20		2.5	1.0	26.4
	23		3.0	1.3	23.3
		9	3.5	1.4	31.9
		16	3.5	1.4	30.2
9	8	9	-	-	-
	8	16	-	-	-
	11	9	3.8	1.4	30.1
	11	16	4.0	2.0	31.1
	14	9	3.5	1.7	34.8
	14	16	3.1	1.0	35.0
	17	9	-[z]	-	-
	17	16	-	-	-
	20	9	-	-	-
	20	16	-	-	-
	23	9	-	-	-
	23	16	-	-	-
16	8	9	-	-	-
	8	16	-	-	-
	11	9	3.8	1.2	33.2
	11	16	3.8	1.0	28.6
	14	9	3.5	1.0	37.0
	14	16	3.7	1.6	32.1
	17	9	--[y]	1.0	--
	17	16	3.0	1.4	24.3
	20	9	2.5	1.0	26.4
	20	16	-	-	-
	23	9	3.0	1.5	22.0
	23	16	3.0	1.2	24.0
Significance					
	Pre-vernalization photoperiod (PVP)		NS	NS	NS
	Vernalization temperature (VT)		*	NS	**
	Vernalization photoperiod (VP)		NS	NS	NS
	PVP x VT		NS	NS	NS
	PVP x VP		NS	NS	NS
	VT x VP		NS	NS	NS
	PVP x VT x VP		NS	**	NS

[z]No plants showed visible inflorescence within 150 d of forcing (one dash).

[y]Flower buds aborted (two dashes).

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

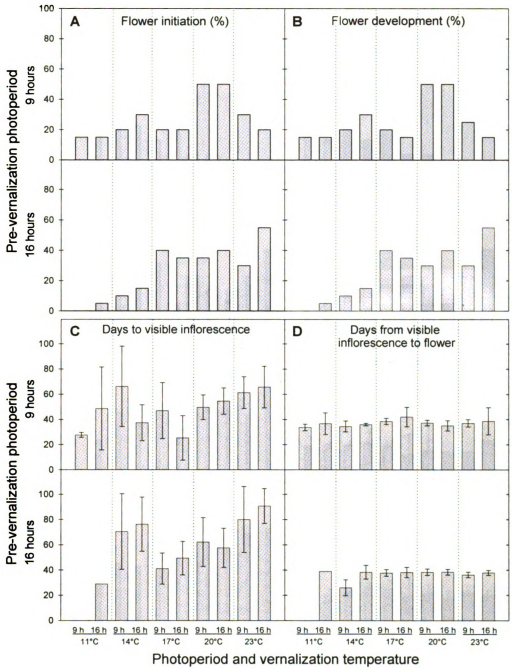

Figure 1. Responses of *Brassia* Rex 'Sakata' forced at 23 ºC under a 16-h photoperiod after 9- or 16-h prevernalization photoperiods for 8 weeks and vernalization (11, 14, 17, 20, or 23 ºC) under 9- or 16-h photoperiods for 8 weeks. Error bars represent 95% confidence intervals. Data for days to visible inflorescence (VI) are for the period from the end of vernalization until VI.

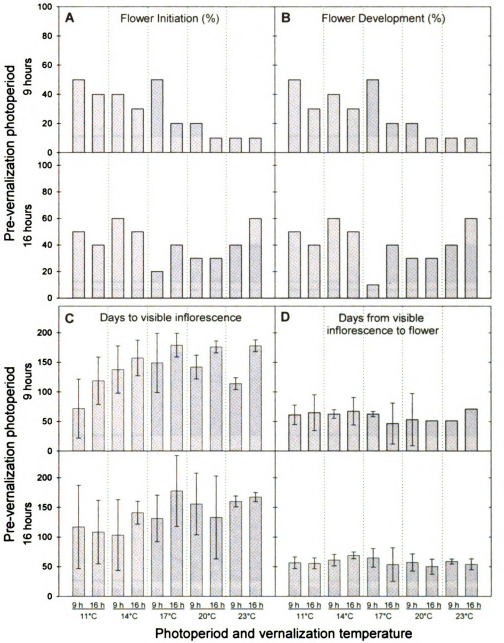

Figure 2. Responses of *Degarmoara* Winter Wonderland 'White Fairy' forced at 23 °C under a 16-h photoperiod after 9- or 16-h prevernalization photoperiods for 8 weeks and vernalization (11, 14, 17, 20, or 23 °C) under 9- or 16-h photoperiods for 8 weeks. Error bars represent 95% confidence intervals. Data for days to visible inflorescence (VI) are for the period from the end of vernalization until VI.

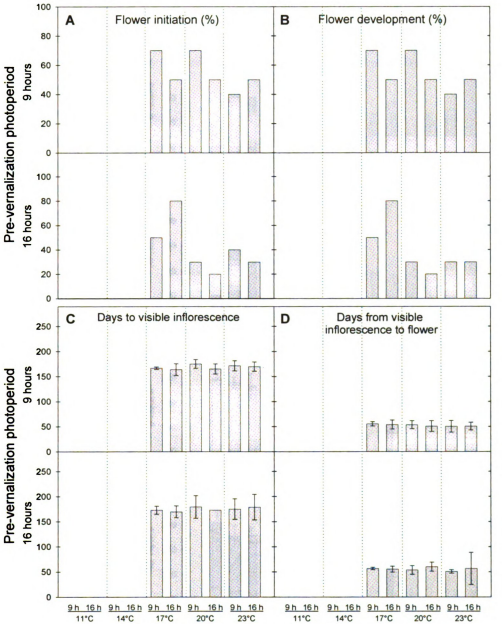

Figure 3. Responses of *Miltassia* Charles M. Fitch 'Dark Monarch' forced at 23 ºC under a 16-h photoperiod after 9- or 16-h prevernalization photoperiods for 8 weeks and vernalization (11, 14, 17, 20, or 23 ºC) under 9- or 16-h photoperiods for 8 weeks. Plants in the 11 and 14 ºC vernalization treatments were discarded due to severe chilling injury. Error bars represent 95% confidence intervals. Data for days to visible inflorescence (VI) are for the period from the end of vernalization until VI.

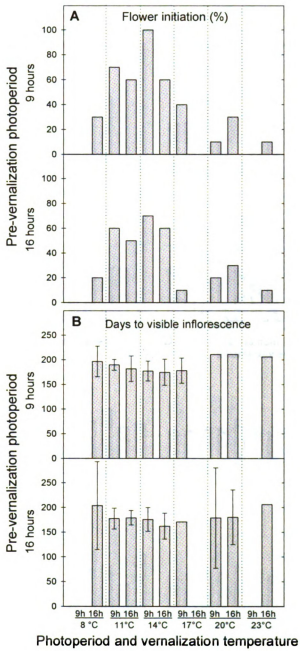

Figure 4. Responses of *Miltoniopsis* Augres 'Trinity' forced at 23 °C under a 16-h photoperiod after 9- or 16-h prevernalization photoperiods for 8 weeks and vernalization (8, 11, 14, 17, 20, or 23 °C) under 9- or 16-h photoperiods for 8 weeks. Error bars represent 95% confidence intervals.

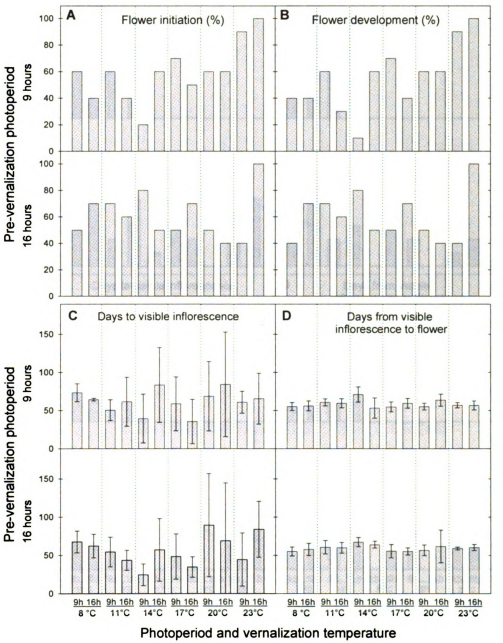

Figure 5. Responses of *Odontocidium* Tiger Crow 'Golden Girl' forced at 23 ºC under a 16-h photoperiod after 9- or 16-h prevernalization photoperiods for 8 weeks and vernalization (8, 11, 14, 17, 20, or 23 ºC) under 9- or 16-h photoperiods for 8 weeks. Error bars represent 95% confidence intervals. Data for days to visible inflorescence (VI) are for the period from the end of pre-vernalization until VI.

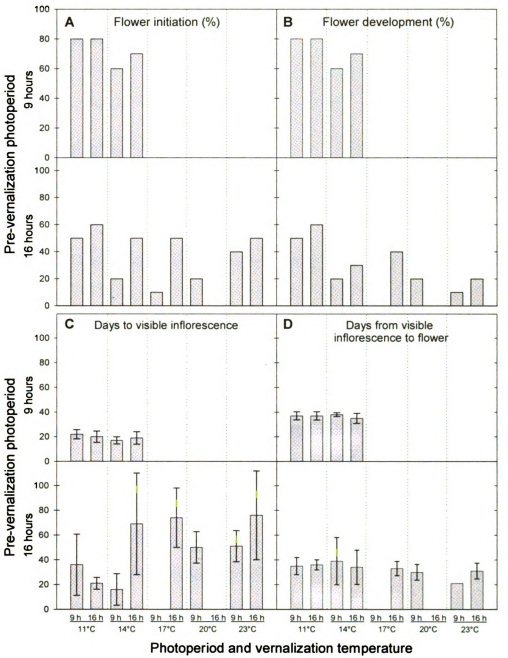

Figure 6. Responses of *Zygopetalum* Redvale 'Fire Kiss' forced at 23 °C under a 16-h photoperiod after 9- or 16-h prevernalization photoperiods for 8 weeks and vernalization (11, 14, 17, 20, or 23 °C) under 9- or 16-h photoperiods for 8 weeks. Error bars represent 95% confidence intervals. Data for days to visible inflorescence (VI) are for the period from the end of vernalization until VI.

SECTION IV

PHOTOPERIOD AND VERNALIZATION INFLUENCE FLOWERING OF MILTONIOPSIS AUGRES 'TRINITY' ORCHID

Photoperiod and Vernalization Influence Flowering of *Miltoniopsis* Augres 'Trinity' Orchid

Roberto G. Lopez[1] and Erik S. Runkle[2]

Department of Horticulture, Michigan State University, East Lansing, MI 48824

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[1]Graduate Student.

[2]Assistant Professor and Extension Specialist, to whom reprint requests should be addressed (E-mail: runkleer@msu.edu).

Introduction

Orchids have become the second most valuable flowering potted crop in the United States (USDA, 2003). According to the American Orchid Society, over 75% of all orchids sold in the United States are *Phalaenopsis* or the moth orchid (Griesbach, 2002). Currently, the wholesale price of flowering *Phalaenopsis* in a 15-cm pot is $8 to $12, with a retail price of $15 to $25 on the mass market (Wang, 2003). As the market becomes saturated with *Phalaenopsis* due to the widespread knowledge of its flower induction requirements, the price will likely decrease and the plant may become a commodity. Consequently, commercial growers and retailers are already seeking other potentially profitable orchids that have consumer appeal and can be programmed into flower for specific markets dates.

Miltoniopsis, or the pansy orchid, produces inflorescences that are adorned with three to six flat and large (7 cm), fragrant and showy flowers, which range in color from cream to pink, magenta, scarlet, and yellow. The flowers often last on the plant for four to eight at temperatures from 14 to 20 °C (Robinson, 2002). Due to these attributes, *Miltoniopsis* has become the fifth most valuable potted orchid produced commercially in the Netherlands, with 797,000 pots sold in 2001 (Barendse, 2002).

In its native habitat, *Miltoniopsis* is an epiphytic and lithophytic genus of six species distributed throughout the wet cloud forest regions (610 to 2,100 m) from Costa Rica to Peru (Baker and Baker, 1993; Morrison, 2000). The sympodial growth habit of this compact plant is distinguished from *Miltonia* by the

presence of a single leaf at the pseudobulb apex, which is surrounded by distinct leaf-like sheaths.

Several outstanding hybrids have been developed in recent years, which are mainly enjoyed by hobbyists. However, little or no information is currently available on how to induce *Miltoniopsis* into flower. In a previous study (Section III), flower initiation of *Miltoniopsis* Augres 'Trinity' was uniform and complete only when plants were exposed to 9-h photoperiods before and during vernalization at 11 to 14 °C. A similar response was observed in *Cattleya warscewiczii, C. gaskelliana,* and C. *mossiae;* flower induction occurred under continuous 9-h photoperiods at 13 °C, and flowering was inhibited when the photoperiod was 16 h (Rotor, 1952; Rotor, 1959). Both *Cattleya* and *Miltoniopsis* are epiphytes native to the humid and wet forests of Central and South America.

Further investigation is necessary to minimizing the production time of *Miltoniopsis* and to understanding at what development stage a pseudobulb van be induced to flower. Casual observations indicate that *Miltoniopsis* is only capable of producing an inflorescence when its pseudobulb(s) are swollen and mature. However, it is not known during what developmental stage (height or node number) an immature pseudobulb can be induced to produce an inflorescence once it becomes mature.

This information will assist greenhouse growers with developing a production schedule for complete, rapid and uniform flowering of *Miltoniopsis* Augres 'Trinity'. We performed experiments to determine how varying durations of two pre-vernalization photoperiods and how vernalization duration, influences

flowering of *Miltoniopsis*. Other objectives of this study were 1) to determine if short days are critical prior to vernalization; and 2) to determine if pseudobulb maturity influence flower characteristics such as flower initiation percentage or time to visible inflorescence.

Materials and Methods

Plant material. *Miltoniopsis* Augres 'Trinity' were grown by a commercial greenhouse (Calif.) at 16 to 26 °C under natural photoperiods (37 °N lat.) with a maximum photosynthetic photon flux (*PPF*) of ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. They were planted in a bark and perlite-based media in June 2002 (10-cm pots) and were received in East Lansing, Mich. on 22 July 2002. Plants were maintained at ≈23 °C in a glass-glazed greenhouse until experiments began. The photoperiod was a constant 16 h, consisting of natural daylengths (42 °N lat.) with day-extension lighting from high-pressure sodium (HPS) lamps, which delivered a supplemental *PPF* of ≈50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height [as measured with a line quantum sensor (Apogee Instruments, Inc., Logan, Utah)]. Light transmission through the greenhouses was reduced using a permanent woven shade curtain that reduced light by ≈55% (OLS 50; Ludvig Svensson, Charlotte, N.C.) and by applying whitewash (up to 50%) to the glass as needed so that the maximum *PPF* was ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Pseudobulb maturity experiment (Expt. 1). Plants were grown at 20 °C under 9-h short days (SD) or 16-h long days (LD) beginning on 26 July 2002 for 0, 4, 8, 12, or 16 weeks in a glass-glazed greenhouse. Photoperiods were

created by pulling opaque blackout cloth over plants between 1700 and 0800 HR, and for the 16-h photoperiod, light from incandescent lamps (delivering ≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was provided between 1700 and 2400 HR. From 0800 to 1700 HR, HPS lamps provided a supplemental *PPF* of ≈70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level when the ambient greenhouse *PPF* was <120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The plants were then cooled for eight weeks at 14 °C under SD in a controlled-environment chamber. The photoperiod was provided by a combination of cool-white fluorescent (VHOF96T12, Philips, Bloomfield, NJ) and incandescent lamps from 0800 to 1700 HR at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After the cooling treatment, plants were grown at 20 °C under a 16-h LD in the greenhouse

Vernalization duration experiment (Expt. 2). Plants were grown at 20 °C under SD or LD (as described above) beginning on 26 August 2002 for eight weeks. The plants were then cooled for 0, 3, 6, 9 or 12 weeks at 14 °C under SD in a glass-glazed greenhouse. After the cooling treatment, plants were grown at 20 °C under LD in the greenhouse. Light conditions were controlled as described above.

Greenhouse temperature and irradiance control. Temperature on each bench was measured by a thermocouple in an aspirated chamber every 10 s, and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, Utah). Average daily air temperature and light integral (DLI) were determined for each experiment from the beginning of the pre-vernalization photoperiod until the end of forcing (Table 1 and 2).

Plant culture. Plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special, Greencare Fertilizers, Chicago, IL).

Data collection and analysis. Ten plants were randomly assigned to each treatment. Immature and mature pseudobulb count, and height and node number of immature pseudobulbs, were recorded for Expt. 1 at the beginning of cool treatments. Immature pseudobulbs were between one and twenty-five centimeters in length (measured from the soil level to the apex). For Expt. 1 and 2, the date at which the first inflorescence was visible (visible inflorescence, or VI) without dissection and the number of inflorescences were recorded for each plant. The percentage of plants that initiated flowers and days to VI were calculated. The few plants that died during the experiments were discarded and not included in the results. A complete randomized design was used and data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED).

Results

Pseudobulb maturity experiment (Expt. 1). Flower initiation was most complete and uniform when plants were exposed to four or eight weeks of SD prior to vernalization (Table 3). Flower initiation never increased above 60% when plants were placed under LD prior to vernalization for any duration. Half of plants placed directly into the 14 °C chamber for eight weeks initiated flowers.

Under short days before vernalization, days to VI from the end of vernalization increased as pre-vernalization photoperiod duration increased. Inflorescence count was not influenced by any treatment provided.

The maturity of a pseudobulb significantly influenced the time to VI. For a mature pseudobulb, time to VI occurred in 29 d compared to 122 to 158 d for an immature pseudobulb (Table 4). Immature pseudobulbs < 7 cm did not initiate flowers within 30 weeks, when the experiment ended.

Vernalization duration experiment (Expt. 2). Increasing the duration of cold increased flower initiation percentage until 9 weeks of cooling (Table 5). Flower initiation was greatest (≥ 80%) when plants were placed under SDs prior to vernalization and vernalized for ≥ 9 weeks at 14 °C. Flower initiation was ≤ 40% when plants were not exposed to a 14 °C temperature treatment. Time to VI was not influenced by pre-vernalization photoperiod. As vernalization duration increased from 0 to 12 weeks, time to VI decreased from 165 to 67 d if SD were provided before cold. Inflorescence count was not influenced by photoperiod prior to vernalization or vernalization duration.

Discussion

The most complete, rapid and uniform flowering of *Miltoniopsis* Augres 'Trinity' occurred when plants with pseudobulbs that had just begun to swell and mature were placed under SD for four to eight weeks of SD then cooled at 14 °C for eight to twelve weeks under SD. These results are in agreement with results from a previous experiment (Section III) in which plants placed under eight weeks of SD and eight weeks of cooling had the highest flower initiation percentage

(100%). In a separate study, 90 to 100% of *Miltoniopsis* Augres 'Trinity' placed in environmental chambers with constant temperatures of 14, 17 or 20 °C under 9-h SD eventually flowered (Robinson, 2002). Robinson (2002) also found that time from VI to flower at 14, 17 and 20 °C under a 9-h photoperiod was 71, 70 and 61 days, respectively. Our results indicate that, production time can be reduced by four weeks, since four weeks of SD prior to cooling is adequate for rapid, complete and uniform flowering of *Miltoniopsis.*

These results also illustrate the importance of providing plants with SD before cold and SD during vernalization. Unfortunately, neither photoperiod nor cooling can be substituted or eliminated. Sixty percent or less of plants initiated flowers when not exposed to SD before cooling (Table 3), and only 30% flowered when not provided with a cool temperature treatment (Table 5). The importance of SD before cooling has also been observed in *Hatiora*, where plants exposed to LD before cooling (7.5 to 12.5 °C for four weeks) flowered poorly (58-73% flowering). *Hatiora* plants exposed to SD prior to cooling had 93 to 100% flowering (Rohwer, 2002).

Similar to other flowering plants, a juvenile phase exists in orchids and plants must reach a certain stage of growth before attaining the capacity to flower. Thus, sexual reproduction is delayed until plants reach a size sufficient to maintain the energetic demands of flowering and seed production. This period of juvenility varies among species and cultivars. In *Phalaenopsis,* for example, the inflorescence normally emerges from the third to fifth node below the apical leaf (Sakanishi et al. 1980; Yonda 1985).

In *Miltoniopsis*, pseudobulb maturity significantly influences flower initiation and time to VI in *Miltoniopsis.* Time to VI increases ≈100 d if the only inducible pseudobulb on a plant is immature. If an immature pseudobulb ≤ 6 cm in height is placed in an inductive treatment, according to our results, it is incapable of initiating flowers. However, immature pseudobulbs ≥ 7 can initiate flowers. Consequently, it is important that growers have uniform plant with nearly mature pseudobulbs to achieve the most uniform flowering of *Miltoniopsis.*

Miltoniopsis Augres 'Trinity' (*Miltonia* Pam-pam x *Miltonia* Alger) is a cool growing hybrid with optimal vegetative growth at temperatures between 20 to 23 ºC, although it can tolerate temperatures from 8 to 26º C. Flower size and quality were adversely affected by high light (> 400 $\mu mol \cdot m^{-2} \cdot s^{1}$) and high temperatures (> 20 ºC) (Robinson, 2002).

The numerous positive attributes of *Miltoniopsis* Augres 'Trinity' merit its consideration as a potted flowering plant for winter and spring holidays (Christmas, Valentine's Day, Easter and Mother's Day) when temperatures are not excessive for shipping and storage to preserve flower quality. Sales at these times would also facilitate production schedules for greenhouse growers in Northern climates as growers could utilize the natural short days and cool temperatures of autumn and winter for flower induction.

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Table 1. Average daily temperature and light integral during pre-vernalization, vernalization, and forcing of *Miltoniopsis* Augres 'Trinity' (Expt. 1).

Pre-vernalization duration (weeks)	Setpoint temperature (°C)					
	Pre-vernalization 20	Vernalization 14	Forcing 20	Pre-vernalization	Vernalization	Forcing
	Average temperature during each treatment (°C)			*Average daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)*		
0	-[z]	13.8	21.0	-	--[y]	5.4
4	22.8	13.8	21.0	7.9	--	5.2
8	22.8	14.1	21.1	5.9	--	5.8
12	22.5	14.0	21.0	4.6	--	6.4
16	21.8	14.0	21.1	4.0	--	6.8

[z]Pre-vernalization treatment not utilized (one dash).
[y]Not measured (two dashes).

Table 2. Average daily temperature and light integral during pre-vernalization, vernalization, and forcing of *Miltoniopsis* Augres 'Trinity' (Expt. 2).

Vernalization duration (weeks)	Setpoint temperature (°C)			Average daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)		
	Pre-vernalization	Vernalization	Forcing	Pre-vernalization	Vernalization	Forcing
	20	14	20			
	Average temperature during each treatment (°C)					
0	20.8	-[z]	21.1	8.2	-	6.0
3	20.8	14.4	20.9	8.2	2.0	6.1
6	20.8	14.1	21.0	8.2	2.1	6.3
9	20.8	14.1	21.0	8.2	2.5	6.6
12	20.8	14.1	21.1	8.2	2.5	7.0

[z]Vernalization treatment not utilized.

Table 3. The effect of pre-vernalization photoperiod and duration on days to visible inflorescence (VI) and inflorescence count of *Miltoniopsis* Augres 'Trinity' (Expt. 1). The pre-vernalization photoperiod was delivered at 20 °C. Plants were then vernalized for eight weeks at 14 °C under a 9-h photoperiod and subsequently forced at 20 °C under a 16-h photoperiod.

Pre-vernalization				
Photoperiod (h)	Duration (weeks)	Flower initiation (%)	Days to VI	Inflorescence count
-[z]	0	50	84	1.0
9	4	90	39	1.2
	8	100	65	1.3
	12	60	88	1.5
	16	60	89	1.5
16	4	40	61	1.3
	8	60	90	1.0
	12	60	64	1.3
	16	60	136	1.5
Significance				
Pre-vernalization photoperiod (PVP)			NS	NS
Pre-vernalization photoperiod duration (PVPD)			*	NS
PVP x PVPD			NS	NS

[z]Plants did not receive a photoperiod treatment prior to vernalization.
[NS, *]Nonsignificant or significant at $P \leq 0.05$.

Table 4. The effect of pseudobulb maturity and immature pseudobulb height on days to visible inflorescence in *Miltoniopsis* Augres 'Trinity' (Expt. 1).

Pseudobulb maturity	Immature pseudobulb height (cm)	Number of Plants	Flower initiation (%)	Days to visible inflorescence(VI)
Immature	6	15	0	-[z]
	7 - 12	30	47	158 ± 27
	13 - 25	25	52	122 ± 30
Mature	--[y]	56	57	29 ± 11
Significance				
Pseudobulb maturity				***
Immature pseudobulb height				***

[z]No pseudobulbs at this height initiated flowers.
[y]Height of mature pseudobulbs not measured.
***Significant at $P \leq 0.001$.

Table 5. The effect of pre-vernalization photoperiod and vernalization duration on days to visible inflorescence and inflorescence count of *Miltoniopsis* Augres 'Trinity' (Expt. 2). Pre-vernalization photoperiods were provided at 20 °C for eight weeks. Plants were then vernalized at 14 °C under a 9-h photoperiod and subsequently forced at 20 °C under a 16-h photoperiod.

Pre-vernalization photoperiod (h)	Vernalization duration (weeks)	Flower initiation (%)	Days to visible inflorescence	Inflorescence count
9	0	30	165	1.7
	3	30	184	1.3
	6	60	111	1.2
	9	90	82	1.4
	12	80	67	1.6
16	0	40	182	1.3
	3	30	169	1.3
	6	60	143	1.2
	9	60	154	1.3
	12	60	97	2.1
Significance				
Pre-vernalization photoperiod (PVP)			NS	NS
Vernalization duration (VD)			***	NS
PVP x VD			NS	NS

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

SECTION V

THE EFFECT OF TEMPERATURE ON LEAF AND FLOWER DEVELOPMENT AND FLOWER LONGEVITY OF ZYGOPETALUM REDVALE 'FIRE KISS' ORCHID

The Effect of Temperature on Leaf and Flower Development and Flower Longevity of *Zygopetalum* Redvale 'Fire Kiss' Orchid

Roberto G. Lopez[1] and Erik S. Runkle[2]

Department of Horticulture, Michigan State University, East Lansing, MI 48824

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[1]Graduate Student.

[2]Assistant Professor and Extension Specialist, to whom reprint requests should be addressed (E-mail: runkleer@msu.edu).

Introduction

In the United States, potted flowering plants are commonly purchased for holidays such as Christmas, Mother's Day, Easter, and Valentine's Day. Thus, greenhouse growers must be able to produce flowers (either for cut flowers or as potted plants) to meet specific market dates. Plants that do not have flowers, or have flowers that are too immature (e.g., only flower buds) or mature (e.g., flowers are all open), are often not sold or sold for a lower price.

Potted flowering orchids are produced in vast quantities throughout the world. In 1995, the world demand for potted orchids was estimated at 1.22 billion units of plant stock with a predicted increase of four percent over the next five years (Hew and Yong, 1997). Orchids are the second most valuable flowering potted crop in the United States, with an estimated wholesale value of approximately $105.6 million (USDA, 2003). However, the flowering process of the majority of orchid species is understood poorly, or not at all. Some notable exceptions include *Phalaenopsis*, *Cattleya*, *Cymbidium*, and *Dendrobium*. In these genera, low temperatures, short photoperiods, or both, regulate the flowering process (Rotor, 1952; Sakanishi et al., 1980; Ichihashi, 1997).

The *Orchidaceae* is the largest and most diverse of all plant families. Approximately ten percent of all flowering plant species are orchids, which comprise 20,000 to 25,000 species grouped into 725 genera (Griesbach, 1985). In addition to the natural abundance of orchids, there are over 100,000 man-made hybrids. The commercial potential of the vast majority has not yet been explored. Important considerations for the introduction of new floricultural crops

include showiness of flower display, ease and length of production, crop uniformity and size, compact size, consumer appeal, and post-production flower longevity.

Zygopetalum, or the ladybird orchid, is a sympodial terrestrial and epiphytic South American genus comprised of 20 species. They are native to neotropic mid-elevation mountains (1,300 to 1,700 m) of Brazil, Guiana, Venezuela and Peru (Rose, 1993). *Zygopetalum* Redvale 'Fire Kiss' is moderately compact (25- to 40-cm tall) and produces several broad and leathery leaves atop mature green pseudobulbs. Four or more leaf-like sheathing bracts, which grow from the base, protect the immature pseudobulbs but these bracts become dry and fibrous with age (Baker and Baker, 1991). The erect inflorescences emerge from the third, forth or fifth sheathing bract of immature pseudobulbs. Lime-green and dark maroon sepals and petals, featuring broad three-lobed labellums in deep magenta and white, characterize the exotic, fragrant and waxy flowers.

Scheduling a crop such as *Zygopetalum* to flower on specific market dates requires knowledge of the relationship between temperature and time to flower. Temperature is one of the critical factors controlling plant developmental processes, such as flowering. Consequently, modeling plant development could help greenhouse growers with scheduling of flowering crops. At temperatures below a species-specific minimum, known as the base temperature (T_b), time to flower (days) is infinite. As temperature increases rise above an optimum

temperature (T_{opt}) flowering is delayed. Base and optimum temperatures vary within and between species and are related to climatic origins.

The reciprocal is often taken for developmental processes such as leaf unfolding or time to flower. The relationship between mean temperature and rate of development towards flowering (1/days, where days is the days to flower) is often linear between T_b and T_{opt} (Roberts and Summerfield, 1987) and can be described as follows:

$$1/\text{days} = b_o + b_1 T \quad [1]$$

Using constants b_o and b_1, T_b and the thermal time required for flowering (degree-days, or °days) can be calculated as follows:

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

$$^{o}\text{days} = 1/b_1 \quad [3]$$

Temperature not only influences time to flower, but also plant architecture and flower longevity. For example, in *Antirrhinum majus* L. 'Jackpot', time from germination to flower initiation, visible bud, first floret, and harvest increased as temperature decreased from 21 to 5 °C (Maginnes and Langhans, 1961). Flowers of *Lysimachia congestiflora* Hemsl. lasted longer when grown at 18 °C compared to those at 26 °C (Zhang et al., 1995). Node development of Dahlia increased linearly as temperature increased from 10 to 24.6 °C with a maximum leaf pair unfolding rate of 0.29 leaf/day at 24.6 °C (Brøndum and Heins, 1993).

Information on the time required to reach a developmental stage is critical to developing crop production schedules. The objectives of this study were to

determine the relationship between temperature and 1) leaf unfolding rate (LUR), 2) leaf expansion, 3) time to flower, 4) flower longevity of the first open flower and 5) inflorescence longevity of *Zygopetalum* orchids.

Materials and Methods

Plant material. *Zygopetalum* Redvale 'Fire Kiss,' plants were received and grown by a commercial greenhouse (Calif.), transplanted into 38-cell plug trays in June 2000, and then into 10-cm pots in April 2001. In Calif., plants were grown at 16 to 26 °C under natural photoperiods (37 °N latitude) with a maximum photosynthetic photon flux (*PPF*) of ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Five hundred plants in a bark and perlite-based media (10-cm pots) were received in East Lansing, Mich. on 6 May 2001. They were maintained at ≈23 °C in a glass-glazed greenhouse until experiments began. The photoperiod was a constant 16 h (0600 to 2200 HR), consisting of natural daylengths (42 °N latitude) with day-extension lighting from high-pressure sodium (HPS) lamps, which delivered a supplemental *PPF* of ≈50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height [as measured with a line quantum sensor (Apogee Instruments, Inc., Logan, Utah)].

Plant culture. In Year 1, Plants were irrigated as necessary with well water (containing 95, 34, and 29 $mg \cdot L^{-1}$ Ca, Mg, and S, respectively) supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 125 K, 13 Ca, 1.0 Fe, 0.1 B and Mo, and 0.5 Mn, Zn, and Cu (MSU Special, Greencare Fertilizers, Chicago, IL). Water was acidified with H_2SO_4 to a titratable alkalinity of ≈140 $mg \cdot L^{-1}$ $CaCO_3$. In Year 2, plants were irrigated as

necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B and 0.1 Mo.

Environmental chamber temperature and photoperiod control. All experiments were performed in walk-in controlled-environment chambers with constant temperature set points of 14, 17, 20, 23, and 26 °C. In Year 1 of Expts. 1 and 3, an additional chamber set at 29 °C was used. In Expt. 1, Year 1, the 23 °C treatment was provided in a greenhouse. Air temperature in each chamber was measured by an aspirated thermocouple every 10 s, and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, Utah). The average daily air temperatures for all experiments were calculated (Table 1). For Expt. 1, Year 1, each chamber was divided in half with black plastic and two photoperiods were created: 9-h of light with or without a 4-h (2200 to 0200 HR) night interruption (NI). The 9-h base photoperiod was provided by a combination of cool-white fluorescent (VHOF96T12, Philips, Bloomfield, NJ.) and incandescent lamps from 0800 to 1700 HR at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Night interruption lighting (≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) was provided by incandescent lamps. In Expt. 1, Year 2, only a 9-h photoperiod was provided since photoperiod did not influence leaf development in Year 1. In Expt. 2 and 3, only 9-h photoperiods were provided.

Leaf development (Expt.1). The experiment was replicated in time, beginning on 12 June 2001 (Year 1) and 19 February 2002 (Year 2). Experimental treatments were identical between years unless otherwise noted.

Ten plants were assigned randomly to the SD and NI sections of the walk-in controlled-environment chamber for Expt. 1. At the beginning of the experiment, one immature pseudobulb was identified and leaf unfolding was recorded weekly by counting the number of leaves from the soil level to the terminal end of the pseudobulb. The leaf length of a marked immature leaf was measured weekly and leaf expansion rate was calculated. Plants were grown for 20 weeks, except for those at 29 °C, in which observations were terminated after 13 weeks due to declining growth and plant mortality.

Flower development (Expt. 2). The experiment was replicated in time, beginning on 12 April 2002 (Year 1) and 19 March 2003 (Year 2) with ten plants per treatment. Experimental treatments were identical between years unless otherwise noted. Plants were grown in a glass greenhouse and on the date that VI was first visible (< 1 cm long), plants were transferred to one of the five chambers until ten plants were in each chamber. The date each plant was placed in the chamber and the date of the opening of first and subsequent flowers were recorded for each plant. At flowering, the number of flower buds and nodes on immature pseudobulbs below the inflorescence were counted and inflorescence length was measured. Days to flower and air temperature during that period were calculated for each plant.

Flower and inflorescence longevity (Expt. 3). The experiment was replicated in time, beginning on 8 August 2001 (Year 1) and 14 February 2002 (Year 2). Experimental treatments were identical between years unless otherwise noted. Plants were grown in a glass greenhouse and on the date that

the first flower of an inflorescence opened on each plant, it was transferred to one of the chambers. The date that each plant was placed in the chamber and the date of flower mortality (senescence) of the first and subsequent flowers were recorded for each plant. Individual flower and inflorescence longevity were calculated for each plant at each temperature.

Data analysis. The experimental designs for both years were completely randomized. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED) for analysis of variance and linear models procedure (PROC REG) for regression models. Data were pooled in each experiment for all measured characteristics. Base temperature (T_b) and thermal time (°days), were calculated (Roberts and Summerfield, 1987). The mean time to flower or leaf unfolding rate (LUR) and flower and inflorescence longevity at each temperature were converted to rates by taking the reciprocal (1/days). The relationship between rate of progress to flowering, leaf development or senescence (1/days) and mean temperature *T* in °C were determined by Equations 1, 2, and 3. Within each developmental stage, slopes and intercepts were computed.

Results

Leaf development (Expt. 1). Photoperiod (9-h or NI) had no significant influence on leaf unfolding or leaf expansion and thus data were pooled. Leaf unfolding increased as temperature increased to ≈25 °C, and then began to decrease (Fig. 1). The days required to produce one leaf averaged 46 d at 14 °C

and 19 d at 25 °C. The leaves of plants unfolded as a linear function until ≈25 °C (Table 2). The base temperature for LUR was estimated at 6.2 °C and the °days required to develop one leaf was 357 °C·d^{-1} (Table 2).

Leaf expansion was significantly influenced by temperature (significantly different at $P \leq 0.001$). As temperature increased from 13 to 29 °C, leaf expansion increased (Fig 2). Leaves became mature earlier at warmer temperatures and, thus the increase in leaf length slowed earlier at the higher temperatures. For example, leaf expansion at 28.6 °C slowed earliest, after less than ≈80 d, and at 13.4 °C, ≥ 120 d elapsed following the start of the experiment before expansion slowed.

Flower development. Time from VI to anthesis decreased with increasing temperature (Fig. 3). Time from visible inflorescence to flower averaged 73 d at 14 °C, and 28 d at 26 °C. Increasing the temperature by 2.1 °C from 17.4 to 19.5 °C accelerated flowering more than increasing the temperature 2.5 °C from 19.5 to 22.0 °C. For example, days from visible inflorescence to flower decreased from an average of 67 to 43 d (24 d or 11.4 d per degree) as temperature increased from 17.4 to 19.5 °C, but only decreased from 43 to 33 d (10 d or 4 d per degree) as temperature increased from 19.5 to 22.0 °C.

Rate of progress from VI to flowering was linear within the range of temperatures tested (Fig. 3). The base temperature was estimated at 6.4 °C for time from VI to flower and estimated thermal time was 556 °C·d^{-1} degree-days (Table 2). Flower bud count of each inflorescence was not significantly

influenced by temperature (data not presented). Average inflorescence length varied among the temperatures, but there were no apparent trends (Fig. 4).

Flower and inflorescence longevity. Flower and inflorescence senescence occurred more rapidly as temperature increased (Fig. 5). As temperature increased from 14 to 29 °C, longevity of the first open flower decreased from 37 d to 13 d. Inflorescence longevity followed a similar pattern, decreasing from 38 d at 14 °C to 15 d at 29 °C. Flower longevity of the second and subsequent flowers decreased by one to three days in comparison to the longevity of the first open flower at all temperatures studied (data not presented).

Flower and inflorescence longevity data were converted to rates, and a thermal time model was developed to predict senescence at different temperatures. Rate of progress to senescence of individual flowers and inflorescences was linear within the range of temperatures tested (Table 2). The base temperatures were estimated at 3.7 and 3.6 °C and the accumulated thermal time lifespan was 370 and 385 $°C \cdot d^{-1}$, respectively.

Discussion

As temperature increased from 14 to 26 °C, there was an increase in the rate of progress to flowering or senescence (and therefore a decrease in time to flowering or flower longevity). Higher temperatures also caused more rapid LUR and leaf expansion. Relative growth rate increased linearly to about ≈25 °C, declining thereafter. The optimum temperature for *Zygopetalum* appears to be ≈25 °C for both LUR and time to flower. However, at this temperature some

flower buds acquired necrotic lesions and aborted soon after. Overall, plants forced at cooler temperatures ≤ 20 °C were developmentally slower, had taller inflorescences, and were more aesthetically attractive.

Similar results with *Phalaenopsis* were observed by Robinson (2002) who found that as temperature increased from 14 to 26 °C, the rate of flower, leaf, and bud development for several cultivars increased linearly. The optimal temperature for this orchid, which is native to tropical and subtropical areas of the South Pacific Islands and Asia, appeared to be 26 °C, but plants performed best at ≈23 °C (Robinson, 2002). The base temperature for a given phenophase of *Phalaenopsis* was between 8 and 12 °C, which is 4 to 6 °C higher than that reported here for *Zygopetalum*. This is not a surprise, since *Zygopetalum* are native to neotropic mid-elevation mountains (1,300 to 1,700 m) that have cooler climates.

Zygopetalum Redvale 'Fire Kiss' can be flowered and scheduled for specific market dates by manipulating greenhouse temperature. Leaf development and flower longevity were modeled as a function of temperature and were found to accurately predict the time of these developmental processes. To complete a developmental process, a plant must experience a specific number of units of degree-days or thermal time above the base temperature characteristic of that process (Roberts and Summerfield, 1987). Thermal time is often used to schedule planting and harvesting of fruit and vegetable crops. It can also be utilized in commercial greenhouse environments, which often have fluctuating temperatures, to predict flowering dates or leaf development. If the

average daily temperature is T_a, the days necessary to complete a developmental process can be calculated by °days/(T_a -T_b). Using this method, the time required to complete a developmental stage can be calculated for a species when the average daily temperature is known. For example, time from visible inflorescence to flower for *Zygopetalum* forced at 14 °C (T_b = 6.4 °C) is estimated at (556 degree-days/7.6 °C) = 73 d. In comparison, actual time from visible inflorescence to flower for plants forced at 14 °C was 75 d. Flower longevity is also accurately predicted with a model; plants grown at 14 °C (T_b = 3.7 °C) have an estimated accumulated thermal time lifespan of (370 degree-days/10.3 °C) = 36 d. Actual flower longevity observed was 37 d.

The life span of orchid flowers varies considerably among genera. *Phalaenopsis* flowers may last for months, while other orchid flowers are ephemeral, lasting only one day. The full bloom period of an inflorescence depends partly on the number of flowers and the longevity of individual flowers. Factors such as air pollution, physical damage to the plant or flower, slight disturbances to the pollinia or anther caps, pollination, temperature and ethylene (C_2H_4) can considerably accelerate the senescence of flowers (Goh and Arditti, 1985).

Thermal time and base temperatures can be used to predict and help maximize the flower life of potted flowering plants for consumers. Achieving optimal shipping, storage and handling temperatures between production greenhouses and floral shops, grocery stores or nurseries could significantly improve flower longevity (personal observation). This information could then be

conveyed to consumers to give them optimal satisfaction with their purchase. In this experiment, temperatures in the range of 14 to 29 °C were tested, the T_{opt} for flower longevity was not observed and thus is < 14 °C. Potted *Zygopetalum* shows signs of chilling injury at temperatures ≤ 8 °C (Section III) and thus, the T_{opt} is likely between 8 and 14 °C. When plants were transferred from cool temperatures (≤ 17 °C) to warmer temperatures (≥ 23 °C), the floral labellum often wilted within a few hours and reduced the aesthetic value and beauty of the inflorescence . Consequently, sudden and large changes in temperature are not recommended for *Zygopetalum* as they can adversely affect flower quality.

In summary, *Zygopetalum* perform best when grown and forced at temperatures between 20 to 23 °C. Although plants grown at ≈23 °C produced leafs approximately two days slower than those grown at 26 °C, overall plant appearance was visually enhanced at 23 °C as leaves did not have necrotic lesions. Forcing temperatures above 23 °C are not recommended as flower quality is adversely affected. Flower and inflorescence longevity of *Zygopetalum* placed in the average home or office (≈20 °C) is estimated at ≈23 d if prior conditions (shipping and retail handling) were not extreme and light and water are adequately provided.

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Table 1. Actual average air temperatures of environmental chambers or (greenhouse) for each experiment.

Experiment	Year	Setpoint temperature (°C)					
		14	17	20	23	26	29
		Average air temperature during experiments (°C)					
1	1	13.4	16.7	19.5	22.4	24.8	28.6
	2	13.4	17.3	19.5	22.4	25.5	-[y]
2	1	13.5	17.4	19.5	22.0	25.5	-
3	1	13.4	17.1	19.5	25.9[x]	25.4	28.6
	2	13.3	17.4	19.6	22.3	25.5	-

[z]NI = 9-h photoperiod plus 4-h night interruption.
[y]Temperature not utilized.
[x]Plants were forced in the greenhouse.

Table 2. Parameters of linear regression analysis relating forcing temperature to rate of progress for leaf development, from visible inflorescence to flower, flower longevity of the first flower and inflorescence longevity in *Zygopetalum* Redvale 'Fire Kiss'. Intercept and slope were used in Eqs. [1] and [2] to calculate base temperature (T_b) and thermal time to complete a particular developmental stage (°C·d^{-1}).

Developmental stage	Intercept (b_o) (1/days)	Slope (b_1) (1/days)/ °C	T_b (°C)	°C·d^{-1}	r^2
Leaf unfolding	-0.0174 ± 0.0038[z]	0.0028 ± 0.0002	6.2	357	0.60[***y]
Visible inflorescence to flower	-0.0116 ± 0.0023	0.0018 ± 0.0001	6.4	556	0.85[***]
Longevity of first flower	-0.0100 ± 0.0047	0.0027 ± 0.0002	3.7	370	0.66[***]
Inflorescence longevity	-0.0094 ± 0.0042	0.0026 ± 0.0002	3.6	385	0.69[***]

[z]Standard error.
[y] [***]Significant at $P \leq 0.001$.

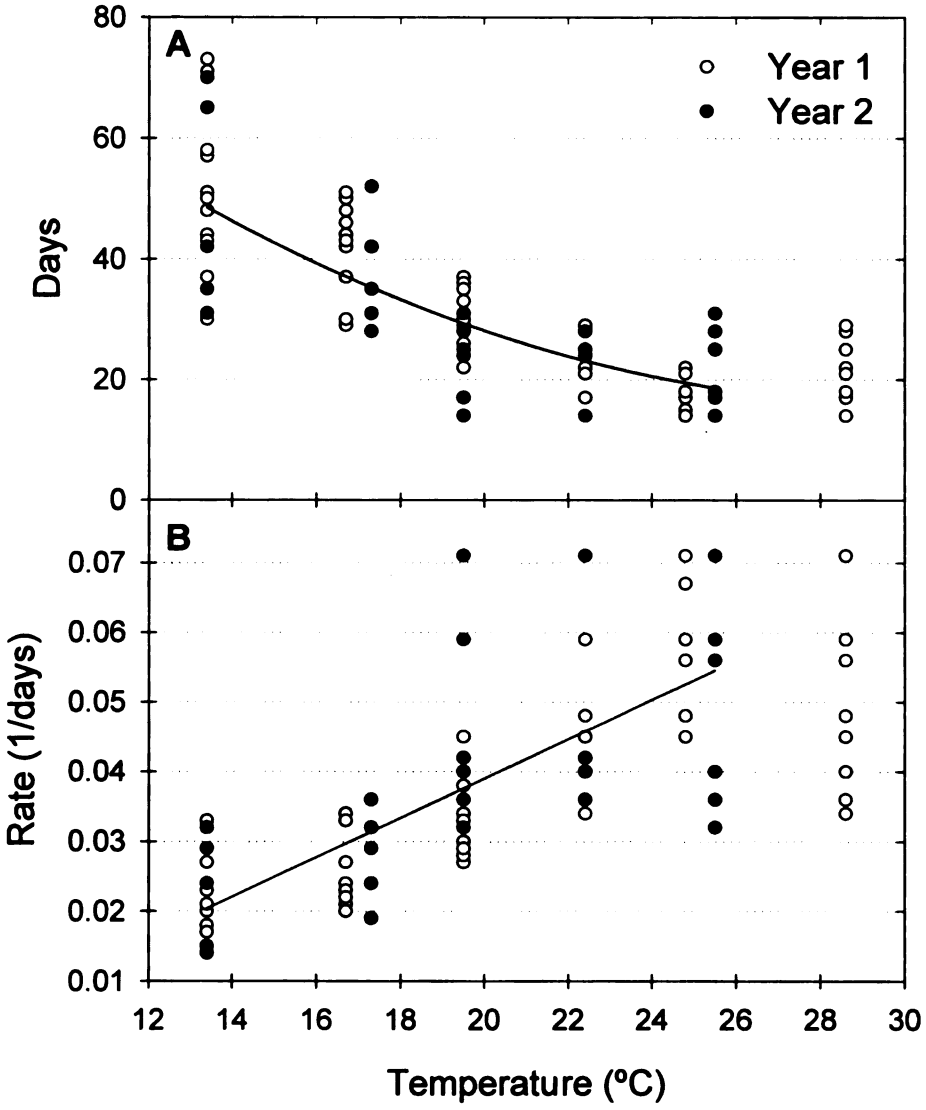

Figure 1. Influence of forcing temperature on leaf unfolding in *Zygopetalum* Redvale 'Fire Kiss'. Each symbol represents individual plants. (**A**) shows days for the indicated developmental stage. The solid lines represent the regression equation using pooled data for Year 1 and 2. Data at 28.6 °C were not included in the regression equation. Lines in (**B**) represent predicted values for the rate of progress (1/days) to leaf unfolding, based on linear regression. Statistical analysis is presented in Table 2.

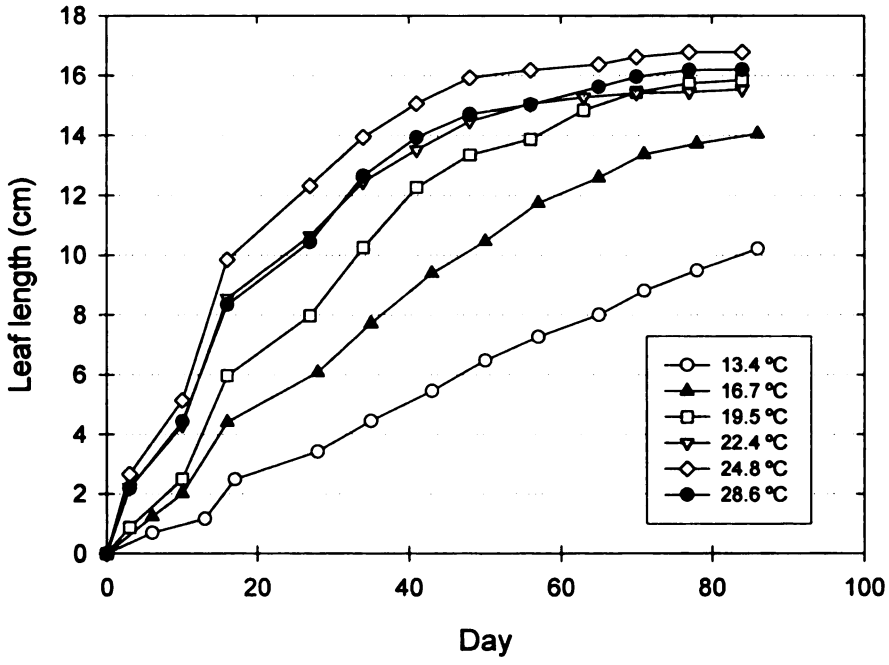

Figure 2. Influence of forcing temperature on daily increase in leaf length (cm) in *Zygopetalum* Redvale 'Fire Kiss'. Each symbol represents the average of 20 plants.

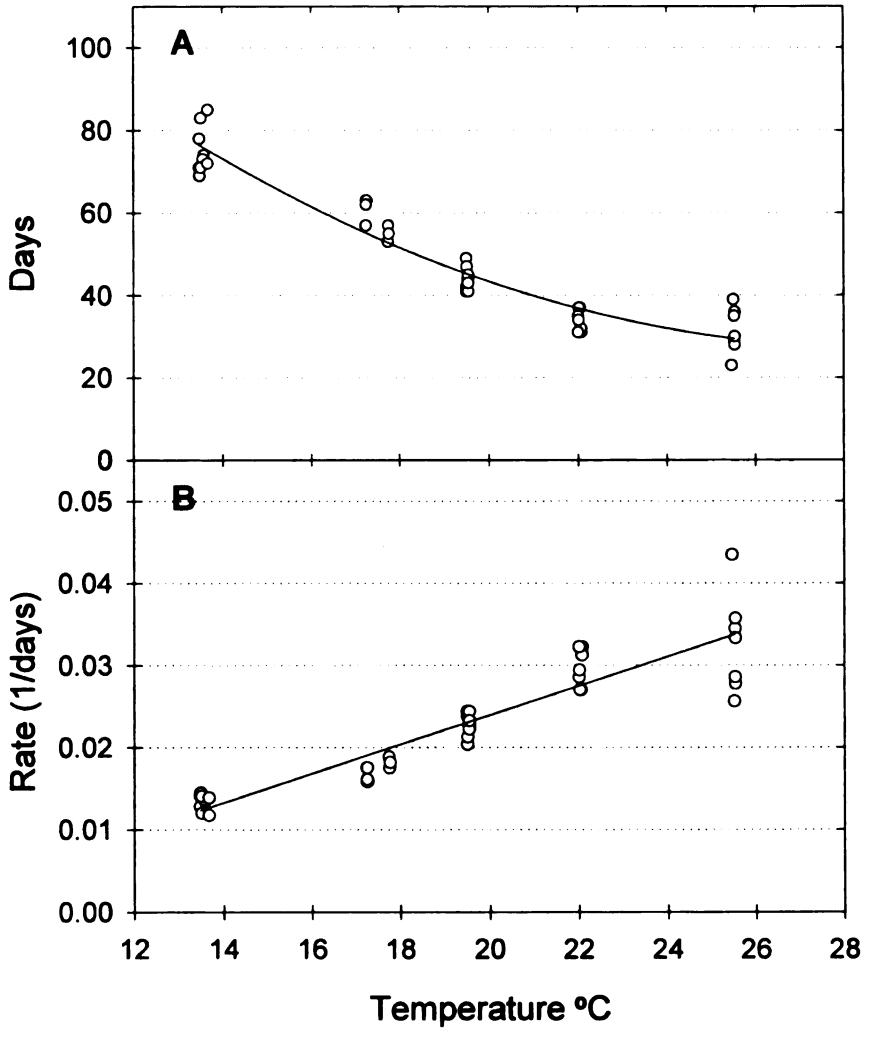

Figure 3. Influence of forcing temperature on time from visible inflorescence to flower in *Zygopetalum* Redvale 'Fire Kiss'. Each symbol represents individual plants. (**A**) shows days for the indicated developmental stage. The solid lines represent the regression equation using pooled data for Year 1 and 2. Lines in (**B**) represent predicted values for the rate of progress (1/days) to flowering, based on linear regression. Statistical analysis is presented in Table 2.

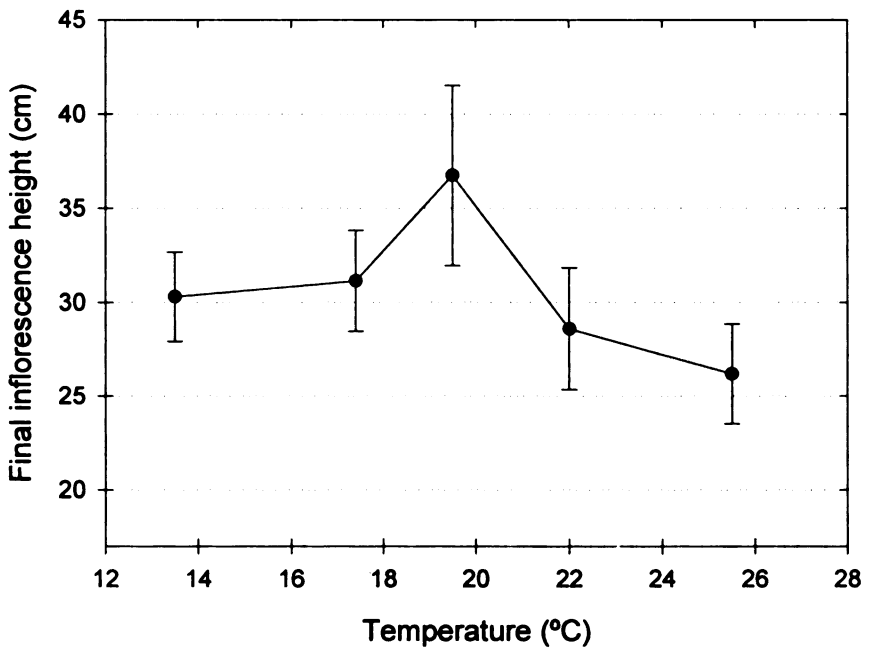

Figure 4. Influence of forcing temperature on inflorescence length (cm) in *Zygopetalum* Redvale 'Fire Kiss'. The symbol represents the average of 10 plants. Error bars represent 95% confidence intervals.

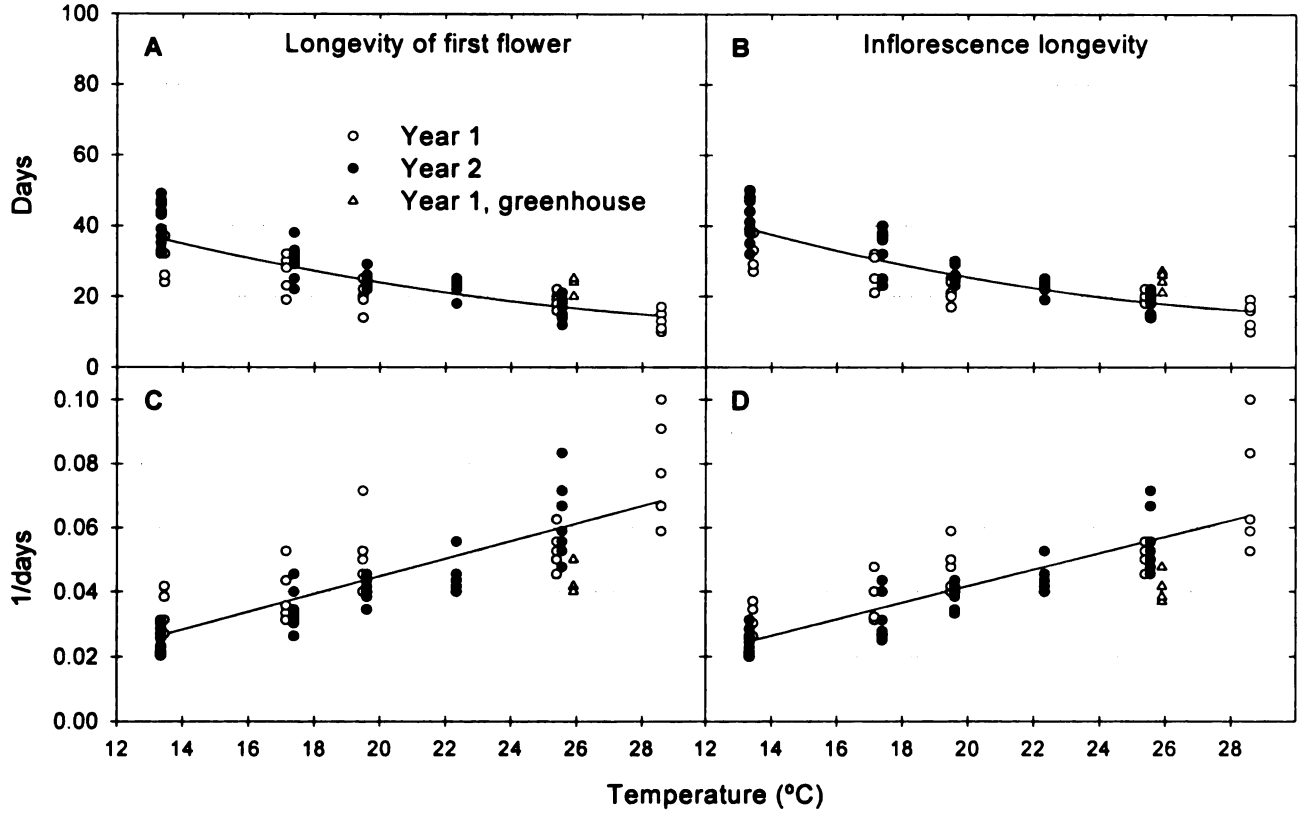

Figure 5. Influence of forcing temperature on longevity of the first open flower and inflorescence of *Zygopetalum* Redvale 'Fire Kiss'. Each symbol represents individual plants. (**A**) and (**B**) show days for the indicated developmental stage. The solid lines represent the regression equation using pooled data for Year 1 and 2. Lines in (**C**) and (**D**) represent predicted values for the rate of progress (1/days) to the indicated developmental stage based on linear regression. Statistical analysis is presented in Table 2.

APPENDIX A

THE EFFECT OF TEMPERATURE ON FLOWER AND INFLORESCENCE LONGEVITY OF MILTASSIA CHARLES M. FITCH 'DARK MONARCH' ORCHID

Research Objective

The research objective of this investigation was to determine the flower longevity of the first open flower and total inflorescence longevity of *Miltassia* Charles M. Fitch 'Dark Monarch' at temperatures from 14 to 26 °C.

Materials and Methods

Plant material. *Miltassia* Charles M. Fitch 'Dark Monarch' were grown by a commercial greenhouse (Calif.), transplanted into plug liners in June 1998, and into 10-cm pots in June 2000. In Calif., plants were grown at 16 to 26 °C under natural photoperiods (37 °N latitude) with a maximum photosynthetic photon flux (*PPF*) of ≈350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants in a bark and perlite-based media (10-cm pots) were received in East Lansing, Mich. on 6 May 2001. They were maintained at ≈23 °C in a glass-glazed greenhouse until experiments began. The photoperiod was a constant 16 h, consisting of natural daylengths (42 °N latitude) with day-extension lighting from high-pressure sodium (HPS) lamps, which delivered a supplemental *PPF* of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height [as measured with a line quantum sensor (Apogee Instruments, Inc., Logan, Utah)].

Plant culture. Plants were irrigated as necessary with well water (containing 95, 34, and 29 $mg \cdot L^{-1}$ Ca, Mg, and S, respectively) supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 125 K, 13 Ca, 1.0 Fe, 0.1 B, and Mo, and 0.5 Mn, Zn, and Cu (MSU Special, Greencare

Fertilizers, Chicago, IL). Water was acidified with H_2SO_4 to a titratable alkalinity of 140 $mg \cdot L^{-1}$ $CaCO_3$.

Environmental chamber temperature and photoperiod control. The experiment began on 9 October 2001 and was performed in walk-in controlled-environment chambers with constant temperature set points of 14, 17, 20, 23, and 26 °C. The average daily air temperatures during the experiment were 13.3, 17.3, 19.6, 22.5, and 25.5 °C. A 9-h photoperiod was provided by a combination of cool-white fluorescent (VHOF96T12, Philips, Bloomfield, NJ.) and incandescent lamps from 0800 to 1700 HR at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Flower and inflorescence longevity. Plants were forced in a glass greenhouse and on the date that the first flower of an inflorescence opened, each was transferred to one of the chambers. The date the plant was placed in the chamber and the date of flower mortality (senescence) of the first and subsequent flowers were recorded for each plant. Individual flower and inflorescence longevity were calculated for each plant at each temperature.

Data analysis. The experimental design was completely randomized. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED) for analysis of variance and linear models procedure (PROC REG) for regression models. Base temperature (T_b) and thermal time, or degree-days, were calculated (Roberts and Summerfield, 1987). Flower and inflorescence longevity at each temperature were converted to rates by taking the reciprocal, and the relationship between the rate of progress to senescence (1/days) and the mean temperature T in °C was determined by

$$1/\text{days} = b_0 + b_1 T \quad [1]$$

where b_0 and b_1 are constants. Once b_0 and b_1 were calculated, the base temperature, T_b, was calculated as

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

and thermal time or degree days (°C d), was determined by

$$^{\circ}\text{days} = 1/b_1 \quad [3]$$

Within each developmental stage, slopes and intercepts were computed.

Results and Discussion

Flower and inflorescence senescence occurred more rapidly as temperature increased (Figure 1A and B). As temperature increased from 17 to 26 °C, longevity of the first open flower decreased from 66 to 27 d, respectively. Inflorescence longevity followed a similar pattern, decreasing from 69 d at 17 °C to 32 d at 26 °C. Flower longevity of the second and subsequent flowers decreased in comparison to the longevity of the first open flower across all temperatures (data not presented). Plants at 14 °C were not included in the regression analysis due to severe chilling injury that affected both the plant and the flowers.

Flower and inflorescence longevity data were converted to rates, and a thermal time model was developed to predict flower senescence at different temperatures. Rate of progress to flower and inflorescence senescence was linear within the range of temperatures tested (Table 1). The base temperatures

were estimated at 12.6 (flower) and 11.4 °C (inflorescence) and accumulated thermal time lifespan of 385 and 455 °C·d^{-1}, respectively.

Flower longevity is accurately predicted with the model; plants grown at 26 °C have an estimated accumulated thermal time lifespan of 29 d. Actual flower longevity observed was 27 d.

In this experiment, the longevity of *Miltassia* flowers and inflorescences was greatest at 17 °C. However, potted *Miltassia* shows signs of chilling injury (water soaked lesions) at temperatures ≤ 17 °C (Section III) and thus, T_{opt} is likely between 18 and 20 °C. Consequently, it is important that *Miltassia* be shipped and stored at 20 °C for optimal flower and inflorescence longevity and to avoid damage to the sensitive foliage.

Base temperature and thermal time information is important for both greenhouse growers and hobbyists to obtain maximum flower longevity, as they often grow several species within the same environmental conditions. An example of two orchids with completely different minimum temperature limits (T_b) is that of *Zygopetalum* Redvale 'Fire Kiss' and *Miltassia* Charles M. Fitch 'Dark Monarch'. *Zygopetalum* is an epiphytic orchid from the neotropic mid-elevation mountains of South America with a base temperature for flower longevity of 3.7 °C, while the warm season orchid, *Miltassia* has a base temperature of 12.6 °C.

Table 1. Parameters of linear regression analysis relating forcing temperature to rate of progress to flower longevity of the first flower and inflorescence longevity in *Miltassia* Charles M. Fitch 'Dark Monarch'. Intercept and slope were used in Eqs. [1] and [2] to calculate base temperature (T_b) and thermal lifespan ($°C \cdot d^{-1}$).

Developmental stage	Intercept (b_o) (1/days)	Slope (b_1) (1/days)/ °C	T_b (°C)	$°C \cdot d^{-1}$	r^2
Longevity of first flower	-0.0329 ± 0.0059[z]	0.0026 ± 0.0003	12.6	385	0.83[***y]
Inflorescence longevity	-0.0251 ± 0.0055	0.0022 ± 0.0003	11.4	455	0.80[***]

[z]Standard error.
[y] [***]Significant at $P \leq 0.001$.

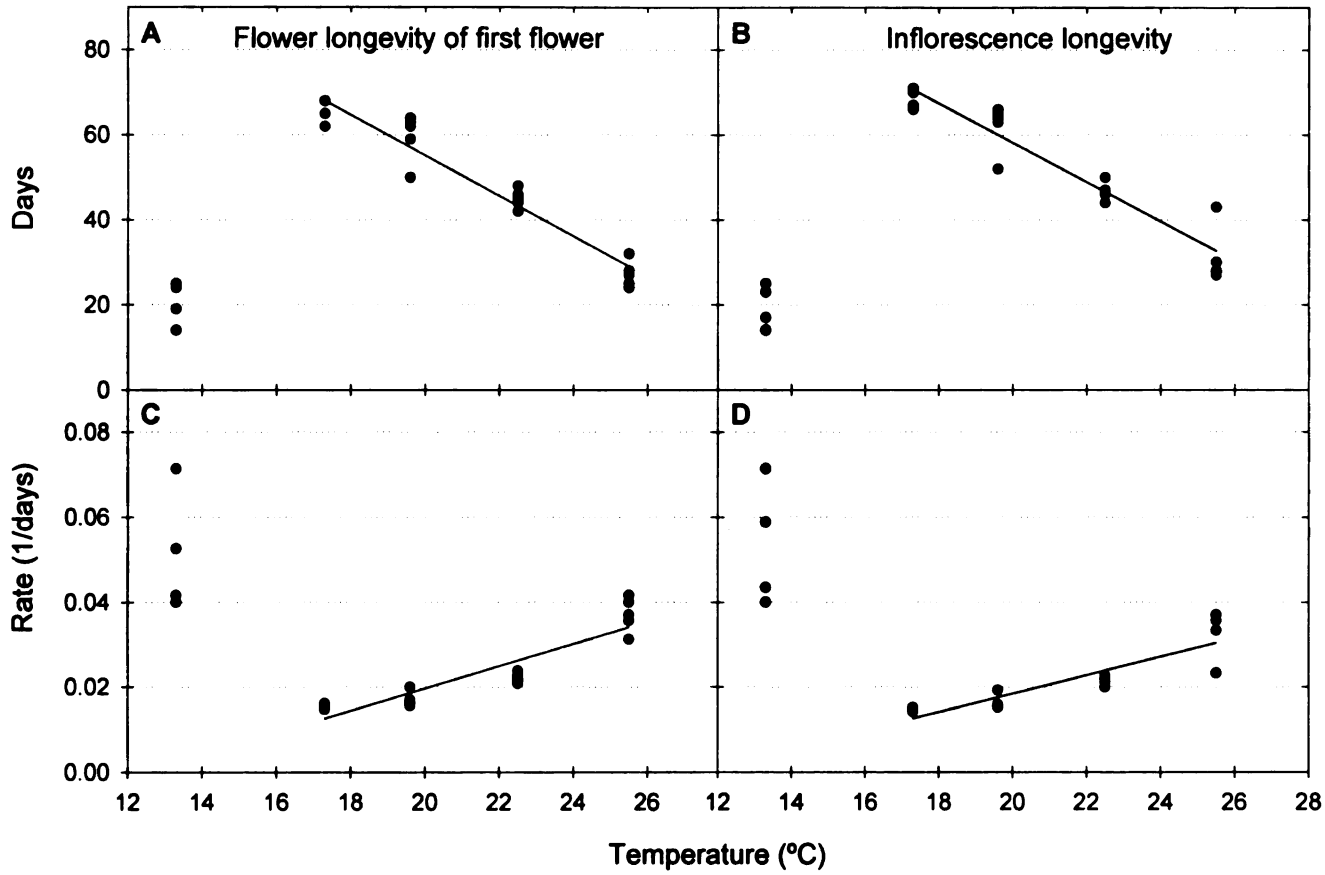

Figure 1. Influence of forcing temperature on longevity of the first open flower and inflorescence of *Miltassia* Charles M. Fitch 'Dark Monarch'. Each symbol represents individual plants. (**A**) and (**B**) show days for the indicated developmental stage. The solid lines represent the regression equation. Lines in (**C**) and (**D**) represent predicted values for the rate of progress (1/no. days) to the indicated developmental stage, based on linear regression. Statistical analysis is presented in Table 1.

APPENDIX B

THE EFFECT OF TEMPERATURE ON NODE DEVELOPMENT OF BRASSIA REX 'SAKATA'

Research Objective

The research objective of this investigation was to quantify how temperature influenced leaf unfolding rate and leaf expansion of *Brassia* Rex 'Sakata'.

Materials and Methods

Plant material and culture. Plants were propagated and grown as described in Appendix A, unless other wise noted.

Environmental chamber temperature and photoperiod control. The experiment began on 11 June 2001 and was performed in controlled-environment chambers with constant temperature set points of 14, 17, 20, 23, 26, and 29 °C. The average daily air temperatures during the experiment were 13.4, 16.7, 19.5, 22.4, 24.8 and 28.6 °C. Each chamber was divided in half with black plastic and two photoperiods were created: 9-h of light with or without a 4-h (2200 to 0200 HR) night interruption (NI). The 9-h base photoperiod was provided by a combination of cool-white fluorescent (VHOF96T12, Philips, Bloomfield, NJ.) and incandescent lamps from 0800 to 1700 HR at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Night interruption lighting (≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) was provided by incandescent lamps.

Leaf development. Twenty plants were assigned randomly to each walk-in controlled-environment chamber, and ten were placed under each photoperiod.

At the beginning of the experiment, one immature pseudobulb was identified and leaf unfolding was recorded weekly by counting the number of leaves from the soil level to the terminal end of the pseudobulb. The leaf length of a marked leaf was measured weekly and leaf expansion rate was calculated

Data analysis. The experimental design was completely randomized. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED) for analysis of variance and linear models procedure (PROC REG) for regression models. Base temperature (T_b) and thermal time, or degree-days, were calculated as described in Appendix A.

Results and Discussion

Photoperiod (9-h or NI) had no significant influence on leaf unfolding or leaf expansion and thus data was pooled. Leaf unfolding increased as temperatures increased from 14 to 26 °C (Fig. 1). At 29 °C leaf unfolding began to slow down. The days required to produce one leaf averaged 75 d at 14 °C, and 12 d at 26 °C. The leaves of plants unfolded as a linear function within the range of temperatures tested (Table 1). The base temperature for LUR was estimated at 11.6 °C with 179 °C·d^{-1} degree-days (Table 1).

Leaf length was significantly influenced by temperature (significantly different at $P \leq 0.001$). As temperature increased from 13 to 29 °C, leaf expansion increased (Fig 2). Leaf expansion was similar at temperatures from 22.4 to 28.6 °C. After 80 d, at the various temperatures, leaf length at 28.6 °C was 3.4 times longer than of plants at 13.4 °C.

Higher temperatures caused more rapid LUR and leaf expansion. Relative growth rate increased linearly to ≈25 °C, indicating that the maximum temperature may be < 29 °C. These results are not surprising since *Brassia* Rex 'Sakata' was bred in Hawaii to tolerate warm temperatures. The calculated, T_b is low (11.6 °C), but *Brassia* only exhibits signs of chilling injury at temperatures ≤ 8 °C (Section V).

Table 1. Parameters of linear regression analysis relating forcing temperature to rate of progress to leaf development in *Brassia* Rex 'Sakata'. Intercept and slope were used in Eqs. [1] and [2] to calculate base temperature (T_b) and thermal degree-days ($°C \cdot d^{-1}$).

Developmental stage	Intercept (b_0) (1/days)	Slope (b_1) (1/days)/ °C	T_b (°C)	$°C \cdot d^{-1}$	r^2
leaf unfolding	-0.0651 ± 0.0161[z]	0.0056 ± 0.0008	11.6	179	0.37[***y]

[z]Standard error.
[y] [***]Significant at $P \leq 0.001$.

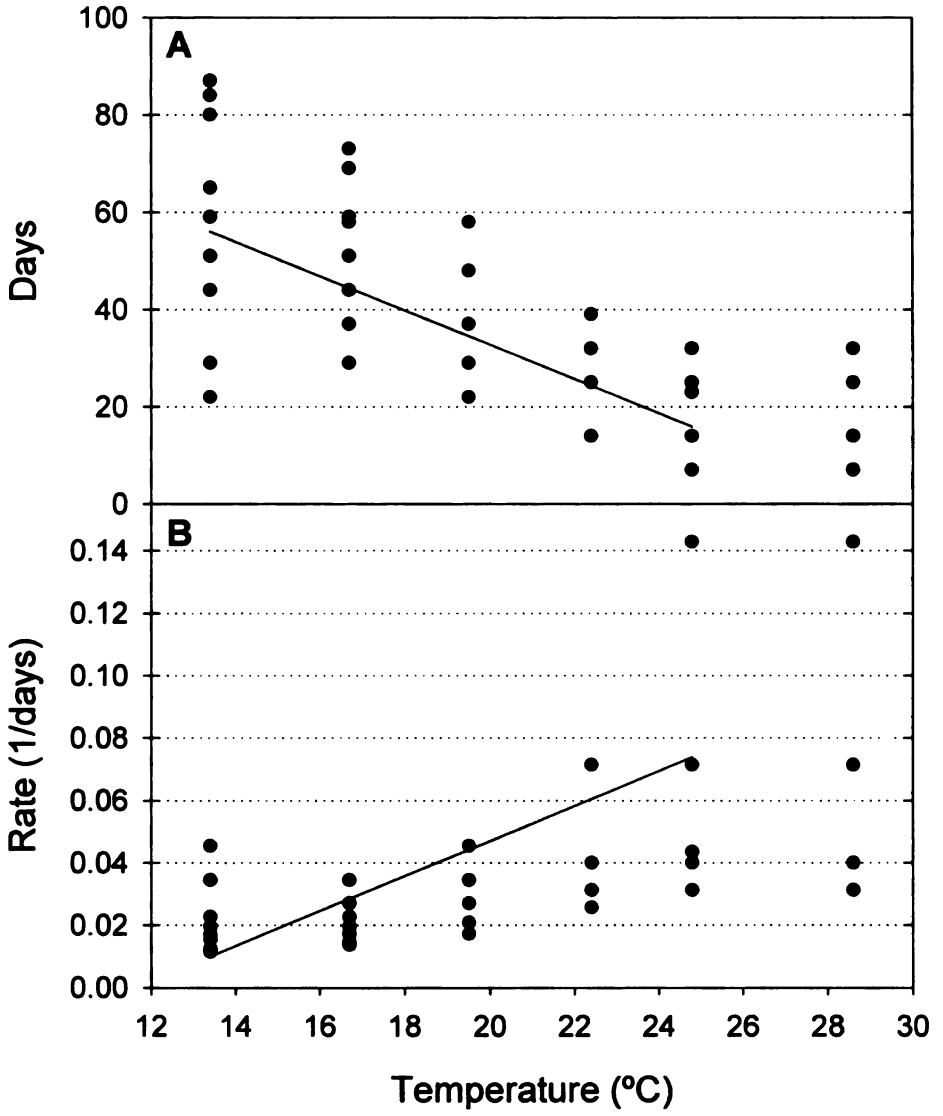

Figure 1. Influence of forcing temperature on leaf unfolding in *Brassia* Rex 'Sakata'. Each symbol represents individual plants (total of 20). (**A**) shows days for the indicated developmental stage. The solid lines represent the regression equation. Lines in (**B**) represent predicted values for the rate of progress (1/days) to leaf unfolding based on linear regression. Statistical analysis is presented in Table 1.

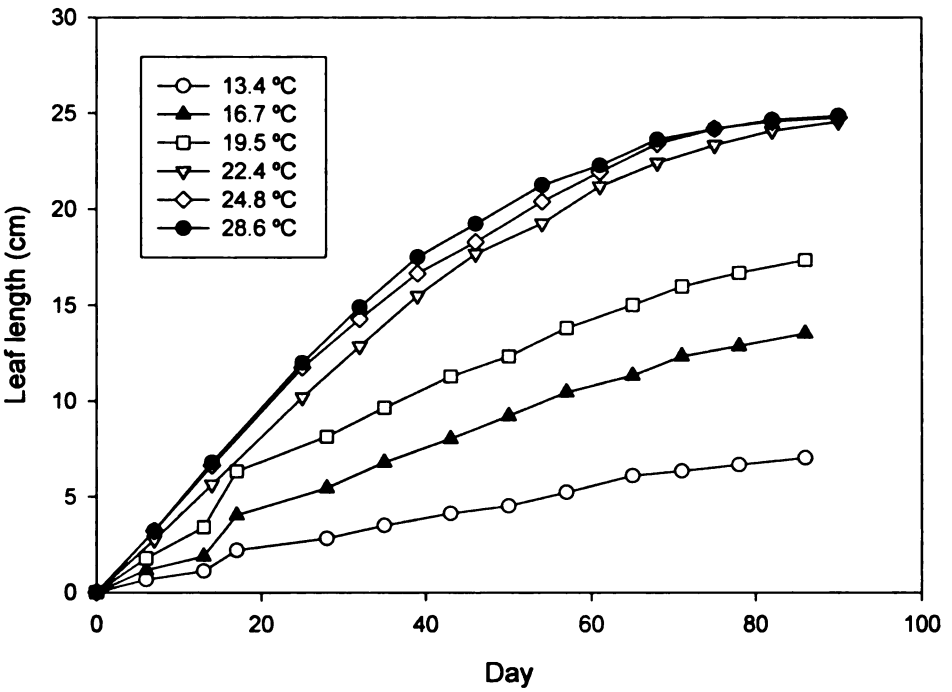

Figure 2. Influence of forcing temperature on daily increase in leaf length (cm) in *Brassia* Rex 'Sakata'. Each symbol represents the average of 20 plants.

APPENDIX C

THE EFFECT OF LIGHT QUANTITY ON GROWTH OF FOUR ORCHIDS

Research Objective

The research objective of this investigation was to determine if daily light integral (DLI) influences vegetative growth and flowering of *Brassia* Rex 'Sakata', *Degarmoara* Winter Wonderland 'White Fairy', *Miltassia* Charles M. Fitch 'Dark Monarch' and *Zygopetalum* Redvale 'Fire Kiss'.

Materials and Methods

Plant material. Plants were grown by a commercial greenhouse (Calif.), *Brassia*, *Degarmoara*, and *Miltassia* were transplanted into plug liners in May and June 1998, and into 10-cm pots in May and June 2000. *Zygopetalum* were transplanted into plug liners in June 2000 and into 10-cm pots in April 2001. plants were grown as previously described in Appendix A. Plants were grown in a glass greenhouse with a constant temperature setpoint of 23 °C. Air temperature in each greenhouse was measured by a thermocouple in an aspirated chamber every 10 s, and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, Utah). Average daily air temperature during the experiment was 25.5 °C.

DLI Experiment. Five plants were placed under five different DLI treatments. Light transmission through the greenhouses was reduced using permanent woven shade curtains that reduced light by ≈15, 27, 50, 75 and 90% (OLS 50; Ludvig Svensson, Charlotte, N.C.) to create the different DLI treatments. Average daily light integral for each treatment from the beginning of

the experiment until the end of forcing was estimated by calculating the DLI in the greenhouse without any shade multiplied by the shading percentage (Table 1).

Data collection and analysis. Inflorescence and flower bud count, final immature and mature pseudobulb count, final plant height, SPAD values (chlorophyll content), and dry shoot and root weights were recorded. The percentage of plants that initiated flowers was calculated. The few plants that died during the experiment were discarded and not included in the results. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED).

Results and Discussion

Brassia. Daily light integral did not influence inflorescence, flower bud and mature pseudobulb count, plant height, shoot, and root dry weights of *Brassia* Rex 'Sakata'. As DLI increased from 5.5 to 0.6 $mol \cdot m^{-2} \cdot d^{-1}$ immature pseudobulb count decreased from 5.2 to 1.7. Chlorophyll content was also influenced by DLI, as DLI decreased, SPAD values increased from 58.1 to 69.6.

Degarmoara. Inflorescence, flower bud and immature pseudobulb count, plant height, shoot or root dry weights of *Degarmoara* Winter Wonderland 'White Fairy' were not influenced by DLI. Plants under the low DLI treatment had significantly fewer mature pseudobulbs than those at DLI > 1 $mol \cdot m^{-2} \cdot d^{-1}$. As daily light integral decreased from 7.2 to 0.6 $mol \cdot m^{-2} \cdot d^{-1}$, chlorophyll content increased from 44.2 to 55.6.

Miltassia. Daily light integral did not influence inflorescence, flower bud immature and mature pseudobulb count and root dry weights of *Miltassia* Charles M. Fitch 'Dark Monarch'. Plant height decreased slightly as DLI decreased from 7.9 to 0.6 $mol \cdot m^{-2} \cdot d^{-1}$. Chlorophyll content was also influenced by DLI, as DLI decreased, SPAD values increased from 51.0 to 63.3. Shoot dry weight decreased from 52.6 to 32.7 g as DLI decreased from 7.2 to 0.6 $mol \cdot m^{-2} \cdot d^{-1}$.

Zygopetalum. Flower bud, immature pseudobulb count, and plant height of *Zygopetalum* Redvale 'Fire Kiss' were not influenced by DLI. As DLI decreased from 7.2 to 0.6 $mol \cdot m^{-2} \cdot d^{-1}$ inflorescence and pseudobulb count both decreased. SPAD values were greatest at 0.6 $mol \cdot m^{-2} \cdot d^{-1}$. Shoot and root dry weights were highly influenced by DLI. As DLI decreased from 7.2 to 0.6 $mol \cdot m^{-2} \cdot d^{-1}$, both shoot and root dry weight decreased almost three fold.

Interestingly, in all the orchid species investigated as daily light integral decreased from 7.9 to 0.6 $mol \cdot m^{-2} \cdot d^{-1}$, SPAD values increased. Consequently, plants allocated more resources into cholorophyll production than to other sinks due to lower light levels. However, inflorescence and flower buds, which are resource sinks, were not significant influenced by DLI. In all the species, as DLI decreased, flower initiation decreased and at 0.6 $mol \cdot m^{-2} \cdot d^{-1}$ few or no plants initiated flowers.

Table 1. The effect of estimated daily light integral (DLI) on inflorescence and flower bud count, number of mature and immature pseudobulbs, plant height, SPAD value, and dry shoot and root weights of *Brassia* Rex 'Sakata' grown at 23 °C under a 16-h photoperiod.

Shade (%)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Flower initation (%)	Inflorescence count	Flower bud count	Pseudobulbs		Plant height (cm)	SPAD value[z]	Shoot dry wt. (g)	Root dry wt. (g)
					Mature	Immature				
15	7.9	60	1.0	9.7	5.0	3.8	29.6	58.1	47.5	12.8
27	7.2	20	1.0	7.0	4.0	3.2	30.5	53.3	40.0	13.3
50	5.8	40	1.0	7.5	5.4	5.2	27.5	64.7	43.5	15.5
75	1.5	0	0.0	0.0	4.2	2.6	28.7	69.0	37.6	11.9
90	0.6	0	0.0	0.0	3.8	1.7	29.2	69.6	31.4	10.5
Significance										
DLI			NS	NS	NS	**	NS	***	NS	NS

[z]Chlorophyll content.

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

Table 2. The effect of estimated daily light integral (DLI) on inflorescence and flower bud count, number of mature and immature pseudobulbs, plant height, SPAD value, and dry shoot and root weights of *Degarmoara* Winter Wonderland 'White Fairy' grown at 23 °C under a 16-h photoperiod.

Shade (%)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Flower initation (%)	Inflorescence count	Flower bud count	Pseudobulbs		Plant height (cm)	SPAD value[z]	Shoot dry wt. (g)	Root dry wt. (g)
					Mature	Immature				
15	7.9	80	3.3	-[y]	9.6	6.4	37.7	45.1	49.5	15.3
27	7.2	60	1.3	8.0	9.2	4.4	35.9	44.2	45.2	11.7
50	5.8	60	2.0	12.0	7.4	6.4	39.9	51.7	42.9	12.7
75	1.5	60	2.7	14.7	8.6	4.8	37.9	55.5	48.6	12.4
90	0.6	20	1.0	8.0	5.7	2.7	36.9	55.6	30.7	8.9
Significance										
DLI			NS	NS	*	NS	NS	**	NS	NS

[z]Chlorophyll content.

[y]Flower bud count could not be determined at the end of the experiment.

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

Table 3. The effect of estimated daily light integral (DLI) on inflorescence and flower bud count, number of mature and immature pseudobulbs, plant height, SPAD value, and dry shoot and root weights of *Miltassia* Charles M. Fitch 'Dark Monarch' grown at 23 °C under a 16-h photoperiod.

Shade (%)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Flower initation (%)	Inflorescence count	Flower bud count	Pseudobulbs		Plant height (cm)	SPAD value[z]	Shoot dry wt. (g)	Root dry wt. (g)
					Mature	Immature				
15	7.9	100	4.8	34.4	9.0	3.8	37.0	52.4	46.1	11.6
27	7.2	100	4.6	28.0	9.4	3.0	32.1	51.0	52.6	9.9
50	5.8	100	3.8	18.0	7.6	4.6	32.7	63.9	37.6	12.2
75	1.5	80	3.8	23.8	9.5	2.3	34.9	65.7	39.8	10.4
90	0.6	40	2.5	11.0	9.3	2.3	31.2	63.3	32.7	8.8
Significance										
DLI			NS	NS	NS	NS	*	***	*	NS

[z]Chlorophyll content.
NS, *, ***nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

Table 4. The effect of estimated daily light integral (DLI) on inflorescence and flower bud count, number of mature and immature pseudobulbs, plant height, SPAD value, and dry shoot and root weights of *Zygopetalum* Redvale 'Fire Kiss' grown at 23 °C under a 16-h photoperiod.

Shade (%)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Flower initation (%)	Inflorescence count	Flower bud count	Pseudobulbs		Plant height (cm)	SPAD value[z]	Shoot dry wt. (g)	Root dry wt. (g)
					Mature	Immature				
15	7.9	100	2.2	5.5	2.2	2.4	26.2	49.2	9.2	4.9
27	7.2	100	3.0	6.4	2.4	2.2	25.9	43.0	12.2	6.7
50	5.8	80	2.8	5.3	1.8	2.6	27.3	53.9	10.2	6.2
75	1.5	60	1.3	2.3	1.8	3.2	25.0	47.4	9.5	5.2
90	0.6	0	0.0	0.0	1.0	1.8	26.1	51.3	4.5	2.2
Significance										
	DLI		*	NS	*	NS	NS	***	***	***

[z]Chlorophyll content.

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